

Book of Abstracts

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RECOVERY PROCESSES OF FROG OLFACTORY EPITHELIUM FOLLOWING ZnSO_4 TREATMENT. Gloria D. Adamek and Robert C. Gesteland. Northwestern University, Evanston, IL 60201

ZnSO_4 solutions can be used to cause chemical ablation of the olfactory epithelium. Perfusion of the olfactory cavity of mice and frogs leads to differing effects. In mice, regeneration is patchy and often incomplete. In frogs, however, there is an orderly sequence of regeneration which has been studied using scanning electron microscopy (SEM), differential interference contrast observation of living tissue and measurements of the electro-olfactogram (EOG). After the olfactory epithelium is sloughed off, the region becomes covered by respiratory epithelial cells, whose cilia move in a characteristic, coordinated fashion. By day 12, when short, motile olfactory cilia are first visible, the EOG is measurable. Using differential interference contrast optics or SEM, short olfactory cilia are seen in patches among the respiratory cilia. Young receptor cells bear short, active cilia. As the cells mature, olfactory cilia increase in number and length. Spontaneous ciliary motility slows and changes in movement pattern. Cilia on the mature, synaptically connected receptor neurons are long and immotile.

In both normal and regenerating epithelia white eminences can be seen with a stereo microscope. These are particularly prominent in the regenerating epithelium which is much thinner than the normal epithelium. Histological stains show these structures to be Bowman's glands. In the regenerating epithelium, the glands are at the surface rather than deep with a long duct. This observation facilitates the search in the regenerating epithelium for single unit activity because it allows visually directed placement of the electrode in the clear areas between the glands where the newly differentiated receptor neurons appear. The orderly way in which the frog epithelium is reconstituted and the ease with which regions of active neurons can be found makes this preparation useful for the study of responses to odors of cells in an immature state prior to the time that synapse formation occurs.

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SUPPRESSION OF BITTERNESS OF QHCL IN MIXTURES: POSSIBLE MECHANISMS. Linda M. Bartoshuk and John P. Seibyl. John B. Pierce Foundation Laboratory, 290 Congress Ave., New Haven, CT 06519.

When two substances with different taste qualities are mixed, one or both of the individual component tastes are often suppressed. A series of experiments provides some hints about the mechanisms underlying this mixture suppression.

In the first experiment, two component mixtures were constructed from NaCl, sucrose, HCl, and QHCl (quinine hydrochloride). Mixture suppression of one component was generally released when the tongue was adapted to the other component prior to tasting the mixture. However, the NaCl-QHCl mixture proved an exception to this generalization and provided an interesting contrast to the sucrose-QHCl mixture. The bitterness of quinine was reduced in both mixtures. Adaptation to sucrose released the suppression of QHCl such that the sucrose-QHCl mixture tasted like an unmixed QHCl solution. Adaptation to NaCl did not release the suppression of bitterness in the NaCl-QHCl mixture.

The work of Zotterman and his colleagues on adaptation in the human chorda tympani suggest an explanation of these results. They found that during prolonged stimulation the chorda tympani response was successively reduced such that it had returned to baseline at about the time adaptation would occur psychophysically. Thus after adaptation to one of the components of a mixture, there is little if any neural response (and thus no CNS response) to that component in the mixture. If the suppression of QHCl is central, then adaptation to the other component should release that suppression because adaptation prevents the other component's response from getting to the CNS. If suppression of QHCl is peripheral, then adaptation would not be expected to release suppression. By such reasoning, our data support a central locus for the suppression of the bitterness of QHCl by sucrose (which agrees with the conclusions of Lawless based on a different experiment) and a peripheral locus for the suppression of QHCl by NaCl.

As a further test of this possibility, subjects were adapted to the sucrose-QHCl and NaCl-QHCl mixtures and tested with QHCl. QHCl was essentially tasteless after adaptation to the sucrose-QHCl mixture. These results are also consistent with a predominantly central locus for suppression of QHCl by sucrose and a predominantly peripheral locus for suppression of QHCl by NaCl. The failure of adaptation to the NaCl-QHCl mixture to adapt QHCl suggests the possibility that the presence of NaCl actually interferes with the binding of QHCl since if the QHCl does not bind effectively in the first place, it cannot produce adaptation.

AGE DEPENDENT CHANGES IN SCENT MARK CONSTITUENTS OF A PRIMATE, THE TAMARIN *SAGUINUS FUSCICOLLIS*. Anne M. Belcher, Gisela Eppie, James G. Kostelc and Amos B. Smith, III. Monell Chemical Senses Center and the Chemistry Department, University of Pennsylvania, Philadelphia, PA 19104.

In the saddle back tamarin, scent marking with large glands in the circumgenital-suprapubic area plays an important role in socio-sexual communication. Scent marks produced by males and females appear to identify species, subspecies, gender and individual, and may communicate information on the reproductive and social condition of the individual.

Information on the approximate age of the scent may also be encoded in the marks. Behavioral experiments have shown that scent marked objects lose some of their attractiveness for the monkeys by the time they have been maintained under room conditions for 24 hours. The attractiveness of the scent further decreases as it ages and by 3-4 days of age scented marked objects are not investigated more frequently by the tamarins than are unscented objects or those carrying control odors.

About 96% (by weight) of the volatile constituents of the scent marks of males and females are comprised of 15 esters of n-butyric acid and of squalene. The remainder consists mainly of a large number of more highly volatile compounds, i.e., those which elute by GC before the first ester, cetyl butyrate.

We report here that qualitative as well as quantitative changes occur in the scent mark composition as the material ages. GC monitoring of individual animals' scent mark profiles indicates that changes in the relative concentrations of compounds present in the fresh scent occur as the marks age. Moreover, aged scent may contain highly volatile material not present in the fresh mark. GC-MS analysis of pooled scent mark material is currently in progress and will result in identification of specific compounds as well as changes in their concentrations.

Changes in the concentration of certain highly volatile components as well as new compounds which may be formed during the aging process, may serve as signals which transmit information on the age of the material. Selective loss of other components may lead to inability of the animals to translate encoded messages concerning species, subspecies, gender and other individual characteristics.

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SENSORY DEPRIVATION AFFECTS THE SYNAPSES BUT NOT THE SIZE OR NUMBER OF MITRAL CELLS. T.E. Benson and J.W. Hinds. Sect. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

Unilateral neonatal naris cauterization results in a functional deprivation of the ipsilateral olfactory bulb (OB) and a smaller volume of its laminae (excepting the ventricular/subependymal zone) as compared with non-deprived (ND) or control OB's (Benson & Hinds '80 Neurosci. Abstr., 6:637). Meisami ('78 Prog. Brain Res. 48:225) has reported a 26% reduction in the number of mitral cells (MC) due to unilateral deprivation in 25-day-old rats. For 30-day-old CD-1 mice we have determined the number of MC's, their nuclear and soma size, the relative number of nucleoli per nucleus, and lateral olfactory tract cross sectional areas from deprived (D) and ND OB's in coronal 1 μm plastic sections. For soma sizes electron micrographs were used as well.

We have found no differences between the D and ND sides in the number of MC's (37,606 D, 38,355 ND; n=4 mice; $P=0.5824$), MC nuclear radius (5.28 μm D, 5.26 μm ND; n=4; $P=0.8311$) MC soma cross sectional area (193 μm^2 D, 198 μm^2 ND; n=6; $P=0.6160$), the number of nucleoli per nuclear cross section (1.05 D, 0.98 ND; n=4; $P=0.2953$), or lateral olfactory tract cross sectional area (0.152 mm^2 D, 0.163 mm^2 ND; n=6; $P=0.2652$). Our MC totals derive from raw counts of all MC nuclei which were corrected by the Floderus equation. We have used plastic sections because in our experience the identification of small MC's in thick paraffin sections was unreliable. We identified MC's by cytological criteria such as a pale nucleus and patterns of Nissl substance and not by "the special miter shape" which Meisami required for counting. These different procedures may explain the different results.

In contrast to these results, our electron microscopic, stereological, blind analysis of MC associated soma synapses has shown a markedly lower number per surface area (N_s) of both MC-to-granule cell (GC) and GC-to-MC synapses on the D side. For MC-GC synapses $N_s=0.0812 \mu\text{m}^{-2}$ D, 0.1255 μm^{-2} ND (n=4; $P=0.0005$). For GC-MC synapses $N_s=0.049 \mu\text{m}^{-2}$ D, 0.104 μm^{-2} ND ($P=0.0034$). Since soma area was the same for the D and ND side, there must be fewer synapses per MC soma on the D side.

A chi-square analysis of the MC-GC/GC-MC ratios showed that the D side was higher, as would be expected at an earlier developmental stage (Hinds & Hinds '76 J. Comp. Neur. 169:15).

Thus functional deprivation seems to affect MC and GC synapses but not MC soma size or the number of these prenatally generated cells. One could hypothesize, in light of no difference in lateral olfactory tract size, that soma size chiefly reflects axon development, which may not be affected by the deprivation at postnatal day 30.

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MODELING THE CONVERGENCE OF GUSTATORY NEURONS. Stephen L. Bieber and David V. Smith. Depts. of Statistics and Psychology, Univ. of Wyoming, Laramie, WY 82071.

Taste-responsive fibers in the hamster chorda tympani (CT) nerve are relatively narrowly tuned, especially those that respond best to sucrose. The breadth of responsiveness of hamster taste neurons to anterior tongue stimulation increases systematically at the levels of the nucleus tractus solitarius (NTS) and parabrachial nuclei (PbN). Response profiles within a best-stimulus class of neurons are relatively homogeneous at each of these levels. Thus, it should be possible to model the nature of the convergence process leading to the increase in breadth of responsiveness at each level.

Responses (impulses/5 sec) to each of the four basic taste stimuli (0.1 M sucrose, 0.03 M NaCl, 0.003 M CH₃I and 0.001 M quinine hydrochloride) in 78 CT, 57 NTS and 53 PbN neurons were used to classify these cells into S-, N-, H- and Q-best categories, depending upon which of the four stimuli elicited the maximum response in each neuron. The values of the average response profiles of each best-stimulus class of fibers in the CT were used as the independent variables in a multiple regression analysis to predict the values of the average response profiles of each best-stimulus category of cells in the NTS. Similarly, the values of the NTS profiles were used to predict the profiles of cells in each best-stimulus class in the PbN. This approach estimates the convergence from one synaptic level to the next (i.e., it calculates the proportional contribution of each best-stimulus class). For example, reproduction of the average response profile of S-best neurons in the NTS requires a convergence ratio of approximately three S-best CT fibers to each H-best fiber, with the contribution of N-best CT fibers being essentially zero. In this regression, the predicted average response profile to NTS S-best neurons is: 86.98 impulses/5 sec to sucrose, 31.87 to NaCl, 28.79 to HCl and 10.31 to quinine hydrochloride. The actual response profile of this class of neurons in the NTS was: 87.50 impulses/5 sec to sucrose, 29.20 to NaCl, 30.15 to HCl and 10.35 to quinine.

