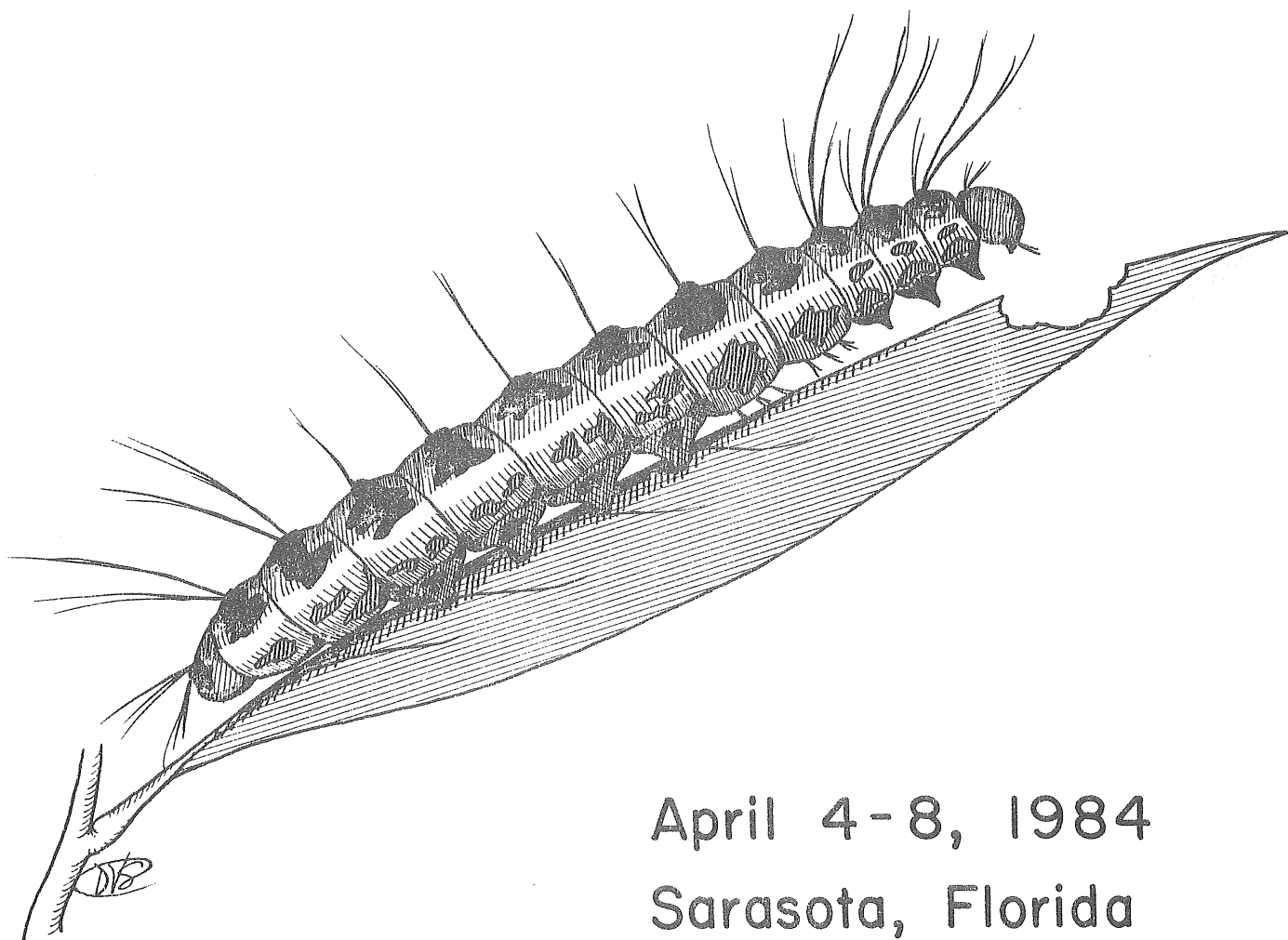


# ACChemS VI Abstracts

The Sixth Annual Meeting of the  
Association for  
Chemoreception Sciences



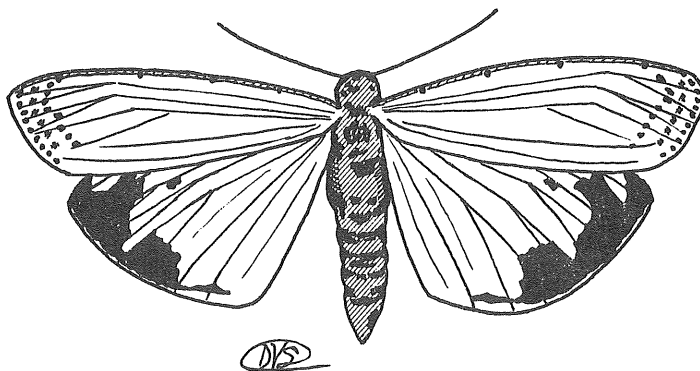
April 4-8, 1984  
Sarasota, Florida

## 6th Annual Givaudan Lecture

1

The Chemical Basis of Obnoxiousness: Survival in Animals and Plants. THOMAS EISNER (Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853).

Many animals and plants are protected by toxins, repellents, or distasteful agents, which render them obnoxious to their enemies. Much has been learned in recent years about such defensive substances, which are extraordinarily diverse in both chemical structure and mode of action. Insects in particular are well protected chemically. While many synthesize their own defensive substances, others appropriate them from plants, raising interesting questions about the use of exogenous chemicals (including perfumes) by humans.



# SYMPOSIUM: Ethological and Evolutionary Perspectives on Chemosensory Function in Reptiles

2

## Evolution and Ontogeny of Reptile Chemoreception.

GORDON M. BURGHARDT (University of Tennessee)\*

Squamate reptiles (lizards and snakes) have diverse means of obtaining information from the environment. The chemical senses are prominent among these, especially the vomeronasal system and associated tongue movements. This presentation will review recent work from our laboratory on the use of chemical cues in feeding, defensive, and social behavior. Particular attention will be paid to the behavior of neonatal animals.

Neonate reptiles have been shown to recognize prey, enemies and conspecifics via chemical stimuli most likely mediated by the vomeronasal system. Snakes reared in isolation of similar natural stimuli also possess such recognition capabilities, although experience can play an important role. A particularly interesting system is that involving responses of snakes that prey upon other snakes and the prey snakes' response to their ophidian enemies.

Tongue movements and the details of their topography and rates vary across species, families, and contexts. However, even lizards with typically low rates of tongue flicking will show surprisingly high rates in some contexts, such as novel environments, as we have shown in neonate and adult green iguanas in both laboratory and field.

Reptiles are ideal organisms for the study of many behavioral, physiological, genetic, and ecological aspects of chemoreception. Yet the advantages they possess have only been marginally exploited to date.

\* Supported in part by NSF research grant BNS 82-17569. Many students and colleagues aided in the studies to be reported.

4

Behavioral and Physiological Ecology of Den Location by Neonatal Prairie Rattlesnakes: Skin-derived Chemical Signals as an Adaptive Mechanism. David Duvall, Brent Graves, Michael King, Geoffrey Carpenter (University of Wyoming).

Female prairie rattlesnakes (*Crotalus viridis*) in Wyoming give birth to live young near, but not at, den entrances in late summer. How neonates find the den they have never visited previously has been enigmatic. Our field studies have verified that they do. A thermally stable chemical signal might provide a mechanism, and we have found that nonvolatile lipids extracted from skin facilitate behavioral actions (i.e., locomotion, tongue flicking, mouth gaping, aggregation) in lab studies that could mediate this event in the field. These exudates are likely expressed after skin shedding, and passively deposited by other adult snakes that return to the den just prior to neonate arrival. Our field work suggests that this hypothesis is reasonable, and the importance of young finding the den may be the function that has resulted in the effect of pregnant (but not nonpregnant) females remaining near their dens consistently from spring to fall. Finally, since these skin lipids are also involved in modulating rates of cutaneous water loss, it is possible that their role in neonate den aggregation has been derived secondarily.

Our work on this problem has been supported in part by funds from the National Geographic Society and the College of Arts & Sciences, and we continue to thank Dr. Paul Weldon for advice on extraction methods.

3

Phylogeny and Ontogeny of Strike-induced Chemosensory Searching and Trailing Behavior. David Chiszar (National Science Foundation and University of Colorado).

Striking rodent prey releases a sustained, high rate of tongue flicking in rattlesnakes. Called strike-induced chemosensory searching, this phenomenon has many properties of a modal action pattern and it contributes to the snake's ability to follow the trail left by the envenomated rodent. The latter point has been verified under lab and field conditions. This pattern of behavior has recently been observed in many other viperid species, and several elapids have exhibited similar behaviors. Accordingly, it is concluded that these behaviors are characteristic of rodent-specializing venomous snakes. Ontogenetic analyses indicate that the behaviors are innate and that they do not deteriorate during periods of disuse in captivity. Our data coupled with recent findings of Grove and Pough suggest that specialization upon rodents by early viperids gave rise to an ambush style of predation which, in turn, placed importance on the predator's ability to capitalize upon any prey wandering into striking range, including large ones. This need was probably the selective force behind many viperid characteristics such as wide heads and stout bodies (to accommodate large bolus), tendency to release rodents after envenomation (to reduce risk of injury from teeth and claws), and excellent trailing ability (to reduce risk of losing prey). This combination of anatomical and ethological evidence creates an evolutionary perspective which sees the commitment to ambushing as the critical step leading to the selection of a suite of synergistic characters organized around the formidability of rodent prey.

5

Is the Vomeronasal System Important for Maintaining the Reinforcing Value of Prey Stimuli? MIMI HALPERN (Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, N. Y. 11203).

A functional vomeronasal (VN) system is critical for discriminated responding to chemical stimuli arising from prey (Burghardt & colleagues, 1966-1975; Kubie, Halpern & colleagues, 1976-1983). Is the VN system also involved in evaluating prey stimuli for their reward value? We (Kubie & Halpern, 1975) have previously demonstrated that garter snakes (genus *Thamnophis*) will accurately follow prey trails (earthworm wash-EW) for food rewards (earthworm bits). Following VN nerve section EW trailing falls to chance levels (Kubie & Halpern, 1979). Whereas postoperative trailing accuracy falls immediately, worm bit ingestion extinguishes over several trials or sessions. We imagine that VN-mediated cues which are both critical for unconditioned prey attack and are rewarding without prior conditioning become associated, during training, with nonVN-mediated cues in the apparatus. Snakes can be trained to anticipate the delivery of worm bits to a conditioned stimulus (feeding dish) and to attack and ingest the food bit. Following VN nerve cuts this conditioned prey attack (CPA) is not disrupted. Snakes trained in both the trailing task and the CPA situation do not extinguish feeding behavior in the trailing task although trailing accuracy falls to chance levels immediately following surgery. Three stages of sensory control of feeding behavior can be postulated: 1. with no conditioning prey attack is dependent on a functional VN system and the VN stimulation is rewarding; 2. nonVN-mediated cues can become associated with prey and these cues may be sufficient to elicit prey attack in the absence of VN stimulation, but are susceptible to extinction; 3. with sufficient pairing, and by a mechanism we cannot explain at present, nonVN-mediated cues become more resistant to extinction. Thus although the VN system may be important normally for maintenance of the reinforcing value of food reward, if the reward has become conditioned to nonVN-mediated sensory cues, prey attack and ingestion will not be disrupted following VN lesions.

Supported by NIH Grant NS11713.

# SYMPOSIUM: Physical Chemistry of Stimulus Access and Removal

6

Hydrodynamic and Surface Chemical Effects of Chemosensory Stimuli. JOHN A. DESIMONE (Department of Physiology and Biophysics, Box 551, MCV Station, Richmond, VA 23298)

Unlike vision and audition where the stimulus can be presented as a square wave of energy at the receptor cells, the wave form of the stimulus in the chemosenses is always determined by convection and diffusion. Consequently the concentration of the stimulus reaching the reactive cell regions and its rate of arrival will be governed initially by hydrodynamic variables. The final approach of the stimulus to the surface will, however, always be limited by diffusion. The thickness of the "diffusion boundary-layer" can be reduced by increasing the convective fluid velocity, or decreasing the fluid viscosity. The influence of stimulus application variables on various properties of the response is well-documented in both olfaction and taste. The latency in both olfaction and taste, and the phasic part of the gustatory response are flow rate dependent. Analysis shows that increasing the stimulus concentration, and the fluid velocity can shorten the latency. The theory also provides a mechanistic method of describing the early phasic rise in the gustatory neural response profile. In the case of salt and acid stimuli there are additional surface chemical effects which may arise due to the interaction of bound surface charge with the mobile stimulus anions and cations. Lateral surface pressures may cause either expansion (increased membrane fluidity) or condensation (decreased fluidity). Whether the stimulus results in one or the other effect depends on the nature of the surface moieties, surface pH, ionic strength, and charge density. Failure to deal with the dynamics of the stimulus may result in serious misinterpretations of the physiological response variables.

8

On Knowing Stimulus Intensity at the Receptor. ROBERT C. GESTELAND (Northwestern University, Evanston, IL 60201)\*

Studies on olfactory and gustatory neural responses generally specify the concentration of the stimulus substance in the bathing solution or in the air stream issuing from the stimulator. Conclusions about relative sensitivity of the neural unit to different stimulus substances expressed in these terms are behaviorally relevant because they describe the reaction of the organism to stimuli as they are encountered in its world. In studies on the biophysical and biochemical processes which constitute stimulus reception and transduction, however, a measurement or an estimate of the stimulus concentration at the receptor is required. This is difficult to acquire, even with the simplest assumption that the stimulus is maintained for a long enough period for the concentration at the receptor to be in equilibrium with that in the surrounding medium. Processes which intervene between stimulus delivery and stimulus action include the following: a, partition of the stimulus between air (or water) and extracellular fluid (mucus or saliva); b, partition between mucus, membrane lipid, membrane surface protein, and (if present) stimulus-specific membrane receptors; and c, establishment of equilibrium between stimulus and cellular removal processes. If the stimulus is of short duration, equilibrium or steady-state is not achieved and regional distribution differences and diffusion times in the several phases will also strongly affect the concentration of the stimulus at the receptor at any instant. Recently, as a result of rapid advances in computation power and of successful modeling of relations between functional groups and physical-chemical parameters, it has become possible to predict vapor pressures, solubilities of sparingly-soluble compounds, and partition and diffusion coefficients. Users of the data base system need specify only the molecular formulas of the solute and of the solvents. Our experience using the data base will be discussed.

\*Supported by by NIH Grants NS18490 and NS14663 and NSF Grant BNS-8117075.

7

Some Counter-Intuitive Properties of Diffusion and Their Role in Understanding Chemoreception in Insects and Other Organisms R. P. FUTRELLE (University of Illinois, Urbana)\*

Pheromone chemoreception in insects shows remarkable specificity and sensitivity. In the silkworm *Bombyx mori* the cells in the sensory hair produce a spike for each single molecule that gets to them. We describe what is known currently about the pathway of the pheromone molecule: its movement in the air, diffusion to the sensillum surface, capture, diffusion to the pore and the dendrite, and its final degradation.

Small odorant molecules move at the speed of sound and traverse complex, tortured paths. The combination of correlated and random properties which these paths display leads to some unusual consequences. These have been explored by Berg and Purcell for cell chemoreception and by the author for chemotaxis and pheromone reception (Trends in Neurosciences, 1984). We will explain, for example, how the shape and sensor distribution of an insect sensillum can lead to extraordinary efficiency in pheromone reception, and how such conclusions follow from the properties of molecular diffusion.

\*Supported in part by NSF83-02985.

9

Do Odorants Need To Penetrate The Olfactory Cell Membrane To Gain Access To Their Receptors? Robert C. MacDonald, Northwestern University, Evanston, IL 60201

Like passive membrane permeability and the actions of many membrane-active drugs, olfactory sensitivity, particularly within a congeneric series, increases with increasing oil/water partition coefficient. One interpretation of such a correlation is that the site of action of the molecule in question is either within a membrane or shielded by a membrane. Such correlations can also come from hydrophobic surfaces on hydrophilic proteins; however, in the case of alcohols, which have been particularly well studied, the data indicate that these molecules become completely immersed in a hydrophobic domain. In the hope of identifying possible mechanisms of transduction or revealing new experimental approaches, some consequences of cell membrane penetration by an odorant will be examined. One such consequence, at least for molecules with dielectric constants higher than that of a lipid bilayer core, is that they would tend to congregate around any charged group that were immersed within or near the surface of the membrane. This conclusion, which follows from electrostatics, would apply to essentially all molecules containing atoms other than C and H. Thus, most odorants, if they partition significantly into the membrane, would increase the solubility of ions by acting, in effect, as dissociable ionophores. Also, charged amino acid residues near the internal surface of the membrane surface would be less forcibly restricted to the aqueous interface. Odorants could therefore affect membrane conductance either by increasing the number of charge carriers and/or by affecting the conformation of a channel-controlling protein. Another potential consequence of entry of an odorant into a membrane is a perturbation of interfaces within the membrane itself. Hydrophobic odorants containing a functional group are often sufficiently amphiphilic to accumulate at surfaces. Such effects have been described for the polar-nonpolar interface of lipid bilayers and a similar accumulation at lipid-protein contact area may be expected. Such internal surface activity, even at forces as small as a few tenths of a dyne/cm, could be sufficient to alter the equilibrium of a two-state channel protein with a gating energy of a few kcal/mol.

## VOLUNTEER ABSTRACTS

10

Feeding Stimulants for Herbivorous Fish. MICHAEL A. ADAMS and PETER JOHNSEN (MONELL CHEMICAL SENSES CENTER, 3500 MARKET ST., PHILADELPHIA, PA 19104)\*

*Tilapia zillii* is an herbivorous fish highly prized for food and widely cultivated in aquaculture throughout the world. To determine which plant-derived substances induce fish to feed, Romaine lettuce, a plant that *Tilapia* find acceptable as food, was chemically fractionated into several portions by functional group. These fractions included: a protein-depleted extract, a lipid-containing extract, a lipid- and protein-depleted extract, an organic acids and sugars fraction and an amino acids fraction. Finally, sugars as a group were separated from organic acids. Taste stimuli were incorporated into cylindrically shaped agar disks and were suspended along with unflavored agar blank disks in test tanks. The fish were then allowed to feed *ad libitum* for a fixed period of time. Afterwards, the agar disks were removed from the tanks and weighed. Weight loss in a stimulus disk, when corrected for consumption of the blank, was used as a measure of preference for a particular substance.

Analysis of the results revealed that the amino acid fraction contained stimulatory activity; this activity was mainly due to glutamic acid. The order of amino acid preference generally followed the order of abundance of amino acids in the lettuce. In addition, malic and citric acids, two organic acids occurring in Romaine lettuce, were tested for activity, even though the organic acids fraction was found to be inactive. While *Tilapia* were indifferent to malic acid, they showed a strong preference for citric acid. Subsequent bioassays showed that the fish would consume significantly greater amounts of lettuce-flavored agar to which citric acid had been added than they would agar flavored with lettuce alone.

\*This work was supported by a grant from the Rockefeller Foundation (GA COH 8117).

12

Peripheral-type Benzodiazepine Receptors in the Central Nervous System: Localization to Olfactory Nerves. ROBERT R.H. ANHOLT, KENNETH M.M. MURPHY, GREGORY E. MACK and SOLOMON H. SNYDER (Johns Hopkins Univ., School of Medicine, Dept. of Neuroscience, Baltimore, MD. 21205).

We have used Ro5-4864, a ligand selective for peripheral-type benzodiazepine receptors, to demonstrate the presence of a high density of these receptors on primary olfactory neurons originating in the nose and synapsing in the olfactory bulb. Binding levels of [<sup>3</sup>H]Ro5-4864 are substantially higher in homogenates of the olfactory bulb than in the rest of the brain. Among peripheral tissues evaluated high levels of [<sup>3</sup>H]Ro5-4864 binding are found in the nasal epithelium. Drug displacement studies show that these binding sites are pharmacologically of the peripheral-type. Their presence in the nasal epithelium and in the olfactory bulb can be demonstrated in several mammalian species. Autoradiographic studies of murine nose reveal a bipolar staining pattern around the cell bodies of the olfactory receptor cells, suggesting the presence of peripheral-type benzodiazepine receptors on both processes of these bipolar neurons. In the brain a high density of [<sup>3</sup>H]Ro5-4864 binding sites occurs in the nerve fiber and glomerular layers of the olfactory bulb. Throughout the rest of the brain [<sup>3</sup>H]Ro5-4864 associated silver grains are diffusely distributed with intense staining over the choroid plexus and along the ependymal linings of the ventricles. Both the distribution and the ontogenic development of the peripheral-type benzodiazepine receptors differ from the central-type receptors. Intranasal irrigation with 5% ZnSO<sub>4</sub> results in a 50% reduction of peripheral-type benzodiazepine receptors in the olfactory bulb without affecting the density of central-type benzodiazepine receptors. Thus, [<sup>3</sup>H]Ro5-4864 binding sites in the olfactory bulb appear in large part to be localized to olfactory nerves which originate in the nasal epithelium. We speculate that the high density of these sites on primary olfactory nerves may be related to the continuous turn-over of these cells and the great regenerative capacity of the olfactory epithelium.

11

Voltage Clamping the Receptor Potential in Olfactory Receptor Cells. Peter A. V. Anderson and Barry W. Ache (C. V. Whitney Laboratory, University of Florida)\*

The antennules of decapod crustaceans such as crabs and lobsters bear bipolar receptor cells which innervate aesthetasc sensilla and have morphological, physiological and functional features in common with the olfactory receptors of terrestrial arthropods and vertebrates. These are generally assumed to be the olfactory receptors of crustaceans. We applied the whole cell patch clamp recording technique to record intercellularly from these neurons, using both isolated-cell and *in situ* preparations.

All cells have high input impedance with pronounced outward rectification, and have long time constants. When depolarized to above -20 to -25 mV, they produced fast, overshooting action potentials. Receptor cells *in situ* exhibited a prolonged depolarizing receptor potential and spiked in a dose-dependent manner to chemical stimulation (extract of crab muscle tissue). The magnitude of the receptor potential was dependent on the resting potential, with one cell exhibiting a positive calculated reversal potential, suggesting that an inward positive current underlies the receptor potential. This view was verified by voltage clamping cells to a holding potential of -60 mV and noting a slow inward current on chemical stimulation. The amplitude of the inward current varied with holding potentials; the relationship between current amplitude and holding potential showed reversal at +40 mV.

Our results confirm that neurons innervating the aesthetasc sensilla of decapod crustacea are indeed chemosensory and, more importantly, demonstrate that the receptor potential of a primary olfactory neuron can be voltage clamped *in situ*. In doing so, they pave the way for detailed analysis of the ionic mechanism(s) underlying the receptor potential.

\*Supported by NSF grants BNS-5209849 and BNS-8308120

13

Olfactory Responses of Terrestrial and Aquatic Tiger Salamanders to Amino Acids. ADAM H. ARZT (Monell Chemical Senses Center, Philadelphia, PA 19104 and Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102), WAYNE L. SILVER and J. RUSSELL MASON (Monell Chemical Senses Center, Philadelphia, PA 19104).\*

Volatile odorants conveyed in air stimulate olfactory receptors of terrestrial vertebrates, whereas olfactory stimuli for aquatic species are present in water and are not necessarily volatile (e.g., amino acids). Whether terrestrial vertebrates respond to non-volatile compounds in solution remains unclear. In this investigation, terrestrial and aquatic olfaction were compared using the aquatic larval (neotenic) and terrestrial adult forms of the tiger salamander (*Ambystoma tigrinum*). The underwater electro-olfactogram (EOG) was used to examine the responses of both forms to various concentrations of amino acid solutions.

Both neotenic and adult salamanders responded to all amino acids tested (L-arginine, L-cysteine, L-glutamate, L-isoleucine, L-alanine and D-alanine). Response magnitudes increased exponentially with logarithmic increase in amino acid concentration, even at the highest concentrations (0.01 M). Mean threshold values were lower in aquatic salamanders than in adults for five of the amino acids tested (*p*'s < 0.02), L-cysteine being the exception (*p* > 0.50). In addition, the slopes of the curves in the log concentration vs. log response plot for L-glutamate and L-arginine were greater in adult than in neotenic salamanders. L-arginine was the most effective compound tested in both neotenes and adults; L-isoleucine and D-alanine were the least effective. L-glutamate was three times as effective in adults than in neotenes (relative to a 0.01 M L-alanine standard), and was the only amino acid for which relative effectiveness changed (*p* < 0.05).

These results indicate that aquatic salamanders are more sensitive than adults to amino acids presented in solution, suggesting that for tiger salamanders the change from an aquatic to a terrestrial environment is accompanied by a change in olfactory function. The nature of the environment surrounding the olfactory receptors may therefore affect their ability to respond to olfactory stimuli.

\*This work was supported in part by NINCDS Grant #1 ROI NS19424-01 to Drs. T. H. Morton and J. R. Mason.

Chemical Noise: Effects of Modified Amino Acid Backgrounds on Responses to Single Amino Acid Stimuli in the Lobster, *Homarus americanus*. JELLE ATEMA, MARILYN SPALDING, and LINDA HANDRICH (Boston University Marine Program).

Raw sea water contains free amino acid concentrations in the pico- to nanomolar range; ammonia occurs in micromolar quantities. This is the normal noise background for lobster chemoreception. Lobsters have prominent populations of receptor cells which are narrowly tuned for single amino acids and ammonia. Such cells are found both in smell and in taste organs. One might expect, therefore, that elevating the normal background for one amino acid should raise its detection threshold to this new level, but should not interfere with the reception of another. We used eight one-year-old lobsters of about 13 mm carapace length in 50 ml centrifuge tubes. We counted antennular-flicking rate to measure their responsiveness to 2 ml stimuli injected into the sea water background flow of  $0.6 \text{ ml} \cdot \text{s}^{-1}$ . We obtained dose-response curves for L-proline, L-glutamine and ammonia from  $10^{-12}\text{M}$  to  $10^{-3}\text{M}$  in single log steps.

In elevated backgrounds of  $10^{-8}\text{M}$  and  $10^{-6}\text{M}$  proline in sea water, the proline threshold shifted up to the new background levels, but the entire curve also dropped, indicating that even at high stimulus concentrations responses were suppressed in elevated backgrounds. This was also seen for glutamine and ammonia. Similarly and unexpectedly, the glutamine and ammonia curves dropped in elevated proline backgrounds; also, the proline response at  $10^{-6}\text{M}$  was completely suppressed by a  $10^{-6}\text{M}$  glutamine background. We conclude that the peripheral receptor cells converge centrally such that here their narrow tuning is not used behaviorally. In normal and elevated backgrounds lobsters responded to both higher and lower stimulus concentrations, indicating that sudden temporary dilution of only one amino acid in the whole background mixture cannot only be detected but can constitute a behaviorally significant stimulus.

Supported by Whitehall Foundation and NSF (BNS-82104434).

Taste and Aging. BARTOSHUK, L.M., MARKS, L.E., STEVENS, J.C. (John B. Pierce Foundation Lab), and RIFKIN, B. (Colgate-Palmolive).

Taste thresholds rise with age. To assess the possibility that suprathreshold function remains normal, we used a relatively new psychophysical method, magnitude matching, which produces cross-modality matching functions showing the dB levels of sound that match each taste concentration. Matching functions from control individuals can be compared to those of individuals whose sensory function is in question.

Magnitude matching functions and detection thresholds for sound (low frequency band of noise) and tastes (sucrose, NaCl, citric acid, and quinine hydrochloride) were obtained from 18 elderly (mean: 83 years) and 18 young (mean: 24 years) subjects. The detection thresholds for the elderly subjects were elevated about one log step for both taste and sound. The magnitude matching functions were similar for the highest taste concentrations. The only abnormality was that the elderly matched the dilute taste solutions (including water) to abnormally loud sounds).

Since many elderly individuals are known to have hearing losses, the choice of audition for the control modality may seem puzzling. However, audition produces stable magnitude matches; not all continua do so. Further, the effects of auditory loss were minimized by avoiding the high frequencies and low intensities known to be most severely affected by age. Thus although we believe that this question is far from settled, our evidence is consistent with the conclusion that the elderly show little if any taste loss for the higher suprathreshold concentrations. The elderly's elevated intensity judgments of low concentrations are consistent with a chronic dysgeusia that adds to the tastes of weak stimuli.