The response profiles of all best-stimulus classes of cells at both the NTS and PbN could be predicted quite well from various combinations of converging input from the preceding level. This analysis suggests a way in which the increased breadth of responsiveness of brainstem taste neurons could occur from converging input from fungiform papillae on the anterior portion of the tongue.

Supported in part by NINCDS Grant NS10211 and Research Career Development Award NS00168 to D.V.S. Chorda tympani (CT) responses were provided by DR. Marion Frank.

PERSISTENCE OF SWEETNESS AND SALIVARY CONCENTRATIONS OF SWEETENERS: EVIDENCE FOR LOCALISED CONCENTRATION

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Four to six subjects each tasted glucose, saccharin and neohesperidin dihydrochalcone solutions of different concentrations and, at intervals after tasting, the salivary concentrations of the three sweeteners were determined (after deproteinisation with perchloric acid) by the Boehringer GOD/perid method, the phenol/sulphuric acid method and UV absorbance assay at λ 220 nm respectively.

Salivary concentrations of each sweetener dropped rapidly after tasting, reaching sub-threshold levels within 15-45 seconds. Some subjects exhibited zero salivary concentrations of sweetener while sweetness sensation was strong and total persistence time of detectable sweetness lasted from 20 - 147 seconds. These results are consistent with the view that persistence of sweetness is due to a localised concentration of stimulus molecules at or near to the receptor. In a separate experiment the inhibition of intensity and persistence of sweetness of sucrose and saccharin by different concentrations of gymemic acid was determined. Whilst gymemic acid inhibited both intensity and persistence of sweetness, inhibition of intensity was always greater than inhibition of persistence. This result is again consistent with localised concentration of stimulus molecules and might be explained by a two-stage model of taste chemoreception.

CHANGES IN OLFACTION WITH AGE

Anne Billing, Steve Van Toller & George Dodd, Warwick Olfaction Research Group, University of Warwick, U.K.

Though it is commonly observed that olfactory acuity declines with age, there have been few comprehensive studies of the phenomenon.

In this work we have studied ten highly purified odorants from distinct odour categories and which are relevant both to flavours and the lives of old people. The odour classes represented include the following; fishy, minty, floral, cheesy, musky, urinous, putrid. For each odorant we have measured the threshold, the odour associations, the odour profile and emotional responses for groups of subjects (N=90-100) between the ages of 20-85.

The data from the experiments is still being analyzed - the following results are already clear:

- 1) The olfactory thresholds show a high variability but for each odour a significant linear regression with age was found confirming the decline in sensitivity with age. No dramatic differences were found between the different odour categories.
- 2) The quality of perception of the odours changes with age in a complex way and it is possible to identify some of the term odour descriptors which undergo a change with age.

The results will be discussed in relation to the distinctive habits of older people.

This work is made possible by a grant from the British Foundation for Age Research.

SALT TASTE RESPONSES IN RATS DEPLETED OF NaCl DURING EARLY DEVELOPMENT. R.M. Bradley, Dept. Oral Biol., Sch. Dent., D.L. Hill and C.M. Mistretta, Center for Human Growth & Devel. and Dept. Oral Biol., U. Mich., Ann Arbor, MI 48109.

Recently we demonstrated that there are developmental changes in taste responses to NaCl, LiCl, NH₄Cl and KCl recorded from the rat chorda tympani nerve. We have now attempted to manipulate these changing responses by depleting NaCl in developing rats. Eleven pregnant rats were fed a sodium-free diet from 3 days of gestation until 12 days after birth; thereafter, they received the same diet with 1% NaCl added. Only 3 rats delivered live young to yield a total of 13 sodium-depleted pups. Six pregnant control rats were fed the 1% NaCl diet and were treated in the same manner as experimental animals. Thirty-four control pups were delivered.

All pups were weaned at 30 days, maintained on the 1% NaCl diet, and at 58 days were preference-tested with water versus 0.001 to 0.5M NaCl (6 concentrations, tested on alternate days). Since depleted rats were smaller than controls, all ingestion values were corrected for body weight. Salt-depleted rats exhibited a decreased preference score for NaCl across concentrations ($p < 0.05$); however, they drank more NaCl ($p < 0.05$) and more water ($p < 0.0001$) than controls. To account for the decreased preference relative to controls, it is obvious that water intake in depleted rats was proportionately greater than NaCl consumption.

After 12 days of preference-testing, neurophysiological responses were recorded from the whole chorda tympani nerve in 13 depleted rats and 15 control rats. Stimuli were 0.1M NaCl, LiCl, NH₄Cl, KCl, and concentration series of 0.01 to 0.5M NaCl, NH₄Cl and KCl. To compare responses to 0.1M salts among animals, integrated, steady-state responses were measured and ratios were calculated for each salt relative to a 'standard' salt (NaCl, NH₄Cl or KCl). Whether responses were expressed relative to NaCl, NH₄Cl or KCl as a standard, no differences were found between sodium-depleted and sodium-fed groups for any of the salt responses ($p > 0.10$). Furthermore, there were no differences between groups for NaCl, NH₄Cl or KCl response-concentration functions ($p > 0.10$).

Therefore when rats undergo NaCl depletion during early development, peripheral taste nerve responses to various salt stimuli recorded at two to three months of age are not altered. However, salt taste responses may have been altered at an earlier age (near the time of depletion) and may have then returned to normal values. This remains to be determined. Also, more data are needed to establish whether the sex of depleted animals is related to salt responses. (Supported by NIH Grant NS17404 and NSF Grant BMS90-15737)

FLUORESCENT MONITORING OF ION-TASTE EPITHELIAL INTERACTIONS. Joseph G. Brand^{1,2}, Stephen J. Kron¹, and David M. Senseman², Monell Chemical Senses Center¹, and School of Dental Medicine² University of Pennsylvania, Philadelphia, PA 19104.

We are monitoring the interaction of salts with taste epidermis of catfish barbel using fluorescent dyes. Albino catfish are immobilized with gallamine triethiodide and perfused through the mouth with tank water. The fish is set in a chamber designed to permit physical isolation of the intact barbel from the body of the animal. This allows the barbel to be exposed to flowing stimuli independent of the rest of the body of the animal. The barbel to be studied is immobilized on a Sylgard base using tungsten loops. These loops surround the barbel but do not pierce or injure the epidermis. The chamber containing the fish is then set under a fluorescence microscope. The microscope is equipped with a Xe arc source, appropriate filters for separation of excitation and emission light, and a photomultiplier for quantitating fluorescence. A 40X water immersion objective is focused onto a taste bud-rich region of the barbel. It is possible to observe blood flow through the barbel, and we routinely employ flow as a criterion of viability. An iris immediately before the photomultiplier is stopped down to a point where light from only a single taste bud is being read. When a 10mM solution of 2-p-toluidinylnaphthalene-6-sulfonate (TNS) is flowed over the barbel a fluorescence increase is observed. Addition of NaCl, KCl, CaCl₂, NaNO₃, or Na₂SO₄ to the TNS medium cause additional concentration dependent increases in TNS fluorescence. Inclusion of 1-10mM CaCl₂ in the bathing medium inhibits the additional rise in TNS fluorescence due to high monovalent cation concentrations. Concentration studies indicate that the increased fluorescence is due to the cationic species. Unlike parallel experiments using liposomes, the magnitude of fluorescence is not dependent upon the position of the cation in the lyotropic series. Removal of these ions from the bathing medium causes TNS fluorescence to return to pre-stimulus levels. Removal of TNS from the bathing medium results in almost complete return of fluorescence to pre-TNS levels, suggesting that little if any TNS is internalized. If 10mM KCN is included in the bathing medium with TNS, the fluorescence increase due to NaCl is observed but the decrease in TNS fluorescence following NaCl removal is not observed.

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CLINICAL EVALUATION OF OLFACTION. William S. Cain, Janneane F. Gent, & Karen Friend. John B. Pierce Foundation Laboratory and Yale University, 290 Congress Ave., New Haven, CT 06519.

Approximately 80% of the patients seen in the first six months of operation of the Connecticut Chemosensory Clinical Research Center complained of olfactory problems, primarily anosmia. Each patient who came to the clinic was given two tests of olfactory functioning, a test of absolute sensitivity to the odorant butyl alcohol and a test of odor identification. The patient's task for each trial of the test of absolute sensitivity was to pick which of two bottles contained odorant and which contained water (two-alternative forced-choice). An ascending sequence of butyl alcohol concentrations was presented in order to minimize the desensitizing influence of adaptation. For the test of odor identification the patient was given a list of 20 items, 10 of which were test items. On each trial, the patient was given a test substance to sniff and identify. Seven of these are primarily olfactory stimuli (baby powder, chocolate, cinnamon, coffee, mothballs, peanut butter, and soap) and three are trigeminal stimuli (ammonia, Vicks Vaporsteam, and methyl salicylate [wintergreen]). In general, the two tests gave compatible results. That is, patients with hyposmia or anosmia exhibited depressed or indeterminate absolute sensitivity and poor or absent ability to identify the olfactory stimuli. The average performance of hyposmics or anosmics on the odor identification task equalled 16%, whereas the average performance of patients with normal olfactory function equalled 87%. Most of the anosmics and hyposmics who have visited the clinic appear to suffer from obstruction in some portion of the nasal passages rather than from a disruption of neural functioning. Clinical and self-report factors important in this diagnosis included a history of polyps, allergic rhinitis, and periodic remission of symptoms.

INFLUENCE OF VARYING CONCENTRATION, TEMPERATURE AND DURATION OF STIMULI ON GUSTATORY PERSISTENCE. Amalia M. Calviño. Laboratorio de Investigaciones Sensoriales, CONICET, Facultad de Medicina, Universidad de Buenos Aires, C.C. 53, 1453 Buenos Aires, República Argentina.

The gustatory persistence magnitude could possibly be related to a number of different variables: substance, concentration, temperature and duration, either singly or in a variety of different sets. The purpose of this work was to evaluate the influence of both combinations: concentration-temperature and concentration-duration on gustatory persistence.

Sucrose, urea or caffeine, NaCl and citric acid were presented at three levels of concentration (these levels were in 2:1 ratios), three levels of temperature (10°, 37° and 50°C) and two levels of duration (1 and 3 seconds). In each experiment total persistence time was recorded when subjects judged that no taste was perceived.

The relative persistence of four qualities support the notion that there is a correlation between persistence and reaction time. Thus, sour and salty compounds, that show reaction times lower than sweet and bitter substances, also show the lowest values of total persistence time.

Previously, the influence of temperature on gustatory persistence has been reported and quantitative persistence-concentration relationships have been derived at each temperature. Thus, citric acid showed an increase of persistence magnitude when temperature raised, NaCl showed the opposite effect of temperature and sweet and bitter compounds showed a maximal persistence at the highest temperature (50°C). However these results were obtained when duration of taste stimulation was 3 seconds. In order to assess the effect of duration on persistence-temperature relationship other experiments using a brief stimulus duration (1 second) have been completed. Citric acid and NaCl continued to show temperature-dependent shifts in gustatory persistence. This means that there is no influence of duration on gustatory persistence magnitude of these qualities. In other hand, the results of sucrose and caffeine show that there is no difference in gustatory persistence magnitude when changes in temperature were made. It can be concluded that this independence is due to the varying duration of taste stimulation.

These effects were confirmed when persistence-concentration relationships were obtained varying the time of taste stimulation. Citric acid as well as NaCl showed no difference in the gustatory persistence magnitude evaluated at 1 or 3 seconds. However sucrose and caffeine showed a significant increase in gustatory persistence magnitude when taste stimuli stay 3 seconds in oral cavity.

DEGENERATION OF THE DISTAL STUMP OF THE OLFACTORY NERVE SEVERED FROM THE CELL BODIES

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After being crushed at 1.5 from the cell bodies, the garfish olfactory nerve degenerates from the crush site toward the synaptic area. The proximodistal degeneration was followed by monitoring the decrease in the weight of consecutive 3 mm nerve segments and by examining electron micrographs of segments taken at various distances from the site of injury. The proximodistal rate of degeneration increases linearly with temperature and ranges from 1.6 mm/d at 14°C to 11.4 mm/d at 31°C. Similar results were obtained when further regeneration was prevented by removing the mucosa containing the olfactory cell bodies.

Since the rates of degeneration are identical to the velocity of the slow phase of axonal transport in regenerating fibers, it can be hypothesized that slow flow is still moving in axons detached from their perikarya. However, since new material is no longer provided by the cell bodies, the result is a depletion of the axonal components inducing a direct or indirect degeneration of the axons. This raises several questions: For how long is slow flow maintained in fibers severed from their cell bodies? What is the velocity of slow flow in the peripheral stump? These problems are presently being investigated and their answer should provide a more complete understanding of the mechanisms underlying axonal degeneration. (This study was supported by NIH grant 17198)

INVESTIGATION OF AND PREFERENCE FOR CONSPECIFIC ODORS BY GERBILS TREATED WITH PSYCHOTROPIC DRUGS. MaryLou Cheal, Neuropsychology Laboratory, McLean Hospital and Harvard Medical School, Belmont, Massachusetts 02178.

Gerbils will reliably investigate odors of conspecifics. If bedding, soiled by gerbils, is hidden under one of five holes in the floor, gerbils show initial preference for the odor hole and then rapidly habituate. This behavior has proven to be very robust in gerbils from four weeks to four years of age. Male and female gerbils respond similarly and a variety of surgical insults do not change preference provided olfaction is spared. Odor preference is also unaffected by treatment with many psychotropic drugs. Gerbils were treated with a range of doses of a variety of drugs that alter neurotransmission: dopamine agonists, amphetamine (single injection of 0, 2, or 3 mg/kg; 15 daily injections of 0, .5, 1, 2, or 3 mg/kg), L-DOPA (0, 3, 10, 30, or 100 mg/kg), piribedil (0, 10, 30, 100, or 300 mg/kg), apomorphine (0, .1, .3, 1, 2, or 3 mg/kg); dopamine antagonist, pimozide (0 or 1 mg/kg); norepinephrine agonists, clonidine (0, .01, .03, .1, or .3 mg/kg), desmethylimipramine (0, 10, 30, or 100 mg/kg); acetylcholine agonist, physostigmine (0, .01, .03, or .1 mg/kg); acetylcholine antagonist, scopolamine (0, .01, .1, 1, or 10 mg/kg); serotonin antagonist, parachlorophenylalanine (0 or 300 mg/kg). There was virtually no responding to any of the holes following the largest doses of amphetamine (single injection of 3 mg/kg), L-DOPA (100 mg/kg), or apomorphine (1, 2, or 3 mg/kg), thus no preference could be shown. However, at all other doses, gerbils spent more time at the hole with odor than at any of the holes without odor following treatment with all drugs except apomorphine. After injections of .1 or .3 mg/kg apomorphine, reliable preference could not be demonstrated even though the gerbils investigated the holes. Some gerbils showed preference and some did not. It appears that motivational and/or attentional factors are disrupted by apomorphine that may interfere with selective investigation of a normally preferred odor.

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HISTAMINE RESPONSE BY THE PROTOZOAN *TETRAHYMENA*

THERMOPHILA

Theresa Cheng and M. Levandowsky, Haskins Laboratories of Pace University, New York, NY

A behavioral assay for chemosensory response by *Tetrahymena thermophila*, using flat capillaries, is described. Cells aggregate in response to histamine and histidine in the range 1 μ M-10 mM. Among analogs, the H_2 -blocker cimetidine causes aggregation and the H_1 -blockers triphenylamine and diphenylamine cause dispersal. The effects of several histamine methyl transferase (HMT) blocking agents are discussed. Kinetics of the behavioral response are described.

Salivary Sodium Levels and Salt Perception Carol Christensen, Mary Bertino, Gary Beauchamp & Mahvash Navazesh, Monell Chemical Senses Center, Philadelphia, PA 19104.

Support for the relationship between salivary composition and taste perception comes chiefly from several studies relating salivary Na concentration and salt perception. For example, Morino and Langford (1978) compared salivary Na levels and salt recognition thresholds and found a sizeable positive correlation (0.64). Gustatory adaptation is the mechanism hypothesized to account for these effects.

The relationship between saliva and salt perception was reexamined using a comprehensive set of salivary and taste measures. Forty-three untrained subjects, 17 to 25 years of age, have been tested. Whole mouth resting and stimulated whole mouth and parotid salivary flow rates and Na levels were measured. Detection thresholds for NaCl were obtained using a staircase method. Subjects used category scales to judge the intensity and pleasantness of the saltiness of strained low-sodium vegetable soup containing six added concentrations of NaCl. Deionized water rinses occurred between each threshold and suprathreshold trial. A sip and spit method was used and all trials were self paced.

There was wide individual variation in both salivary flow rates and Na levels. Resting whole mouth salivary flow rates varied more than 10-fold among subjects and salivary Na concentrations varied more than 3-fold. Stimulated flow rate ranges were not as large (5-fold). Salivary Na levels increased when salivary flow was stimulated. In this study, stimulation produced an average 3-fold increase in whole mouth salivary Na levels. A significant positive correlation (0.61) was found between parotid flow rates and sodium levels among subjects; a similar but non-significant trend was evident with whole mouth measures.

There was a significant negative correlation (-0.33) between stimulated whole mouth sodium concentrations and NaCl detection thresholds. Threshold values were not significantly correlated with other salivary sodium measures or with flow rate and suprathreshold measures were not significantly correlated with any salivary measures. The negative correlation between Na levels and taste indicates that there was a tendency for individuals with higher salivary Na levels to have lower NaCl thresholds; a finding which contrasts with earlier reports. It is difficult to explain these results based on gustatory adaptation. Salivary Na levels are probably continually variable both because of varying states of oral stimulation and variable residues of taste and rinse solutions. A significant correlation only between stimulated sodium levels and taste is not surprising because the oral movements associated with the sip and spit procedure would likely stimulate salivary flow. (Study partially funded by University of Pennsylvania Dental GCRC, NIH 1-MO1-RR01224, and U.S.D.A. 59-3204-03.)

RADIOIMMUNOASSAY OF OLFACTORY MARKER PROTEIN IN ORGAN CULTURES OF RAT OLFACTORY TISSUES. Meng-Inn Chuah, Albert I. Farbman, Northwestern University, Evanston, Illinois.

A marker protein found exclusively in olfactory receptor neurons has been identified, isolated and a specific antibody raised against it. This molecule called olfactory marker protein (OMP) is a soluble, acidic protein found throughout the entire cytoplasm of mature olfactory receptor cells including the axon terminals in the bulb. In a previous study we used an immunohistochemical technique to show that OMP is present in organ cultures of embryonic olfactory mucosa grown either alone or in combination with presumptive olfactory bulb. Because the latter group of cultures appeared to contain more OMP positive cells, we initiated the present study to make quantitative comparisons of the amounts of OMP in the 2 groups of cultures. Specimens were taken from rat embryos on day E15 (day E1 is when the dam is sperm positive) and explanted onto collagen coated Millipore filter rafts supported by stainless steel grids in organ culture dishes. Cultures were grown in serum-free Waymouth's MB752/1 medium supplemented with 0.30mg/ml L-ascorbic acid and 0.10mg/ml Gentamicin at 35°C in an atmosphere of 5% CO₂ in air. After 7 days, cultures were harvested, pooled and homogenized in 100mM Tris; the homogenates were centrifuged and radioimmunoassays* were performed on the supernatants. The results showed that cultures of olfactory mucosa grown in combination with bulb contained nearly twice as much OMP as cultures of olfactory mucosa grown alone. These results suggest that olfactory bulb enhances the synthesis of OMP by receptor cells in culture. The significance of these data is that they are the first to show that the developing primary olfactory pathway may behave like other developing neural systems in the sense that the postsynaptic targets may influence the maturation of the presynaptic receptor neurons.

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PERCEPTION OF NASAL PUNGENCY IN SMOKERS AND NONSMOKERS. J. E. Cometto-Muniz* & William S. Cain, John B. Pierce Foundation Laboratory & Yale Univ., 290 Congress Ave., New Haven, CT 06519.

Two experiments explored nasal pungency evoked in smokers and nonsmokers. The first experiment took advantage of the finding that high concentrations of the pungent stimulus carbon dioxide inhaled through the nose will trigger a reflex, momentary interruption of inhalation with short latency (700-800 msec). Our findings confirmed our earlier results that smokers ($n=21$) yield a higher threshold for the reflex than do nonsmokers ($n=20$). The findings also indicated an acute as well as a chronic effect of smoking: the threshold for smokers rose even higher after smoking one or two cigarettes in a short interval, whereas the threshold did not change for nonsmokers or for smokers who did not smoke during the interval.

The second experiment inquired whether the relative magnitudes of odor and pungency varied from smoker to nonsmoker. This experiment derived psychophysical functions for the pungency of carbon dioxide and the odor of iso amyl butyrate using the method of magnitude estimation. Participants (17 smokers and 16 nonsmokers) judged a scrambled series of the odorant and the irritant at various concentrations. The comparative judgments of odor intensity and irritation in the two groups of participants implied that smokers perceive pungency less keenly than nonsmokers. This impairment, which was uniform across concentrations of carbon dioxide, fell into register with the difference between smokers and nonsmokers measured in the study of the reflex. The data support the conclusion that the reflex can actually offer an objective index of functional status of the common chemical sense.

*Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas, República Argentina, on leave from the Laboratorio de Investigaciones Sensoriales (CONICET-Fac. de Medicina, UBA), Buenos Aires, Argentina.

SPECIES DIFFERENCES IN TESTOSTERONE EFFECTS ON SOCIAL ODOR INVESTIGATION. Catherine A. Cornwell-Jones & Kathleen R. Zahs, Department of Psychology & Department of Biology, Princeton University, Princeton, NJ 08544.

The effects of castration and testosterone (T) replacement on investigation of conspecific odors were compared in adult male Sprague-Dawley rats and Mongolian gerbils. Subjects were housed in groups with females from weaning until surgery on postnatal Day 80. Eight-9 males were in each surgical group for each species. Sham operates received bilateral subcutaneous implants of empty silastic tubing (i.d. = 1.57mm, o.d. = 3.18mm). All other subjects were castrated and were either implanted with empty tubing or received T treatment. T-treated rats were implanted with T-filled capsules either 15 or 30mm long. T-treated gerbils were implanted with T-filled capsules 2 or 10mm long, or received 3 weekly injections of 100µg of testosterone propionate (TP).

Twenty-seven to 30 days after surgery, subjects were given four 5 min tests in an emergence apparatus consisting of a start cage connected by a tunnel to a stimulus cage which was separated from a stimulus tunnel by a wire screen. The stimulus cage contained a wire screen resting on shavings taken from the nest of a lactating gerbil or rat. The stimulus tunnel was either empty or contained an anesthetized female conspecific. A 'T' effect was inferred if emergence behavior of castrates and shams was significantly different and if high T doses restored the behavior of hormone-treated castrates to sham levels.

Treatment effects were greatest with a female in the tunnel and the other species' bedding in the stimulus cage. Under this condition, T increased the duration of the longest single entry into the stimulus cage and the total time in the stimulus cage for rats, but decreased these measures for gerbils. Gerbils in the high T groups also had higher latency scores than low-T groups. When conspecific shavings were in the stimulus cage and the stimulus tunnel was empty, T increased entrance latency for gerbils, and also increased the longest entry duration and total time in the stimulus cage for rats. In summary, T increased investigation of social odors for rats, but decreased such investigation by gerbils.

The rat data are consistent with previous evidence that T increases social investigation in this species. In contrast to colonial rats, gerbils may form bonded pairs in the wild which attack strange intruders. Our data suggest that high T levels reduce the probability that male gerbils will risk approaching females in novel olfactory territory.

CHANGES IN OLFACTORY EPITHELIUM FOLLOWING BULBECTOMY IN HAMSTER. Richard M. Costanzo and Pasquale P.C. Graziadei, Dept. Physiol. Medical College of VA, Richmond, VA. 23298 and Dept. Biol. Sci. Florida State Univ., Tallahassee, FL. 32306

Previous descriptive studies have reported replacement of cell populations in the olfactory epithelium following bulbectomy. In this more quantitative study, we examined a large number ($n=63$) of histological preparations to provide analysis of the time course and extent of change in the epithelial cell populations. Following unilateral bulbectomy, we examined a minimum of 3-4 animals at postoperative days 0,1,2,3,4,5,7,10,15,25,35,60, and 120. Measurements of cell number, epithelial thickness, and cell density (experimental side/control side) showed an immediate degeneration of cells in the olfactory epithelium, decreasing to 39% of control side by day 4. During days 4 through 15 there was an increased growth period resulting in a new population of cells which was maintained at a level 60-70% of control through days 15-120. Epithelial thickness decreased to 60-70% during the degeneration period, but there was no further recovery during subsequent days 4-120. Although cell density decreased to 54% by day 4, it recovered to control levels during the growth period and remained high through May 120.

An analysis of the epithelial cell population by cell type (supporting, receptor, basal cells) shows that most of the change is in the larger receptor cell population, whereas in the smaller supporting and basal cell populations, there appears to be no change. These results are consistent with observations made in a separate set of experiments ($n=33$), in which we examined the olfactory epithelium (days 0-94) using scanning electron microscopy.

Recovery of cell density to control levels suggests that density may be an important factor regulated by the olfactory epithelium. Although the number of replacement receptor cells does not reach control levels, the replacement process occurs in a thinner supporting epithelium, which perhaps limits the total number of receptors. This suggests that one possible regulatory mechanism underlying the regenerative process of the olfactory epithelium is to maintain some preferred level of receptor density.

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DISTANCE CHEMORECEPTION IN ADULT *STYLOCHUS ELLIPTICUS*.

Daniel, Peter, Daniel Rittschof and Timothy Cole. Horn Point Environmental Laboratories, Univ. of Md. Cambridge, MD. 21613 and College of Marine Studies, Univ. of Del., Lewes, DE. 19958.

Marine predators capable of specializing on certain prey often develop search images characterized by distance chemoreception. *Stylochus ellipticus*, a turbellarian polyclad, is known to specialize on oyster spat (*Crassostrea virginica*) or barnacles (*Balanus eburneus*). *Stylochus mediterraneus* specializes on mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*) and has been found to be attracted by the effluent from its prey. Therefore we postulate that *Stylochus ellipticus* may be attracted to its prey by distance chemoreception.

In order to determine the presence or absence of this behavior a variety of instruments currently used for chemoreception studies were employed. A modified design of the most sophisticated of these instruments, which was developed for studying *Urosalpinx cinerea*, was used first. No significant response to prey effluents could be detected. Under similar conditions *U. cinerea* responded dramatically. Second, we duplicated without success the Y-maze and methods used to determine chemoreception in *S. mediterraneus*. Finally, we introduced the adult barnacle-conditioned *S. ellipticus* to a long term prey choice system and obtained strong responses to barnacles but none to oysters. The length of the experiment may explain why the other time-limited designs did not work.

These results suggest that adult *Stylochus ellipticus* are not good subjects for laboratory investigations of distance chemoreception. It is further suggested that distance chemoreception may play a greater part in the earlier life history of the animal, such as larval settlement and/or early adult development. This is supported by contrasting the distribution of prey of *S. ellipticus* and that of other predators exhibiting strong chemoreception. Further studies should concentrate on the earlier life stages.

TRANSSYNAPTIC CONTROL OF SUBSTANCE P AND DOPAMINE EXPRESSION IN THE MAIN OLFACTORY BULB OF THE HAMSTER. B.J. Davis, F. Macrides, R. Kream, T. Kawano and F.L. Margolis. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Roche Institute of Molecular Biology, Nutley, NJ 07110.

Expression of dopamine and tyrosine hydroxylase by intrinsic neurons of the olfactory bulb has been demonstrated in mice and rats to be transsynaptically regulated by carnosine-containing peripheral receptor neurons. We now report that expression of substance P by olfactory bulb neurons is under similar control in the hamster. Substance P-like immunoreactivity (SPLI) is present in external tufted cells and centrifugal afferents of the main olfactory bulb (MOB). Tyrosine hydroxylase-like immunoreactivity (THLI) is present in a heterogeneous population of external tufted, middle tufted, and periglomerular cells that is thought to synthesize dopamine, and in norepinephrine-containing centrifugal afferents. Peripheral deafferentation of the MOB by intranasal irrigation with a zinc sulfate solution results in almost total loss of neuronal SPLI in immunocytochemically-processed MOB tissue. SPLI persists in the centrifugal afferents. This tissue also shows substantial reductions of neuronal THLI. Biochemical analyses in peripherally deafferented hamsters show reductions in the MOB content of carnosine and dopamine, but not norepinephrine. These findings extend our prior evidence for transsynaptic regulation of dopamine expression to the hamster, and to include expression of substance P by intrinsic MOB neurons. Afferent neuron regulation of target neuron gene expression in the olfactory bulb thus appears to be a phenomenon of broad influence. It may play a role in processing chemosensory information as well as offering a system in which to study neuronal plasticity.

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HORMONE-INDUCED CHANGES IN PERIPHERAL RECEPTOR SENSITIVITY. Edward E. Davis, SRI International, Menlo Park, CA 94025

It is generally accepted that the role of the peripheral sensory system is to provide the CNS with a flow of information that varies only as the stimulus activating the sensory system varies. It has also been demonstrated that hormones may act on CNS processes to modulate or modify the behavior of an organism. No cases have been reported to date in which a hormone modulates the behavior of an animal by acting on a peripheral receptor system.

Female mosquitoes that have taken a blood meal will not engage in further host-seeking behavior. One component of the inhibition of host-seeking behavior accompanies oocyte development, appearing 24 to 30 h postblood meal (PBM) and reaching maximal effect between 36 and 72 h PBM. Normal host-seeking activity is restored within 24 h following oviposition. It has been shown that a hemolymph-borne factor(s) is responsible for these behavioral changes. We have examined the electrophysiological activity of one peripheral chemosensory neuron to lactic acid (LA), a normal host-attractive substance, before and after a blood meal and after oviposition. We found that the sensitivity of the LA-excited neuron to LA was depressed beginning about 24 h PBM--coincident with the inhibition of host-seeking behavior. Following oviposition, the sensitivity of this type neuron recovered towards pre-blood meal levels just as the behavior does. Furthermore, by transfusing hemolymph from blood-fed female mosquitoes into non-blood fed females, we found the sensitivity of the LA-excited neurons to LA was depressed just as it was in blood-fed mosquitoes 24 to 30 h PBM. We conclude that the sensitivity of the peripheral LA-excited neuron is modulated by a hemolymph-borne factor associated with oocyte development and suggest that the decrease in receptor sensitivity may mediate the inhibition of host-seeking behavior of female mosquitoes following a blood meal.

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Pleasantness of Odors: Some Psychometric Observations with Odorants Presented by Microencapsulation

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Odor quality can be characterized by a number of relatively discrete attributes. Of these attributes, the pleasantness of odors has the dominant influence on judgments of similarity among odors. Therefore, an efficient index of odor perception may be based on judgments of pleasantness. The use of pleasantness judgments to screen persons clinically will require a reliable instrument that distinguishes among frank perceptual aberrations, measurement artifacts, and idiosyncratic individual differences in odor perception.

Data have been obtained for odor lists of 20 odorants with over 1000 persons. Reliability of single subject data has been determined within single test sessions, over successive test sessions, and over repetitions of judgments. Reliability >.7 for individual subject's can be achieved, which is sufficient to support clinical inferences based on single test results.

Pleasantness perception has been evaluated globally, 20 odors spanning the perceptual space, and locally, 7 odors located in various restricted regions, i.e., all very unpleasant. The fine grain features in the small list scales resembled the structure of the long form, which supports the conclusion that odor pleasantness perception measured by the "paper & pencil" tests used here is stable enough to exploit clinically.