Supported by NIH grants AG 01331 and NS 16993

Extinction of Response to Urine Odor as a Consequence of Vomeronasal Organ Removal in Male Guinea Pigs. GARY K. BEAUCHAMP, CHARLES J. WYSOCKI, JUDITH L. WELLINGTON, (Monell Chemical Senses Center)\*

In mammals, the vomeronasal organ (VNO)/accessory olfactory system (AOS) plays a central role in neuroendocrine events. However, the role of these structures in regulating behavior is more mixed. We previously found that removal of the VNO in male guinea pigs disrupted investigatory responses to conspecific odors but this disruption appeared time-dependent; immediately following surgery, behavior appeared almost normal whereas several months following surgery, animals became almost totally unresponsive to conspecific odor. This curious time-dependent effect has also been observed in other species. We postulated that in the absence of a functional AOS, the main olfactory system (MOS) was capable of maintaining response to conspecific odor but that this response extinguished following repeated exposures. However, post-surgical change in the CNS, unrelated to exposure to the bioassay, remained a possible explanation. To test an extinction hypothesis, three groups of adult male guinea pigs were formed. For two groups (A and B) the VNO was surgically removed, while the third group (C) experienced sham surgery. Beginning 1-2 weeks following surgery, males in groups A and C were given 2 standard urine-response tests/week for 24 weeks during which investigation time was recorded. Initiation of testing of group B animals was delayed until week 16 following surgery. If a form of extinction was occurring, the following was predicted: 1) responses from group A would decline relative to group C; 2) at the first post-surgery test, group B would be as responsive to urine as group C and much more responsive than group A; 3) a decline in responses for group B should occur during tests after week 16. In contrast, if the decline resulted from change in the CNS following VNO removal, responses of group B should resemble those of group A during tests at week 16 and thereafter. The data strongly supported the extinction hypothesis. The MOS is therefore capable of eliciting a high level of investigatory behavior in response to female urine odors but, in the absence of the VNO, this response wanes, perhaps due to a loss of reinforcing properties associated with VNO/AOS stimulation.

\*Supported by the National Science Foundation (BNS 82-01759).

The Response Profile of the Glossopharyngeal and Chorda Tympani Nerves of the Mouse (C57BL/6J) to Sugars. L.M. BEIDLER and M.S. NEJAD (The Florida State University).

The analysis of single fiber responses to selected sugars have shown that multiple receptor sites for sugars exist (Beidler and Tonosaki, 1983). The purpose of this research was to demonstrate the simultaneous neural response profile of the glossopharyngeal and chorda tympani nerves to 12 selected sugars in the mouse (C57BL/6J). The integrated relative neural responses of the glossopharyngeal and chorda tympani nerves to half molar concentrations of sucrose, glucose,  $\alpha$ -methyl-d-glucoside, fructose, mannose, sorbose, xylose, galactose, rhamnose, maltose, arabinose and 0.3 molar lactose were simultaneously recorded ( $n=9$ ). A solution of 0.2M  $\text{NH}_4\text{Cl}$  was used as the reference and all the integrated responses were normalized with respect to 0.2M  $\text{NH}_4\text{Cl}$ . Examination of the normalized data showed that the relative magnitude response profiles of the chorda tympani (front of the tongue) and glossopharyngeal (back of the tongue) to the chosen sugars were different. The relative response profile of the chorda tympani nerve to  $\text{NH}_4\text{Cl}$  and 12 sugars was:  $\text{NH}_4\text{Cl} \gg \text{sucrose} \gg \alpha\text{-methyl-d-glucoside} \gg \text{fructose} \gg \text{mannose} \gg \text{sorbose} \gg \text{xylose} \gg \text{galactose} \gg \text{glucose} \gg \text{rhamnose} \gg \text{arabinose} \gg \text{lactose} \gg \text{maltose}$ . Whereas, the relative response profile of the glossopharyngeal nerve to  $\text{NH}_4\text{Cl}$  and 12 sugars was:  $\text{NH}_4\text{Cl} \gg \text{rhamnose} \gg \text{galactose} \gg \text{fructose} \gg \text{arabinose} \gg \text{mannose} \gg \text{lactose} \gg \alpha\text{-methyl-d-glucoside} \gg \text{sorbose} \gg \text{sucrose} \gg \text{xylose} \gg \text{glucose} \gg \text{maltose}$ . Sucrose was the high stimulating sugar for the front of the tongue, whereas, rhamnose, galactose and fructose were the high stimulating sugars for the back of the tongue. Maltose was the least stimulating sugar for the front and as well as the back of the tongue of the mouse. It was concluded that the sensitivity of the front and back of the tongue to sugars vary and it was indicated that sugar receptor sites were not totally the same in the front and back regions of the tongue.

Electron Microscopic Deoxyglucose Autoradiography of Quick Frozen Olfactory Bulb. T.E. BENSON\*, P.E. PEDERSEN\*, G.D. BURD\*, D.M.D. LANDIS#, and G.M. SHEPHERD\* (\*Yale Univ., @Rockefeller Univ., #Massachusetts General Hospital)

We have employed the quick freezing method of Heuser, Reese and Landis (Cold Spr. Harb. Symp. Quant. Biol., 40:17, '75) in the development of a technique for 2-deoxy-<sup>3</sup>H-glucose (2DG), electron microscopic (EM) autoradiography (ARG).

Twelve-day rat pups were injected intraperitoneally with 150 µCi/g 2DG and either suckled or were exposed to amyl acetate odor for 60 minutes. Slabs of freshly dissected olfactory bulb were frozen against a liquid He chilled copper plate and the frozen tissue simultaneously acetone substituted and osmicated. Tissue was embedded in plastic/silicone. Sections were cut on a dry knife and spread on anhydrous glycerol for light microscopic (LM) ARG. Eighty nm sections were cut onto glycerol for EM ARG. Photographic emulsion was placed over carbon-coated sections with an expanding loop (Telford & Matsumura, Stain Technol., 44: 259, '69).

Quick freezing yielded ultrastructural preservation of all bulbar laminae. Scintillation counting indicated that tracer washout was negligible and that label did not diffuse into glycerol used for sectioning. EM ARG corroborated these findings. It was also apparent that tracer translocation did not occur during emulsion application: At the tissue/plastic interface there were high grain densities over bulbar tissue, but only background or "crossfire" grains over the plastic.

LM ARG showed patterns of label uptake similar to those observed with more conventional freezing (Lancet et al., Proc. Natl. Acad. Sci. USA 79:670, '82). In preliminary observations of EM autoradiographs, silver grains were seen over terminals and dendritic cytoplasm in the neuropil of the external plexiform layer of the olfactory bulb.

Because of the apparent absence of tracer washout or translocation, this method appears to be suitable for the analysis of the incorporation of 2DG (and, perhaps, other diffusible substances) at the level of cellular processes and cell organelles.

(USPHS NRSA #5 F 32 NS06990-02 & NS 16993)

Stopped-Flow Analysis for Reaction Kinetics and Intensity-Time Models of Chemoreception. G.G. BIRCH and F.F. MORPETH, Food Studies, University of Reading, Whiteknights, Reading, Berks. RG6 2AP, UK.

The technique of stopped-flow analysis is well established (1) for enzymic characterisation, reaction rate determination and identification of transient metabolic compounds.

We now report the use of a Hi-Tech Stopped-Flow system for following the time course of reaction between proteins and sugars. The time course may be conveniently recorded (e.g. 295 nm) which represents a conformational distortion of the proteins on binding with sugar. A typical first order rate constant for 0.3 mM bovine serum albumin reacting with 0.56 M D-glucose at 20°C was  $57.5 \pm 1.8 \text{ sec}^{-1}$ , in phosphate buffer, pH 7.0. The apparatus is designed to allow a choice of sweep times between 10 msec and 10 sec but, under the above conditions, the reaction between bovine serum albumin and glucose appears to be complete within 20-30 msec. This time interval resembles those reported (2) for rat neurophysiological recordings rather than human psychophysical data (3,4) for the reaction time or persistence of taste response.

- 1) Morpeth, F.F. and Massey, V. *Biochemistry* 21, 1313-1317 (1982).
- 2) Marowitz, L.A. and Halpern, B.P. *Chem. Senses and Flavor* 2, 457-460 (1977).
- 3) Birch, G.G., Latymer, Z. and Hollaway, M. *Chem. Senses* 5, 63-78 (1980).
- 4) Ogunmoyela, O.O. and Birch, G.G. *J. Agric. Fd. Chem.* 30, 77;81 (1982).

Taste Response in People with Chinese or European Background. MARY BERTINO (Monell Chemical Senses Center), MABEL CHAN (Dept. of Home Economics and Nutrition, New York University).

Using category scales, previous work has demonstrated differences in sweetness and saltiness taste responses between individuals of Chinese vs. European backgrounds. To expand upon this work, we have tested new groups of individuals using magnitude matching to map the intensity functions.

Sixty students from New York University served as subjects. Thirty were Caucasians with European backgrounds and thirty were Chinese from the People's Republic of China. Each subject was required to estimate the sweetness of a concentration series of sucrose in water and the saltinesses of: (a) a concentration series of NaCl in water and (b) a concentration series of NaCl in low sodium chicken broth. Magnitude matching to the loudness of a series of 1000hz tones was used. Subjects also rated the pleasantness of the taste of each concentration using a category rating scale. Using category scales they also rated the pleasantness of cookies and of crackers with varying concentrations of sucrose and NaCl, respectively. All instructions were given in English and, for most of the Chinese, in Chinese.

After normalization to the 1000hz tones, no significant differences in the slopes or intercepts of the psychophysical functions were found for any of these tastants. Concentration dependent group differences in the rated pleasantness of sucrose in water, salt in water, sucrose in cookies and salt in crackers were found. In particular, the Chinese rated sucrose solutions as tasting more pleasant, which confirms previous work.

This work was supported by NIH-1715. We thank Campbell Soup Company for providing us with low sodium chicken broth and crackers.

Nicotinic (N) and Muscarinic (M) Cholinergic Receptors are Segregated and Coincide with Acetylcholinesterase (AChE) Localization in Rat Olfactory Bulb. G. Blaha, W. Blair, W.T. Nickell and M.T. Shipley (University of Cincinnati College of Medicine).

We report that the adult rat olfactory bulb has a distinctive pattern of AChE staining. High levels are present in the glomerular layer, the deep half of the external plexiform layer (epl) and the internal plexiform layer (ipl). Levels are slightly lower in the superficial half of epl and in parts of the granule cell layer. There are no intrinsic cholinergic (Ch) neurons in the bulb; the major source of Ch input arises in the diagonal band (DB) (Macrides, et.al., '81; Van Ooteghem, et.al., '83). In DB at least 90% of all AChE + neurons contain ChAT, strongly indicating that they are cholinergic.

Receptor sites were localized using the autoradiographic technique of (Young and Kuhar, '79). The ligand for N receptors was [<sup>125</sup>I]-α-bungarotoxin (α-BT); ligands for M receptors were [<sup>3</sup>H]-scopolamine (Scop) and [<sup>3</sup>H]-QNB.

α-BT binding is almost exclusively restricted to the glomeruli. In sections incubated prior to fixation there was occasionally α-BT binding in the deep half of epl. By contrast, Scop and QNB binding was low in glomeruli and very high throughout epl and ipl with some binding in the granule cell layer (gcl).

These results suggest that nicotinic receptors are mainly in the glomeruli while muscarinic receptors are present in epl, ipl and in some parts of gcl. Such strict laminar segregation of N and M receptors within the same neural structure has not been reported for any other part of the brain. DB-Ch inputs may act upon two fundamentally different receptors ("fast" and "slow") at two different levels of functional processing in MOB. The distribution of N and M receptors precisely matches the distribution of AChE indicating that this enzyme is a reliable marker for Ch synapses in the bulb.

Supported by: NIH NS 19730, NINCDS 18490; US ARMY DAMD-82-C-2272 and DOD DAA G-83-G-0064.

Effect of Dietary Sodium Restriction on Taste Responses to Salted Soups. CHRISTINA BLAIS, R. M. PANGBORN, M. F. FERRELL and N. BORHANI (Depts. Food Science and Technology, Nutrition, and Community Health, University of California, Davis, CA 95616) and R. PRINEAS (Div. of Epidemiology, University of Minnesota, Minneapolis, MI 55455).

Tests were conducted among 53 participants in the Hypertension Prevention Trials at the University of California and the University of Minnesota to determine whether long-term reduction of dietary sodium alters perceived intensity and hedonic response to salty stimuli in pre-hypertensive adults.

Subjects followed one of four supervised regimes consisting of (1) 1600 mg Na<sup>+</sup>/day (n=14); (2) 1600 mg Na<sup>+</sup> and 3200 mg K<sup>+</sup>/day (n=14); (3) 1600 mg Na<sup>+</sup>/day plus caloric restriction (n=12); (4) caloric restriction only (n=13); and (5) no intervention (16 age- and sex-matched controls). Subjects were tested at 0, and after 1, 3, 6, 8, 10, 13, 16, and 24 weeks of the diet.

Degree of liking for saltiness in a cream soup was measured by (1) ad libitum addition of NaCl to maximum liking, and (2) scaling of hedonic responses to a seven-sample concentration series (0 - 1.1% added NaCl), on a 10-cm graphic scale. Perceived intensity of saltiness was rated on a similar graphic scale. Sodium content of the ad libitum mixes was analyzed with a sodium ion-selective electrode.

Dietary sodium intake was assessed at 0, 12 and 24 weeks using randomly assigned 24-hour food records and by analysis of sodium and creatinine excretion in overnight urine collections.

There was a trend toward decreased liking for higher concentrations of NaCl over time, but little or no change in response to lower concentrations. Hedonic ratings for all concentrations of NaCl varied more across time for subjects on sodium restricted diets than for the control or weight-loss only groups.

A Peripheral Neural Correlate of the Human Fungiform Papillae Salty, Insipid Sensations. BOUDREAU, J.C. (Sensory Sciences Center, University of Texas at Houston).

Neurophysiological research in several different laboratories has revealed the existence of a Na, Li specific peripheral taste system in mammalian herbivores and omnivores, but not carnivores. The presence or absence of this system is readily detectable in the behavior of the animals toward NaCl solutions. It is proposed that the human has an identical system, and that activity within this system is largely, but not entirely, responsible for the elicitation of the human sensations of salty and insipid. Under normal conditions, neurons in this system display high levels of spontaneous activity due in large part to salivary Na. Solutions with Na levels greater than salivary Na will be perceived as salty. Solutions with Na levels less than that of the saliva will inhibit the neurons and be perceived as insipid. The existence of this system in humans is attested by the fact that human behavior toward NaCl is similar to that seen in the rat and sheep. The stimuli eliciting the human salty sensations are, in most respects, similar to those active in the animal experiments with one glaring exception: KCl, the chloride salt most inactive on animals, elicits a fairly strong salty sensory component. To resolve this discordance, it is suggested that the human salty sensation is the result of higher order neural activity that involves another peripheral taste system.

This research was financed in part by National Science Foundation Research Grants.

What is the Role of Narrow Tuning in Lobster Feeding? PAOLA F. BORRONI, LINDA HANDRICH, and JELLE ATEMA (Boston University Marine Program)

In the lobster, *Homarus americanus*, the first two pairs of walking legs are essential for feeding behavior. These taste organs contain several different populations of chemoreceptor cells, including at least 6 types of narrowly-tuned cells that respond maximally and often exclusively to only one compound. Their behavioral function is not known.

To measure leg taste responses we selected dactyl clapping during 5 min presentations of agar cubes containing stimulus solutions. A 21-compound mix of amino acids and amines, as found in mussel tissues, was as stimulatory as a complete extract of mussel tissue. From this mix, the 6 compounds for which populations of narrowly-tuned chemoreceptors exist were tested individually, in single log steps from 10<sup>-6</sup> M to their solubility limit. Of these, ammonia was the most stimulatory, but only in the middle of the dynamic range tested; in contrast, responses to the other 5 compounds were seen only at the highest concentrations tested. A mixture of the 6 compounds gave far lower responses than the sum of individual compound responses would have predicted, even lower than the response to ammonia alone.

This last behavioral result resembles the physiological finding of "mixture suppression" (Gleeson and Ache, Neuroscience Abstract #295.22, 1983; Johnson and Atema, Neuroscience Abstract #295.23, 1983). However, since the 21-compound mix is highly stimulatory, there appear to be specific, yet unknown combinations of compounds which are effective behavioral stimuli. The 6 compounds that stimulate the narrowly-tuned receptors, however, are not such a combination.

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An Apparatus for the Continuous Monitoring of Feeding by Caterpillars. ELIZABETH BOWDAN (University of Massachusetts, Amherst, MA 010030).

In the apparatus to be described the caterpillar completes a circuit when it comes into contact with the food. Before beginning to eat the animal explores the food for some seconds. This activity generates a series of erratic movement artefacts. As the animal begins to eat the movement artefacts become quite regular and highly characteristic. Each movement artefact corresponds to a single bite. Analysis of the feeding of tobacco hornworm caterpillars on tomato leaves (a normal host plant) indicates that these animals spend far less time eating than I had thought. Even the most voracious spent less than half the time eating. The percentage of time devoted to eating is correlated with the weight of the animal.

This work was supported by duPont grant # 5-20685 awarded to Dr. V. G. Dethier.

**Quantitative Analysis of Lingual Taste Buds and Papillae Over the Life Span of the Rhesus Monkey.** Robert M. Bradley, Hazel M. Stedman and Charlotte M. Mistfetta. (Dept Oral Biol, Univ Michigan, Sch Dentistry, Ann Arbor MI 48109.

The availability of tongue specimens from rhesus monkeys of known age has made possible a quantitative study of age-related differences in the taste organs of a species of monkey often used in taste research. Fifteen tongues from monkeys in five different age groups (3 animals each at 4,8,13,24 and 31 years) were fixed by immersion in formalin at sacrifice. Fungiform papillae were observed over the anterior two thirds of the tongue. Four to five circumvallate papillae were arranged in an inverted V, with two to three papillae at the apex and one at the end of each limb. On each posterior, lateral border was a single foliate papilla. The tongues were coated in a water soluble dye and the papillae counted. Next taste pores in the fungiform papillae were stained with 5% Ponceau S red and counted. The circumvallate papillae at the apex of the V were dissected, serially sectioned, and the taste buds counted. The entire foliate papilla from the left side was excised, sectioned and the taste buds counted. The results of these counts are presented below.

Total Number of Lingual Taste Buds as a Function of Age and Percentage of Total Taste Buds for Each Papilla Type.

Age (years)	Fungiform	Circumvallate	Foliate	Total
4	1273 (16%)	2104 (28%)	4667 (56%)	8044
8	1701 (16%)	3304 (32%)	5646 (52%)	10650
13	1698 (22%)	2712 (34%)	3644 (44%)	8053
24	949 (11%)	3727 (42%)	3959 (47%)	8632
31	859 (12%)	2630 (38%)	3554 (50%)	7044

It can be concluded that the majority of lingual taste buds in the rhesus monkey are on the posterior tongue. Statistical analysis of the taste bud counts as a function of age have demonstrated that there are no significant age-related differences. Supported in part by N.S.F. grant BNS 83-11497 and N.I.H. grant DE05782.

**Effect of Amiloride Concentration on Reduction of Chorda Tympani Responses to Alkali Chlorides.** J.G. BRAND, J.H. TEETER, W.L. SILVER (Monell Chemical Senses Center, Phila., PA 19104).

The diuretic amiloride is a reversible inhibitor of epithelial sodium transport in a variety of tissues. It reduces Na<sup>+</sup> flux in dorsal lingual epithelium of dog (DeSimone et al., J. Gen. Phys., in press) and partially blocks human taste responses to Na and Li salts (Schiffman et al., PNAS 80, 6136). Amiloride also reduces chorda tympani responses to Na and Li in rats and gerbils (Heck et al., Science, in press; Teeter et al., Soc. Neurosci. 9, 295.14). We have examined the effect of amiloride on integrated whole nerve - chorda tympani responses in the rat to LiCl, NaCl, KCl and RbCl (0.001-1M) and the effect of varying the concentration of amiloride (1 $\mu$ M-500 $\mu$ M) on the response to varying NaCl concentrations (20mM-500mM). The tongue was continuously washed with either deionized water or the appropriate amiloride concentration in water. The salt stimuli were delivered to the tongue in 1ml aliquots during these washes. Experiments were also performed comparing the inactivation of the tonic response to 500mM NaCl after a water rinse with a rinse containing 500 $\mu$ M amiloride plus 500mM NaCl. Amiloride treatment (500 $\mu$ M) resulted in a 60 to 90% reduction in amplitude of the responses (both phasic and tonic) to NaCl and LiCl (>than 50mM), but no appreciable reduction in responses to KCl, RbCl or sucrose. On a percentage basis, responses evoked by higher concentrations (50mM-1M) of NaCl and LiCl were affected more than those by lower concentrations. Amiloride itself did not elicit a response. Identical first-order decays of inactivation of the tonic NaCl response were exhibited for both a water rinse and an amiloride-NaCl rinse, but a residual response remained during the amiloride rinse which could be eliminated by a subsequent water rinse. The effects were readily reversible. Comparing the NaCl-evoked responses (20mM-500mM) in the presence of several amiloride concentrations suggests that amiloride is classically noncompetitive only at the highest concentration tested (500 $\mu$ M). Below this the inhibition exhibits complex (non-linear) kinetics.

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**Why Does the Clinical Odor Identification Test Work?** WILLIAM S. CAIN (John B. Pierce Foundation and Yale University), JANNEANE GENT, and FRANK A. CATALANOTTO (University of Connecticut Health Center)

The odor identification test used at the Connecticut Chemosensory Clinical Research Center contains ten common items (seven olfactory, three trigeminal) which patients seek to identify with the aid of a list of names. The test discriminates among patients with normal and reduced functioning just as keenly as a threshold test. The identification test places the functioning of the hyposmic patient at a seemingly correct position on a continuum between the extremes of normosmia and anosmia. How does this test of quality become a test of quantity? An item analysis was performed on the data from 69 hyposmic patients who made between one and six errors on the seven olfactory items. (Since normosmics commonly make no errors and anosmics make seven errors, their data offer no insight into the inner workings of the test.) Errors could occur in three ways 1) inability to perceive an item (I don't smell it.), 2) ability to perceive an item, but admitted inability to identify it (I smell it, but it is too weak to identify.), and 3) misidentification. Some items discriminated normal from depressed functioning far better than others. Cinnamon, for instance, discriminated quite well. Based on the high frequency of the responses of "I can't smell it" or "I can smell it, but it is too weak to identify," cinnamon appears to discriminate by means of its low perceived intensity. Other items, such as peanut butter, discriminated quite well through misidentifications. It in particular was confused with other oily items. An analysis of the various items one by one reveals that the identification test discriminates the normosmic from the hyposmic by a number of means, not all readily anticipated. An understanding of these means permits a more systematic choice of stimuli and of format and can help resolve differences in types of hyposmia.

**Videotape Analysis of Insect Feeding Suppression by an Extract of Ziziphus jujuba.** PETER J. CANNEY (Neurobiol. & Behav.), BRUCE P. HALPERN (Psychology and Neurobiol. & Behav., Cornell University, Ithaca NY 14853)

**PROCEDURE:** High magnification (56X), 20 min videotapes of individual, 6 hr starved, southern armyworm (*Spodoptera eridania*, Lepidoptera: Noctuidae) larvae feeding on hostplant (*Phaseolus vulgaris*) leaves were made after 3 treatments of mouthparts: None (n=9), distilled water (n=10), or 0.9% w/v aqueous-ethanol Z. jujuba extract (n=10). Treatment was a 3 min immersion of mouthparts in 10ul drop of distilled water or extract, then a 10 sec, 100ul flowing distilled water rinse. 1st 15 min of behavior was analyzed for sequence and duration of behaviors. **RESULTS:** No significant treatment effects on number of changes in behavior [none = 12 $\pm$ 2.4; water = 5 $\pm$ 2.06; extract = 11.5 $\pm$ 2.35 (median  $\pm$  s.e.)] or number of feeding bouts [none = 4 $\pm$ 1.32; water = 2.5 $\pm$ 0.44; extract = 4.5 $\pm$ 0.59 (Kruskal-Wallis, p > 0.1)]. However, significant differences did occur for total feeding duration. Duration of feeding by extract-treated larvae was << water or non-treated larvae [none = 696.83 $\pm$ 69.7 sec; water = 728.59 $\pm$ 41.85 sec; extract = 384.41 $\pm$ 80.11 sec]. The distribution of feeding durations for extract-treated larvae resembled longer (9-24hr) extract-in-diet consumption tests. **CONCLUSIONS:** The suppression of feeding duration suggests a taste modifier action on chemoreceptors by ziziphins in the Z. jujuba extract. Absence of other behavioral changes excludes general physiological effects. Similarity of extract-altered feeding duration to longer consumption data suggests a common underlying phenomenon and prediction of long-term behavior from this brief assay.

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Moderated Dietary Salt Reduction and Salt Taste Perception of Normotensive Individuals with Family History of Hypertension<sup>1</sup>. MABEL M. CHAN, JENENE G. GAREY (Department of Home Economics and Nutrition, New York University), IRMA TERPENNING (AT&T Bell Laboratories, Murray Hill, NJ).

The object of this study is to examine the effect of moderate reduction in dietary salt consumption on salt taste perception in normotensive, non-institutionalized individuals with family history of hypertension. Twelve subjects (5 males and 7 females) between 18 and 24 years of age were instructed to follow a reduced sodium diet regime for a period of 10 weeks. The following data were collected during the prediet period, at the sixth and tenth week of the implementation of the sodium reduction diet: body weight, blood pressure, 24 hour urine for Na, K and creatinine analysis, whole saliva for Na and K analysis, 3-day dietary record and salt taste perception tests. Salt taste intensity and preference responses were collected using a line scale with anchor point at each end. A series of seven concentrations of salt in water or chicken broth in equal log steps varying from 0.06 M to 0.85 M were used as stimuli. Five concentrations of salt in rice (0.5 to 3.3%) provided the third stimulus. The data were analyzed using analysis of variance. No significant change with time was observed in any of the variables. It is possible that the testing period is not sufficiently long for any response to occur and the level of salt reduction may not be low enough to produce any significant change. The only significant finding was that subjects with high baseline dietary calcium rated lower salt concentrations more pleasant and saltier than subjects with low baseline dietary calcium. McCarron and his coworkers recently reported the correlation of low dietary Ca with hypertension.<sup>2</sup> It would be interesting to further study the relationship between Ca status and salt taste.

<sup>1</sup>This study was supported by the Dean's Development Fund, SEHNAP, New York University.

<sup>2</sup>McCarron, D.A., et al., Dietary Calcium in Human Hypertension, Science, 217:267-9, 1982.

Saliva Alters the Oral Perception of Acids. CAROL M. CHRISTENSEN (Monell Chemical Senses Center, Philadelphia, Pa.), DANIEL MALAMUD (School of Dental Medicine, Univ. of Pennsylvania), JOSEPH G. BRAND, and ELI DWECK (Monell Chemical Senses Center).

In a previous study, we found that thresholds for citric acid were significantly lower following salivary flow reductions produced by administration of the anticholinergic, atropine. Because atropine reduces the concentration of bicarbonate, the principal salivary buffer, it was hypothesized that the increased sensitivity to citric acid was attributable to reduced buffering of the tastant by saliva. The possibility that saliva buffers acid tastants, and thereby affects the perception of acids was tested.

In the first experiment, the pH of a series ( $10^{-3}$  to  $10^{-6}$  M) of acetic, citric and hydrochloric acid solutions was measured both before and after the solutions were held briefly in the oral cavity (3 sec). Subjects ( $n = 14$ ) sipped and expectorated 4 and 20 ml volumes of the solutions. The results demonstrated dramatic changes in the pH of acid solutions held briefly in the oral cavity; the pH usually increased 1-2 units, with greater changes in the smaller volume of solution. In the second experiment, recognition thresholds and magnitude estimates of taste intensity were obtained separately for 4 and 20 ml volumes of acetic and hydrochloric acids, and sucrose ( $n = 9$ ). If salivary changes in the pH of acids affect sour perception, then thresholds should be lower and the perceived sourness greater for 20 ml volumes of acid because the pH changes would be less. These predictions were confirmed. Thresholds for both acids were significantly lower for 20 ml volumes of tastant, whereas sucrose thresholds were not affected by volume; e.g., acetic acid thresholds were  $2.3 \times 10^{-4}$  M for 4 ml and  $7.1 \times 10^{-5}$  M for 20 ml of solution. The perceived intensity of all three tastants was significantly affected by volume; 20 ml quantities were perceived to be more intense.