EVIDENCE FOR AN AMILORIDE-SENSITIVE ION CHANNEL IN CANINE LINGUAL EPITHELIUM. John A. DeSimone, Gerard L. Heck and Shirley K. DeSimone. Department of Physiology, Medical College of Virginia, Richmond, Va. 23298.

We have demonstrated the existence of an active transport system for ions in the dorsal lingual epithelium of the dog (Science 214, 1039, 1981), with properties that suggest a possible role in taste transduction. When the epithelium is placed between lucite chambers, with mucosal and serosal surfaces bathed in oxygenated Krebs-Henseleit buffer, a transepithelial potential develops ($E_{V_{sc}}$). This potential can be shorted out by passing an external current. The short-circuit current (I_{sc}) equals the magnitude of the active ion current. Average values for $n = 16$ are: $E_{V_{sc}} = 17.2$ mV (serosa positive), $I_{sc} = 31.4 \mu A/cm^2$, and $R = 576 \text{ ohm-cm}^2$. When the mucosal compartment contains NaCl at concentrations ranging from 1 mM-1M, the electrical properties reflect the salt concentration. Typically, salt concentrations of about 30 mM reduce I_{sc} to zero, and a large, ouabain-sensitive, increase in current is seen at NaCl concentration above 0.15, saturating between 0.5 and 1 M. This range of response is not seen in ventral lingual epithelia. The response to salts can be significantly reduced by treating the mucosa with 10^{-4} M amiloride. This is good evidence that the response to salts involves a sodium current through an amiloride-sensitive channel. In addition to salts, the preparation responds to sugars and acids. However, the response pattern differs from that of salts in that sugars cause increased current and potential at relative constant resistance and acids cause very high potentials at high resistance. Thus various classes of tastants produce characteristic response "signatures". In the dog, at salt concentrations above 0.2 M, the response order is $Na^+ > K^+ > NH_4^+ > Li^+$. Monosodium glutamate usually gives higher potentials and resistances than corresponding NaCl concentrations. These results suggest that an amiloride-sensitive ion channel may be involved in transduction.

CONVERGING TASTE RECEPTOR INPUTS TO THE HAMSTER SOLITARY NUCLEUS. J. David Dickman, Robert D. Sweazey and David V. Smith. Dept. of Psychology, University of Wyoming, Laramie, WY 82071.

Taste receptors are distributed over various parts of the tongue and intraoral cavity. Only about 15% of the rat's taste buds are located in fungiform papillae on the anterior portion of the tongue (Miller, 1977). Most electrophysiological studies of taste however, have restricted stimulation to the anterior tongue. Processing of taste information through the hamster brainstem is understood only with respect to this anterior receptor area. In the rat, responses can be elicited in the nucleus tractus solitarius (NTS) by both anterior tongue and posterior oral stimulation, with considerable overlap of multi-unit responsiveness to both fields (Halpern and Nelson, 1965). In the rat pons, many of the same neurons can be driven by both anterior and posterior stimulation (Norgren and Pfaffmann, 1975).

The present investigation examines the overlap in sensitivities to anterior tongue and posterior oral gustatory stimulation in the hamster NTS. Stimuli (0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl and 0.001 M QHCl) were delivered to the anterior area through a glass chamber and to the posterior area through an inserted tube both at a rate of 3.6 ml/sec.

Multi-unit responses could be recorded from the anterior lateral NTS over a span of about 400-500 μ m in the rostral-caudal and about 200-250 μ m in the medial-lateral dimension. Although there was some tendency for the most rostral penetrations to respond primarily to stimulation of the anterior portion of the tongue, the majority of sites responded to both anterior tongue and posterior oral stimulation. This overlap suggests, but does not demonstrate, the convergence of single peripheral fibers onto individual medullary neurons. However, of the several neurons isolated thus far, most were driven by both anterior and posterior stimulation. Often the pattern of responsiveness of a neuron was different to stimulation of these two receptor fields. For example, one neuron responded well to sucrose delivered to either the anterior or posterior fields, but to NaCl following only posterior stimulation. Another responded exclusively to HCl on the anterior tongue and to quinine delivered to the posterior oral cavity. Additional cells possessed similar sensitivities to both anterior and posterior stimulation.

Since the response profile of an NTS neuron may differ, depending upon the receptors stimulated, characterization of the sensitivities of these cells may be more complex than when considering anterior tongue input alone. Complete understanding of taste information processing by brainstem neurons depends upon input from divergent receptor areas.

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TASTE CODING OF ETHYL ALCOHOL: ELECTROPHYSIOLOGICAL AND BEHAVIORAL DATA. Patricia M. Di Lorenzo, Stephen W. Kiefer, Anne G. Rice and John Garcia. Department of Psychology, University of California at Los Angeles, Los Angeles, California, 90024.

Single-unit responses to gustatory stimuli were recorded in the nucleus of the tractus solitarius (NTS) of the anesthetized rat. Solutions of NaCl, HCl, sucrose, QHCl, 6% and 9% ethyl alcohol (ETOH) were bathed over the rostral tongue through a flow chamber. Results revealed that over half of the taste-related units in the NTS responded to 9% ETOH. Where present, a response to ETOH consisted of a brief initial burst of activity with no sustained elevation of the rate of firing. The presence of a response to ETOH in a given unit was not related to the profile of responses to the 4 "basic" tastants.

In a second experiment, a conditioned taste aversion (CTA) was produced to 3%, 6% and 9% ETOH in different groups of rats. Subjects were subsequently presented with each of the 4 basic tastants on separate days. All tests were conducted in the home cage with a one-bottle test. No generalization of the CTA to any of these test stimuli was found. However, rats that were conditioned to avoid 6% and 9% ETOH did appear to generalize the aversion to a mixture of sucrose and QHCl but not to a mixture of NaCl and HCl.

INGESTION OF GLUCOSE, 3-O-METHYL GLUCOSE AND 2-DEOXY-D-GLUCOSE IN THE RAT. Richard J. DiRocco, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

The phenomenon of dietary obesity demonstrates that taste can be a major determinant of an organism's energy balance. Lean rats will overeat and become obese if they are provided with a variety of highly palatable foods in addition to their standard lab chow. One of the basic rules organisms follow in using taste as a predictor of food utility is that sweetness implies energy. Using glucose, 3-O-methyl glucose (3OMG) and 2-deoxy-D-glucose (2DG), we have attempted to violate this rule in order to examine the interaction between taste and metabolism. Glucose, 3OMG and 2DG compete equally for glucose transport across membranes of erythrocytes and blood-brain barrier (BBB), providing some basis for the notion that they taste equally sweet. However, unlike glucose which can be metabolized to yield energy in the form of ATP, 3OMG is not metabolized and 2DG inhibits the metabolism of glucose.

Rats were trained to drink 0.8 M solutions of sucrose around noon each day. After 7 days, they were divided into 3 groups which received 0.8 M solutions of glucose, 2DG or 3OMG in finely calibrated burettes. Cumulative intake was measured every minute for the first 5 minutes, again at 10 and 60 minutes and finally at 24 hours. No other food or water was available during the entire interval. At ten minutes, rats had consumed 6.7 ml of glucose, 1.4 ml of 3OMG and 0.5 ml of 2DG. Twenty-four hour consumption levels were 155 ml (glucose); 57 ml (3OMG) and 1.3 ml (2DG).

These data indicate that these three sugars taste different. Humans tasting 0.8 M solutions of 2DG report a bitter to bitter-sweet quality whereas tasters of 3OMG report a more complete sweetness. These modifications of the glucose molecule produce major differences in its sweetness. The results presented here suggest that the taste system is more like the glucose transport system of intestinal epithelium, which actively transports glucose and 3OMG but not 2DG, than the glucose transport system of erythrocytes and BBB.

Further studies will examine the effects of pairing a sweet taste with a metabolic suppression by infusing glucose orally and 2DG directly into the blood through intracardial catheters.

ODOR PERCEPTION IN CHILDREN IN RELATION TO NASAL OBSTRUCTION Richard L. Doty, S. Nasrin Ghorbanian & Jack L. Paradise. Clinical Smell and Taste Research Center, University of Pennsylvania; Ambulatory Care Center, Children's Hospital of Pittsburgh; Departments of Pediatrics and Community Medicine, University of Pittsburgh, Pittsburgh, Pa.

To determine whether nasal obstruction results in impaired olfactory function in children, olfactory detection thresholds were established in 45 boys and 33 girls, 65 of whom exhibited at least some degree of nasal obstruction due to adenoid hypertrophy, allergic rhinitis, upper respiratory infection, or a combination of these conditions. Nasal obstruction ratings, determined from clinical estimates of mouth breathing and hyponasality, were clearly related to olfactory detection thresholds for phenyl ethyl alcohol established by a single staircase procedure. Twenty-one of 28 subjects with nasal obstruction attributed to enlarged adenoids exhibited both decreased olfactory thresholds and nasal obstruction ratings following adenoidectomy. Olfactory thresholds tested across equivalent time periods in control subjects who received no intervening operation evidenced no systematic change in either of these measures. These data demonstrate, for the first time, that even minor nasal obstruction in children results in a decreased ability to smell. In addition, this study is the first to demonstrate that adenoidectomy can result, in some children, in an increase in olfactory sensitivity.

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COMMUNICATION OF GENDER FROM HUMAN BREATH ODORS: RELATIONSHIP TO PERCEIVED INTENSITY AND PLEASANTNESS. Richard L. Doty, Paul A. Green, Carol Ram & Samuel L. Yankell. Clinical Smell and Taste Research Center, University of Pennsylvania School of Medicine; Department of Oral Medicine and Clinical Research Center, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pa.

During five consecutive daily test sessions, 10 men and women rated the relative intensity and pleasantness of breath odors from 14 males and 19 females on a no-oral-hygiene regimen. In addition, the likely gender of the donor of each odor was also estimated. The breath odors of males were rated, on the average, as more intense and less pleasant than the breath odors of females. Women consistently gave lower pleasantness ratings to the odors than did men. Both the male and female judges assigned the breath odors to the correct gender classes at a frequency unlikely due to chance, although the females were more accurate in this regard. An inverse relation between breath odor intensity and pleasantness was noted. Systematic changes in the rated intensity and pleasantness of the odors were present across the five days of the study period. These data suggest that differences exist between the breath odors of men and women, and that humans, like many other mammals, may be capable of assessing gender from oral odors. However, such assignments conceivably reflect the strategy of assigning stronger and less pleasant odors to the male category and weaker and less unpleasant odors to the female category, regardless of the odor donor.

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OLFACTORY TESTING IN THE CLINIC: FINAL DEVELOPMENT OF THE UNIVERSITY OF PENNSYLVANIA SMELL IDENTIFICATION TEST (UPSIT). Richard L. Doty, Paul Shaman & Michael Dann, Clinical Smell and Taste Research Center, University of Pennsylvania School of Medicine; Department of Statistics, University of Pennsylvania.

As a first step in our Center's development of a clinically-useful battery of standardized tests for evaluating olfactory function, we have developed the University of Pennsylvania Smell Identification Test (UPSIT). This 40-item test incorporates microencapsulated odorants for the evaluation of the general ability of patients to detect, recognize and identify a wide range of both single- and multiple-component odorants. In this presentation, the rationale for the development of this test will be discussed, along with data from a series of experiments used in its refinement and validation. Included will be results from a number of clinical trials on patients with syndromes associated with smell dysfunction, including Kallmann's syndrome, Korsakoff's syndrome, and Parkinson's disease. A special discussion of olfactory problems in the elderly will be presented.

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EMPIRICAL EVALUATION OF HEAD-SPACE CONCENTRATIONS OF SNIFF BOTTLES USING LASER-INDUCED FLUORIMETRY AND MULTIPHOTON IONIZATION PROCEDURES. Mark M. Dostader, Richard L. Doty, and Michael Topp. Clinical Smell and Taste Research Center, University of Pennsylvania School of Medicine; Department of Chemistry, University of Pennsylvania

For practical reasons, liquid-dilution sniff bottles are more useful than air-dilution olfactometers in determining both threshold and suprathreshold measures of olfactory function. This is particularly true in clinical settings, where continued monitoring of olfactometers is not practical. Sniff bottles are easy to prepare, clean, and store, and allow for the testing of several odorants within the same general time period. Unfortunately, complex interactions between odorants and diluents do not allow for direct and accurate assessments of head-space concentrations in such bottles, and little is known as to how long liquid concentration series can be used before their concentrations depart significantly from their initial values. In the present work, an empirical assessment was made of head space concentrations of several liquid dilution series (e.g., phenyl ethyl alcohol in propylene glycol) using both laser-induced fluorimetry and multiphoton ionization procedures. Non-linearity from theoretical calculations based upon ideal gas laws was present across wide ranges of the concentrations at several temperatures. The present results allow for the calculation of linear concentration series for use in standardized smell test paradigms.

Supported by a grant from the National Institute of Neurological and Communicative Disorders and Stroke (NS 16365).

CHEMOSENSORY INHIBITORS PRODUCED BY BLUE MUSSELS, A. B. Ducharme and D. Rittschof, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Newly hatched oyster drills, *Urosalpinx cinerea* (Say), exhibit a positive response to chemoattractants produced by barnacles (*Balanus* sp.). This response can be significantly reduced or eliminated when inhibitory substances produced by a second prey species, the blue mussel (*Mytilus edulis* Linne), are present. Here we report preliminary findings as to the molecular size of these inhibitors and attempts to desalt and concentrate the substances from seawater. Solutions containing the inhibitory material were prepared by incubating *M. edulis* (fresh weight of 200-400 g) in 4.5 L of seawater for 4 or 20 hours. After filtration to remove particulates, inhibitor strength was determined by measuring the response of newly hatched oyster drills to a standard chemoattractant preparation in the presence and absence of the inhibitors. There appears to be a minor and major component to the inhibitory activity. The minor component is not produced in detectable quantities after 4 hours, but is detectable after 20 hour incubation. This inhibitor is adsorbed by Amberlite XAD-7 resin, but not XAD-4 or XAD-2. Inhibitory activity has not been recovered from the XAD-7 resin by elution with distilled water or methanol. The major component of the inhibitors is present after both 4 and 20 hour incubations. Pressure dialysis indicates a molecular weight of less than 5000 Daltons. Unlike barnacle stimulus and the minor inhibitory component, this component is not adsorbed by Amberlite XAD-7 resin. Other non-ionic resins tested also did not adsorb the activity. At the present time we are determining the characteristics of the inhibitory response and examining alternatives for desalting and concentrating the inhibitors. This research was supported by Sea Grant.

EOG VARIATIONS DURING $ZnSO_4$ INDUCED DEGENERATION AND SUBSEQUENT REGENERATION OF OLFACTORY RECEPTORS IN THE CHANNEL CATFISH. Jay R. Erickson and John Caprio. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The destructive effects of $ZnSO_4$ solutions on the olfactory epithelia of the channel catfish (*Ictalurus punctatus*) were shown to be dependent upon concentration and length of exposure (Cancalon, AChemS abstract 1981). Selective degeneration of olfactory receptor cells and destruction of sustentacular cell microvilli could be attained by brief epithelial irrigation with $ZnSO_4$ solutions of low to moderate concentrations. In this ongoing study, the underwater electro-olfactogram (EOG) is being used to monitor olfactory receptor activity during the $ZnSO_4$ induced degeneration and subsequent regeneration of channel catfish olfactory receptor cells. The epithelial surface changes concurrent with this process are being observed using scanning electron microscopy (SEM).

The treatment consists of briefly flushing one of the paired olfactory mucosae with 6 ml of 7% $ZnSO_4$; the untreated mucosa serves as a control. Following irrigation the fish are returned to the tank at 21°C.

EOGs are recorded daily from both treated and control mucosae for seven days following $ZnSO_4$ treatment (day 0). $10^{-4}M$ concentrations of L-alanine, L-arginine, L-glutamic acid, L-cysteine, L-glutamine, L-glutamic acid- γ -methyl ester and L-norleucine are used as test stimuli. After each recording session, control and treated mucosae are excised and prepared for SEM.

Although the magnitudes of EOG responses to a standard stimulus compound ($10^{-4}M$ L-alanine) differed greatly between fish (values ranging from 2-12mV), the recorded responses to the same compound varied little between the paired olfactory epithelia in an untreated fish. Preliminary results showed no significant magnitude differences between EOGs recorded from the treated and control mucosae for each of the compounds tested during the first three days after $ZnSO_4$ irrigation. On day four, however, a relative decline in the EOG magnitude of the treated mucosa was evident. The EOGs recorded from the treated mucosa were seen to approach control values during the fifth day. During days six and seven the magnitudes of the EOGs recorded from the treated mucosae were seen to approximate the control values. (Supported in part by NIH Grant NS 14819)

POSTNATAL CAFFEINE EXPOSURE THROUGH MATERNAL DIET: EFFECTS ON CAFFEINE PREFERENCE AND DEVELOPMENTAL MEASURES IN RATS. Fay Ferrell and Lisa Gullberg. Department of Nutrition, University of California, Davis, CA 95616

Exposure to a specific dietary flavor early in life has been shown in several species to enhance subsequent preference for that flavor. Flavor cues in the diet eaten by a lactating mammal and transmitted to the young through her milk can influence the offspring's food choices during weaning. Weanling rats preferentially ingest the diet consumed by their mother during the nursing period even if that diet is relatively unpalatable compared to others.

Caffeine, a pharmacologically active component of coffee, tea, cocoa, and cola beverages, has recently received interest as a teratogen in rats and as a risk factor for various health problems in humans. The FDA has advised pregnant women (but not lactating women) to eliminate or limit their consumption of caffeine containing products. Caffeine rapidly enters breast milk, although in low concentrations. Newborns, however, which eliminate caffeine significantly more slowly than adults, might ingest high concentrations through nursing.

This study used rats to examine effects of postnatal caffeine exposure via nursing on postweaning preference for caffeine solution. Eighteen dams were assigned to one of three groups: Control, Lo Caf, or Hi Caf. Beginning on the day of parturition, drug treated dams received either low (~ 23 mg/kg/day) or high (~ 94 mg/kg/day) "pharmacologic" doses of caffeine dissolved in distilled water as the sole source of fluid. Control dams received distilled water. Litters were reduced to six pups, and nursing was the sole fluid source prior to weaning.

At weaning, pups and dams were housed individually, and a series of 24-hour two-bottle preference tests for water vs. caffeine solution were conducted over a 21-day period for each pup and dam. On Test Day 1, Hi Caf, but not Lo Caf pups preferred caffeine to water more than did Controls ($p < 0.05$). Hi Caf pups showed a cyclic pattern of acceptance/rejection of caffeine. Initially Hi Caf and Lo Caf dams had higher preferences for caffeine over water than did Control dams, but differences were not significant. Lo Caf dams showed the cyclic trend observed in Hi Caf pups. Hi Caf pups gained less weight over the study and their auditory startle reflex appeared slightly but reliably sooner. Results suggest that caffeine ingested through mothers' milk during the nursing period can influence caffeine intake patterns for at least a time after weaning in the rat.

COMMON CHEMICAL SENSE IN THE FISH, PRIONOTUS: SPINAL SENSORY PATHWAYS AND NEUROPEPTIDES, Thomas E. Finger, Dept. Anatomy, Univ. Colorado Medical School, 4200 E. 9th Ave., Denver, CO 80262.

The common chemical sense as defined by Parker ('22) is a chemosensory modality mediated via trigeminal or spinal nerves which are not associated with specialized end organs. The greatest development of this sensory system occurs in the fin rays of certain teleosts, among which are the sea robins, *Prionotus*. These fish use the first three fin rays of the pectoral fin to locate food chemically. The fin rays are innervated only by spinal nerves, and possess isolated chemosensory cells within the epidermis (Whitaker, '71). These large spinal nerves end in accessory lobes, three paired enlargements of the dorsal horn of the spinal cord (Herrick, '07).

In order to trace the connections originating from the fin rays, horseradish peroxidase (HRP) was used as an anterograde and retrograde neuronal tracer. Transganglionic transport of HRP from the fin ray nerves reveal that each fin ray is represented in a single accessory lobe. The nerve from the most ventral fin ray ends in the caudal accessory lobe; that from the dorsal fin ray in the rostral lobe. The pectoral fin itself, which lies dorsal to the free fin rays, is represented in the spinal cord lying rostral to the accessory lobes.

Large neurons of the accessory lobes project anteriorly to the cerebellum. Most efferent cells of the accessory lobes project to a lateral funicular nucleus lying at the spinomedullary junction. The lateral funicular nucleus projects, in turn, to a thalamic target tentatively identified as n. preglomerulosus. This spino-funicular-thalamic pathway is the major ascending channel for the fin ray, common chemical, sense.

The primary sensory fibers terminating in the accessory lobes do not exhibit substance P-like immunoreactivity (SPLI) although SPLI does occur in the dorsal horn proper. Despite the absence of substance P input to the accessory lobes, a population of neurons in the lobes shows enkephalin immunoreactivity.

A CLINICAL TEST FOR OLFACTION, María Rosa García-Medina, Laboratorio de Investigaciones Sensoriales, CONICET, Facultad de Medicina Universidad de Buenos Aires, C.C. 53, 1453-Buenos Aires, República Argentina.

A test for qualitative and quantitative measurement of olfactory alterations in clinical practice is proposed here. The first part consists of an odor quality matching test and the second is a threshold test. Patients are instructed to match odors to odors. Seven different odorivectors are presented. In order to measure threshold a 13 one-third step dilution series of a 4ml V/V butanol is used with a forced choice method.