These results strongly suggest that the proximal stimulus in whole mouth taste testing of acids is not solely the prepared stimulus but rather the stimulus altered by saliva.

Taste Preferences in Aged Male and Female Gerbils. MARYLOU CHEAL, ANN MARIE FOLEY, AND J. JAY BRAUN (Arizona State University).

A common complaint of aged humans is the loss of flavor of foods in comparison to their memory of the same foods at an earlier age. Several sensory systems may be altered by the aging process to lead to such changes in perception. One of these systems is taste. As a basis for developing a model of changing taste perception with age, male and female gerbils were tested for taste preference for the four common tastants using a single bottle 15 min test. Concentration series at half log steps were used: sucrose and sodium chloride, .001 to 1.0 M; hydrochloric acid, .0001 to .1 M; and quinine hydrochloride, .00001 to .01 M. Two groups of gerbils of each sex ( $n = 8$  per group) were tested: males and females that ranged in age from close to two years of age, and males and females that were between 6 and 12 months of age. All groups preferred .1 and .3 M sucrose solution, in comparison to water, but the peak was flattened for the older gerbils, particularly the old males. There were no age differences in responses to NaCl. All groups had a mild preference for .1 M and a strong aversion for 1.0 M NaCl. There were clear aversions to .003 and .01 M QHCl and to .01, .03, and .1 M HCl. In addition to the data on taste preference, we also found that old male gerbils had much less tolerance for water deprivation than old female or younger gerbils. Four of eight old male gerbils suffered irreversible dehydration as a result of the drinking schedule while none of the other groups were adversely affected.

Olfactory Bulbectomy Suppresses the Testicular Response to Short Daylength in the Hamster. A.N. CLANCY, F. MACRIDES, B.D. GOLDMAN, AND A. BARTKE (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545).

A system of LHRH-immunoreactive fibers and cell bodies extends into the olfactory bulbs and peduncles and may be involved in hormonal responses to environmental stimuli. However, olfactory bulbectomy does not produce long-term changes in circulating T levels in males or in accessory sex organ weights, suggesting that responsiveness to odors *per se* and the integrity of this LHRH system are not necessary for the maintenance of androgenic activity. The present study examined the effect of olfactory bulbectomy on the testicular response to shortening of daylength. Hamsters initially maintained on a 14:10 hr light-dark cycle were either sham-operated or had their olfactory bulbs removed bilaterally by aspiration and were subsequently housed on a 10:14 hr light-dark cycle. Testicular length was measured at weekly intervals over a 5 month period. Sham-operated animals exhibited the normal pattern of testicular regression and eventual recrudescence. Testicular regression was significantly reduced in bulbectomized animals. Many of these animals showed no regression; others exhibited a reduced degree and/or shortened duration of regression. Radioimmunoassays of serum LH, FSH, prolactin, and T demonstrated elevations of circulating FSH in bulbectomized hamsters on long daylength but no significant changes for the other hormones. On short daylength, the bulbectomized animals showed the normal decline in circulating prolactin. The suppression of the testicular response can be attributed to a failure of short daylength to produce a prolonged decline in gonadotropin secretion below the levels capable of maintaining testes function. The aspiration removed the main and accessory olfactory bulbs and rostral portions of the anterior olfactory nucleus. Damage to these olfactory structures and to the LHRH-immunoreactive fibers and cell bodies present in them thus can affect the endocrine responsiveness of hamsters to changes in daylength.

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Sensory Cues Used by Starlings in Discriminating  
Among Plant Species Used During Nest Construction. L.  
CLARK and J. R. MASON (Monell Chemical Senses Center).

The use of nest cavities by *Sturnus vulgaris* declines as a function of cavity age. This is presumably due to increased pathogen and parasite load associated with long term use of cavity sites. Those starlings that continue to use cavities incorporate fresh green vegetation into the dry nest matrix. The incidence and magnitude of this behavior increases as a function of cavity age. The plants included represent a biased sample of available vegetation. Preliminary evidence indicates that these plants may exert insecticidal effects on at least some ecto-parasite populations (e.g. *Menacanthus spinosa*). Starlings are capable of discriminating among plant extracts and an inverse relationship exists between preference ratio scores and the frequency with which plants are chosen in the field. Here, we report on the possible chemo-sensory cues used by starlings in discriminating among plant species used during nest construction.

Odor Pleasantness Perception Related to Sensitivity for  
Two Odors, Cyclohexanone and Pyridine. RICHARD G. DAVIS  
(Veterans Administration Medical Center, Lexington, KY)

Measures of odor sensitivity, commonly absolute thresholds, are frequently used in clinical evaluations to support inferences about human odor perception, and general olfactory system integrity. Previous investigations have shown that consensually valid judgments of odor pleasantness can be furnished by individuals whose fundamental odor sensitivity varies widely. These earlier studies were based on a single odorant estimate of odor sensitivity, and hence could have been limited by focusing on just one odorant. The present study evaluated odor sensitivity to cyclohexanone and pyridine. Pleasantness judgments were also obtained for a set of 22 odorants. Olfactory thresholds were obtained by ascending series of odors delivered by squeeze bottle. The odors were diluted in distilled water. Pleasantness judgments were obtained by odor delivery in microencapsulation technology. Persons (N=114) were recruited from undergraduate courses in Psychology at the University of Kentucky to perform the three tests. The results for cyclohexanone repeat the results found previously. The results for pyridine were essentially the same as for cyclohexanone. Specifically, individuals provide generally the same consensually valid perception of odor pleasantness independent of their absolute threshold sensitivity to cyclohexanone and to pyridine. These findings generalize the previous results to two odors, and reinforce the conclusion that odor perception of pleasantness is a very robust property of the olfactory perceptual system. Odor pleasantness perception is immune to the effects of sensory deficits relating to basic odor sensitivity.

Cytochrome Oxidase (CO) Activity in the Olfactory System:  
Ontogeny and Response to Peripheral Deafferentation. R.  
Costanzo (Medical College of Virginia), M.T. Shipley and S.  
Van Ooteghem (University of Cincinnati College of Medicine).

The distribution of CO corresponds to the location of major synaptic fields in the olfactory bulb and piriform cortex (Shipley et.al., this meeting). If CO levels reflect synaptic activity, the ontogenetic development of CO should parallel the development of synaptic systems in the bulb and cortex. Removal of specific synaptic inputs might decrease CO activity. In the visual system CO is reduced in functional anatomical loci by sensory stimulation, deprivation and eye removal (Wong-Riley, '79; Horton and Hubel, '81) but there have been no previous studies of the development of CO activity.

Pre- and post-natal rats were perfused and stained for CO. Adult hamsters were stained for CO 4-10 days after olfactory nerve (ON) transection. At E-15 there are no 1° synapses in glomeruli and there is no discernible, organized CO activity in the bulb. At E-18, some 1° synapses have formed (Farbman, et.al., '80) and CO was distinctly visible in tiny glomeruli. At birth (P-1) glomeruli were larger, contained more CO and CO was present in the external plexiform layer. From P-1 there is a progressive increase in CO; the pattern of maturation is being studied as is the development of CO in retrobulbar areas.

ON transection sharply reduced CO activity in all bulb layers. Glomeruli became smaller and the amount of CO/unit area was less than on the normal side indicating that the decline in CO is not due to the reduced size of the glomeruli alone. There was a marked reduction in layer I of AON and piriform cortex. Thus, the present method reveals transsynaptic changes in CO.

CO histochemistry shows changes in metabolic activity associated with normal synaptic development and deafferentation. It may be possible to detect bulbar and transsynaptic responses to different odor conditions.

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An HVEM Autoradiography Study of Cell Turnover in the  
Mouse Vallate Taste Bud. R.J. DELAY, J.C. KINNAMON, and  
S. ROPER (University of Colorado Health Sciences Center)

Utilizing the technique of High Voltage Electron Microscopy (HVEM) autoradiography we have been studying turnover cell in the mouse vallate taste bud and have been investigating whether Light (type II) and Dark (type I) are two different classes of cells or represent developmental stages of a single cell type. Young adult mice were given a single injection of tritiated thymidine (4  $\mu$ m/gm.body weight). The animals were then sacrificed at fixed time intervals (1 hr-10 days) and processed for EM autoradiography. Light cells were identified by the presence of a large spherical nucleus, abundant smooth endoplasmic reticulum and lightly-staining cytoplasm. Dark cells were distinguished by their smaller invaginated nucleus with patchy heterochromatin, abundant rough endoplasmic reticulum, and dense cytoplasm. Cells which could not be unambiguously classified as either Light or Dark cells were not studied further. Cells were identified in a double-blind analysis using coded micrographs. Our pilot data indicate that the first cells to incorporate label (6 hr-2 days after injection) were Dark cells. At 3 days after injection, most labelled cells could not be identified unambiguously as Light or Dark. Four days after injection, however, labelled Light cells appeared. By 7 days after injection, the majority of labelled cells were Light cells. These results suggest that the first cells to enter the taste bud are Dark cells and that these gradually mature into Light cells over a period of about a week, confirming earlier hypotheses (e.g., Heidenhain, 1914) that the two putative cell types are indeed different developmental stages of one and the same cell type.

Supported in part by grants from Procter & Gamble CO and NIH NS20382.

Purinergic Olfactory Receptors: Electrophysiological and Behavioral Evidence. CHARLES DERBY, W. E. S. CARR, and BARRY ACHE (C. V. Whitney Laboratory, University of Florida)\*

Purinergic cells, stimulated by adenosine and adenosine nucleotides, are found internally in the brain, heart, and other organs of vertebrate animals. We have found that the olfactory systems of two marine invertebrates also possess purinergic receptor cells that exhibit response specificities and antagonistic effects characteristic of one of the type of purinoceptors found internally in higher animals.

Electrophysiological recordings from single olfactory cells in the antennules of the spiny lobster, together with behavioral measurements of the effectiveness of chemoattractants for a shrimp, have revealed that the chemosensory systems of both species are highly sensitive to adenosine 5'-monophosphate (AMP). Tests with AMP analogs, plus a purinergic antagonist, reveal very close correlations between results obtained physiologically and behaviorally in the two species. In both cases, (1) AMP is clearly the most effective purine tested; (2) there is a potency sequence of AMP > ADP > ATP or adenosine; (3) though all changes in the structure of the AMP molecule lead to a significant diminution in activity, the greatest diminution accompanies changes in the ribose phosphate moiety; and (4) the response to AMP is antagonized by theophylline. Collectively, these results indicate that both invertebrates have olfactory purinoceptors closely related to the P<sub>1</sub>-type (= R-type) purinoceptors found internally in vertebrates. It is becoming increasingly evident that the olfactory and gustatory systems of lower organisms contain several of the receptor types found internally in higher organisms, including purinergic, taurinergic, glutamatergic, glycinergic, and GABAergic receptors.

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The Transcellular and Paracellular Routes of Ion Translocation Across the Lingual Epithelium and Their Role in Transduction. JOHN A. DESIMONE, (Medical College of Virginia) GERARD L. HECK, SHEELLA MIERSON, AND SHIRLEY K. DESIMONE, (Dept. of Physiol. & Biophys., Box 551, MCV Station, Richmond, VA 23298)

An *in vitro* preparation of mammalian dorsal lingual epithelium has led to fresh insights into the early events surrounding gustatory transduction. The epithelium of both canine and rat tongues can be placed between lucite chambers and changes in potential, current, and resistance can be monitored. Changing the composition of the solution in the chambers, or the addition of pharmacological agents produces important changes in these electrical parameters. The arrangement also allows for radioisotopic ion-flux determinations. It is easily proved that both dorsal and ventral epithelium transport Na<sup>+</sup> and Cl<sup>-</sup> actively between symmetrical solutions of Krebs-Henseleit buffer. In addition the dorsal surface contains a special transport system which is especially activated by hyperosmotic NaCl or LiCl, but not KCl. This activation can be blocked by the Na-channel blocker, amiloride. These results suggest that this special transport system may be the means by which Na-Li taste transduction is initiated. This hypothesis is supported by the effects of amiloride (10<sup>-6</sup>M) on the summated chorda tympani response of the rat to NaCl and KCl. The response to NaCl is sharply reduced, whereas the response to KCl is hardly affected. The results enable us to conclude that Na (and Li) entry into the taste-bud cells across the apical barrier is via a specific route and is part of the causal chain leading to the neural response. On the other hand the route of K-entry is different. *In vitro* studies on ouabain-treated lingual tissue suggest that K may utilize a paracellular as well as a cellular transepithelial pathway. Thus a new paradigm of taste transduction has emerged supported by epithelial and neurophysiological studies. The new paradigm unites gustatory physiology with the methods, concepts, and models of G-I and renal physiology and should serve as a basis for continued progress in the study of each of the taste modalities. Supported by NIH Grant NS 13767 and NSF Grant BNS-8309135

Preliminary Evidence for Taste Buds in the Prairie Rattlesnake, *Crotalus viridis viridis*. J. DAVID DICKMAN, DAVID DUVAL and DAVID V. SMITH (Departments of Psychology and Zoology/Physiology, University of Wyoming, Laramie, WY 82071).

Previous investigations have concluded that snakes lack gustatory receptors, based largely upon histological examination of the tongue (Nonoyama, 1936; Payne, 1945; see Bradley, 1971). However, Uchida (1980) reported taste buds along the dental arch of the rat snake with no taste buds being located on the tongue. Since chemosensory cues are known to be essential for prey trailing and other behaviors in rattlesnakes (Chiszar et al., 1983; Duval et al., 1980), it is possible that taste information may be used. The purpose of the present investigation was to examine the oral cavity of the rattlesnake for the possible existence of taste receptors. Coronal sections of the oral epithelium of prairie rattlesnakes were cut and stained with standard hematoxylin and eosin techniques. Light microscopic examination revealed structures in the stratified epithelium that are morphologically similar to what have been described as taste buds in other reptilian species (Uchida, 1980). The apical portion of the structures ends with an indentation that appears to be open to the oral cavity. However, no feature characteristic of a "taste pore" has been identified in the structures to date, similar to findings in other snakes (Uchida, 1980). To correctly classify these structures as taste buds, additional types of evidence are necessary. First, if these structures function as taste receptors, there should be evidence of innervation by sensory afferents. Second, the receptor cells should be open to the oral cavity so that chemical stimulus access is possible. In order to demonstrate the existence of these features, further research, including silver impregnation and transmission electron microscopy, is now being conducted.

Responses to Olfactory Stimuli in the Gustatory Area of the Parabrachial Pons of the Rat. PATRICIA H. DILONE'RO (Department of Psychology, Smith College, Northampton, MA 01063), JOHAN CARCIA (UCLA, Los Angeles, CA 90024)

Electrophysiological responses to gustatory and olfactory stimuli were recorded in the parabrachial nucleus of the pons (PbN). NaCl (1M) was presented initially as a gustatory probe stimulus to identify the taste-responsive part of the PbN in rats (under flaxedil). Thereafter, responses to Na Saccharin (1%) as a gustatory stimulus and to vanilla, almond and ethyl alcohol as olfactory stimuli were recorded. Analysis of both single and multiunit recordings demonstrated the existence of neural elements within the PbN that are responsive to both gustatory and olfactory stimuli. These elements most often responded selectively to one or two of the olfactory stimuli. Responses to olfactory stimuli varied in latency, temporal pattern and magnitude from responses to gustatory stimuli. In some cases, ethyl alcohol as a tastant was also tested but produced a response only when presented as an olfactory stimulus. The location of the gustatory-olfactory elements was determined after examination of histological material. These elements were recorded from an area in the lateral portion of the taste-responsive part of the PbN, just below the brachium conjunctivum. The area extended at least 0.5mm in a dorso-ventral plane. The present results suggest that the PbN receives input from both olfactory and gustatory pathways. Furthermore, these pathways may converge on the same neural elements within the PbN. This convergence may be part of a neurophysiological substrate for the perception of flavor.

CCK-8 Decreases the Preference for Sweet Tastes in Rats. PATRICIA M. DI LORENZO (Department of Psychology, Smith College, Northampton, MA 01063), DIANA L. RALPHE (Department of Psychology, Smith College, Northampton, MA 01063)

In two experiments the consumption of aqueous solutions of sweet tastants was measured after CCK-8 injections. All rats were adapted initially to a 23.75 hour water deprivation schedule; food was available ad libitum throughout both experiments. On alternate days animals were injected with either CCK-8 (1.8ugm/kg;i.p.) or isotonic saline (2.0cc/kg;i.p.). After 1/2 hour, sweet solutions were presented for 15.0 minutes in their home cages. Each rat served as its own control. In Experiment 1, solutions of sucrose (.10, .20, .40 or .60M) were presented in a pseudorandomized schedule. Analysis of the results showed that rats consumed significantly less sucrose after CCK-8 than after saline injections. Results were consistent for all sucrose concentrations tested. Because previous investigators have suggested that CCK-8 produces satiety in hungry rats, it was hypothesized that the present data may have been confounded by the post-ingestional effects of sucrose as a nutrient. Therefore, the effect of CCK-8 on consumption of NaSaccharin (.05, .10, .20, .40%) solutions was studied in Experiment 2. In addition, the possible effect of CCK-8 on water intake was also examined. A significant decrease in NaSaccharin intake was observed after CCK-8 across all concentrations tested. Water intake was not altered after CCK-8. Results of these experiments suggest that CCK-8 may alter taste preferences for foods regardless of their nutritional content. This effect may, in part, account for the previously reported effects of CCK-8 on food intake.

Detection of Sugars by Fiddler Crabs. G. DUNKEL and D. RITTSCHOF (Duke University Marine Laboratory, Beaufort, NC 28516)

Fiddler crabs feed on exposed intertidal sands by sifting microscopic organisms, algae, protozoans, and diatoms from the inorganic substrate (Crane, 1975). Many micro-organisms secrete organic compounds, including polysaccharides composed primarily of glucose, galactose, mannose, and rhamnose. These compounds could serve as chemical cues to direct *U. pugilator* to organic-rich substrate. We tested the ability of fiddler crabs to detect sugars in the laboratory. Fiddler crabs feed preferentially in fresh intertidal sand or organic-free sand with sucrose or glucose added. Weak responses were also observed in comparisons between organic-free sand and organic-free sand supplemented with galactose, rhamnose, or sorbose. Crabs did not respond to fructose or mannose. Carbohydrate and glucose concentrations of seawater in fresh intertidal sand were determined. Total carbohydrate levels were at and above  $ED_{50}$  for crab responses to glucose or sucrose. Free glucose was below the limit of detection by crabs. Exopolymers containing glucose secreted by microorganisms could direct fiddler crab feeding.

Smell Identification Ability: Changes with Age. RICHARD L. DOTY, STEVEN APPLEBAUM, AND PAUL SHAMAN (Smell and Taste Center, University of Pennsylvania)\*

Smell identification ability was assessed in 1677 healthy persons ranging in age from 4 to 99 years using the 40-odorant University of Pennsylvania Smell Identification Test (UPSIT). Following a normalization of the response data, mean test scores were calculated at half-decade intervals. A marked monotonic decline in average test scores was present across the later age range, whereas peak performance was achieved by the late teenage years. Females performed better than males at all ages, including prepubertal ones. The latter finding suggests that sex differences in odor identification ability are unlikely the result of differences in concurrent levels of circulating gonadal hormones. A striking similarity between the changes in UPSIT scores and measures of central visual function across the age continuum (e.g., critical flicker frequency, the minimum dark-adapted light threshold, and visual acuity) reveals that age-related changes in these two senses are similar. Probable causes for the changes in olfactory function are discussed.

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The Intensity of Flavor. M.P. ENNS and D.E. HORNUNG, (St. Lawrence University, Canton, NY 13617)

The integration of odorants and tastants in producing the sensation of flavor (overall intensity) was studied using the Mixed Modulus Delivery System (Hornung and Enns, Chemical Senses, in press). First, subjects used the method of magnitude estimation to scale the intensity of the smell and taste of four almond extract concentrations. Then, these subjects scaled the overall intensity of all olfactory and gustatory combinations of distilled water and four almond concentrations. When the mean magnitude estimation of overall intensity was plotted as a function of the log concentration of the odorants, five parallel lines emerged. These curves represent the effect of increasing the concentration of the tastant. Five parallel lines were also found when the magnitude estimation of overall intensity was plotted as a function of the tastants. These curves represent the effect of increasing the concentration of the odorant. For both sets of curves, as the concentration increased, the Y intercept also increased. Therefore, subjects apparently considered the strength of the olfactory and gustatory signals and then added these together to produce the overall intensity. However, for all combinations of tastants and odorants, the overall intensity was slightly less than the sum of the intensity of the taste and the intensity of the smell. The data further suggest that when determining the overall intensity, the magnitude assigned to the olfactory and gustatory sensations is less than the magnitude that would have been assigned if the odorant or tastant had been scaled alone. This conclusion seems justified since while smelling distilled water and tasting almond, subjects reported an overall intensity that was less than what they had previously reported to be the intensity of the taste itself. Likewise, overall intensity was less than the intensity of the smell itself when subjects smelled almond while tasting distilled water.

Early development of the olfactory nerve in the rat.  
ALBERT I. FARBMAN and LYNN M. SQUINTO (Northwestern University, Evanston, IL).

Early development of olfactory receptor axons was examined with the transmission electron microscope in rat embryos from the 13th to the 17th embryonic day (E13-E17). As is generally the case in other developing neurons, axonal outgrowth from the olfactory receptor neuron occurred before dendritic differentiation became evident. This was indicated in sections which showed axons extending from perikarya of cells containing several dividing centrioles within the cytoplasm near the nucleus. These centrioles, in a later stage, migrate to the distal end of the cell and form the basal bodies of olfactory cilia. Their presence in the perikaryon of the receptor neuron is an indicator that the dendrite is not yet ciliated, therefore not fully differentiated. The earliest evidence of olfactory receptor axons leaving the sensory epithelial compartment was seen on day E14. These axons were accompanied by epithelial cells which also migrated into the connective tissue. Two morphologically different types of migrating epithelial cells were seen. One contained dense cytoplasm, many free ribosomes and a variable number of centrioles, up to six. The other cell type had a lighter cytoplasm, more rough endoplasmic reticulum, larger mitochondria, and also contained several centrioles. The latter cell type was related to axons in a manner similar to that of Schwann cells. After E14, there were rare examples of the first cell type. It is tentatively concluded that the first cell type may be a younger version of the second, and that the second is a precursor to the Schwann cell. This is significant in that it suggests that olfactory nerve Schwann cells, unlike Schwann cells of other nerves, are not derived from neural crest, but are derived from olfactory placode.

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Vagal and Facial Nerve Gustatory Nuclei have Different Reflex Connections in the Brainstem. THOMAS E. FINGER and YASUHIRO MORITA (Univ. Colo. Sch. Med., Denver, CO 80262).

The vagal and facial nerves innervate taste buds which lie in different locations and which appear to serve different functions. The facially innervated taste buds are concerned with food selection while vagally innervated taste buds are implicated in swallowing and digestive reflexes. This distinction is especially clear in ictalurid catfish wherein the facial nerve innervates external taste buds and the vagus nerve innervates oropharyngeal taste buds. These gustatory nerves terminate centrally in distinct facial and vagal gustatory lobes in the medulla. HRP was injected into either the facial or vagal lobe and the connections of these structures were compared. The facial lobe projects to the spinal funicular nuclei, descending trigeminal nucleus, the contralateral facial lobe and the ipsilateral glossopharyngeal and vagal lobes. In addition, the facial lobe projects directly to the medial reticular formation in the rostral medulla as well as sparsely to the vicinity of the trigeminal and facial motor nuclei. The vagal lobe shares few of these connections. The vagal lobe projects to the commissural nucleus of Cajal, the contralateral vagal lobe, and the ipsilateral facial lobe. More importantly, the vagal lobe maintains a large efferent projection to the area of the lateral reticular formation in which lie the dendrites of the vagal and glossopharyngeal motor nuclei. In summary, the facial gustatory system projects to spinal and trigeminal sensory nuclei and the medial reticular formation of the rostral medulla; the vagal gustatory system is connected to the vagal-glossopharyngeal motor complex and the lateral reticular formation of the mid-medulla. Furthermore, the facial gustatory pathway has more extensive long ascending connections to isthmus and thalamic gustatory nuclei. These connections are consistent with the concept that the facial nerve gustatory system serves as an exteroceptive system in conjunction with spinal and trigeminal systems while the vagal nerve taste system mediates reflex swallowing.

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Chorda Tympani Responses to Salts in Dahl Na Sensitive and Na Resistant Rats Fed High- or Low-NaCl Weaning Diets. FAY FERRELL and SARAH D. GRAY. (Depts. Nutrition and Human Physiology, University of California, Davis, CA 95616).

We (Ferrell & Gray, 1982) and Priehs, Bernard & Maas (1983) have independently observed decreased neural gustatory sensitivity to NaCl relative to KCl and CaCl<sub>2</sub> in Spontaneously Hypertensive (SHR) compared to normotensive (WKY) rats. Dahl rats differ from SHR in physiological mechanisms mediating their hypertension and in preference for NaCl. The Dahl Salt-sensitive rat is genetically predisposed to become hypertensive when fed a high-sodium diet, whereas the salt-resistant rat remains normotensive. SHR show enhanced preference for NaCl solutions but Dahl salt-sensitive rats avoid it. We fed weanling salt-sensitive (NaS) and resistant (NaR) rats an 8.0% (Hi Salt) or 0.4% (Lo Salt) diet for four weeks. Then, with continued feeding of the assigned diet, we recorded electrophysiological responses from the whole chorda tympani nerve to 0.1M NaCl, KCl, CaCl<sub>2</sub>, LiCl and NH<sub>4</sub>Cl and to concentration series of 0.01M to 0.5M NaCl, KCl and CaCl<sub>2</sub>. Recordings were made over a three-week period. Integrated steady-state responses were measured 10 seconds after stimulus onset and ratios were calculated for each stimulus relative to 0.1M NaCl. Preliminary results suggest a nonsignificant but reliable trend for increased K/Na, Li/Na and NH<sub>4</sub>/Na ratios with both NaS genotype and high dietary NaCl. Increased Ca/Na ratios seem to be associated only with high dietary NaCl. No detectable differences were seen as a function of duration of maintenance past 28 days on the high NaCl diet. Evidence that a high-salt diet might alter the effectiveness of Li relative to Na is interesting because those two salts are similar in physical properties, taste quality and nerve stimulatory effectiveness within a given species. Single fiber studies are needed to determine magnitude and direction of neural changes in response patterns to Na, K and Ca, all of which are involved in blood pressure regulation. Supported by Grant #83-S147, Am. Heart Assn. CA Affil.

Temporal Properties of Impulses in Sucrose-Responsive Hamster Taste Neurons. MARION E. FRANK & THOMAS P. HETTINGER (Univ. of Connecticut Health Center, Farmington, CT 06032).

Sucrose-sensitive hamster chorda tympani fibers respond in a characteristic way to application of sucrose solutions to the tongue. Following a latency of about 0.3 sec, bursts of up to 50 spikes occur in succession, often with a rhythmic period of about 0.7 sec. Neither the latency nor the interburst interval is determined by the chorda tympani fibers themselves, since responses to strong salt solutions in these units have shorter latencies and are not periodic. The interburst frequency is nearly independent of sucrose concentration, while the number of impulses per burst increases rapidly with increase in sucrose concentration up to 0.1 M, followed by smaller increases up to 2 M sucrose. The interspike intervals in the initial burst following stimulus application tend to be the same, about 7-10 msec, at all sucrose concentrations; the intervals increase slightly during a single burst and in subsequent bursts during a given stimulus application. Fructose and sodium saccharin also give bursting responses in sucrose-sensitive units. The difference in temporal patterns of responses to NaCl and sucrose implies that stimulation by these two substances occurs by essentially different mechanisms. NaCl can directly produce changes in receptor-cell potential, whereas sucrose stimulation requires the intermediacy of binding to specific receptor sites. The latency and bursting patterns of responses to sucrose are thought to be due to properties of the sucrose receptor cells in which slow regenerative electrical events may occur with a rise time corresponding to the latency of the neural response and at a rate equal to the interburst frequency of the nerve fibers.

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The Classification of Simple Taste Stimuli by PTC Tasters and Non-Tasters: A Reaction Time Analysis. Robert A. Frank (University of Cincinnati), Debbie Korchmar (University of Cincinnati).

In an attempt to further characterize gustatory sensitivity in phenylthiocarbamide (PTC) tasters and non-tasters, the reaction times of 40 subjects (20 PTC tasters and 20 non-tasters) were measured during the classification of five tastants and distilled water into five taste categories. Three concentrations of sucrose, NaCl, HCl and quinine sulfate and four concentrations of PTC were used as stimuli. Subjects were classified as PTC tasters or non-tasters based on their responses to PTC stimuli administered during the test and upon a pretest of PTC sensitivity with six concentrations of PTC ranging from  $10^{-5}$  to  $10^{-3}$  M. During testing, subjects classified the stimuli into one of five categories labelled sweet, salty, sour, bitter or no taste. Reaction times and category responses were recorded by a microcomputer for each trial. Following each trial, subjects spit the stimulus into a nearby sink and rinsed any residual taste from their mouths with tap water. The inter-trial interval was 30 sec. There were no significant differences in reaction times between tasters and non-tasters for any of the stimuli tested (although the difference for PTC approached significance,  $p < .07$ ). However, an examination of classification errors revealed that in addition to categorizing PTC as tasteless, PTC non-tasters committed 16 of the 18 classification errors involving sucrose or sweet taste. The errors for other types of judgments were evenly distributed between the two groups. The sweet-related errors are not accounted for by a few pathological individuals since 12 of the 20 non-tasters made at least one error of sucrose classification or judging another substance as sweet. These results indicate that in addition to a deficit in sensitivity to bitter substances, PTC non-tasters also demonstrate a tendency to misperceive sucrose or identify other basic tastants as sweet. This finding is consistent with other results showing differences in sucrose sensitivity between PTC tasters and non-tasters when absolute thresholds or intensity magnitudes are assessed.

The Review and Post-Award Process at the National Institutes of Health. THOMAS V. GETCHELL, ANDREW HAMILL, MICHAEL F. HALASZ and JACK PEARL (Department of Anatomy, Wayne State University, Detroit, MI 48201, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20205, and Division of Research Grants, National Institutes of Health, Bethesda, MD 20205).

The initial review process will be described by a chemosensory scientist who is chairman of the Sensory Disorders and Language Study Section and by the Executive Secretary of the study section. Changes in policy related to the second phase of the review process and the budget will be described by the project officer for the chemosenses at the National Institute of Neurological and Communicative Disorders and Stroke. The post-award policy related to budgetary and administrative changes for grants will be discussed by a grants management specialist.

Cholinergic Control of Glandular Secretion in the Olfactory Mucosa of the Salamander. Marilyn L. Getchell & Thomas V. Getchell (Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201).

We have examined the effects of the cholinergic agonist methacholine on acinar cells of superficial Bowman's glands (sBG) and deep olfactory glands (dG) in the olfactory mucosa. A 1 ml aliquot of solution containing 5 mg/ml methacholine in 0.15M saline was introduced over a 1 min period into the nasal sacs through the external nares. After a 15 min incubation period, the animal was perfused and the tissue fixed, plastic-embedded and stained using standard techniques. Computer-assisted morphometric techniques were employed to analyze the agonist's effects. Areas of the 20 acini studied were comparable. Methacholine caused a significant decrease in the secretory granule content of cells in both sBG and dG. Granule areas were reduced by 56% in sBG and by 46% in dG ( $p=0.001$ ). Secretory granules occupied a significantly smaller fraction of the cell area in both sBG ( $p=0.001$ ) and dG ( $p=0.05$ ). Granule heights were reduced by about 40% in both sBG and dG ( $p=0.001$ ). Secretory granules occupied a significantly smaller fraction of the cell height in both sBG ( $p=0.001$ ) and dG ( $p=0.01$ ). Methacholine reduced the granule area, granule/cell area and granule/cell height to a significantly greater extent in sBG than in dG. This suggests a greater affinity for and/or access of the agonist to the receptor sites of sBG. Cell areas in sBG were unaffected but were reduced by 27% in dG ( $p=0.001$ ). Cell heights were unaffected. Methacholine also caused an increase in lumen area (sBG, 92%; dG, 156.5%) and perimeter (sBG, 54%; dG, 93%); these changes, indicative of secretion, were statistically significant in dG ( $p=0.01$  and  $0.05$ , respectively). The results suggest a cholinergic input to the olfactory glands and/or blood vessels in the olfactory mucosa, indicating a role for the parasympathetic nervous system in the control of the secretory process.

Supported by NIH-NS-16340.

Human Olfactory Discrimination of Mouse Strain and H-2 Phenotype. AVERY NELSON GILBERT, KUNIO YAMAZAKI AND GARY K. BEAUCHAMP (Monell Chemical Senses Center)\*

Human ability to discriminate the odors produced by other mammals is rarely investigated since the human nose is often thought to be poor. Yet anecdotal reports indicate that researchers can smell a difference between strains of laboratory mice. Mice are able to discriminate strains by olfaction, even when the genetic differences between strains are limited to genes of the major histocompatibility complex (MHC) on chromosome 17. We have explored the human ability to make these olfactory discriminations, using as stimuli intact mice, their urines, and their feces. People can easily distinguish such distantly related mouse strains as BALB/c from C57BL/6. Discrimination becomes increasingly difficult as the genetic difference between strains is made progressively less. Very few persons can distinguish between  $F_2$  homozygous segregants of MHC congenic strains (strains which differ genetically only in the MHC). Urine and feces also appear to contain cues sufficient for strain discrimination.

\*Supported by NIH 5 T32 NS07176-03 and NIH 1 F32 NS07353-01.

Simultaneous stimulus and neuron solutions in multidimensional scaling spaces. GILL, J. M. II and ERICKSON, R. P. (Duke University)

It has proven essential in the non-chemical senses to plot neural responses in the context of the relevant stimulus domain; photoreceptor sensitivities across the wavelength dimension, somesthetic neurons across the skin surface, etc. As presently used, the technique of multidimensional scaling (MDS) has very usefully demonstrated arrangements of neurons or stimuli, but not both together; this problem is due to the fact that only differences between neurons or between stimuli have been obtainable. What is lacking for a simultaneous solution including both neurons and stimuli are measures of the differences between a particular neuron and each stimulus, and vice-versa. For this we simply used (the inverse of) the amount of response of each neuron to each stimulus. The resulting matrix from which the MDS space was computed contained comparisons between all objects, neurons and stimuli (neuron-neuron, stimulus-stimulus, neuron-stimulus). The method was validated with data of known solutions, such as color vision (Monte Carlo). With taste data, the solutions were congruent with individual solutions for stimuli or neurons alone, and placed neurons in clearly meaningful positions; i.e. neurons particularly sensitive to fructose were placed near that stimulus, etc. We feel that the validity of the solutions may be greater than for neurons or stimuli alone since more constraint or information goes into the placement of each point. Neural response functions (the response of a neuron across the dimension) for each neuron may be directly derived from these solutions. The benefits of such solutions would be the same as those for the display of data in other sensory systems in which neurons and stimuli are placed in the same space.

Supported by grants from the NSF and PHS.

Developing olfactory receptor cells grow axons in tissue culture. FEDERICO GONZALES and ALBERT I. FARBMAN (Northwestern University, Evanston, IL)

We report preliminary results on the successful growth of chick and rat olfactory axons in tissue culture. Epithelium and underlying mesenchyme of the nasal pits of 5 day chick embryos or 17 day fetal rats were dissected from surrounding structures and incubated in a medium containing collagenase. This enzyme treatment resulted in removal of most of the mesenchyme. In the remaining nasal epithelial sheet, most of the non-olfactory region was cut away and discarded. The sensory epithelium was: 1) explanted whole, 2) minced into very small pieces and then explanted, or 3) dissociated in EGTA or trypsin-EDTA solution before explantation. Tissue fragments or dissociated cells were placed in culture dishes and grown in Waymouth's medium, containing horse serum and embryo extract, at 37°C in an atmosphere of 5% carbon dioxide/air. A minimum volume of medium was used in order to hold fragments of tissue against the bottom of the culture dish by surface tension. Within 24 hours after explantation, most of the non-dissociated cultures, i.e., those in groups 1 & 2, began to sprout axons. Axons tended to form into fascicles and some left the fascicles, thus often forming complex networks around the explants. Individual axons were exceedingly thin, beaded along most or all of their length, and ended in small growth cones. Some axons grew to a length of more than 2 mm after 9 days in culture. Receptor cell bodies remained intimately associated with each other and with the non-neuronal elements in the epithelium. The results suggest that growth of axons from olfactory receptor cells in tissue culture is dependent on some degree of contact with adjacent epithelial cells.

Supported by NIH Grant NS-06181.

Clinical Laboratory Screening Tests for Patients With Chemosensory Dysfunction as a Primary Complaint. R.B. GOODSPEED, J.F. GENT, F.A. CATALANOTTO, L.M. BARTOSHUK, W.S. CAIN, J.O. DONALDSON, A. FERRIS, G. LEONARD (Conn. Chemosensory Clinical Research Center [CCCRC]).

Screening tests, routinely used in clinical medicine as an aid in finding disease processes, are most useful for diseases with a prevalence of 2 to 10%. A positive result on a good screening test increases the probability of the presence of a particular disease to a range where a diagnostic test is most effective. A negative result effectively eliminates that disease as a diagnostic possibility. Forty (40) commonly employed clinical laboratory screening tests were selected to be part of the routine evaluation of patients at the Taste and Smell Clinic of the CCCRC. These tests were selected to screen for a variety of systemic diseases that have been reported to be associated with taste and smell disorders, e.g., thyroid abnormalities, diabetes mellitus, etc. Analyses based on the first 237 patients showed that abnormally elevated blood eosinophil counts among patients with olfactory deficits were more commonly associated with a nasal or sinus disease etiology than with a post-viral-like illness etiology. A similar association was found for elevated serum IgE levels. There were no other significant group findings. In addition, our data indicate that the prevalence of diseases reported to be associated with chemosensory disorders is below the range where our laboratory screening tests can be most effective. The chemosensory tests and clinical examinations can function most effectively as screening tests that place patients into Chemosensory Diagnostic Categories (CDC). Appropriate diagnostic tests can then be used in each CDC to determine an etiologic diagnosis and hence determine treatment options, e.g., for patients with confirmed anosmia, sinus x-rays should be ordered to check for signs of nasal disease. In conclusion, routine clinical laboratory screening tests are not recommended for patients whose primary complaint is chemosensory in nature. Instead, appropriate diagnostic tests should be selected after patients have been placed into a CDC by chemosensory testing, clinical history and physical examination.

Quantitative Study of Morphological Development of Rat Taste Bud. David S. Gottfried, Charlotte M. Mistretta, Robert M. Bradley (Oral Biology, Dentistry, Univ. of Michigan, Ann Arbor MI 48109) and David L. Hill (Psychology, Univ. of Toledo, Toledo OH 43606).

We have made a quantitative study of morphological development of the rat taste bud to compare with data on functional maturation. Anterior tongues from 31 rats aged one through 100 days postnatal were serially sectioned and stained with hematoxylin and eosin for light microscopy. All fungiform papillae and taste buds were counted and each taste bud was classified in one of four developmental stages based on morphological characteristics. Classification criteria included orientation of cells, presence of more than one cell type, distinction of taste bud cells from surrounding epithelial cells, and presence of a taste pore. By 5 days postnatal 95% of the adult number of fungiform papillae were observed and by 15 days, 99% of papillae contained the usual complement of one taste bud each. However, morphological maturation of taste buds continued after 15 days. Up to 30 days postnatal there were changes in percentages of taste buds in the two, most mature developmental stages. The developmental function for acquisition of morphologically mature taste buds relates closely to maturation of neurophysiological, salt taste responses.

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Electrophysiological and Morphological Examination of Antennal Sensilla on the Corn Earworm Moth

A. J. Grant, M. S. Mayer, and R. W. Mankin  
(Insect Attractants, Behavior, and Basic Biology Laboratory, U. S. Dept. Agriculture)

The pheromone-detecting abilities and antennal morphology of the male corn earworm moth, *Heliothis zea* (Boddie) were examined by single-sensillum recording techniques and scanning electron microscopy (SEM). The antenna comprises approximately 80 subsegments that support several types of sensillar structures. Electrophysiological investigations were limited to the *s. trichodea* arranged in lateral rows along the proximal 40 to 50 subsegments of the male antenna. The recordings indicate that these sensilla are innervated by at least two cells, one of which is sensitive to (Z)-11,hexadecen-1-al, a major component of the pheromone system. In the poster, attention is given to a new stimulation system that permits accurate quantification of the odor presentation. A concentration-response relationship and several SEM micrographs are presented. Observations are made concerning the nature of the neural response of the pheromone detection system.

Connectivity of the Olfactory Axons with Forebrain Neurons following Partial Bulbectomy.  
GRAZIADEI, P.P.C. and MONTI GRAZIADEI, G.A.  
(Department of Biological Science, Florida State University, Tallahassee FL 32306)

Following subtotal bulbectomy, the olfactory sensory axons from a newly reconstituted population of sensory neurons regrow and form glomerular structures not only in the remaining portions of the olfactory bulb but also in the subependymal layer of the lateral ventricle and in the anterior olfactory nucleus. The regrowing axons form glomeruli in unconventional areas of the remaining bulb (external plexiform layer, mitral cell layer, granule cell layer) and the large neurons of the olfactory bulb reorient their dendrites to branch into these glomeruli. The axons that regrow into the anterior olfactory nucleus also form glomeruli, often in proximity of the lateral olfactory tract. The relationships of the anterior olfactory nucleus neurons with the glomeruli will be reported. These observations indicate that the olfactory axons do not preferentially invade their target when mechanical disruption of the olfactory pathway is experimentally induced in postnatal animals.

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Taste Responsivity of Neonates as a Function of Maternal and Infant Size. JOEL A. GRINKER and ADAM DREWNOWSKI (Human Nutrition Program, The University of Michigan)\*

Randomly selected full-term normal neonates (n=36) were tested 36 to 72 hrs post delivery. Infant weights averaged 3382 g and maternal pre-pregnancy weights averaged 60.8 kg. Indices of infant and maternal fatness included triceps skinfold (infant 5.4; maternal 17.08) and midarm circumference (infant 11.2; maternal 26.0). As expected, maternal size was related to infant size ( $r=0.33$ ). The microstructure of taste responsivity for a subsample of infants (n=13) to different concentrations of dextrose in distilled water (1/16, 1/8, 1/4, and 1/2M) was measured using an artificial nipple and pressure transducer. The pattern of sucking responses was recorded on magnetic tape for subsequent computer analysis. Infants in this group averaged 3504 g in weight, 5.3 triceps skinfold, and 10.6 midarm circumference. Parameters of taste responsivity, including the number of sucking bursts, the number of sucks, and response rate, differed marginally between a water standard and all concentrations of dextrose. Significant between-subject variability was observed in the patterns of response to sweet taste, and was correlated with infant size. The results are discussed in terms of techniques used to measure "taste responsivity" in neonates, as well as theories relating chemosensory responsiveness to parameters of infant growth and development.

\* Supported by NIH Grant AM32944.

Effect of Multiple-Sip Drinking Upon Judged Taste Intensity. BRUCE F. HALPERN, DIANE L. BARSKY, CYNTHIA F. KANNUS (Department of Psychology and Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853)

**GENERAL PROCEDURE:** A magnitude estimate of total taste intensity (modulus assigned before the beginning of the series) of the colorless liquid in 8 drinking glasses was requested 0.5 sec after 1st swallows (detected by a laryngophone). Sips from successive glasses were cued by tones at 0, 10, 22, 35, 51, 70, 89, and 111 sec from the beginning of the multiple sip series. Each subject participated in 3 identical sessions; 1st = practice. Modulus liquid and glasses 1 to 7 were 180 mM sucrose in 10 mM citric acid ("lemonade"). **EXPERIMENTS 1 & 3:** For 7 and 9 subjects, all liquids were "lemonade"; modulus liquid was held in the mouth until subjects could determine intensity, then swallowed. Judged intensity decreased across the 8 glasses ( $p < 0.005$ , Friedman ANOV); inten. of glasses 5 - 8 were significantly  $<$  glasses 1 & 2 (Wilcoxon MPSR). **Exp. 1:** % max. inten. =  $87(e^{-0.014 \text{ sec}})$ ,  $r = .83$ ; median inten. was 25% of max. at 92 and 114 sec. **Exp. 3:** % max. =  $73 - [(3.436) \ln(\text{sec})]$ ,  $r = .53$ ; median inten. was 50% of max. at 92 sec; 67% at 114 sec. **EXPERIMENT 2:** For 9 subjects, glass 8 was distilled water; modulus was assigned 0.5 sec after 1st modulus swallow. Judged intensity showed no signif. change across glasses 1 - 7 ( $p > 0.1$ , Friedman ANOV); there were no significant differences between glasses ( $p \geq 0.09$ , Wilcoxon MPSR). Glass 8 intensity differed from all other glasses,  $p < 0.001$ . **SUMMARY:** Change in judged intensity during multiple-sip drinking may be related to the standard used; no change was found with a modulus matched to the time of subsequent intensity judgements.

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Intracellular Recordings in the Olfactory Bulb of the Tiger Salamander: Initial Results.  
K.A. HAMILTON and J.S. KAVER (Tufts Univ., New England Medical Center, Neurosurgery Dept., Boston, MA).

By intracellularly recording the activity of neurons and subsequently staining them using the horseradish peroxidase technique, it is possible to identify circuit elements and examine how the elements interact. We hope to clarify integrative mechanisms in the olfactory bulb of the tiger salamander by recording *in vivo* responses to electrical stimulation and controlled odorant application and by morphologically identifying the neurons from which intracellular recordings are obtained.

Most of our recordings have been obtained from neurons which spike actively. In one neuron, electrical stimulation of the olfactory nerve or medial olfactory tract resulted in sustained hyperpolarization and suppression of spiking; the suppression elicited by stimulating the nerve was preceded by a brief depolarization and a burst of impulses. Staining of this neuron indicated that it was a mitral/tufted cell that had processes in at least two glomeruli.

Other types of neurons spike less actively and their responses appear to be more variable and more predominantly depolarizing. The location and spiny processes of one neuron which was stained indicated that it was a granule cell. Another neuron, which was not stained, responded to stimulation of the nerve with a strong burst of impulses; it did not appear to respond to stimulation of the tract.

We are in the process of characterizing the responses to odorants and the morphological diversity of stained neurons.

Supported by USPHS grant NS-20003 to JSK.

Bitter Electric Taste in Hamsters. M. SCOTT HERNESS (The Rockefeller Univ., NY, NY 10021) and CARL PFAFFMANN (The Rockefeller Univ., NY, NY 10021)\*

Electric taste has produced stimuli which can mimic the qualities of salty ( $\text{Na}^+$ ), sour ( $\text{H}^+$ ), and sweet (Saccharin-). We present here evidence for several anions which may serve as bitter electric taste probes, thus completing the spectrum of taste qualities. Responses were recorded from the hamster chorda tympani to stimulation of the anterior tongue with microamperes of current through 0.001 M solutions of the following sodium salts: NitrobenzeneSulfonic Acid (NBSA), Picrate, Nitrobenzoic Acid (NBA), and Cholate. Cathodal responses (delivering anions to the receptors) produced the following efficacy series:

NBSA > Picrate > NBA > Cholate

The most efficacious stimuli saturated at the highest response level and had the lowest threshold. Anodal responses (delivering  $\text{Na}^+$  to the receptors) were virtually identical for the different salts. Twenty-four hour two bottle preference tests were also conducted to determine if these salts (all of which are bitter to humans) are avoided by hamsters. With the exception of NaNBSA, all salts were avoided at concentrations of 0.01 M and above. NaNBSA was not avoided or preferred even at a 0.1 M concentration. Animals with taste aversions formed against NaNBSA and tested against the four standard taste qualities generalized the aversion to sucrose. Picrate, NBA, and Cholate are thus considered as good candidates for bitter electric taste with cathodal currents in the hamster. However, since all of these salts are bitter to humans, they may all be considered as potential bitter electric taste probes in humans.

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Vago-gustatory Interactions in the Parabrachial Nucleus of the Rat GERLINDA HERMANN (Brain Res. Inst., UCLA) and RICHARD ROGERS (Dept. Physiol., Northwestern Univ.)

Behavioral data indicate that visceral/vagal afferent signals can strongly bias taste preference and elementary ingestive behavior. Indeed, anatomical, electrophysiological, as well as recent behavioral data suggest that the brainstem is a principal locus of visceral and gustatory afferent control over ingestive phenomena. Our previous anatomical and physiological data revealed that the "gustatory" division of the parabrachial nucleus (PBN) is a site of considerable overlap and convergence of visceral and gustatory input. However, to date, there have been few studies on the response of identified gustatory-PBN neurons presented with simultaneous vagal and gustatory afferent signals. In order to assess the effects of vagal afferent signals on taste afferent processing in the PBN, we superimposed stimulations of vagal afferents and gustatory afferents onto single identified PBN units. We did this by attaching an axial, bipolar stimulating electrode to the left cervical vagal trunk and using anodal stimulation of the tongue, respectively. Chemospecificity was verified by application of various taste solutions to the tongue. Latencies of PBN unit response to either cervical vagal or tongue (electrical) stimulation alone was determined. Based on these latencies, the onset of the gustatory stimulus was delayed for an optimal period (approx. 15msec) relative to the onset of the cervical vagal stimulation. This spatial summation of vagal and gustatory afferent activity onto convergent PBN units revealed that the threshold for gustatory activation of these units, on a background of constant vagal activity, could be substantially reduced. All gustatory-PBN units examined at this time (restricted to the middle and caudal zones of the PBN), have demonstrated convergence with vagal afferent information.

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Development of Salt and Sugar Responses in Hamster Chorda Tympani Nerve. DAVID L. HILL (Dept. Psychology, Univ. Toledo, Toledo, OH 43606).

To determine whether changes in salt and sugar responses occur during development in the hamster, whole-nerve responses were recorded from the chorda tympani nerve in hamsters aged 15-73 days postnatal. Ten hamsters were studied aged 55-73 days, eleven in hamsters aged 25-35 days and nine in hamsters aged 15-20 days. Chemical stimuli applied to the anterior tongue were 0.1M and 0.5M  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ ,  $\text{LiCl}$  and  $\text{KCl}$ . Responses were also recorded to a concentration series of sucrose, fructose, maltose, glucose (0.01M-1.0M) and lactose (0.01M-0.5M). Steady-state response amplitudes were analyzed.

When salt responses were expressed relative to the 0.1M  $\text{NaCl}$  response, increases occurred to 0.1M and 0.5M solutions of  $\text{NH}_4\text{Cl}$  and  $\text{KCl}$  and to 0.5M solutions of  $\text{NaCl}$  and  $\text{LiCl}$  during development. In contrast, only responses to 0.1M  $\text{NaCl}$  and  $\text{LiCl}$  changed when expressed relative to 0.1M  $\text{NH}_4\text{Cl}$  responses. Changes also occurred to sugars. When compared to the respective 0.5M response, responses to the 0.75M and 1.0M monosaccharides, glucose and fructose, were largest in young hamsters; no differences were found for the disaccharides sucrose, maltose and lactose.

These results show that integrated salt responses in the hamster during development change in the opposite direction compared to rat and sheep. That is, response ratios of  $\text{NH}_4\text{Cl}$ , and  $\text{KCl}$  to  $\text{NaCl}$  increase during development for hamster and decrease in rat and sheep. Additional changes in hamster taste responses occur to monosaccharide but not disaccharide sugars. These results suggest that taste receptor events in hamster are different than rat during development, and that different developmental processes occur between monosaccharide and disaccharide stimulation.

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Inhibition of the Gerbil's Electrophysiological Sucrose Taste Response by Para-nitro-phenyl- $\alpha$ -D-glucopyranoside and Chloramphenicol<sup>1</sup>. WILLIAM JAKINOVICH (Lehman College), VASILIKI VLAHOPOULOS (Lehman College)

In our search for sweet taste inhibitors we have discovered that the gerbil's whole nerve electrophysiological response to sucrose is suppressed by para-nitro-phenyl- $\alpha$ -D-glucopyranoside (PNP-GLU) and chloramphenicol (CA). In these experiments, we prepared mixtures of PNP-GLU or CA with sodium chloride, potassium chloride, hydrochloric acid and sucrose. The following were observed:

- (1) Neither PNP-GLU nor CA alone stimulate the gerbil's taste nerve.
- (2) The taste responses produced by sodium chloride, potassium chloride and hydrochloric acid were unaffected by the presence of either PNP-GLU or CA.
- (3) The sucrose response is inhibited by these substances.
- (4) In both cases inhibition was surmounted by a high concentration of sucrose.
- (5) CA is a more potent inhibitor than PNP-GLU. Moreover, both of these inhibitors are more potent than our other gerbil sucrose taste inhibitor, methyl 4,6-dichloro-4,6-dideoxy- $\alpha$ -D-galactopyranoside.
- (6) The inhibition occurs only when PNP-GLU and CA are mixed with sucrose; it is short lived, and ceases when the mixtures are rinsed from the tongue.

This experiment indicates that finding new and more potent inhibitors is possible. However it also indicates that we cannot predict which compounds will inhibit, making this work more difficult.

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Pheromone Uptake and Processing on Moth Antennae. K.E. KAISLING & S. KANAUIA (Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen, FRG).

Freshly isolated antennae of the male moth of *Antheraea polyphemus* adsorbed about 25% of <sup>3</sup>H-labelled pheromone molecules (E-6,Z-11 hexadecadienyl acetate) from an air stream passing the antenna. About 40% of molecules desorbed if the antenna was exposed to a fresh air stream for 30 min. More than 75% of the adsorbed molecules were found on the long olfactory hairs (sensilla trichodea). The distal half of the hairs caught about twice as many molecules as the proximal half. A fraction of the adsorbed molecules migrated from the hairs towards the antennal branch (see also Steinbrecht and Kasang, 1972). Immediately after adsorption the molecules could be eluted from the antenna by pentane. With increasing incubation time the fraction eluted in pentane decreased. The molecules could still be eluted by more polar solvents (toluene, chloroform, methanol) except for a residual fraction which reached 20% of the total radioactivity per antenna after 30 min of incubation (see also Kasang and Kaissling, 1972). Most of these processes are characteristically altered in vacuum-dried antennae.

G. KASANG and K.E. KAISLING, in Int. Symp. Olfaction and Taste, ed. D. Schneider, Wiss. Verlagsgesellsch., Stuttgart, 1972, 200-206.

R.A. STEINBRECHT and G. KASANG, *ibid.*, 193-199.

Taste Receptor Cells in the Lobster, *Homarus americanus*, Are Narrowly Tuned Even at High Stimulus Concentrations. BRUCE R. JOHNSON, RAINER VOIGT, PAOLA F. BORRONI and JELLE ATEMA (Boston University Marine Program).

The taste receptor cells on the walking legs of the lobster *Homarus americanus* have remarkable spectral specificity when tested at stimulus concentrations of approximately  $3.5 \times 10^{-6}$  M (Derby, C.D. and Atema, J. J. Comp. Physiol. 146:181, 1982). However, lobster taste receptors may encounter substantially higher concentrations of stimuli, as when the walking legs are ripping apart mussel flesh which contains  $10^{-4}$ - $10^{-1}$  M amino acids (Mackie, A.M. et al. J. Fish. Biol. 16:701, 1980). Since knowledge of response specificity is important to understand the nature of the sensory code, we re-examined the taste receptor specificity in *Homarus* at high but biologically relevant stimulus concentrations. In addition to tuning breadth measurements, we investigated how exposure to high stimulus concentration affects the dynamic response range of the walking leg glutamate receptors.