Ten patients participated. They complained of olfactory problems as a result of: head injury (2), nasal wall trauma followed by plastic surgery (3), long term allergic sinusitis (3), long term use of nasal drops (1) and industrial exposures (1).

Preliminary data show that in spite of the cause of the olfactory loss, when the complaint was only olfactory impairment patients could match correctly all the odors they were able to smell. When the complaint was parosmia in addition to olfactory loss, one or more odors that the patient was able to smell were wrongly matched. The mistakes do not seem to be attributable to an inability to smell since the patient confused strong and weak odors (i.e. musk and formaldehyde) yet the threshold test indicated hyposmia.

The case of a young female who had suffered head trauma is presented. Her complaints of hyposmia and parosmia were verified by the test.

CLINICAL EVALUATION OF TASTE: THREE CASE STUDIES. Janneane F. Gent, Linda M. Bartoshuk, Janet Hooper, & John Seibyl, John B. Pierce Foundation Lab., 290 Congress Ave., New Haven, CT 06519.

The psychophysical evaluation of taste function for patients that come to the Connecticut Chemosensory Clinical Research Center consists of 26 randomly presented concentrations of: NaCl, sucrose, citric acid, quinine HCl, 6-n-propylthiouracil, and water. In addition a 1000 Hz tone at one of 10 loudness levels (30 dB - 102 dB) is presented every few trials. Each stimulus is presented twice. Patients are instructed to give nonmodulus magnitude estimates of each of the stimuli presented. They are instructed to use the same intensity scale for both sensory modalities. If the patient's history indicates something unusual about the sensory innervation to the two halves of the tongue, a brief filter paper test is also given. In this test the patient is asked to identify the quality and rate the intensity of a small piece of solution soaked filter paper.

Each patient's taste results are evaluated in terms of the auditory intensity function produced. That is, the decibel equivalent is found for the intensity of each tastant. This cross-modality matching scheme permits us to evaluate the patient's perception of taste intensity relative to the perception of loudness.

Three case studies are presented that have been chosen to illustrate diagnoses of hypogeusia (weaker than normal sense of taste), dysgeusia (a persistent unpleasant taste, often sour or bitter), and normal taste in a patient claiming an inability to taste or smell.

Case 1. Mrs. V., age 49, came to the clinic with the complaint that she has not been able to taste or smell normally since 1963. Twenty years ago she moved into a house whose prior occupant had kept 20 cats. Shortly thereafter she suffered her first asthma attack. Since then she has had three polypectomies which have helped her breathe, but not smell or taste.

Case 2. Mrs. W., age 43, reports that her inability to smell started following a bad cold in 1981. Since then she has been diagnosed as having allergies, a sinus infection, and nasal polyps.

Case 3. Mrs. P., age 83, arrived at the clinic complaining that she was unable to smell and that "most foods have no taste except sweet, sour, [or] salty."

TASTE ADAPTATION IN THE HAMSTER CHORDA TYMPANI. Janneane F. Gent & Marion E. Frank, John B. Pierce Foundation Lab., New Haven, CT & Univ. of Connecticut Health Center, Farmington, CT.

Adaptation in taste has been described for both neural responses of the chorda tympani of rats and psychophysical responses of humans. An accurate appraisal of adaptation depends upon precise maintenance of a constant stimulus to a fixed number of taste receptors.

With a flow method in animal neural preparations, constant stimulation of a specified receptor population is achieved by enclosing a portion of the tongue in a chamber. The neural response to a constantly flowing, moderately intense NaCl solution consists of a large, rapid phasic component that is reduced by half in 0.5 s, and a smaller slower component that decays half way to a steady state in 15 s.

Human taste adaptation has not been successfully studied with flow methods. Greater control over a stimulus is attained when it is applied to the tongue with a small piece of solution-soaked filter paper. A sensation elicited by a moderately intense NaCl solution on filter paper decays exponentially, is reduced to half its initial size in less than 30 s, and approaches zero in 90 s or more. This is a very different time course of adaptation than is observed neurally.

In order to make a comparison and assess the peripheral contribution to the adaptation observed psychophysically, the filter paper method is used in the present experiment to deliver solution of NaCl, sucrose, Na saccharin, D-phenylalanine, citric acid, and NH_4Cl to the water-rinsed hamster's tongue. The integrated response of the chorda tympani nerve is recorded every 200 ms during 120 s of stimulation. Initial results show the time course of a response to a moderately intense NaCl solution introduced on filter paper differs from a response to a solution flowing over the tongue: the phasic component is slower, decaying to half its maximal size in 2 s, and an apparent steady state is attained by 40 s. These results suggest that gustatory neural responses can decay quite differently with time but still convey the same information about stimulus quality to the central nervous system, even after reaching a steady state.

RESPONSE PROPERTIES OF HAMSTER PONTINE TASTE NEURONS TO A BROAD RANGE OF SAPIID STIMULI. J.M.Gill II, M.Conley, F.W.Maes, and R.P.Erickson. DUKE UNIV., DEPT. of PSYCH., DURHAM, NC 27706

As its taste system is quite sensitive to sweet and bitter stimuli, the hamster has become the object of study in sensory coding experiments in gustation. VanBuskirk and Smith (1981) have recorded neural responses to taste stimuli in the PTA of this species. Their measure was the number of responses occurring over a 5 second interval. As it has been reported that rats can make discriminations within 1 second, the data here were analyzed over 1 second intervals.

In an attempt to describe the anatomical relations of this area WGA-HRP was iontophoretically injected into the recording site. Retrogradely labeled cells were seen ipsilaterally in the rostral NTS, and CNA and bilaterally scattered throughout the medullary reticular formation. Anterogradely labeled terminals were seen bilaterally in Vpm-pc and ipsilaterally in the bed nucleus of the stria terminalis, and the CNA. This distribution of label suggests that the PTA of the hamster is anatomically similar to that of the rat (Norgren, 1981).

In order to determine whether the neurons or stimuli fell into groups, hierarchical cluster analysis was performed on the correlations for the first, and fifth second intervals as well as the mean of the 5 sec. response. In distinction to the results of VanBuskirk and Smith, who used factor analysis, no evidence of grouping was found. The results of factor analysis of these data will be discussed.

The correlations among the 30 stimuli related well to the behavioral data (Nowlis and Frank, 1977). Multidimensional analyses suggested that 3 dimensions account well for the data, in agreement with Scott's (1976) data for the rat PTA. The correlations also suggest that similar stimuli are more discriminable after the first second, especially sugars and acids.

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CHEMICAL CHARACTERIZATION OF THE SEX PHEROMONE OF *CALLINECTES SAPIDUS*: INITIAL RESULTS. Richard A. Gleeson and Michael A. Adams. Monell Chemical Senses Center, University of Pennsylvania and Whitney Marine Laboratory, University of Florida.

Although many studies have examined the chemical nature of pheromones in a wide variety of insect species, no comparably definitive work has been performed with their aquatic counterparts, the crustaceans. Indeed very few chemical signals have been structurally elucidated in marine and freshwater organisms generally. A pheromone system in the blue crab, *C. sapidus*, is currently being investigated as a model to better understand the chemical nature and evolution of signal compounds in aquatic arthropods.

Previous work demonstrated the presence of a pheromone in the urine of pubertal female crabs which releases courtship behavior in males. In the present study urine collected from these females is used as a raw material source of active pheromone, and fractions of same are surveyed for activity in a bioassay system which capitalizes on the specific courtship response of male crabs. A test-sample delivery system permits relatively precise control over the temporal and spatial presentation of stimuli to pheromone receptor sites on freely behaving animals and also minimizes the fraction volume required for assay.

Our initial studies have demonstrated that the active material is non-volatile and very polar with a molecular weight in the range of 300-600 based on gel filtration chromatography. It is stable with heating and unaffected by various protease digestion regimes. Sephadex G-15 and LH-20 column chromatography has been used in the isolation of the pheromone from urine, and reverse phase HPLC of the active fractions from these columns has resolved several UV absorbing substances. These fractions are currently undergoing further purification.

Kittredge et. al (Fishery Bulletin 69: 337-343, 1971) reported that crusteodysone (β -ecdysone) stimulated male courtship in two *Cancer* crabs and *Pachygrapsus*, suggesting that the evolution of pheromone communication in crustaceans may have involved the externalization of molting hormone receptors (Kittredge and Takahashi, J. Theor. Biol. 35: 467-471, 1972). However, our tests using crusteodysone with *Callinectes* proved negative.

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CHARACTERISTICS OF PATIENTS WITH TASTE AND SMELL DISORDERS

R.B. Goodspeed, F.A. Catalanotto, L. Bartoshuk, W. Cain, J. Gent, J. Donaldson, G. Snyder, L. Allen, K.M. Ostrom, J. Schlitzer, M.J. Schierberl, and N. Ryan, Connecticut Chemosensory Clinical Research Center, U of Connecticut Health Center and Yale U.

The Connecticut Chemosensory Clinical Research Center established a Taste and Smell Diagnostic Clinic in August, 1981. Each patient is seen by a multidisciplinary team of investigators including specialists in internal medicine, neurology, ENT, dentistry, nutrition and psychophysicists in taste and smell. This preliminary report describes the first 65 patients seen by our group and includes selected demographic data, chemosensory complaints and diagnoses, and correlated biomedical data.

The 65 patients included 26 males and 39 females; average age for males was 47 years (range 16 to 79) and for females 52 years (range 25 to 83). Thirty-eight patients presented with disorders in both taste and smell; 9 patients reported only a taste problem while the remaining 18 patients reported a problem only with olfaction. The patients' subjective reports of their chief complaints did not accurately describe their problems as evaluated by objective measurements of taste and smell; indeed, 4 patients had no measurable problem in taste or smell. Our testing indicated anosmia to be the primary chemosensory problem; 35 of our 65 patients were anosmic. This included 23 patients with anosmia only and 12 patients with anosmia plus some taste disorder. An additional 17 patients had hyposmia, either alone or with some additional taste problem. Only 9 patients demonstrated an isolated taste problem, including hypogeusia, dysgeusia, or a combination of both.

There were a number of associated medical conditions that occurred commonly in these patients that were either reported by the patients or revealed by history and examination. The major categories of conditions included infection, generally an upper respiratory infection; central nervous system trauma, which might have included neurosurgery; nasal surgery, usually for polyps; or dental surgery.

It has been suggested that there is a relationship between the trace nutrient zinc and chemosensory function; therefore, we measured zinc levels in all patients. The mean plasma zinc value was 89 ug per 100 ml (range 66 - 137), well within the normal limits. Mean red blood cell zinc, a measure of total body burden, was 1.4 ug/10⁹ cells (range 0.96 - 2.74), also within normal limits. Additional data will be presented comparing the distribution by disorder with age- and sex-matched control values.

A comparison between data on the first 65 patients and other published data on patients with chemosensory disorders will be presented. Supported by NS-16993.