Single receptors from the first two pairs of walking legs were identified electrophysiologically with a search stimulus (SS) composed of 15 compounds, then tested individually at  $3 \times 10^{-4}$  M to determine the response spectrum for a particular cell. Of 60 cells analyzed 37% responded best to glutamate; 15% to hydroxyproline; 13% to ammonium chloride and 13% to betaine. The other cells responded best to a variety of the remaining test stimuli, some with great specificity. Although the response specificity varied from cell to cell, the glutamate and ammonium chloride-best populations contained most narrowly tuned cells; the betaine and hydroxy-proline best cells showed much broader response breadth. As expected, prior exposure to high stimulus concentrations does affect glutamate cells by reducing their subsequent sensitivity to low stimulus concentrations.

Our study demonstrates that at least the glutamate and ammonium chloride cells remain narrowly tuned at high stimulus concentrations. They may thus represent a true labeled line for the presence of glutamate and ammonium chloride.

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Topographic arrangement and response properties of gustatory neurons in the vagal lobe of the catfish. JAGMEET S. KANWAL and JOHN CAPRIO. (Louisiana State University, Baton Rouge, LA 70803).

The vagal lobe (VL) is the primary center for gustatory input from the oropharyngeal region of teleosts. However, in Cyprinidae and Ictaluridae, an additional structure, the facial lobe, receives visceral input from facial (VII) nerve branches innervating the extra-oral epithelium. The VL in these fishes is analogous to the caudal portion of the nucleus solitarius of mammals and receives afferents from the glossopharyngeal (IX) and vagal (X) nerves. In the present study, mechanical and chemical (amino acids and quinine hydrochloride) stimulation of the oral cavity were employed to map the representation of the oropharyngeal sensory epithelium in the VL of the channel catfish, *Ictalurus punctatus*. These data revealed that the antero-posterior axis of each lateral half of the oral cavity is represented ipsilaterally and antero-posteriorly in the VLs. Neuroanatomical studies employing transganglionic transport of HRP also indicated an antero-posterior pattern of projection of the peripheral (IX-X) neurons. Electrophysiological mapping showed that specific structures, (palatal organ, gill arches and tongue region) are represented in somewhat diffuse and overlapping domains. Also, mechanical stimulation (ipsilateral and bilateral) of the upper lip and proximal portion of the maxillary barbels produced neural responses in an anterior region of the VL. Peripheral recordings indicated the presence of phasically responding taste and tactile units and tonically firing proprioceptive units in the IX and X nerves. (Kanwal & Caprio, 1983). The three types of excitatory units characterized peripherally were also observed within the VL; in addition, some units were inhibited by either mechanical or chemical stimulation of the oral epithelium. Thus, the VL preserves, in general, the spatial organization and electrophysiological nature of the gustatory input.

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Voltage-Sensitive Dye Recording from the Olfactory System of the Tiger Salamander. J.S. KAVER (Tufts-N.E.M.C., Boston, MA), D. SENSEMAN (Univ. Texas, San Antonio, TX), L.B. COHEN (Yale Medical School, New Haven, CT)

We have used a voltage-sensitive dye to monitor neuronal activity in the intact salamander olfactory bulb after odor stimulation of the mucosa. Certain dyes, when incorporated into neuronal membranes, change their fluorescent properties with voltage change across the membrane. By recording the optical signals generated by the dyes, one can measure voltage changes in neuronal tissue without the use of invasive electrode penetrations. By using an array of optical recording devices, signals from a number of sites can be measured simultaneously with a time resolution of 0.7 msec (review-Cohen and Salzberg, 1978, Rev.Physiol.Biochem. Pharm. 83:35).

In the present study we extended the experiments of Ohrbach & Cohen, 1983 (J.Neurosci. 3:2251) by using the styryl dye RH414 to observe activity in the olfactory bulb after stimulation of the nose with controlled odor pulses. A square array of 124 detectors was centered over the bulb to record fluorescence changes with both electrical and odor stimulation. In this way an array of large signals was recorded which was aligned parallel to the bulbar layers. Unlike the mammal, the cell layers in the salamander are stacked in a planar configuration and extend to the surface of the bulb so that the fluorescence signals could be correlated directly with the bulbar strata. These studies afford the possibility for observation of the spatial distribution of neuronal activity in the olfactory pathway over a short time course and thus are an important adjunct, providing improved temporal resolution, to the 2-deoxyglucose method.

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A Quantitative Comparison of Dorsal Flow (Open) and Closed Taste Delivery Systems. STEVEN T. KELLING, BRUCE P. HALPERN (Department of Psychology and Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853).

**PROCEDURE:** Conductivity of stimuli delivered by open and closed (tongue forms part of wall) systems was compared after an artificial tongue (AT) or no-tongue (I) (open system only). Single 100, 200, 500, and 1000 msec pulses of NaCl at 50, 100, 300, and 500 mM, or Na-Saccharin at 2 mM, were preceded and followed by distilled water. Flow rate was 5 ml/sec for the open system; 10 ml/sec, closed. **MEASURES:** Stimulus duration at "recognition" (10 mM NaCl, 83  $\mu$ M NaSac) and "detection" (1 mM NaCl, 40  $\mu$ M NaSac) criteria, plus onset (10% max. conductivity to 90%) and removal (90% max. conductivity to 10%) times. **RESULTS: Time Course:** All closed onset times were < 10 msec; open, < 10 msec for NaCl, < 135 msec for NaSac. All open removal times were >> closed times,  $p < 0.001$ , Mann-Whitney U, with no overlap of values. All closed removal times for NaCl were  $\leq 95 \pm 6$  msec (mean  $\pm$  S.E.); for NaSac,  $\leq 49 \pm 3$  msec. All open removal times for NaCl were  $\geq 512 \pm 39$  msec for I;  $\geq 889 \pm 21$  for AT; for NaSac,  $\geq 268 \pm 12$  msec for I,  $\geq 541 \pm 13$  for AT. **Duration:** All open durations were >> intended durations and >> closed durations,  $p < 0.001$ , Mann-Whitney U, with no overlap in AT distributions. For the 1 mM NaCl criterion, closed durations were  $\leq 144 \pm 3$  msec,  $253 \pm 6$ ,  $574 \pm 3$ ,  $1075 \pm 4$ ; open durations were  $\geq 232 \pm 12$  msec,  $349 \pm 15$ ,  $657 \pm 8$ ,  $1154 \pm 11$ , for I;  $747 \pm 14$  msec,  $909 \pm 14$ ,  $1313 \pm 12$ ,  $1744 \pm 15$ , for AT. **CONCLUSIONS:** Closed systems can produce stimulus pulses similar to intended durations and rapidly remove the stimulus pulse. Open flow systems cannot.

Support was from NSF Grant BNS-8213476.

The Application of Interactive Real-Time Computer Graphics to Three-dimensional Modelling of Taste Bud Ultrastructure. J.C. KINNAMON, T. EDWARDS\* T. SHERMAN, S. ROPER (University of Colorado Medical School), (\*Colorado State University)

The generation of three-dimensional (3-D) serial section reconstructions with interactive computer graphics is a powerful approach for studying the ultrastructure of taste buds, taste cells and their synapses. Computer generated 3-D reconstructions can be rotated in the X, Y and Z axes in real-time and the structures maintain their relative positions.

In the present study we have utilized an interactive Evans & Sutherland graphics system in conjunction with a VAX computer to generate 3-D reconstructions from serial thick and thin sections of vallate taste buds from the mouse examined with high voltage electron microscopy and conventional transmission electron microscopy, respectively. By using real time rotations, we observed characteristics of the 3-D structure of taste buds which were not apparent in static 3-D displays. We previously described taste cell synapses to be of two types, macular and bleb-like. Using this new technique we have found that the latter synapses resemble rod and cone synapses to bipolar retinal cells. These invaginated synapses are associated with thin neuronal processes that penetrate into the nuclear region of the taste cell. The active synaptic site corresponds to the invaginated portion of the taste cell membrane apposed to the neuronal process, which may either be cylindrical in shape or constricted at the base. Other synapses are often situated in grooves formed by the taste cell membrane. There was no apparent correlation between synaptic structure and cell type (e.g., Light or Dark).

This study was supported in part by grants from NIH and Procter & Gamble, as well as the Department of Engineering Sciences, Colorado State University.

The Hydrolytic Susceptibility of the Garter Snake Chemoattractant Found in Earthworm Wash. DONALD M. KIRSCHENBAUM, NANCY SCHULMAN and MIMI HALPERN (Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, N. Y. 11203).

Earthworm wash (EW) contains a chemoattractant (CA) for garter snakes. When EW is examined by gel chromatography on G-75 or Aca44 two peaks, F2, of large MW, 65+K, and, F4, of smaller MW, about 3-5+K are separated. F2 contains all the CA activity. These experiments describe changes in CA and structural integrity of EW after heating at 95 degrees C in either 0.1N HCl or 0.1N NaOH for 0, 1, 2, and 3 h. Following heating the samples were cooled, neutralized to pH 5.8, and lyophilized. All lyophilized samples were reconstituted to 5X their original concentration in .15M saline, tested on garter snakes for CA activity, and separated into their constituent peaks on a 44 x 1.6 cm column of Aca 44. The changes in CA activity, OD 280 nm, carbohydrate (CHO) content, and ninhydrin-positive (NP) material content were determined at each time interval. All CA activity was lost after 1 h of heating in either acid or base. The samples, after separation on Aca 44, exhibited different analytic parameters depending on whether they were heated in acid or base. The F2 peak, after heating for 3 h in acid, showed a decrease in OD 280 nm, a decrease in CHO content to zero, and the disappearance of the small amount of NP material originally present. The F4 peak showed almost no change in OD 280 nm, and an increase in heterogeneity of molecular weight. The CHO content increased 5-fold with the major increases seen between 0-1h and 2-3h. The content of NP material remained almost constant until a 5-fold increase was seen in the sample heated for 3h. The F2 peak disappeared completely after 1h heating in 0.1N NaOH as demonstrated by the absence of OD 280nm, the absence of CHO content, and the absence of NP material. The F4 peak obtained after heating in alkali showed an increase in 280 nm absorption and CHO content. The large amount of NP material found at zero time persisted throughout the heating period showing a small decrease, 16%, after 3h.

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# Gustatory Evoked Potentials in Man Elicited by Chemical Stimulation of the Tongue

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Despite numerous attempts, a method of recording gustatory evoked potentials (GEP) has not been found as yet. This study reports a new stimulation technique by which taste receptors can be stimulated selectively.

Chemical stimuli of an approximately rectangular concentration course were presented to 5 subjects. Transition time for any desired concentration level was less than 20 ms. Acetic acid of different concentrations was used as the stimulant. EEG was recorded from standard 10/20-positions referenced to A<sub>1</sub>. Taste receptors were activated in a sufficiently synchronous manner to evoke summated slow wave potentials in the central nervous system. Concordant to other cortical evoked potentials the vertex was the site of the most pronounced deviations of gustatory evoked potentials.

Potential amplitudes increased and latencies shortened with rising concentrations. After application of the local anesthetic tetracaine hydrochloride (10 mg) to the tongue, GEPs and taste sensations were eliminated. In one patient ageusia during radiation therapy (36 Gy) was proved by the attenuation of the GEPs.

Also GEPs related to sweet (fenchon, chloroform), salty (ammonium chloride) and bitter (thujon) taste sensations were recorded for the first time.

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

# Lesions of the Area Postrema in Rat Alter Sensory Processing of Taste Information. THERESE KOSTEN & ROBERT J. CONTRERAS (Dept. of Psychology, Yale University, New Haven, CT 06520).

We reported previously that lesions of the area postrema (AP) and the medial nucleus of the solitary tract (NST) in the rat lead to an enhanced preference for saline solutions. Presently, we investigated the specificity of this effect by measuring the preferences for a few concentrations of several salt solutions (NaCl, KCl, NH<sub>4</sub>Cl, CaCl<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>), quinine, and sugars (glucose, sucrose) in 9 AP lesioned rats and in 6 sham control rats. Preferences were tested in a two-bottle test over 48 hr.

The AP lesioned rats exhibited significantly higher preferences ( $p < .01$ ) and intakes ( $p < .02$ ) for all the salt solutions; the greatest differences were at the isotonic and slightly hypertonic concentrations. There were no group differences in the intakes or preferences for quinine and sugar ( $p > .05$ ). For all the solutions, there were no differences in total intake ( $p > .10$ ). The preference measures were further analyzed using multidimensional scaling. The scaling resulted in two dimensions for both groups. The first dimension was a preference-aversion dimension. The second dimension differed by group: it was a "saltiness" dimension for the control group and a "visceral effects" dimension for the AP lesion group. Solutions that cause illness (quinine, NH<sub>4</sub>Cl) or satiety (sucrose, glucose) tended to be high for the AP lesion group's second dimension.

Thus, lesions of the AP lead to enhanced preference for several salt solutions, but not to increased preference for the other solutions. For AP lesioned rats, we speculate that: 1) salty solutions become more like sweet or palatable substances and; 2) solutions that cause visceral reactions are discriminated but may have more positive connections than they do for intact rats.

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# Non-invasive Recordings of Peripheral Pain-related Electrical Potentials in Man

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Non-invasive methods were employed to record pain-related electrical potentials from the human respiratory nasal mucosa. Within a constantly flowing air stream of controlled temperature and humidity, gaseous stimulants (isoamylacetate, linalool, eucalyptol, carbon dioxide) at painful concentrations were presented by a newly developed stimulating device.

The amplitudes of the potentials correlated with the concentrations of the stimulants as well as with subjective estimations of pain intensity. Depending upon interstimulus interval, paired stimuli led to either adaptation or enhancement of the second response. The local anesthetic tetracaine hydrochloride, and also the systemically administered analgesic pentazocine given prior to painful stimulation resulted in a decreased amplitude of negative potentials.

Analogous to the electroolfactogram, this peripheral response was interpreted as a summated receptor potential of chemical nociceptors.

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# The Fine Structure of Human Circumvallate Taste Buds.

S. H. KUBOWICZ, B. W. JAFEEK, and D. T. MORAN (Depts. of Otolaryngology and Anatomy, Univ. of Colorado Health Sciences Center, Denver, CO 80262).\*

We have used conventional transmission electron microscopic techniques to study human circumvallate taste buds removed by biopsy from surgical patients. Like taste buds in other species, human circumvallate taste buds are ovoid aggregations of specialized cells that extend throughout the lingual epithelial thickness and that present a taste pore at their apical pole by which intragemmal cells contact the oral cavity. Tight junctions isolate the taste pore from the underlying intragemmal intercellular spaces.

At least two and perhaps three cell types are present within the taste buds: dark cells, light cells, and "type III" cells. Dark cells are thin, longitudinally oriented cells with extensive membrane interdigitations and centrally located, irregular, heterochromatic nuclei. They contain small mitochondria, abundant ribosomes and RER, apical accumulations of dense (secretory) granules 80-200 nm in diameter, and an apical neck capped by microvilli that project into the taste pit. Their ultrastructure suggests that they are supporting elements.

Light cells, by contrast, are larger cells with large, spherical, euchromatic nuclei containing one or more nucleoli. They contain larger mitochondria than do dark cells, fewer free ribosomes and RER profiles, a more extensive SER system, multiple Golgi systems, and a perinuclear filamentous sheath; they too directly contact the taste pit. Their ultrastructure suggests that they are the chemoreceptive elements.

Infranuclear accumulations of dense-cored vesicles 70-125 nm in diameter and clear, presumably synaptic, vesicles 30-60 nm in diameter are also seen in some cells. Synapses onto intragemmal nerve fibers have been seen only in association with these vesicle accumulations. Only the clear vesicles have been seen directly apposed to the presynaptic membrane. Whether these vesicles are characteristic of a distinct "type III" cell or are local manifestations of some, but not all, light cells, is as yet unknown.

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High and Low Peri-sniff Air Flows Have Little Effect Upon Stimulus-Response Relationships. D. B. KURTZ (Northwestern University, Evanston, IL), M. M. MOZELL and S. W. SWIECK (Upstate Medical Center, SUNY, Syracuse, NY).

Mozell, Sheeha, Swieck, Kurtz and Hornung (1984, *J Gen Physiol*, in press) have shown, in the frog, that the magnitude of the summated olfactory nerve discharge can be adequately modeled as a multiplicative function of stimulus volume, stimulus duration and the number of odorant molecules. In their experiment, the test sniffs interrupted a 20 cc/min flow of humidified air. Since the flow of humidified air did not instantaneously remove the odorant from the olfactory cavity, the residual odorant molecules represented a possible 'tail' of stimulation and possibly affected stimulus-response relationships. The present experiments examined whether the choice of the flow rate for the humidified air altered the model generated. Sniffs identical to those of Mozell et al. were presented to bull frogs. In the first experiment, the flow of humidified air was raised to match the flow rate of the stimulus presented. Perhaps the higher flow of humidified air would clear the olfactory cavity of odorant faster thereby reducing the impact of the 'tail'. Analysis of responses revealed stimulus-response relationships nearly identical to those generated by Mozell et al. In the second experiment, the flow of humidified air was halted before and after the presentation of the stimulus. Again perhaps stimulus-response relationships would be altered. In particular, it was expected that the exponent on time would be reduced since the duration of the stimulus was short in comparison to the total length of time that the odorant was allowed to interact with the receptors (.35 and .70 sec vs. 8 sec). In addition, the responses were expected to be larger than when the flow of humidified air removed odorant molecules. As expected, the responses generated were larger. Small differences were noted in the exponents of the three variables. However, most striking was that the model remained substantially the same. This indicates that stimulus-response relationships are generated in the initial moments while the stimulus is flowing into the olfactory cavity and that increasing or decreasing odorant clearance mainly alters the relative size of the responses.

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Monoclonal Antibodies Against Unique Surface Glycoproteins of Frog Olfactory Cilia. DORON LANCET, ZEHAVA CHEN (Dept. Membrane Research, The Weizmann Inst. of Science, Rehovot, Israel)\*

We have recently reported that frog olfactory cilia contain seven specific membrane polypeptides, four of which are glycosylated (Z. Chen and D. Lancet, *Proc. Natl. Acad. Sci. [USA]*, 81, 0000 [1984]). Based on the finding that lectins inhibit the electroolfactogram (EOG) response to a range of odorants (Shirley, S., Polak, E. and Dodd, G.H. *AChemS V*, no. 128 [1983]), we suggested that glycosylation may be a "common denominator" property of protein structures involved in reception of many or all odorous stimuli. We hypothesize that lectin binding interferes with signal transfer from the odorant binding site at a putative "variable region" to the invariable transducing component(s).

In order to develop additional probes to common denominator structures of the receptors, and to obtain more information on the specific ciliary proteins, we have raised monoclonal antibodies against the total detergent extract of isolated olfactory cilia. Two of the olfactory-unique membrane glycoproteins, gp95 and gp58, were found to be highly immunogenic, each giving rise to several specific antibodies. The antibodies have been selected by high immunoassay reactivity towards the complete ciliary membrane extract, and could not be directed against minor protein subpopulations. It is therefore expected that if reactive to receptor structures, such antibodies will be specific to determinants shared by all receptor types. At present, we are studying the effect of the monoclonal antibodies, topically applied to frog olfactory epithelial surface, on EOG responses. In parallel, the antibodies are used for protein-structural studies and for tissue and cellular localization.

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Amiloride Does Not Suppress Taste and Olfactory Responses to Amino Acids in the Catfish. CHARLES F. LAMB IV, JOHN DUDEK, HILARY THOMPSON & JOHN CAPRIO (Louisiana State University, Baton Rouge, LA. 70803)

Amiloride, a specific inhibitor of Na<sup>+</sup> transport in epithelial tissue (Benos, 1982), was recently shown to depress gustatory neural activity in the rat to NaCl and LiCl, but not KCl, and reduce the perceived taste intensity of Na<sup>+</sup>, Li<sup>+</sup> and sweeteners in humans (Schiffman et al., 1983). Similar neural effects were also reported in the gerbil (Teeter et al., 1983). In human psychophysical studies, Schiffman et al. (1983) reported that amiloride reduced the perception of sweet and salty, but not bitter, amino acids (AA), and that differential effects of the drug were observed between D (sweet) and L (bitter) stereoisomers of the same AA. Thus, we attempted to determine whether amiloride might be useful as a molecular probe of the highly sensitive AA taste and olfactory receptors of the channel catfish.

We tested AA (0.01mM or 0.1mM) before, during and after presentation of 0.1mM amiloride to the maxillary barbel taste buds and olfactory mucosa of the catfish. Common stimuli for both taste and smell included L-Ala, L-Arg, L&D-His, L&D-Trp and Gly. Additional AA for taste were D-Ala and L-Abu; other stimuli for olfaction included L-Glu, L-CysH and L-Met. The AA solutions were added either to a continuous flow of well-water (before and after amiloride irrigation) or to amiloride (begun 30 min prior to AA testing) that bathed the respective receptors. Interstimulus intervals were 2 min. No significant change in AA relative effectiveness resulted from the amiloride treatment determined from integrated taste and EOG recordings. Amiloride, however, was found to be a moderate olfactory, but a poor gustatory, stimulus. These results indicate that olfactory and gustatory responses to AA in the catfish are not mediated by amiloride-sensitive channels.

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Effects of Oral Chemical Irritation on Taste. H. T. LAWLESS (Monell Chemical Senses Center) and D. A. STEVENS (Clark University).

Intense sensations of oral irritation were induced by rinses with emulsions of capsaicin oleoresin and of piperine, constituents of red and black pepper, respectively. After rinses with these emulsions, the perceived intensities of two concentrations of sucrose, NaCl, citric acid and quinine (representing the four classical taste qualities) were evaluated by magnitude estimation. Comparing taste intensity after rinses with capsaicin and after control rinses with emulsifying agents or water, there were significant decrements in taste intensity of citric acid and quinine, and on one concentration of sucrose, but no effect on salt. The effects of piperine were more broad, with significant decrements in perceived intensity relative to emulsion controls for all substances. These effects point to an inhibitory influence of stimulation of the common chemical sense in the oral cavity on gustatory sensations.

Chemosensory Responses of a Ciliate to Pro-opiomelanocortin Peptide Hormones. M. LEVANDOWSKY (Haskins Labs, Pace Univ., N.Y.), A. S. LIOTTA and D. T. KRIEGER (Mt. Sinai School of Medicine, N.Y.)

A number of peptides previously thought to be derived solely from vertebrate endocrine systems have been shown to be present in unicellular organisms, including the ciliate *Tetrahymena*, but their function in these organisms is not known. We have found that *Tetrahymena* aggregates in capillaries containing such peptides. Adrenocorticotrophic hormone (ACTH), melanocyte-stimulating-hormone (MSH), and alpha-, beta- and gamma-endorphins are active at concentrations below  $10^{-4}$ M; several peptides fragments of these were also active. After correction for adsorption, it appears that levels of dissolved peptides were in the nanomolar range. The opioid antagonist naloxone blocked these and other chemosensory responses.

Suppression of Mitotic Activity in Frog Olfactory Epithelium by Hydroxyurea. M.S. LIDOW, S.J. KLEENE, and R.C. GESTELAND (Northwestern University).

Normal olfactory epithelium contains olfactory receptor cells of different ages. In order to study the cells of a given age, we are trying to obtain olfactory epithelia with developmentally synchronized cell populations. We plan to ablate the epithelium by standard methods and then allow it to regenerate for a short time. We will then continuously suppress mitotic activity in the epithelium to preserve a developmentally synchronized population of cells. We have found the potent antimitotic drug hydroxyurea useful for this; this drug has almost no side effects on other cellular functions. The drug was applied to the nasal cavity of frogs continuously for 48 hrs through polyethylene tubes introduced into the nose and fixed on the head. The amount of  $^3$ H-thymidine incorporated over the same 48 hrs was measured.  $^3$ H-thymidine was injected into the frog lymphatic sac 3 times a day during the 2 days (0.33 mCi per g of weight per injection). The  $^3$ H-thymidine incorporated per 10 ng of epithelial DNA was measured by standard methods. The radioactivity recovered was hydrolyzable by DNase with snake venom phosphodiesterase. To see how hydroxyurea affects mitotic activity in the whole organism, the incorporation of  $^3$ H-thymidine into liver DNA was also measured. As a control, we determined that introduction into the nasal cavity of pure Ringer at flow rates up to 30 cc/day does not change mitotic activity in frog olfactory epithelium.

We found that hydroxyurea can suppress mitotic activity in olfactory epithelium by 92%. At the highest flow rates, mitotic suppression is great for both epithelium and liver. However, at lower flow rates mitotic suppression in olfactory epithelium is higher than in liver. We also found that control levels of mitotic activity in olfactory epithelium and liver and also the effect of hydroxyurea are highly dependent on the animal's state of health.

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Clinical Investigation of Reported Taste Loss in Depressed Patients. NAOMI E. LOHR, PH.D. (University of Michigan), ANDREA L. JACOBS, B.A. (Washington University in St. Louis), and DOUGLASS KING, M.D. (Vanderbilt University)

Reported taste loss, a highly sensitive and specific diagnostic marker for melancholia, is being investigated by means of psychophysical measurement in a group of psychiatric patients.

Data from the laboratory evaluation of taste and olfaction function in depressed patients and age congruent control subjects are presented. Olfaction is assessed both by means of the University of Pennsylvania Smell Identification Test and an odor identification test developed at the John B. Pierce Foundation Laboratory. Results from the two methods appear comparable and support the patients' report of normal olfaction. Taste function is assessed both in terms of threshold sensitivity and suprathreshold discriminations. Thresholds are established for NaCl by means of the Staircase-Forced Choice Method (Bartoshuk, 1978) which requires patients to make a series of judgements between pairs of stimuli, each pair consisting of a cup of NaCl concentration and a cup of deionized water. The first group of depressed patients appear to have higher thresholds for taste quality (NaCl) than non-depressed controls. Suprathreshold function is assessed by means of a Pierce Foundation developed "Magnitude Matching" in which intensities of the four taste qualities are given nonmodulus ratings, and, in addition, are matched to an auditory stimulus of varying decibels. Depressed patients with taste loss complaints do not appear to differ from patients without taste loss complaints in suprathreshold gustatory function. Case illustrations are chosen to contrast melancholic with non-melancholic patients.

Olfactory Placode Transplantation in *Xenopus laevis*. L. MAGRASSI and P.P.C. GRAZIADEI (Department of Biological Science, Florida State University, Tallahassee, FL 32306)

Ectopically transplanted olfactory placodes can, in amphibian embryos, connect with several regions of the C.N.S. where a marked hyperplasia is always present in connection with the termination of the olfactory fibres. We have investigated the transplant of the olfactory placode after removal of the eye vesicle to test the capacity of the olfactory neurons to connect and influence an highly determined structure such as the optic cup and stalk. *Xenopus laevis* embryos at stages 23 to 24 were used as donors and hosts; all the animals were sacrificed at stages 47 to 50. The hosts (n=50) received the transplant of two olfactory placodes connected by a strip of ectoderm, in place of the right optic vesicle. The transplanted placodes fuse with the homolateral host's placode. Two or three olfactory nerves originate from the fused olfactory organ and terminate into the host C.N.S.. One nerve is consistently seen to terminate into the homolateral olfactory bulb. The other nerves terminate at the level of the diencephalon. A protrusion from the lateral wall of the diencephalon is visible at macro- and microscopical levels. The protrusion contains a normal ventricle lined by ependyma and connected with the third ventricle by means of a foramen situated at the level of the recessus preopticus. Glomerular structures are always present at the apical pole of the protrusion where the olfactory axons terminate. The present data support the hypothesis that the olfactory fibres can interfere with and can influence the normal process of regeneration of an highly determined structure such as the optic vesicle and the optic stalk.

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Neurons of the Hippocampal Formation send Collateral Projections to both the Anterior Olfactory Nucleus and Lateral Septum in Hamsters. J.E. MARCHAND, T.A. SCHOENFELD, AND F. MACRIDES (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545).

Previous studies have shown that the main olfactory bulb has a relatively direct input to the hippocampal formation via the entorhinal cortex. The present study characterized a relatively direct input to the main olfactory bulb from the hippocampal formation via pars medialis of the anterior olfactory nucleus. Injections of wheat germ agglutinin-horse-radish peroxidase or fluorescent dyes (nuclear yellow, Evans blue or fast blue) into pars medialis produced extensive retrograde neuronal labeling throughout the subiculum and hippocampal field CA<sub>1</sub>, as well as a modest incidence of retrograde labeling in hippocampal field CA<sub>2</sub>. Extensive retrograde labeling was present in both the dorsal and ventral portions of the hippocampal formation, but was more prevalent in the ventral portion. Both portions are known to project heavily to the lateral septum; therefore, double injections of fluorescent dyes were made. SITS (4-acetamido, 4'-isothiocyanostilbene-2,2'-disulfonic acid) or fast blue were injected into the lateral septum and Evans blue or nuclear yellow were injected into pars medialis. Each of the combinations produced double labeling of neurons in the subiculum and hippocampus. Because Evans blue and SITS are excited by different filter systems, and because SITS does not appear to be taken up by fibers of passage, unequivocal double labeling was best achieved using this combination. The finding of substantial double labeling indicates that neural activity transmitted to the septum from the hippocampal formation is also transmitted to the olfactory system via pars medialis. Furthermore, this component of the anterior olfactory nucleus can be conceptualized as complementary to the entorhinal cortex in mediating relatively direct interactions between the hippocampal formation and the main olfactory bulb. This functional analogy may help to account for previous findings of heavy, reciprocal connections between pars medialis and the entorhinal cortex.

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Chemical Lavage of the Olfactory Epithelium Produces Selective Decrements in Responding to Ketone Odorants by Tiger Salamanders. J. RUSSELL MASON and L. CLARK (Monell Chemical Senses Center) and THOMAS H. MORTON (University of California, Riverside).

Tiger salamanders (*Ambystoma tigrinum*) were trained to avoid cyclohexanone (CH) and dimethyl disulfide (DMDS) by pairing odorant presentations with bright light, as negative reinforcement. Responding to *n*-butanol (BuOH), which was not paired with light, was monitored concurrently. Training continued until avoidance responses were made to 80% of presentations of CH and DMDS and 20% of presentations of BuOH. After training, the animals were anesthetized by immersion in MS-222, and their olfactory sacs were lavaged bilaterally with one of the following: (a) 50mM NaBH<sub>3</sub>CN, (b) 0.5mM ethyl acetoacetate (EAA), (c) 50mM EAA, (d) 0.5mM EAA followed by 50mM NaBH<sub>3</sub>CN. Neither 50mM NaBH<sub>3</sub>CN nor 0.5mM EAA produced changes in responding to the odorants during subsequent tests ( $p > 0.25$ ). Conversely, both 50mM EAA and 0.5mM EAA followed by 50mM NaBH<sub>3</sub>CN produced selective decrements in responding to presentations of CH ( $p < 0.01$ ). Similar results were obtained (a) when (2-methylthio) ethyl acetoacetate was substituted for EAA, or (b) when cyclopentanone was substituted for CH. Bilateral olfactory nerve cuts eliminated responding to odorants, demonstrating that olfaction was specifically affected by the lavage procedure. The selective decrements in responding to CH or cyclopentanone presentations after lavage are consistent with the hypothesis that Schiff bases bind uncharged carbonyl odorants to receptor proteins.

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Diet-Taste Relationships. Mattes, R.D. (Monell Center).

Difficulties in documenting correlations between measures of taste function and dietary habits have been attributed, in part, to the common use of 1) aqueous solutions as tastants, 2) threshold measures as descriptors of overall taste function, and 3) unreliable dietary assessments. To ascertain the validity of these concerns, measures of taste sensitivity, perceived intensity and preference, obtained with aqueous taste solutions and food systems, were correlated with various aspects of food intake measured by two procedures.

Thirty-five healthy, non-smoking, normal weight, male and female, 18-42 year-old subjects who largely controlled the purchase and preparation of the food they consumed participated. Recognition thresholds for aqueous solutions of sucrose and urea were determined by a forced-choice staircase procedure. Thresholds for these tastants in a cherry beverage and tonic water were obtained by randomly presenting 16 concentrations of each system and asking subjects to identify the proportions of sweet, sour, salty and bitter components present. Perceived intensity ratings were determined via a magnitude matching procedure. Preferences were ascertained by allowing subjects to dilute and concentrate samples to their preferred level. A 7-day food record containing each subject's impression of the predominant taste of each item consumed and a food frequency questionnaire provided the dietary information.

No single taste measure obtained with either tastant system correlated with any evaluated aspect of dietary intake. Combinations of taste measures were able to account for 30-36% of the variance in sweet and bitter intake ( $p < .02$ ), though protein, carbohydrate and fat intake could not be predicted at better than chance levels. The strength of different taste attributes as predictors of sweet and bitter intake varied between taste qualities, but not tastant systems. The order was: preference > perceived intensity > sensitivity for sweet and perceived intensity > sensitivity > preference for bitter tastants. If verified and extended to salty and sour tastes in further studies, this latter observation may provide a rational basis for the selection of specific taste tests when assessing diet-taste relationships in selected clinical populations. When combined with other observations in the literature, the present findings indicate such endeavors may reveal associations between measures of taste function and the preferred taste of an individual's diet, but not its nutrient content.

The Sweetness of Binary Mixtures of Sucrose, Fructose, and Glucose. ROBERT L. MCBRIDE (CSIRO Division of Food Research, P.O. Box 52, North Ryde NSW 2113, Australia)

All binary combinations of the natural sugars sucrose, fructose, and glucose were studied using a functional measurement approach, i.e., factorial stimulus designs with category rating as the response measure.

The sweetness of all mixtures was found to conform to simple algebraic additivity up to a certain level; this was evidenced by parallelism in the factorial plots. However, above this level some convergence was noted, signifying a breakdown in additivity. The convergence was considered genuine, and not due to nonlinearity in the response scale, since a previous comparison with sweetness-matching studies had confirmed response scale linearity.

The results also suggest that the phenomenon of synergism, often claimed with binary mixtures of the above three sweeteners, is a measurement artifact: A result of using physical units of measurement when psychological units are more appropriate.

Olfactory cues and pig agonistic behavior: evidence for a submissive pheromone of adrenal origin. JOHN J. MCGLOTHLIN (Dept. Animal Science, University of Wyoming).\*

Previous work, bearing directly on this report, described a detailed ethogram of swine agonistic behavior and found pig aggression and submissiveness to be influenced by topically applied chemicals or natural fluids (urine or plasma). Also, pigs treated with adrenocorticotropin hormone (ACTH) showed increased submissiveness. The objective of this work was to determine at what stage during a fighting bout a pheromone may be used to modulate pig aggression. Urine was collected from pigs treated with 1.0 IU/Kg ACTH (A) and from saline-treated (S) controls. Forty pigs were then randomly assigned, in groups of four each, to be observed under one of the following four treatments: (1) nothing applied; (2) S-aerosolized during early-fight; (3) S-during late fight (4) A-during early fight or (5) A-during late fight. Duration of aggressive behavior did not differ among treatments. Duration of submissive behavior was greater ( $P < .01$ ) for treatment 5 ( $\bar{x} \pm SE$ , respectively;  $.017 \pm .007$ ,  $.010 \pm .008$ ,  $.023 \pm .007$ ,  $.013 \pm .008$ ,  $.058 \pm .008$  minutes). These results fit the hypothesis that, in addition to visual cues, an olfactory cue is released towards the end of a fight to signal submission. Aerosolized A may have interfered with this pheromonal signal.

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Response Patterns to Odor in Olfactory Bulb Output Cells of the Hamster: Effect of Concentration and Involvement of Intra-bulbar Inhibition. MICHAEL MEREDITH (Dept. Biol. Sci., Florida State Univ., Tallahassee, FL 32306)

The genesis of temporally patterned responses to odor stimulation in the olfactory bulb is being investigated in two ways 1) by measuring changes in electrical stimulation and 2) by mapping the spatial distribution of different temporal patterns across the surface of the bulb in response to a range of odor qualities, concentrations and time courses. The anatomical substrate for inhibition in the bulb and the inhibitory consequences of electrical stimulation have been extensively investigated by others but it is not known if periods of suppressed spike activity during odor responses are due to cessation of afferent input or to direct inhibition of bulbar cells. Convergence and glomerular complexity make it impossible to record the input reaching a particular second order cell. The results of electrical stimulation of nerve bundles to drive second order cells (1 above) shows that the stimulation threshold increases during suppressive periods of odor responses, suggesting that the suppression of spike rate is due to direct inhibition of bulbar cells. Analysis of temporal patterns and their distribution (2 above) shows that, as in other species, a wide range of patterns can occur. The successive patterns elicited by pulses of successively higher concentration can show a number of different sequences of different patterns, even for cells in the same animal responding to the same stimuli. For example, if a wide enough range of concentrations is used, a full cycle of response patterns from predominantly excitatory to predominantly suppressive and back to predominantly excitatory may occur with increasing concentrations of the same odor. In another cell, the alternate sequence may be seen, with intermediate concentrations being excitatory. These results are consistent with the general principles of a computer analysis of the effect of spatially restricted afferent input to a simulated neural network modelled after known olfactory bulb circuits.

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The development of ciliated dendritic endings of olfactory receptor cells in rat embryos: A scanning electron microscope study. B. Ph. M. MENCÓ & A. I. FARBMAN (Northwestern University, Evanston, IL 60201).

Because olfactory cilia are the putative sites of odorant stimulation, we decided to determine whether the increase in numbers and lengths of cilia and of the density of olfactory receptor cell endings bearing these cilia, is associated with other signs of receptor cell maturation as the appearance of synapses, of olfactory marker protein and of response selectivity. Fetuses from intra-uterine days E14-E22 and adults were used. At E14 and E15 the majority of dendritic endings bears a single cilium, a "primordial" rather than a true olfactory one. From E15 on there is an average increase in the number of observed cilia per dendritic ending by one per day. The means (cilia/ending) as a function of embryonic age fit a linear regression with a coefficient of determination of 0.99. In E22 fetuses the average number of observed cilia is 8; in adults the number is 11. The numbers range from 0-30. Frequently, one ending within a given area has 10-15 cilia, whereas the ones surrounding it have considerably fewer. A substantial number of endings shows only partial development of cilia; their appearance suggests a common membrane surrounding an axonemal aggregate. During the developmental period from E14-E22, there is also a steady increase in the average lengths of the cilia. However, the carpet of long tapering cilia, as found in adult animals, was never found in the embryos. Densities of ciliated endings remain fairly constant during the examined period, and fluctuate around 4-5 million per  $\text{cm}^2$ . Olfactory supporting and nasal respiratory cells also bear a single primordial cilium during part of fetal life. In contrast to olfactory sensory cilia, cilia of non-sensory respiratory cells sprout almost all at once from E18 onwards. Alignment of the latter cilia suggests that synchronization of their beating pattern begins at E21. Our data show that the developmental patterns of the two types of cilia differ considerably.

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The Canine Lingual Epithelium Actively Transports Ions. SHELLA MIERSON, GERARD L. HECK, SHIRLEY K. DESIMONE, JOHN A. DESIMONE (Medical College of Virginia)

We have proposed a new transport paradigm to explain an early step in gustatory transduction. We have investigated the ion transport properties of the dorsal canine lingual epithelium, and found that it actively transports  $\text{Na}^+$  and  $\text{Cl}^-$  as well as other ions. The *in vitro* preparation was mounted in a lucite chamber to monitor transepithelial potential, current, and resistance. An external current applied to null the electrical potential is the negative of the short-circuit current (Isc). If identical solutions are on both sides of the chamber (no chemical or electrical potential gradient across the tissue), Isc is a measure of active transport by the tissue. We have measured  $\text{Na}^+$  and  $\text{Cl}^-$  fluxes across the tissue using radioactive tracers  $^{22}\text{Na}$  and  $^{36}\text{Cl}$ . Under symmetrical conditions, i.e. Krebs-Henseleit buffer on both sides, for 16 pairs of tissues,  $\text{Isc} = 1.33 \pm 0.10 \mu\text{Eq}/\text{cm}^2\text{-hr}$ , net  $\text{Na}^+$  absorption =  $0.62 \pm 0.10 \mu\text{Eq}/\text{cm}^2\text{-hr}$ , and net  $\text{Cl}^-$  secretion =  $0.19 \pm 0.07 \mu\text{Eq}/\text{cm}^2\text{-hr}$ . The conclusions for symmetrical conditions are (1)  $\text{Na}^+$  absorption accounts for 45% of the Isc; (2) there are two independent transcellular  $\text{Na}^+$  pathways, one amiloride-sensitive and one amiloride-insensitive; (3) ouabain, added to the serosal solution, inhibits both Isc and active  $\text{Na}^+$  absorption. When hyperosmotic (0.25 M) NaCl is placed in the mucosal bath, both Isc and  $\text{Na}^+$  absorption increase, whereas under symmetrical conditions  $\text{Na}^+$  absorption accounted for less than half the Isc, under hyperosmotic conditions net  $\text{Na}^+$  absorption  $>$  Isc. Ion substitution studies show that the tissue transports a variety of larger ions, though not as efficiently as  $\text{Na}^+$  and  $\text{Cl}^-$ . We have shown that the lingual epithelium, like other epithelia of the gastrointestinal tract, actively transports ions. However, it is an unusual epithelium both in its response to hyperosmotic solutions and in the variety of ions it can transport. Both of these unusual properties would be required for ion transport to be an early step in gustatory transduction. Supported by NSF Grant BNS-8309135, NIH Grant NS 13767, and A.D. Williams Foundation Grant 6-48412.

### Taste Responses to Water and Salts in the Rabbit.

INGLIS J. MILLER, JR. (Dept. of Anatomy, Wake Forest Univ., Bowman Gray Sch. of Medicine, Winston-Salem, NC 27103)

Previous reports of chorda tympani taste responses in the rabbit have shown that the system responds to water and that potassium salts give greater magnitudes than sodium salts. These observations were of interest to study the interaction between salts and solvents in comparison to rodent taste systems. In the current experiments, summated responses of the entire chorda tympani nerve have been recorded from a single electrode placed in contact with the uncut nerve in the middle ear. Animals were anesthetized with ketamine, sodium pentobarbital, and urethane. Preliminary observations were obtained from 5 rabbits. Quantitative responses are expressed relative to 0.1 M KCl, and the tongue was rinsed .01 M NaCl. Application of water to the tongue produced a transient response which decayed to a sustained level above that produced by mammalian ringers. The mean relative response to H<sub>2</sub>O was .44±.11 (N=10), and the response to deuterium oxide (D<sub>2</sub>O) was .84±.27 (N=18). Responses to salts of various cations, anions and solvents were characterized by comparison of the area under the concentration-response curve from .001 M to 1.0 M as a ratio to the area from KCl (mean + s.d.). Responses to the salts follow: CaCl<sub>2</sub>D=1.53±.44; MgCl<sub>2</sub>D=1.36±.10; CaAcH=1.33±.10; KClD=1.26±.06; CaCl<sub>2</sub>H=1.21±.05; MgCl<sub>2</sub>H=.97±.11; KBzD=.91±.14; KBzH=.87±.16; KAcH=.80±.55; NaClD=.60±.15; NaAcH=.52±.01; NaClH=.31±.21. Salts in D<sub>2</sub>O yielded greater responses than in H<sub>2</sub>O. Acetate (Ac) salts were greater than Cl for the same cation. Larger cations produced larger responses than smaller ones. Dioxane diminished the response to water. The observations seem to be consistent with the conclusion that water molecules are included in the ionic complex which associates stimulus ions with receptors.

### Paradoxical Sensitivity and Aversion of 3 Day Old Rat Pups to NaCl, Quinine and Ammonium Chloride. KAREN MOE (University of Pennsylvania)

Many mammalian species demonstrate a preference for the taste of sodium chloride when it is offered in low concentrations but they show an aversion to higher concentrations. As reported at this meeting last year, 6 and 12 day old rat pups show these concentration-dependent changes. That is, they show the inverted-U-shaped preference-aversion curve for saline that is characteristic of adult rats. They prefer saline concentrations that adult rats reject, and they do not reject NaCl until it is offered in very high concentrations. Thus, their preference-aversion function is shifted to the right along the concentration axis. This may reflect the postnatal timing of much of the development of the rat's gustatory system.

To continue this line of investigation, consumption of NaCl and other tastants was measured in 3 day old rats. The pups were offered NaCl (0.15, 0.5, 0.9, 1.7 M), quinine (2.8, 5.5 x 10<sup>-5</sup> M), ammonium chloride (0.2, 0.9 M) or deionized water by infusion through an anterior mouth catheter. Intake was measured by recording change of weight following the 30-minute test infusion.

The immaturity of the gustatory system at this age and the findings summarized above led to the prediction that 3 day old pups would either be completely indifferent to NaCl or would show an adult-like preference aversion function shifted even farther along the concentration axis. However, the response of the 3 day olds was very different from the response of the older pups. In general, they seemed more sensitive than the older pups. They showed no preference for any saline solutions offered and rejected a concentration which the older pups either preferred or found neutral. They also rejected a quinine solution to which the older pups were indifferent, and ammonium chloride (preferred or neutral to older pups). One possible explanation of these results is that the responses of the 3 day olds are trigeminally mediated.

### Structural and Ultrastructural Observations on the Nervus Terminalis During Development.

MONTI GRAZIADEI, G.A., CUCCIO, S. and GRAZIADEI, P.P.C. (Department of Biological Science, Florida State University, Tallahassee FL 32306)

The olfactory placode is the anlage of the neurons of the olfactory and vomeronasal organs; there is also evidence that the neurons of the nervus terminalis originate from the same placode. Mouse embryos from E10 to E17 have been processed for light and electron microscopic observations with the intent to study the modalities of development and the morphology of the neurons of the nervus terminalis. Beginning at E10, light microscopic preparations, stained with a silver method (Holmes), show clumps of cellswith the nuclear and cytoplasmatic characteristics of the olfactory nerve cells, leaving the neuroepithelium and entering the underlying mesenchima. These cells are usually arranged along the course of the developing olfactory nerves. At E11 these neuron-like cells can be seen leaving both the olfactory and the vomeronasal epithelia; they are consistently arranged in clumps and connected with the olfactory and vomeronasal nerves. At later stages (E13 to E17) they are associated with the central roots of the nervus terminalis. At ultrastructural level these neuron-like cells can be distinguished on the base of their organelle content from the mesenchimal cells and the sheath cells. They are provided with a prominent Golgi apparatus, cisternae of the rER and free ribosomes. Their nucleus is round and provided with a clear chromatin pattern.

Acknowledgements. Supported by NSF, BNS 8006803

### Olfactory Neurons Transplantation into the Olfactory Bulb of Neonatal rats. MONTI GRAZIADEI, G.A. and GRAZIADEI, P.P.C. (Department of Biological Science, Florida State University, Tallahassee, FL 32306)

The presence of a neurogenetic matrix in the olfactory neuroepithelium persists through the life of the animal, offering the opportunity to transplant this neuroepithelium and to investigate the development of the olfactory neurons in a number of locations. In the present experiments we have transplanted olfactory neuroepithelium from neonatal rat pups in the olfactory bulb of littermates. At different survival times (from 20 to 90 days) the hosts were sacrificed for structural and ultrastructural observations. Some specimens were immunohistochemically stained for the demonstration of the olfactory marker protein (OMP). The transplanted neuroepithelium survives well in this experimental setting and portions of it maintain the typical structure of the organ. In these circumstances bundles of axons originate from the epithelium and run in the surrounding bulbar tissue. In no instance we have observed these bundles to terminate in ectopic glomerular structures nor to direct towards the glomeruli of the host's bulb. Other portions of the epithelium become simple cubic and are formed mostly by supporting cells, due to the migration from the epithelium of the neuronal elements. In these regions the neurons migrate in cords or singularly into the bulb parenchyma where they come in direct contact with the bulbar neurons. With immunocytochemistry we have observed that both the neurons maintaining an intraepithelial position and those migrating are seldom positive to OMP. The possibility that these neurons may require factors other than their target to express OMP is presently investigated, as well as the possibility that metaplastic changes may occur.

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### Olfaction In Rats With Reinnervated Remnants Of Olfactory Bulb.

G.A. Monti Graziadei, P.P.C. Graziadei (Florida State University), B.M. Slotnick (The American University), and A.B. Butler (Georgetown University School of Medicine)

Rats with one olfactory bulb removed when neonates and the second bulb removed when adults were tested on a control (tone-light) discrimination and for their absolute olfactory threshold (amyl acetate). For histological analysis, heads were decalcified and sectioned at 20µm. Stained sections were examined to determine the extent of lesions and growth of new olfactory sensory axons. On the neonatally operated side new olfactory receptor cell axons penetrated the ipsilateral hemisphere where they formed glomerular-like structures in rostral neocortex (14 cases), anterior olfactory nucleus (2 cases), or in a small remnant of previously deafferented olfactory bulb in which normal cytoarchitectonic structure was absent (4 cases). In 6 cases new olfactory receptor cell axons failed to make contact with the forebrain. On the adult-operated side there was extensive scar formation and in-growing sensory axons ended in neuromas outside of the brain. All experimental animals acquired the tone-light discrimination. Those with new axons to a remnant of olfactory bulb tissue acquired the amyl acetate detection task and their detection thresholds were within the range of control (unilaterally bulbectomized) rats. These rats were also able to detect isopropyl acetate vapor and discriminate isopropyl acetate from amyl acetate vapors. The remaining animals failed to acquire the odor detection task, even when tested with a relatively strong (4% of vapor saturation) odor. These results support the following conclusions: 1. In the adult bulbectomized rat extensive scar formation appears to prevent new sensory axons from making contact with the forebrain. 2. In the neonatally operated rat new sensory axons can penetrate into the forebrain and form glomerular-like structures. 3. New sensory axons which terminate in frontal neocortex or in the anterior olfactory nucleus do not appear to support olfaction. 4. However, sensory axons which reinnervate a previously deafferented and morphologically abnormal remnant of olfactory bulb appear to be functional and to allow for olfactory sensitivity which is surprisingly similar to that of control rats.

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Transplant of Olfactory Mucosa in the Brain of Neonatal and Adult Rats. An Ultrastructural Study. MORRISON, E.E. and GRAZIADEI, P.P.C. (Department of Biological Science, Florida State University, Tallahassee, FL 32306)

Olfactory neuroepithelium from neonatal rats has been known to survive when transplanted in the cerebral ventricles as well as in the brain parenchyma. Olfactory neurons continue to differentiate from the epithelial neurogenetic matrix (the so called basal cells) and olfactory axons originating from the transplanted neurons penetrate into the brain. Interestingly, they normally fail to form glomeruli. Previous light microscopic observations have also documented that, in some regions of the transplanted neuroepithelium, the neurons migrate away from the epithelial compartment and penetrate into the brain parenchyma. Our present ultrastructural observations are derived from transplants of olfactory neuroepithelium from donor pups into littermates or adult rats. Survival of the animals varied from 20 days to eight months. During this experimental period new neurons continually form from the neuroepithelial matrix that seems not to exhaust its capacity to form neurons. Several neural elements migrate into the brain parenchyma losing any contact with the other epithelial components. Often the migrating elements are in direct contact with the C.N.S. neurons. The relationships of the migrating neurons with the cellular elements of the brain will be described in detail.

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Differential Laminar projection of Palatal and Gill Arch Taste Buds in the Goldfish. YASUHIRO MORITA and THOMAS E. Finger (Univ. Colo. Sch. Med., Denver, CO 80262).

The vagus nerve in goldfish, *Carassius auratus*, innervates taste buds located on the gill arches and on the palatal organ, a fleshy organ attached to the roof of the mouth. The gustatory portion of the vagus nerve terminates in the large special visceral nucleus of the caudal medulla, the vagal lobe. In cyprinids such as goldfish, the vagal lobe is a complex laminated sensorimotor structure. The sensory nerves terminate in layers 2, 4, 6 and 9 (terminology of Morita et al., '80) throughout the vagal lobe. Small injections or lesions of the vagus nerve reveal that the nerve projects onto the vagal lobe in a somatotopic fashion such that the anterior part of the palatal organ and the first gill arch are represented anteriorly while the posterior parts of the oral cavity are represented posteriorly. The ventromedial portion of the palatal organ is represented ventrally in the vagal lobe and the dorsolateral palatal organ in the dorsal part of the lobe. In addition, HRP injections made selectively into the gill arch nerves and palatal organ nerves reveal a separation of input from these two sources. The palatal organ fibers terminate in layers 6 and 9; the gill arch nerves project heavily to layers 2, 4 and 9. The input to layer 2 enters the vagal lobe in the superficial sensory root while the input to the deeper layers enters with the main sensory root. Thus, layers 2 and 4 receive predominantly gill arch inputs while layer 6 receives palatal organ inputs. In addition to the aforementioned sensory inputs, both the palatal organ and gill arch nerves contain motor fibers arising from the neurons of the motor layers of the vagus nerve.

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Selective Modification of Proteins in the Olfactory Epithelium THOMAS HELLMAN MORTON (Department of Chemistry, University of California, Riverside, CA 92521) and J. RUSSELL MASON (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104)

A chemically selective technique for covalent modification of Schiff base-forming proteins has been devised and examined in vitro and applied in vivo. In vitro studies using pure ketone-binding proteins as chemical models demonstrate that this procedure irreversibly labels the amino groups of lysine residues at binding sites.<sup>1</sup> The sequence of reagents used, acetoacetic ester followed by sodium cyanoborohydride, blocks a protein that binds uncharged ketones, but does not react with other lysine-rich proteins. In particular, enzymes that bind electrically charged ketones exclusively are not affected. Application of the same modification procedure to the olfactory epithelia of tiger salamanders (*Ambystoma tigrinum*) selectively attenuates their olfactory sensitivity to ketone-containing odorants, as measured by a behavioral assay. This assay, which has been previously used to detect selective, partial anosmia<sup>2</sup>, suggests that Schiff base formation may play a role in binding or transduction of ketone-containing odorants.

1. J.R. Mason and T.H. Morton, *Tetrahedron* 1984, **40**, 483-492.
2. J.R. Mason and T.H. Morton, *Physiol. Behav.* 1982, **29**, 709-714.

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Odor Perception in Blind Persons: A Case for Superiority. CLAIRE MURPHY (San Diego State University), WILLIAM S. CAIN (Pierce Foundation Laboratory, Yale University), TARA RACHINSKY and ELIZABETH KONOWAL (Monell Chemical Senses Center), MICHELE J. REED and JEANNE WITHEE (San Diego State University).

There has long been an interest in the non-visual sensory abilities of the blind. The present experiment focused on the olfactory system and addressed two questions. First, do the blind experience heightened olfactory sensitivity? Second, do the blind possess enhanced ability to identify odors? Participants were 42. Twenty-one were blind. The 21 sighted controls were matched for age, gender, smoking status, and general educational level. Olfactory threshold was determined using a series of 11 aqueous dilutions of butanol. Each solution was presented monorhinally. A forced-choice, two-alternative, ascending procedure was used, with 4 threshold determinations per subject. Odor identification ability was assessed using a battery of 80 common odors. Each subject (S) was tested, blindfolded, 3 times with an average of 3 days between sessions. Ss performed a free recall task with verbal feedback as to the veridical identification. Analysis of variance showed no advantage in threshold sensitivity for blind Ss, regardless of the index used: the average, the better, or the poorer of the two nostrils. Threshold showed only a weak association with age. Identification data were subjected to a one-between (blind, sighted), one-within (days 1, 2, 3) analysis of covariance. Blind Ss significantly outperformed sighted Ss on the identification task,  $p < .01$ . The superior performance of the blind is particularly interesting given the lack of any increase in threshold sensitivity to olfactory stimulation. As expected, all Ss increased their identification scores with practice,  $p < .0001$ . There was no interaction of visual status and practice. Finally, the age covariate was highly significant,  $p < .0001$ . Regardless of visual status, a subject's age significantly affects his odor identification ability.

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The Mouse Glossopharyngeal and Chorda Tympani Nerves Response to NaCl, Sucrose, QHCl and Citric Acid. M.S. NEJAD and L.M. BEIDLER (The Florida State University).

The simultaneous response profile of the glossopharyngeal (GL) and chorda tympani (CT) nerves of the mouse (C57BL/6J) to NaCl, sucrose, QHCl and citric acid was recorded and response-concentration functions for both nerves were obtained. The values of  $1/KRs$ ,  $Rs$  and  $K$  were derived and calculated from application of the fundamental taste adsorption equation (Beidler, 1954).  $1/KRs$  is proportional to the response threshold.  $Rs$  is the maximum response and  $K$  is related to the strength of binding of stimulus to the receptor sites (Beidler, 1954, 1978). The response-concentration functions disclosed some contrasting differences between the back (GL) and front (CT) of the C57BL/6J strain of mouse tongue. Considering the calculated values of  $1/KRs$ , the sensitivity of the back and front of the tongue in response to citric acid seemed to be the same. The front of the tongue indicated to be more sensitive to NaCl and sucrose than its back and the back of the tongue appeared to be more sensitive to QHCl than its front. The maximum response ( $Rs$ ) of the glossopharyngeal and chorda tympani nerves to citric acid were about the same. Also, the  $Rs$  of both nerves to NaCl were relatively equal. The  $Rs$  values of GL nerve to QHCl was higher than that of CT and the  $Rs$  value of CT to sucrose was higher than that of GL. The  $K$  values of the glossopharyngeal and chorda tympani nerves in response to citric acid were in close approximations and also, the related  $K$  values of glossopharyngeal and chorda tympani in response to sucrose were the same. In contrast, the corresponding  $K$  values of the glossopharyngeal and chorda tympani nerves in regard to QHCl and NaCl were different: the glossopharyngeal  $K$  value in response to QHCl was higher than that of the chorda tympani and the chorda tympani  $K$  value in response to NaCl was higher than that of the glossopharyngeal nerve.

Role of Food Flavors and Dietary Fat in Cafeteria Feeding: Lipid Deposition in Rats M. NAIM (Hebrew Univ., Fac. Agric., Rehovot, Israel), J.G. BRAND AND M.R. KARE (Monell Chemical Senses Center, Phila., PA 19104).

Normal rats can be induced to accrue fat if offered a variety of foods high in fat and sucrose and varying in flavor (the "supermarket" diet). Since this diet permits no control of the macro and micronutrient intake of the animals, experiments employing it are not nutritionally sound. To resolve this problem we studied the role of flavors in a cafeteria (CAF)-induced obesity model where 12 flavors were imbedded in a nutritionally balanced (NB) semi-purified diet at levels found to be preferred by the rats. The diet was further modified by producing 4 different textures. Rats (S.D. strain, 15 rats/group) fed the NB flavored diets in CAF (NB-CAF) did not consume more calories nor did they gain more weight than controls (NB-CON) fed the unflavored diet for 23 days. However, rats fed a high fat (25% saturated vegetable fat, 5% corn oil, 1% palm oil) -high sucrose (25%) diet flavored as above in CAF (HFHS-CAF) consumed 15% more calories ( $p < .01$ ) and gained 20% more weight than their controls fed the unflavored high fat-high sucrose diet (HFHS-CON). Food intake and body weight gain did not differ among NB-CON, NB-CAF and HFHS-CON groups. Retroperitoneal and epididymal fat pads of HFHS-CAF group rats were heavier by 35% and 25% respectively ( $p < .01$ ) than those of other groups. Neither liver weights nor weight of intrascapular brown adipose tissue differed among any of the four groups. The results suggest that the presence of preferred flavors in a nutritionally balanced diet in a chronic feeding paradigm (i.e., not meal-feeding) designed to maximize variety does not induce hyperphagia. Only if the diet contains lipogenic constituents such as high fat and sucrose do the preferred flavors contribute to the obesity induced by CAF feeding.

IXth Nerve Fibers Routed into the Tongue Musculature Do Not Reinnervate Gustatory Papillae. BRUCE OAKLEY, ELIZABETH KEPPEL and STEPHEN E. HUGHES (University of Michigan)

The IXth nerve (glossopharyngeal) was directed into the muscles of the rat tongue via the distal stump of the XIIth nerve (hypoglossal, tongue motor). Five to nine months later we determined that the cross-regenerated IXth nerve had successfully reached the tongue as indicated by functional motor connections with tongue muscles. However, no action potentials could be recorded from the IXth nerve during oral stimulation (taste, touch, cooling). In addition, the cross-regenerated IXth nerve failed to reform taste buds in the foliate or vallate papillae. Hence, although the gustatory epithelium lay only a few millimeters away, the sensory fibers did not return to it. For mammalian cutaneous targets the route of nerve entry may be critical if normal functional innervation is to be re-established.

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Taste descriptions in English and Japanese.  
MICHAEL O'MAHONY and RIE ISHII (Dept. Food Science and Technology, University of California, Davis, California 95616)

Taste descriptions were obtained for filter papers previously soaked in solutions of NaCl, fructose, quinine hydrochloride, citric acid, MSG and KCl from 103 American subjects, 110 Japanese subjects and two further subgroups of Japanese: 43 taste panelists at Ajinomoto Co. and 54 other workers at the same company. Everyday taste descriptions were obtained without prior presentation of standards to define descriptors nor with any limit nor suggestion as to the words to be chosen. Taste descriptive strategies were similar among the groups except that Japanese had a greater tendency to call citric acid 'sour'. MSG, however, was different. It was generally described as 'salty' by Americans, while the Japanese assigned it a specific highly codable descriptor. This was 'Ajinomoto taste' for Japanese subjects but it was not generally used by Ajinomoto workers and tasters who used the term in the literature: 'umami'. The inconsistency of descriptions indicates the cultural specificity of everyday taste descriptive language.

Rodenticides as Conditioned Stimuli in Food Aversion Learning. RUSSELL F. REIDINGER, JR. (U.S. Fish and Wildlife Service, c/o Monell Chemical Senses Center, Philadelphia, PA), CHARLES N. STEWART (Whitely Psychology Laboratories, Franklin and Marshall College, Lancaster, PA) and J. RUSSELL MASON (Monell Chemical Senses Center, Philadelphia, PA)\*

Some rodenticides are more likely than others to cause bait-shyness. Such variation may be due in part to flavor differences among rodenticides, and to differential effectiveness of such flavors as conditioned stimuli (CSs) for food aversion learning. To investigate the flavor of a rodenticide as a CS, rats (N = 20, Sprague-Dawley derived males) were trained to drink during a 15 min period in the morning and 30 min in the afternoon. For conditioning, experimental rats (N = 10) were allowed to drink up to 10 ml of a rodenticide solution at (a) 0.05 LD-50 per ml, or (b) the limit of solubility if less than a. Control rats (N = 10) drank water. Then, all rats were injected with lithium chloride (102 mg per kg body wt). After 2 days for recovery, animals were presented single choice tests of 4 rodenticide concentrations. Rodenticides tested were warfarin, sodium warfarin, scilliroside, strychnine, calciferol, alpha-chlorohydrin and ANTU. Of interest, rats failed to learn reliable aversions to the purified form of scilliroside (the active ingredient of red squill), consistent with the notion that a contaminant or other ingredient in the bait may be responsible for bait-shyness. In work conducted subsequently, evidence was obtained that warfarin is not usually associated with bait-shyness because the interstimulus interval between ingestion of bait and onset of illness is over 8 hrs.

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Origin of Olfactory Projections to the Hypothalamus and Thalamus  
J.L. PRICE (Washington University), L.S. SASAKI (Grinnell College) and B.M. SLOTNICK (The American University)

Previous anatomical and electrophysiological studies have shown that the olfactory system projects to discrete sites in the thalamus and hypothalamus. The hypothalamic projection may mediate neuroendocrine and autonomic responses, while that to the thalamus has been implicated in odor discrimination learning. The present studies have examined the origin of olfactory fibers to the hypothalamus, and whether olfactory-related cells project to both hypothalamus and thalamus. Using physiological unit responses to olfactory bulb shock as guides, small injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA\*HRP) were made into the lateral hypothalamic area (LHA). Short latency responses were consistently obtained from the posterior part of the LHA, in the region of the nuclei gemini. Fewer responsive sites were found in more rostral hypothalamic areas. Injections of WGA\*HRP into the posterior LHA retrogradely labeled cells in four olfactory areas: 1) the ventroposterior and medial parts of the anterior olfactory nucleus, 2) the deep polymorphic zone of the olfactory tubercle, 3) the deepest layer of the piriform cortex (ventral endopiriform nucleus) and 4) the anterior cortical nucleus of the amygdala. No additional areas of labeled cells were found with very large injections, while more discrete injections in the rostral LHA resulted in only moderate numbers of labeled cells in the anterior cortical nucleus and few labeled cells in the other areas. Because the cells in the olfactory tubercle and piriform cortex which project to the LHA are in the same layers as those which project to the mediodorsal thalamic nucleus (MD) (Price and Slotnick '83), the double retrograde tracing method of injecting different fluorescent dyes (true blue and nuclear yellow) into the LHA and MD was used to determine whether the same cells give rise to both projections. Although most of the retrogradely labeled cells were singly labeled, a substantial number were double labeled with both tracers, indicating that they sent axons to both the LHA and MD. Single and double labeled cells were intermixed, with no evidence of a differential organization. In conclusion, the olfactory projections to the hypothalamus are heaviest to the posterior part of the LHA. These fibers arise from several parts of the olfactory cortex, and have a more widespread origin than the fibers to MD. However, at least some of the cells deep to the olfactory tubercle and piriform cortex have bifurcating axons which go to both of these diencephalic sites.

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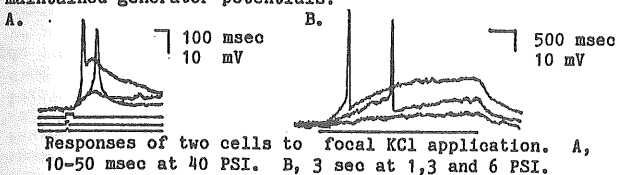
Stimulation of Neuroactive Amino Acid Transport by Insulin: A Possible Mechanism for Neuromodulation in the Olfactory Bulb of the Adult Rat. D. RHOADS AND J.G. BRAND (Monell Chemical Senses Center, Phila., PA 19104)

High affinity, Na<sup>+</sup>-dependent transport systems for neuroactive amino acids and other neurotransmitters are present in nerve ending (synaptosome) preparations from homogenized nervous tissue. Such transport systems may play a central role in neurotransmission since they can function as a presynaptic uptake mechanism to: 1) maintain low extracellular (synaptic) levels of neurotransmitters, and 2) reduce the synaptic levels of neurotransmitters following their depolarization-induced release. Bovine insulin caused a dose-dependent (10<sup>-6</sup> to 10<sup>-5</sup> M) stimulation of  $\gamma$ -aminobutyric acid (GABA) uptake into synaptosomes prepared from adult rat olfactory bulbs. The stimulated uptake, which ranged up to two times the control values, was the result of increases in both the initial rate of GABA uptake and the overall capacity for GABA accumulation. Similar results were obtained with other Na<sup>+</sup>-dependent amino acid transport systems. The effect of insulin on the initial rate of GABA uptake with respect to GABA concentration indicated a 50-70% increase in V<sub>max</sub> (control = 1.1  $\pm$  0.3 nmol/min/mg protein) with no significant effect on the K<sub>m</sub> (con. = 4.1  $\pm$  1.0  $\mu$ M). The stimulatory effect of insulin was not dependent on the presence of glucose or Ca<sup>2+</sup> in the incubation buffer, nor on protein synthesis, but was significantly reduced in the presence of the Na<sup>+</sup>, K<sup>+</sup>-ATPase inhibitor ouabain. As a result of stimulating Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, insulin could enhance the membrane electrical potential and the transmembrane Na<sup>+</sup> gradient, both of which contribute to Na<sup>+</sup>/amino acid symport processes such as the synaptosomal transport system for GABA. Preliminary experiments using merocyanine-540 suggest a slight hyperpolarization of the synaptosomes in the presence of insulin. The presence of insulin and insulin receptors in the olfactory bulb provides a mechanism for modulating the efficiency of secondary active transport and, thus, the synaptic levels of neurotransmitters such as GABA.

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Chemosensory Stimulation in Mudpuppy: KCl Evokes Impulses in Taste Cells. S. ROPER and M. McPHEETERS (University of Colorado Medical School)

Recent experiments have shown that taste cells in some species produce regenerative impulses in response to electrical stimulation (Roper, 1983; Kashiwayanagi, et. al, 1983). The question remains whether chemical stimulation also produces impulses and whether impulses are necessary and sufficient for chemosensory transduction. We have been recording intracellular responses in taste cells of the mudpuppy and found that focal application of KCl near the taste pore reliably evokes generator potentials and impulses in all taste cells in which stable microelectrode penetrations were achieved. (Focal KCl application: pipette contained 0.1-1.0 M KCl; tip diameter = 1-1.5 microns; 10 msec to 5 sec pulses at 10-40 PSI air pressure; pipette approximately 10-50 microns above taste pore). Responses had rapid rise times (<50 ms) and lasted the duration of KCl application. Further, only single impulses were elicited by maintained generator potentials:



Applying KCl to surrounding surface epithelial (SE) cells revealed a striking difference in their chemosensitivity: even doses as high as 5X those which depolarized taste cells were completely ineffective on SE cells. Focal release precluded an accurate measurement of [KCl] at the target, but if the resting potential of SE cells behaves according to Nernst relations, we estimate that [KCl] was < 10mM at the cell surface. Experiments to establish actual [KCl] and to test other tastants are in progress.

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Role of Membrane in Paramecium Chemoreception. STEPHANIE SCHULZ, MARIA DENARO, ROBIN PRESTON, JUDITH VAN HOUTEN. (Department of Zoology, University of Vermont, Burlington, VT 05405)\*

Paramecia detect and respond to soluble chemicals around them. Specific binding of attractants is transduced into changes in membrane potential (usually hyperpolarization), which result in altered ciliary beating and, hence, attraction. At least two of these steps in the chemosensory pathway involve the cell membrane: binding and hyperpolarization. We have used several approaches to examine these roles of the cell membrane in chemoreception. 1) Binding studies with radiolabeled attractant folate have shown specific, saturable binding to normal paramecia ( $K_d$  29  $\mu$ M) and low level, non-saturable binding to mutant d4-534, which is not attracted to folate. Two induced revertants of d4-534 show partial restoration of binding with the restoration of attraction to folate. Since isolated cilia show about 3% of the total cell binding, it is likely that the cell body membrane is the one involved in chemoreception. 2) Normal cells dyed with fluorescein-labeled folate show intense fluorescence, while mutant d4-534 cells show emission barely above background autofluorescence. The fluorescence is specific for folate and can be reduced to background with excess folate. 3) PAGE analysis of cell membranes from normal and mutant cells shows altered mobility of a ~103,000 dalton protein. This is one of several proteins that elute with folate from folate-sepharose affinity columns. 4) The ionic mechanism of the hyperpolarization of cells in attractants is being studied with standard electrophysiological techniques. Mutant d4-534 shows reduced hyperpolarization in folate compared to normal cells. We do not yet know whether the membrane has still other functions in chemoreception. It may be the site of transduction of the chemical cue into an electrical cue, as opposed to an intracellular site of an internal second message; or it may be the site of adaptation to chemical cues, as it is in bacteria.

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Orientation Reactions by the Horseshoe Crab, *Limulus*, Polyphemus to Chemical Stimuli and a Newly Discovered Pheromone  
Jack Schlein (York College) and James Nahirny (Cardozo High School)

The horseshoe crab, *Limulus polyphemus*, mates during the months of June, July and August at the time of the full moon. Mating females are observed in shallow water generally surrounded by two to four males. Although Barlow et al (1982) have shown that vision appears to play a role in the mating behavior of *Limulus* our study indicates that a chemotropic pheromone may also be involved.

A Y-shaped maze which was previously shown to be effective in determining tropic responses to chemical stimuli (Schlein and Lakatos, 1983) was utilized in a series of experiments conducted during the mating season. Specimens were initially run in the maze without any stimuli, to determine statistically the left and right bias for each horseshoe crab. Sea water that had contained female *Limuli*, male *Limuli* and non-sexually active female *Limuli* were each tested as stimuli in the maze, and the subsequent attraction or aversion by the *Limuli* in the maze recorded. Evaluation of the data indicates that females of *Limulus polyphemus* produce a sex attractant pheromone during the mating season.

Barber (1956) has recorded electrophysiological responses from gnathopod spines in the legs of *Limulus* in response to clam (*Venus*) extract, a mixture of amino acids, 0.5M. glycine and HCl at a pH of 0.02. Additional experiments were conducted, using the maze, to determine behavioral correlates to these electrophysiological studies. A positive chemotropic response occurred to clam extract, glycine and the amino acid mixture, as opposed to a negative response to HCl. These data support the hypothesis that chemoreception plays an integral role in orientation during mating as well as in food gathering.

Development of the Olfactory System: Neighboring Nuclei Project to the Bulb at Different Ages. S. Schumacher and M.T. Shipley (University of Cincinnati College of Medicine).

In the adult rat two neighboring basal telencephalic nuclei--the horizontal limb of the diagonal band (HDB) and the nucleus of the lateral olfactory tract (NLOT) project to the olfactory bulb. If the distance between source neurons and their target determines the age at which a neural pathway develops then HDB and NLOT should connect with the bulb at about the same time. We report that the projection from HDB reaches the bulb 4-8 days earlier than that from NLOT.

The tracer wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was injected into the main olfactory bulb of rats at birth, P-1, P-2,3,4,8, and 20. The animals were perfused after 24 hrs. The brains were sectioned at 40-50 $\mu$ m and reacted by TMB histochemistry for HRP.

At P-1 many neurons in HDB are labeled by retrograde transport of WGA-HRP. The neurons are small and tightly clustered. From P-2 - P-4 more neurons are labeled; they become larger and begin to separate from one another. By P-8 the nucleus begins to resemble the adult form. By P-20 the labeling of DB neurons appears to be comparable to adults. By contrast, no neurons are labeled in NLOT during P-1 - P-4. A few labeled cells are present at P-8. By P-20 most of the NLOT cells are labeled as in the adult.

Clearly, distance from their target structure does not alone determine the "connectional birthdates" of these two adjacent nuclei. Perhaps neurons in the two nuclei terminate upon bulb neurons or dendritic segments that develop at different times. Or, they may address post-synaptic receptors that emerge at different ages. It should be possible to evaluate these and other possibilities within the orderly cortical structure of the olfactory bulb.

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Relationships between Taste Reactivity and Intake in the Neurologically Intact Rat. GARY J. SCHWARTZ (Univ. of Penn.), HARVEY J. GRILL (Univ. of Penn.)

To investigate the differences between short-term intake tests and taste reactivity responses to tastes, rats received 1 min. intraoral infusions of tastants. Oral responses were videotaped and analysed in terms of the sequence and number of ingestive and aversive taste reactivity response components evoked; intake was also measured. The number of rats displaying ingestive components and the mean number of ingestive components displayed per rat elicited by sucrose and NaCl increased with increasing concentration; intake was high across all concentrations. HCl infusions elicited alternation between ingestive and aversive response components. The number of rats displaying aversive response components and the mean number of aversive response components displayed per rat elicited by QHCl increased with increasing concentration, while both intake and the median latency to reject QHCl decreased. To determine whether other tastes judged bitter by humans would elicit a quinine-like taste reactivity response in the rat, sucrose octa-acetate (SOA), quinine sulfate (QS) and caffeine (CAF) solutions were examined. Both QS and CAF infusions elicited an increased number of aversive response components with increasing concentration, while intake decreased. SOA infusions elicited alternation between ingestive and aversive response components followed by a display of solely aversive components, and both intake and median latency to reject the infusions decreased significantly with increased concentration. Rats received prolonged infusions of hypertonic NaCl solutions until fluid was seen on the surface of the test chamber, indicating rejection. Prolonged infusions of hypertonic NaCl solutions elicited an initial display of ingestive response components followed by an abrupt shift to aversive response components and fluid rejection. Higher concentrations elicited the shift sooner than lower ones. The results suggest that patterns of taste reactivity response components are good predictors of intake duration and may be useful indicators of quality coding.

Cytochrome Oxidase (CO) Activity in the Olfactory System: Normal Distribution. M.T. Shipley and S. Van Ooteghem (University of Cincinnati College of Medicine) and R. Costanzo (Medical College of Virginia).

CO is an intramitochondrial enzyme essential to aerobic cellular respiration. CO activity is high in auditory and visual structures and the hippocampus in loci where both spontaneous neural activity and 2-Deoxyglucose uptake are high (Kageyama and Wong-Riley, *Neuroscience*, '82). We report the distribution of CO in the olfactory bulb and piriform cortex of normal adult rats and hamsters.

Cryostat (4-16µm) or freezing microtome (40-50µm) sections were reacted for 15-120 min. in a modification of Wong-Riley's (Brain Res. '79) histochemical medium. High levels of CO were present in glomeruli, the external and internal plexiform layers and in the neuropil of the granule cell layer. Activity was low in the olfactory nerve layer and low to moderate in most cell bodies. Different glomeruli had different amounts of CO. The differences did not appear to relate to the size or location of the glomeruli. To evaluate this impression, sections were studied with an image analysis computer system. The results showed that different glomeruli have different amounts of CO activity/unit area than others. The physiological bases for inter-glomerular differences in CO activity remain to be determined. By contrast, there is little variation in CO activity within individual glomeruli. This may imply that from moment to moment some glomeruli are more active than others. CO activity is very high in the molecular layer of the anterior olfactory nucleus and piriform cortex corresponding to the location of the terminals of olfactory bulb efferents.

CO activity demonstrated by enzyme histochemistry corresponds in location and intensity to regions known to contain high concentrations of synapses in olfactory structure. CO and other metabolic enzymes may be useful in unravelling the development and functional organization of the olfactory system.

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Some Observations on Electrogustometry. R.G. SETTLE & A.L. JACOBS (University of Pennsylvania Clinical Smell and Taste Research Center & Philadelphia VA Medical Center)\*

Although electrogustometry (EGM) is commonly used outside of the U.S. in the clinical evaluation of taste function, little is known about the relationship between tastant solution and EGM thresholds. EGM, NaCl and citric acid (CA) thresholds were obtained from 12 males and 11 females (20-73 yrs) by a forced-choice staircase procedure. In the first session EGM thresholds were determined for both the right and left sides of the tongue using a 500 msec pulse from a constant current generator. Two carbon-rubber anodes (d=1.3 cm), straddling the midline, were applied to the anterior-dorsal surface of the tongue. Stimuli were delivered to each side using a Gellerman sequence. The same EGM measures, together with whole-mouth CA and NaCl thresholds, were obtained in a second session approximately a week later. There was a significant correlation between NaCl and mean EGM thresholds ( $r=.47$ ) but not between CA and EGM ( $r=.33$ ) or CA and NaCl ( $r=.35$ ). Females had significantly lower EGM and NaCl but not CA thresholds than did males. There was a significant, age-related increase in EGM and NaCl ( $r=.60$  &  $r=.43$ ) but not in CA ( $r=.16$ ) thresholds. The correlation between EGM and NaCl thresholds and the similar influence of other factors on them support the hypothesis that iontophoresis of extracellular sodium may play a role in electric taste stimulation. Correlations between EGM thresholds for the left and right sides were low to moderate for the first ( $r=.35$ ) and second test ( $r=.60$ ). Test-retest reliability of EGM thresholds were moderate for the left ( $r=.64$ ) and right sides ( $r=.45$ ). Absolute (20 µA) or relative (two fold) left-right differences in EGM thresholds are considered indicative of pathology. Six subjects in the first test and three in the second met both criteria, but only one subject met these criteria in both tests. These data suggest that EGM thresholds are not particularly reliable and that published criteria for pathology are too liberal.

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Nasal Trigeminal Chemoreception: Sensitivity and Response Latencies for Aliphatic Alcohols. WAYNE L. SILVER and J. RUSSELL MASON (Monell Chemical Senses Center, Phila., PA 19104).\*

Nasal trigeminal chemoreceptors of the rat respond to many volatile stimuli (Silver and Moulton, 1982). However, little information is available regarding the sensitivity of the nasal trigeminal system to a homologous series of odorants. In the present experiment, integrated multiunit recordings were obtained from the ethmoid branch of the rat trigeminal nerve in response to 8 n-aliphatic alcohols (methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol). Stimuli were presented via an air dilution olfactometer, at a flow rate of 2 L/min. Air was drawn through the nasal cavity via a nasopharyngeal cannula at a rate of 1 L/min. Responses were obtained to all 8 alcohols, and thresholds decreased with increasing carbon chain length (from approximately 6000 ppm for methanol to 35 ppm for octanol). For individual compounds, response magnitudes increased, and response latencies decreased (e.g., from approximately 2.0 sec at 4200 ppm to 0.16 sec at 55,000 ppm for ethanol), with increasing stimulus concentrations. These results demonstrate that increasing lipophilicity results in increasing stimulus effectiveness. One plausible explanation for these findings is that the more lipophilic a substance, the more easily it can penetrate mucous and epithelial layers to reach chemosensitive trigeminal free nerve endings.

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Voltage Clamp Studies on Dog Lingual Epithelia. S.A. Simon and J.L. Garvin (Duke University Medical Center)

Following the procedure described by DeSimone et al. (Science 241: 1039-1041, 1981), we voltage clamped the dorsal surface of the dog tongue utilizing a region just anterior to the circumvallate papillae. The short circuit current,  $I_{sc}$ , open circuit voltage,  $V_{oc}$ ,  $^{22}Na$ , and  $^{36}Cl$  fluxes were measured. In confirmation of the results of DeSimone et al., we found that the  $I_{sc}$  and  $V_{oc}$  are completely ouabain inhibitable. However, a ouabain insensitive  $Na$  and  $Cl$  flux are present. Under symmetrical Krebs-Hensleit, K-H, solutions, the  $Na$  influx ( $M \rightarrow S$ ) can only account for 30% of the  $I_{sc}$ . The efflux ( $S \rightarrow M$ ) of  $^{36}Cl$  can account for the remainder of the  $I_{sc}$  although most of the  $Cl$  flux is electrically silent.

The lingual epithelium is sensitive to changes in  $NaCl$  and  $HCl$  concentrations in the mucosal solution. Log dose-response curves of  $I_{sc}$  and  $V_{oc}$  vs. the concentration of these compounds show that  $HCl$  is approximately an order of magnitude more effective than  $NaCl$  in stimulating the  $I_{sc}$  and  $V_{oc}$ . Amiloride inhibits approximately 30% of the  $I_{sc}$  in K-H buffer (145 mM  $Na^+$ ) but has no effect on the  $HCl$  stimulated  $I_{sc}$ . These data are in agreement with the taste perception measurements of Schiffman.

The dog tongue is also responsive to glucose when added to the mucosal solution. The response of the  $I_{sc}$  and  $V_{oc}$  to this sugar depends on both the glucose and the  $NaCl$  concentrations. We found that in the absence of  $NaCl$  there is no stimulation of  $I_{sc}$  or  $V_{oc}$  up to 1 M glucose. This result suggests that salt may be necessary for the tasting of glucose.

A Computer-Controlled Automated System For Determining Olfactory Thresholds. BURTON M. SLOTHICK (The American University)

Determination of absolute olfactory thresholds in animals using training methods and air-dilution olfactometers of the type described by Tucker, Moulton, Slotnick and others is a time-consuming and arduous task. We have developed an automated multi-channel odor generator and training and test procedures which considerably simplify olfactory psychophysical studies with animals and humans. The system consists of an Apple computer, interface, 8-channel odor generator, and animal test chamber or sniffing port for human subjects. The odor generator consists of a clean air channel and an over-the-surface saturator whose output is diluted sequentially in log steps in 7 independent channels. Each channel connects to the common port of separate 3-way Teflon solenoid valves. Operation of a solenoid manifolds 300 cc/min of a stimulus into a 2700 cc/min clean air carrier stream. Because all stimuli are generated continuously, rapid changes in stimulus concentration can be achieved and the selection of stimuli can be made interactive with the subject's performance on a trial-by-trial basis. Absolute olfactory thresholds can be determined using methods of limits, constant stimuli, adjustment or titration procedures. A go, no-go, discrete trials procedure is used in which a rat initiates a trial by breaking a photobeam at the air inlet port of a wind-tunnel. Attending behavior is enforced by the requirement that the animal maintain its nose in the photobeam for the first 2-sec of the trial. On S+ (odor) trials, contact with a response bar produces a .05-ml water reward. Except that responses on S- (no odor) trials are not reinforced, there is no punishment for errors of commission or omission. Preliminary training and psychophysical tests are completely automated. Four hundred or more trials may be given in a single session and trained animals complete approximately 4-5 trials/min. During each session trial sequences, responses, and response latencies are displayed on a CRT and at the end of a session fine and coarse grain summaries of performance are printed. The relative advantages of several psychophysical procedures with rats and humans are discussed.

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Effect of Stimulus Volume on  $NaCl$  Taste Threshold

Burton M. Slotnick, Angelina Wittich (The American University), and Robert Henkin (Georgetown University School of Medicine).

In laboratory investigations, optimal conditions and stimulus parameters for assessing taste thresholds can be achieved readily. However, in clinical practice, where an extensive series of taste tests may be used to diagnose or assess treatment of taste disorders, the use of "optimal" test conditions may be impractical because of the amount of time required to obtain a threshold for even one tastant. A rapid and simple taste threshold test which uses small (1-drop) volumes and no inter-trial rinses has been developed by Henkin and has been widely used in clinical investigations. Because both stimulus volume and rinse are known to affect threshold, we investigated these variables in an effort to assess and, potentially, improve this clinical tool. Eight adult subjects were tested using an ascending method-of-limits and a three-alternative forced-choice method on each trial. Stimulus volumes were 0.05 ml (one drop), 0.5 ml, and 0.9 ml. For the rinse condition, the subject's tongue was rinsed with 1 ml of water between trials. Results demonstrated: 1. an inverse relationship between threshold and volume but no significant effects of the rinse condition. Thresholds for the smallest volume (4.3 mN) were significantly different from those of the 0.5-ml volume (2.3 mN) and 0.9-ml volume (1.8 mN). 2. Variability of threshold in repeated tests was significantly higher for the smallest volume. 3. Subjects reported appreciably more difficulty in making judgments with the smallest volume stimulus as compared to the other two. 4. There was a significant practice effect over repeated determinations with all volumes. 5. The absolute values of thresholds obtained were within the range of those reported in laboratory investigations using whole mouth stimulation and intertrial rinses. We conclude that the simple 'three drop' method described by Henkin can provide reasonable estimates of taste thresholds in normal subjects but that the procedure can be improved by the use of slightly larger stimulus volumes and, to minimize evident practice effects, making 2-4 separate threshold determinations for each tastant.

The Innervation and Chemical Specificity of Aesthetasc Hairs in the Lateral Antennule of the Spiny Lobster *Panulirus interruptus*. MARIBETH SPENCER and JAMES F. CASE (Department of Biological Sciences, University of California, Santa Barbara, California 93106)

Light and scanning electron micrographs of the lateral antennule of *Panulirus interruptus* reveal that each aesthetasc hair is innervated by a cluster of approximately 1000 sensory neurons. The dendrites of these neurons extend distally into the aesthetasc hairs and the axons from each cluster of somata extend proximally to the brain. Each cluster is encased in glial sheath, effectively isolating them morphologically and physiologically. Extracellular recordings were made with suction electrodes from the axons of single clusters to determine the responsiveness of individual hairs to amino acids, neurotransmitters, nucleotides, metabolic products, organic acids, and sugars. These experiments indicate that individual hairs are broadly responsive to a variety of stimuli and homogenous in this breadth of responsiveness. Further, the degree of responsiveness of aesthetascs to these stimuli correlates well with the behavioral responsiveness of *Panulirus interruptus* to these same stimuli as reported by Zimmer-Faust et al. (submitted).

The Flehmen Response of the Stallion: A Behavioral, Chemical, and Endocrinological Perspective. C.C. STAHLBAUM (Cornell University Ithaca, NY and Monell Chemical Senses Center, Philadelphia, PA) and K.A. HOUP (Cornell University)

The flehmen response is a behavior exhibited by many ungulates which is characterized by the curling up and back of the upper lip. During the response the head is extended and elevated and respiration is slow and deep. The role of the flehmen response in the behavioral repertoire of the stallion was investigated in both laboratory and field studies. In the laboratory we evaluated both the nature of the chemical stimuli which elicited flehmen behavior and the resultant endocrinological responses. The field study provided objective evaluation of the context in which the flehmen response naturally occurred.

We determined that investigation of mare urine was a more potent elicitor of the flehmen response than investigation of other types of horse excrement or of the mare herself. Chemical analysis revealed that a component in estrous mare urine responsible for eliciting the flehmen response was readily extractable in the organic solvents methylene chloride and butanol. This component was not extracted by nitrogen which revealed its relatively non-volatile nature. Flash chromatography and thin layer chromatography of this component indicated that it was not polar. Nuclear magnetic spectroscopy revealed the presence of aromatic rings in the active fraction. Additionally, the endocrinological study revealed a time-dependent correlation between the flehmen response in the presence of mare urine and plasma hormone levels. Although the flehmen response of stallions in a pasture breeding situation was found to correlate with the rate of mounting they performed, the flehmen response did not necessarily occur proximate to the copulatory act itself. Our results suggest that the flehmen response of the stallion may be involved in the behavioral and/or physiological priming of the stallion for reproduction when mares are most likely to be receptive to his intentions.

Chemical Senses and Aging: Taste vs Olfaction. J.C. STEVENS, and L.M. BARTOSHUK, (John B. Pierce Foundation Laboratory and Yale University).

In another presentation (Bartoshuk et al) it is pointed out that although taste thresholds are higher in the elderly than in the young, suprathreshold magnitudes of common taste substances seems to remain remarkably stable in old age (mean: 83 yrs). Olfactory thresholds also rise with age. In contrast to taste, however, suprathreshold olfactory magnitudes are also frequently (but not always) attenuated in the elderly. One study showed that (on average across all subjects) the elderly perceived odor magnitude of amyl butyrate to be about half as strong in the elderly (20 subjects mean: 72 years) as it was to the young (20 subjects, mean: 22 yrs). Also the nasal pungency of CO<sub>2</sub> (common chemical sense, CCS) was attenuated by about half in elderly people. (There was, however, no correlation between degree of olfactory and CCS degeneration.) These age-related results were obtained by magnitude matching with the loudness of a noise band (150-500 Hz, 50 to 100 dB SPL) whose detection threshold had to meet a criterial level. To compare directly suprathreshold magnitudes of taste (NaCl) and odor (amyl butyrate) we had subjects estimate taste and smell of various concentrations on a common scale of intensity, in the same test session. Three age groups of 20 subjects each took part: 20-25, 65-78, and 80-95 yrs. The disparity between olfactory and taste magnitudes was least for the young, more for the old, and most for the very old, thereby confirming our hypothesis that old age takes a larger toll on olfaction than on taste.

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Behavior-Indicate for Quality of Taste Sensation in Nonhuman Primates.

STEINER, J.E. (Dept. Oral Biology, Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel). GLASER, D. (Dept. Anthropology, University of Zurich, Zurich, Switzerland).

Differential orofacial motor-patterns were found, in earlier studies, to be reliable and quantifiable indicators of taste hedonics. This innate reflectory response to tastants was first described in human infants in perinatal age, and found to be controlled by brainstem structures. Animal studies revealed comparable reactions in rabbit and rat pups, suggesting the cross species appearance of fixed behavior-patterns indicating the quality of taste sensations.

The present study's aim was to explore such reflex coordinations in nonhuman primates. Observations encompassed 12 species, a total of 23 individual animals among them 3 juveniles, sampling both sexes. Results show that supra-threshold sweet triggers in some species high-frequency lapping, lip and finger licking, accompanied by relaxed face and opened sparkling eyes. Other species displayed long-lasting persistent and uninterrupted sipping-sucking of the sweet liquid with repeated low-frequency elevation-relaxation of eyebrows. Naive evaluators read clearly the communicational message or "enjoyment" in these motion-features. Bitter, in contrast, triggers in some species high-frequency, pendular head-shaking, copious salivation and spitting. Juvenile animals display rhythmic rubbing-cleaning of lips and mouth. Other species show sudden, deterred, brusque withdrawal from the drinking-device after experiencing even delicate volumes of bitter. Bitter induced behavior features were also easily interpreted by naive evaluators as signs of aversion, disgust. Some preliminary observations on apes in captivity revealed facial expressive responses to tastants definitely similar to the human gustofacial response. An attempt was made to quantify some parameters applicable to motor-reactions.

Conditioned Taste Aversion Generalization Across Five Different Rodenticides. CHARLES N. STEWART (Franklin & Marshall College, Lancaster PA 17604), J. RUSSELL MASON and RUSSELL F. REIDINGER, JR. (Monell Chemical Senses Center, Philadelphia PA)\*

The taste characteristics of several standard rodenticides was investigated by pairing the ingestion of a small amount of each substance with illness induced in the rodents by means of LiCl injection. The ingestion of rodenticides  $\alpha$ -naphthylthiourea (ANTU), strychnine,  $\alpha$ -chlorohydrin, calciferol and sodium warfarin were contrasted for experimental animals who had the above pairings and controls who had not. While animals were able to develop taste aversions to each of the toxins, only very modest generalization of aversion across rodenticides was observed. This suggests that these toxins have distinctive flavor characteristics to rodents. In the case of at least two rodenticides (ANTU and Na-warfarin) double pairings of illness and taste were required in order to produce reliable and significant aversions. This finding is consistent with field observations that rodents do not readily develop "bait shyness" with these rodenticides.

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Afferent Projections of the Superior Laryngeal Nerve in the Lamb. ROBERT D. SWEAZEY and ROBERT M. BRADLEY. (Dept Oral Biol, Univ Michigan, Sch Dentistry, Ann Arbor, MI 48109.)

To determine the central projections of taste afferents innervating taste buds on the epiglottis, horseradish peroxidase was applied to the cut end of the superior laryngeal nerve (SLN) in 4 lambs aged 30 to 60 days. After 72 hours transverse sections of the brainstem were processed using the tetramethyl benzidine method.

Afferent fibers in the SLN entered the ipsilateral brainstem through a series of vagal rootlets, from the level of the rostral inferior olive caudal to the level of the dorsal motor nucleus of the vagus (DMV). A small number of fibers entered the dorsal spinal trigeminal tract while the majority of fibers entered and ran caudally in the solitary tract (ST). Labeled terminals in the nucleus of the solitary tract (NST) extended from rostral levels of the DMV caudally to the commissural nucleus. At the level of the DMV the majority of the terminations were located in the medial NST. Further caudally, at the level of the area postrema, SLN terminals were located primarily around discrete bundles of ST fibers; a few terminals were located in the medial and ventrolateral NST. At the level of the commissural nucleus terminals were located exclusively in the lateral aspects of the nucleus. No terminals were found in the brain contralateral to the labeled SLN. Finally, a small number of terminals were located in the dorsal-lateral portions of the caudal spinal trigeminal nucleus.

The central projection of the SLN is quite extensive. However, not all of the projection of the SLN is gustatory since this nerve carries temperature, tactile and probably nociceptive afferents from the epiglottis and other areas of the larynx. Only by neurophysiological experiments will it be possible to determine which part of this projection responds to chemical stimulation of the epiglottis.

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Morphological and Electrophysiological Differentiation of Mouse Olfactory Cells in Culture. JOHN H. TEETER (Monell Chemical Senses Center, Phila., PA 19104) and NEIL I. GOLDSTEIN (Immunomedics Inc., Newark, NJ 07103).

The clonal cell line MOE CL1, isolated from primary cultures of adult mouse olfactory mucosa, is presumed to consist of olfactory basal cells (Goldstein and Quinn, *In Vitro* 17, 593, 1981). Early passage MOE cells were characterized by rounded, phase-refractile cell bodies and the presence of carnosine and carnosine synthetase activity. After over 250 divisions in culture, however, these cells lost their bipolar morphology and apparently their carnosine synthetase activity. We have examined the effects of two differentiation promoting agents, dibutyladenosine 3':5'-cyclic monophosphate (DB) and 12-o-tetradecanoylphorbol-13-acetate (TPA), on the morphology, growth and electrophysiological properties of MOE cells. Cells grown in the presence of DB (1mM) or TPA (200ng/ml) often became bipolar, with rounded, phase-bright soma and long neurite-like processes after a lag phase of 72-96 hr. Cells conditioned for at least one passage in DB or TPA, or cells exposed to a mixture of DB (0.5mM) and TPA (100ng/ml), attained a bipolar morphology within 24 hr, showing no lag phase. Cultures grown with DB, TPA or DB+TPA showed significant increases in the percentage of neurite-bearing cells. An increase in population doubling time and decrease in saturation density were also observed in cultures exposed to DB. Morphologically differentiated cells in cultures exposed to DB or DB+TPA tended to have larger resting membrane potentials (-10 to -50mv) and input resistances (40 to 220 M $\Omega$ ) than undifferentiated MOE cells in control cultures (-5 to -20mv; 10 to 50 M $\Omega$ ). In addition, these cells displayed several types of spontaneous changes in membrane potential including slow, regular oscillations; irregular, slow and fast depolarizations; and trains of action potentials. These results indicate that MOE cells can be induced to undergo changes in morphology, growth and electrical properties characteristic of neural differentiation in culture.

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Changes In Catfish Olfactory Bulb Cell Responses To Amino Acids Following Olfactory Tract Transection. HILARY THOMPSON & JOHN CAPRIO (Louisiana State University, Baton Rouge, LA. 70803)

The present study determined the effect of olfactory tract (OT) transection on amino acid induced responses of mitral cells (MC), which were identified by the recording depth and similarity of response to OT unit activity. 720 responses (#spikes/first 5s response minus 5s pre-stimulus count) were recorded from 24 MC in 4 fish. The initial rise of the simultaneously recorded EOG determined the response onset. Two replications each of glutamic acid, arginine, alanine, cysteine and methionine (pH adjusted if necessary), chosen as representatives of acidic, basic, short-chain neutral and long-chain neutral L-amino acids, respectively, were tested at 3 concentrations ( $10^{-4}$  M,  $10^{-3}$  M,  $10^{-2}$  M). Recordings were obtained from an equal number of MC (12) before and after OT transection. MC spontaneous activity ( $4.6\text{Hz} \pm 2.2\text{M} \pm \text{S.E.}$ ) was similar to values found in the burbot (Doving, 1966) and trout (MacLeod, 1976). This activity increased significantly ( $P < .05$ , ANOVA) after OT cutting ( $6.3\text{Hz} \pm 2.2$ ). In addition, the mean responses to alanine, glutamic acid and methionine changed significantly ( $P < .05$ , T test) from negative (suppression) to positive (excitation). The reduced suppression to natural stimuli post OT transection is consistent with the finding in the burbot (Doving, 1966) that OT electrical stimulation suppressed bulb cell activity. Thus, in the present experiments, the removal of central neural input to the olfactory bulb increased MC spontaneous activity and had a facilitory influence on bulbar amino acid responses.

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Latency of Ingestion and Rejection Responses to Sapid Stimuli. J.B. TRAVERS and R. NORGREN. (M.S. Hershey Medical Center, Pennsylvania State University)\*

Tactile and gustatory receptors in the oral cavity provide sensory cues to determine whether an ingestion or rejection response will occur. Previous behavioral studies (Grill and Norgren, 1978) demonstrated that gustatory stimuli produce stereotyped orofacial movements, the observable concomitants of ingestion and rejection. In order to further clarify the relationship between these orofacial movements and ingestion and rejection, we have recorded EMG responses from chronically implanted rats receiving intraoral infusions (50ul) of sucrose (S), QHCl (Q), or water. In a previous report (*Neuroscience Abstracts*, 9:189, 1983), we described differences in the timing relationship between a jaw opener (anterior digastric), a tongue protruder (genioglossus) and a tongue retractor (styloglossus) during ingestion and rejection responses to S and Q respectively. The present analysis reports differences in the latency of several responses during ingestion and rejection. Swallowing was observed following either S or Q stimulation. In the first 10 sec following Q stimulation, however, only half as many swallows occurred as followed S stimulation ( $P < 0.001$ ). In addition, the latency to the first swallow following stimulation with Q ( $\bar{X} = 3.86$  sec) was over twice as long as the latency to the first swallow following S ( $\bar{X} = 1.50$  sec;  $P < 0.05$ ). Prior to the first swallow to Q the animal initiated a rejection response (gapes) with a mean latency of 0.86 sec ( $\text{SD} = 0.18$ ). During this 0.86 sec period rats made an average of 4.9 ( $\text{SD} = .9$ ) tongue movements (licks) that were indistinguishable from those to S stimulation. These initial lick responses are consistent with the hypothesis that bitter stimuli must reach the posterior tongue to initiate a rejection response (Nowlis, 1977).

Supported by grants NS18731 and NS10150.

Chemoreception by the Western Atlantic Ghost Crab *Ocypode quadrata* (Fabricius): Responses to Natural Stimuli from Fresh and Aerobically Decomposing Tissues. THOMAS J. TROTT (Boston University Marine Program).

*Ocypode quadrata* has been described as a predator, scavenger, and deposit feeder. To test this species response to freshly injured versus decomposing tissue, blue crab claw muscle was homogenized and incubated at ambient temperature while being constantly stirred and aerated. Sequential samples of the mixture were taken at 0, 6, 24, and 48 h. After ultrafiltration, the retentates and filtrates were bioassayed using cheliped flexion as the criterion for a response. The majority of the activity of the homogenates was in those filtrates containing substances  $<1,000$  MW ( $F_2$ ), although the activity of retentates containing substances  $>10,000$  MW increased with the age of the samples. Thresholds to  $F_2$ , however, increased with the age of the samples. Acidified sub-samples of  $F_2$  were extracted with ethyl ether to obtain organic acids and neutral compounds. The extracted aqueous phase was made basic and re-extracted to separate amines from amino acids. Thresholds to extracted  $F_2$  revealed that extracts containing amino acids were most active. Furthermore, the thresholds to all extracts increased with age. This increase was more dramatic for extracted amines and neutral compounds. These results suggest that scavenging may be a secondary feeding strategy to predation i.e., freshly injured tissues are more attractive than aerobically decomposing flesh.

Supported by grants from the Explorers Club, Sigma Xi, and the University of Georgia Marine Institute, Sapelo Island, GA to TT.

Dynamic Response Patterns of Taste Receptor Cells in the Lobster, *Homarus americanus*. RAINER VOIGT and JELLE ATEMA (Boston University Marine Program).

To begin to determine the dynamic response patterns of taste receptor cells in the walking legs of the lobster a test chamber was designed which allowed the application of short stimulus pulses (0.1s rise time, 0.3s peak duration, and 1.0s decay to 25% peak concentration) with high reproducibility. Peak concentrations varied by 5% (SEM,  $N=10$ ); similar variance characterized the entire pulse shape. Single units were identified by extracellular recording from small nerve bundles. Since glutamate cells are most frequently encountered in extracellular recordings of single chemoreceptor cells in the lobster leg (37%), we chose this population for further characterization of dynamic properties, here adaptation and disadaptation. We stimulated repeatedly (with standard stimulus pulses;  $3.5 \times 10^{-4}M$ ) and varied the stimulus intervals. Post-stimulus responses were measured as the number of spikes in 500ms time bins beginning with the first spike after introduction of the stimulus in cells without spontaneous activity.

The variability of responses to repeated stimulation was significantly lower in the first 2-3 bins. There was great variation in total response durations. Receptors giving long (3s) responses adapted faster than those with short responses (3s). Furthermore, long responding cells required longer time periods for disadaptation than cells with short responses. Brief exposure to high stimulus concentrations ( $3.5 \times 10^{-4}M$ ) subsequently suppressed responses to lower concentrations; at similar interstimulus intervals subsequent responses to high concentrations were still observed, but those to lower concentrations were abolished. Similar suppression of subsequent responses was seen when other amino acids were used.

Supported by grants from Deutsche Forschungsgemeinschaft to RV and NSF (BNS-82104434) and Whitehall Foundation to JA.

The Ontogenetic Development of Acetylcholinesterase (AChE) Activity in the Rat Olfactory Bulb. S. Van Ooteghem, S. Schumacher and M.T. Shipley (University of Cincinnati College of Medicine).

The development of a pathway involves the growth of fibers to a target structure, the formation of synaptic contact and the expression of molecular mechanisms necessary for synaptic transmission. Little is known about the degree to which these events are temporally correlated or interdependent. We report results which suggest that biochemical maturation of cholinergic (Ch) synapses occurs long (14-20 days) after the fibers reach the bulb.

Specific AChE was localized in pre-, post-natal and adult brains using a copper thiocholine method. Centrifugal afferents to the bulb were retrogradely labeled at the same ages by WGA-HRP injections in the main olfactory bulb (MOB).

The source of cholinergic input to MOB is from neurons in the nucleus of the diagonal band (DB). Neurons in DB are retrogradely labeled in abundance at birth and the number of labeled neurons increases steeply in the first week of life. At the same time, DB neurons are intensely AChE positive. There is a close correspondence among AChE+ and choline acetyltransferase ChAT+ neurons in DB (Eckstein and Sofroniew, '83). MOB is devoid of AChE until nearly two weeks after birth, yet our method does stain AChE terminals from birth onwards in other brain areas. The AChE pattern in MOB is not fully mature until around 50 days.

These results provide evidence for the idea that cholinergic terminals do not become biochemically "mature" until several weeks after they have grown into the bulb. In autonomic ganglia, AChE appears slightly ahead of ChAT and synaptic transmission. Thus, in the brain, synaptic function may lag considerably behind connective formation. It will be of interest to learn what events regulate synaptic biochemical maturation and to determine whether other, transmitter specific projections to the bulb develop similarly to the Ch system.

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Innervation Patterns of Chorda Tympani and Lingual Nerve Fibers in Hamster Fungiform Papillae. MARK C. WHITEHEAD. (Dept. Oral Biol., UConn Dent. Sch., Farmington, CT 06032)

Synaptic endings of the chorda tympani (CT) are apparently restricted to fungiform taste buds while lingual (trigeminal) nerve (LN) endings terminate extragemmally (Whitehead and Beeman, Soc. Neurosci. Abstr., 1983). The present study determines the numbers of CT and LN profiles innervating apical fungiform papillae and the morphologies of individual fibers corresponding to these two nerves.

For quantitative analysis, tangential sections of normal papillae were compared in electron micrographs with CT-denervated papillae. In normal papillae, at levels through the mid-lower bud, nerve fibers surrounding the bud outnumber intragemmal fibers by factors of 2-3 to 1. Comparable levels of CT-denervated papillae contained no intragemmal fibers but the number of fibers surrounding the bud was normal. Apically, few intragemmal endings occur in normal papillae; none in CT-denervated papillae. However, endings within the non-taste epithelium, in normal and experimental papillae were more numerous than at basal levels; some appeared atrophic. The total number of profiles in the hamster at lower bud levels (approximately 100) is one-half that in the rat (Beidler, Olf. and Taste, 1969).

In Golgi preparations we determined that many profiles observed in electron micrographs result from branching of CT and LN fibers below and within the taste bud and non-taste epithelium, respectively. Little branching occurs in basal papillae. LN fibers enter the apical epithelium lateral to the taste bud. After branching, they proceed, superficially and terminate as fine points, blunt (2-4  $\mu m$ ) expansions, or as atrophic, ball-on-stalk retraction bulbs located 5-50  $\mu m$  from the epithelial surface. A few terminate subepithelially and some are unbranched. CT fibers branch from subgemmal swellings (2-5  $\mu m$ ), penetrate the bud and extend apically, while branching further. CT endings are tapered, expanded, or pedunculated.

These data are consistent with the innervation of separate epithelial domains of fungiform papillae by the CT and LN. Individual fibers of both nerves form radially oriented, divergent branches which, for the CT, allows single fibers access to central (presumably mature) as well as peripheral (immature) cells of the taste bud. The variable morphologies of CT and LN terminals may reflect their remodeling during "turnover" of epithelial cells.

(Supported by Grant NS16993 and CT. Research Foundation)

Sniffing Genes for Specific Anosmias. CHARLES J. WYSOCKI (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104)\*

We previously demonstrated that many commercially available inbred strains of mice could smell diluted concentrations of isovaleric acid, which has a sweaty smell to most humans, but mice of the C57BL/6J and C57BL/10J strains could not. However, these isovaleric acid insensitive strains could smell other odorants, i.e., amyl acetate and the musky smelling compound pentadecalactone. A more extensive strain survey, seeking other possible animal models of human specific anosmias, was initiated. Three odorants formed the core of stimuli: amyl acetate, androstene, and Thibetolide. The later two compounds were chosen because of demonstrated variation among humans in their ability to perceive these odorants.

A conditioned odor aversion paradigm was used to assess perception of these odorants in 28 strains of mice. All strains avoided amyl acetate after an initial pairing of the odorant with an injection of lithium chloride. Hence, these mice could detect amyl acetate. Surprisingly, many strains did not avoid androstene after the pairing of injection and odorant presentation. Mice of the CBA/J strain, however, consistently avoided the odorant after conditioning. Hence, at least CBA/J mice could detect androstene whereas many other strains appeared to be anosmic to androstene. Some strains, i.e., PL/J and RF/J, also failed to avoid Thibetolide during testing. Members of these strains may be anosmic to this compound.

If continued tests confirm the preliminary results obtained during this strain survey, then additional animal models will be available for future neuroanatomical, neurophysiological and biochemical investigations into the mechanisms of olfaction.

\*Supported by the National Institutes of Health, grant NS-17580.

Cytochrome Oxidase Histochemistry of the Main Olfactory Bulb (MOB) and Accessory Olfactory Bulb (AOB): Developmental Effects and Species Variation in Metabolism. CHARLES J. WYSOCKI, GARY K. BEAUCHAMP, JUDITH L. WELLINGTON and SHARON GREELEY (Monell Chemical Senses Center, 3500 Market St. Phila. PA 19104)\*

Cytochrome oxidase (CO) is the terminal enzyme electron acceptor in cellular respiration. Variation in mitochondrial CO activity is positively correlated with variation in cellular metabolism. We stained sections of the MOB and AOB of neonatal (24 hr old) and adult rats and adult mice and guinea pigs for CO activity. All animals were individually housed and no attempt was made to isolate subjects from colony odors. Neonates were kept with their mothers and siblings until perfusion.

For the MOB there was variation in staining throughout the glomerular layer in all subjects regardless of age or species: some glomeruli were intensely stained, whereas others were not. Striations also were observed in the granule cell layer of adults.

Developmental differences were evident in the AOB. Neonatal rats possessed high levels of CO in the glomerular layer of the AOB whereas adult rats did not. High levels of CO activity within the neuropil of the neonatal animal may reflect (a) the demands of intense neuronal growth and synaptogenesis which occur during this time or, (b) constant *in utero* stimulation of the vomeronasal organ.

Differences among species in adult AOB activity were also evident. Results from mice were similar to those of adult rats: little CO activity was observed in the AOB. The lack of CO activity in the AOB of adults suggests a quiescent vomeronasal organ. The results of guinea pigs were in marked contrast to those obtained from adult mice and rats. The glomerular layer of the AOB in guinea pigs was moderately to intensely stained. While these species differences are puzzling, the CO activity in the AOB of guinea pigs is consistent with results obtained from <sup>14</sup>C-2-deoxyglucose studies of the AOB in this species.

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A Single Gene Mutation Alters Urine Odor in Mice. KUNIO YAMAZAKI, GARY K. BEAUCHAMP, (Monell Chemical Senses Center), JUDITH BARD, LEWIS THOMAS and EDWARD A. BOYSE. (Memorial Sloan-Kettering Cancer Center)\*

The Major Histocompatibility Complex of genes (MHC) encompasses a region of chromosome 17 in the mouse containing a large number of closely linked genes many of which control graft rejection and other immune responses. We previously found that differences in this complex of genes also affect the mating choices of mice, and that mice can be trained to distinguish arms of a Y maze scented by odors from mice differing only in this genetic region.

As no known gene complex exhibits such vast diversity as the MHC, its potential as a major source for genetically-based signals of individual identity is great. Since the function and identity of all the genes in the MHC are not yet understood, we do not know whether the MHC-determined odor phenotype is constituted by known MHC genes or unknown genes which may reside within the MHC complex and which might have a primary function in sensory recognition. That question is classically decided by determining whether the trait is affected by mutation of a known gene. For this purpose we have studied the ability of mice to distinguish the odor of the C57BL/6 (H-2<sup>b</sup>) strain from that of a congenic mutant strain differing only by a mutation at the K<sup>b</sup> gene, B6.C-H-2<sup>bmi</sup>. Mice were trained by reward in a Y maze to distinguish the odors of urine samples, and the successful distinctions of mutant from non-mutant were confirmed by transfer of training, without reward, to coded samples of urine from genetically equivalent urine donor mice.

Although it is not excluded that the differences in odor phenotypes which distinguish mutant from non-mutant mice are directly related to the structure of the gene products, which are proteins, it is equally possible that MHC related odor phenotypes arise from effects of MHC genetic variation on metabolic pathways either directly, or indirectly through developmental polymorphism.

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