NEUROPHYSIOLOGICAL AND MORPHOLOGICAL DIFFERENCES IN PHEROMONE SENSITIVE CHEMOSENSILLA OF TRICHOPLUSIA NI. Grant, A., Mankin, R.W.*, Mayer, M.S.*, and O'Connell, R.J. The Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545 and *Insect Attractants, Behavior, and Basic Biology Research Laboratory, ARS, USDA, Gainesville, FL 32604.

Concurrent investigations of the external morphology of specific types of chemosensilla on the antenna of *Trichoplusia ni* (Hübner) and the neurophysiological responses of the two olfactory receptor neurons that they contain are presented. Electrophysiological studies identified two groups of sensilla. They are differentiated by their spontaneous activity and by their relative sensitivity to the species's own pheromone (Z)-7-dodecen-1-ol acetate (Z7:12Ac). Their morphology is sufficiently distinct to allow them to be reliably selected for study at the light microscope level (approximately 500x). The first group of sensilla has relatively high levels of spontaneous activity (an average of 2.96 impulses/second for the A neuron and 3.46 for the B neuron). The A neurons in this class of sensilla are reliably excited by low doses of pheromone. The B cells on the other hand are not as reliably excited by this substance but when they are, low doses of Z7:12Ac are effective. The second group of sensilla has a relatively low level of spontaneous activity (an average of 0.10 for the A cell and 0.43 for the B cell). The A neurons in this class of sensilla are unresponsive to Z7:12Ac even when the concentration is raised 10,000 fold. The B neuron on the other hand is reliably excited by doses of Z7:12Ac approximately 1000 times larger than those that are reliably effective in the A neuron of the high spontaneous class of sensilla. Morphological examination of sensilla, whose electrophysiological properties are known, with scanning electron microscopy has revealed several features which may be correlated with response properties. These features include the length, degree of taper, number of surface annulations and number of pores/square micron of surface.

Topographic and Structural Changes of Mitral Cells Following Subtotal Bulb Removal. P.P.C. Graziadei and G. A. Monti Graziadei, Florida State University, Tallahassee, Florida

Previous observations have shown that total bulbectomy in neonatal and adult mice is followed by regrowth of the olfactory axons into the spared forebrain. Here, the sensory axons form glomerular structures and establish synaptic contacts with the dendrites of "local" neurons. The latter undergo, consequently, structural changes to adapt to the new sensory input. When one third to two thirds of the bulb is removed the sensory axons form new glomeruli in all the layers of the spared bulb and the mitral and tufted cells show dramatic reorientation of their dendrites towards these new ectopic glomeruli. In order to observe the reaction of the mitral cells when most of their environment has been removed, we have ablated large portions of the olfactory bulb, leaving only inconspicuous fragments. A series of neonatal mice 5-10 days old were subtotally bulbectomized and after 60-90 days survival their heads were prepared for histological examination. Serial sections were stained with a modification of the Bodian silver method. The regrowth of the olfactory axons has been observed in all the animals studied. The sensory input invades large regions of the forebrain, reaching at instances the head of the caudate nucleus. The fragments of the olfactory bulb are totally invaded and their histological structure disarranged. Large neurons are observed, either singularly or in groups of a few elements, among the regrowing olfactory bundles. The dendrites of these neurons expand in many directions and terminate, branching, in globose glomerular formations. While the large cells seem to have several structural characteristics of the mitral cells, their arrangement is profoundly modified. These cells have obviously lost their primitive location and interneuronal contacts and, during surgery, both their axons and dendrites must have suffered considerable damage. In several instances, similar cells, contacting the glomerular structure with their dendrites, are located inside the forebrain among cortical neurons. The origin and nature of these latter elements is presently investigated. The interest of the finding resides in the survival of these neurons and their rearrangement in a totally new environment. The role of the primary olfactory input upon the survival and the rearrangement of these neurons deserves further study.

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Aberrant Olfactory Projection to the Telencephalon in a Bullfrog. P.P.C. Graziadei and M.M. Mozell* Florida State University, Tallahassee, Florida 32306 *SUNY, Upstate Medical Center, Syracuse, New York 13210

An adult bullfrog routinely dissected for electrophysiological recording was observed to have an uncommon anatomical organization of the olfactory sensory projections to the telencephalon. A morphological illustration of the uncommon finding will be provided.

It is known that both in amphibians and mammals experimental ablation of the olfactory bulb induces ectopic projections of the olfactory input to several regions of the forebrain. In this frog, purchased from a Louisiana supplier and not previously operated, we have observed that the right olfactory nerve ends in a large irregularly-shaped olfactory bulb which is continuous with the anterior poles of the two cerebral hemispheres. The left olfactory nerve bypasses the bulbar structure and terminates on the lateral aspect of the left telencephalic hemisphere, where it penetrates. Histologically, the left olfactory nerve has been seen to form, on the left telencephalic wall, a bulbar-like structure with glomeruli and associated neurons. The exceptional organization will be illustrated at the macro and microscopic level. This frog seems to show that the organization induced by experimental manipulation can indeed occur, however exceptionally, in nature.

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MITRAL CELL DEGENERATION IN THE NEUROLOGICALLY MUTANT MOUSE PCD.
Charles A. Greer and Gordon M. Shepherd. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT. 06510

Purkinje Cell Degeneration (PCD) is a mutant gene in mice which, when present in the homozygous recessive condition, has several postnatal effects on the nervous system. These include the rapid loss of cerebellar Purkinje cells and slower degenerative losses of photoreceptors, some thalamic nuclei, and mitral cells of the olfactory bulb. Mitral cell degeneration, assessed with Fink-Heimer methods on the lateral olfactory tract, was not pronounced until 10 mon. postnatal. We have studied olfactory bulbs of PCD mice to establish more precisely the timecourse of mitral cell degeneration and the consequent effect upon the functional organization within the bulb.

C57BL/6J mice carrying the *pcd* gene at one or both loci were obtained from Jackson Laboratories and at either 4 or 8 months postnatal were either sacrificed for routine histological examination or tested for functional organization with the ¹⁴C-2-deoxy-D-glucose (2DG) technique. The heterozygous (*pcd/+*) littermates of affected (*pcd/pcd*) mice were employed as controls since our early studies demonstrated normal olfactory systems in the heterozygous genotype.

At 4 months postnatal *pcd/pcd* had only 19% of the number of mitral cells found in *pcd/+*. By 8 months only 4% remained. In parallel with the loss of mitral cells the thickness of the external plexiform layer (EPL) decreased. This may reflect the decline in number of the mitral cell primary and secondary dendrites. Similarly, the diameter of individual glomeruli was reduced. The olfactory nerve and granule cell layers in *pcd/pcd* did not exhibit any gross morphological changes. Interestingly, on gross inspection, the population of tufted cells in the EPL did not appear to be subject to the influence of the *pcd* gene. Examination of the functional organization in the olfactory bulb with the 2DG technique revealed essentially normal spatial density patterns within the glomerular layer of the *pcd/pcd* mice following odor stimulation.

These results demonstrate that the degenerative loss of mitral cells in the olfactory bulbs of *pcd/pcd* mice is occurring earlier than previously suggested. Also, the 2DG analyses raise the possibility that the olfactory receptor input is functionally normal despite the loss of mitral cells. Consequently, this murine mutant would seem to be a promising model for studying the role of mitral cells in olfactory bulb function, the effect of mitral cell loss on synaptic interactions of remaining elements, and plasticity and reorganization within the olfactory system.

EFFECTS OF TRYPAN BLUE AND ANILINE ON DEVELOPMENT, HATCHING, AND PREY CHEMORECEPTION OF THE PREDATORY MURICID GASTROPOD UROSALPINX CINEREA. G. L. Gruber, A. B. Ducharme, and D. Rittschof, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Inexpensive, reliable, reproducible, and quantitative methods should be developed for screening chemicals and chemical wastes for toxic effects. An assay employing encapsulated embryos was used to test the effects of aniline and trypan blue on development, hatching, and chemoreception. Aniline is a known toxic chemical; whereas trypan blue has not been tested for toxic effects. Six groups of 25 egg capsules containing late veligers were continuously exposed to 10, 1, and 0.1 ppb trypan blue and aniline in filtered seawater. Two control groups were exposed to filtered seawater. In each group on every third day, development was examined in four egg capsules that had layer 1 removed, and hatched juveniles were counted and stored at 14°C in capped jars containing corresponding concentrations of toxicants. Chemoreception by these hatched juveniles from each group was assayed with unfiltered seawater and concentrated barnacle stimulus (*Balanus* spp.) diluted 1:3000 and 1:500 with unfiltered seawater. No gross morphological abnormalities were observed in experimental groups. Total hatching of experimental groups decreased 25-30% except for 0.1 ppb aniline where it increased 22%. Time of maximum hatching was delayed 2-4 days at 10 and 1 ppb trypan blue and 1 ppb aniline; the magnitude of hatching at this time decreased 28% at 10 and 1 ppb aniline. Tests with juveniles of trypan blue groups showed a significant increase ($p = 0.005$) assay response to control seawater. Juveniles that had developed in 1 ppb trypan blue showed a significantly ($p = 0.05$) increased response to 1:500 dilution of barnacle stimulus when compared to juveniles from control conditions. However, the responses of other trypan blue groups to stimulus was similar to control groups. Juveniles that had developed in 10 ppb aniline showed no increased response to control or barnacle stimulus compared to hatched juveniles of control groups. Increased control and barnacle stimulus response suggests that chronic exposure to trypan blue late in development may induce hyperactivity. Techniques from this initial study may be applicable to simple, short-term studies of sublethal effects of chronic exposure of developmental stages of encapsulated marine invertebrates to pollutants. This research was funded by an institutional grant from the National Institutes of Health to the University of Delaware.

PRELIMINARY CHARACTERIZATION OF RESPONSE-ELICITING COMPONENTS OF EARTHWORM EXTRACT: A NEW BIOASSAY.
Mimi Halpern, Lorraine Reformato, Donald M. Kirschenbaum. Downstate Medical Center. 450 Clarkson Avenue, Brooklyn, New York 11203.

Fifteen garter snakes (*Thamnophis sirtalis*) reliably spent more time and tongue flicked more frequently at a dish containing earthworm extract than a dish containing distilled water when these were presented simultaneously for two minute intervals. The discriminability of the earthworm extract was directly related to its concentration.

Garter snakes with their vomeronasal ducts sutured closed did not respond differentially to earthworm extract and water under these test conditions. Thus their ability to discriminate earthworm extract from water in this bioassay was dependent upon a functional vomeronasal system.

Earthworm extract retains its biological activity after boiling at 100°C for 15 minutes and after lyophilization. Its effectiveness is not altered by changes in pH. Snakes continued to respond differentially to extracts with pH 2, 5-6 or 11. Chloroform extractions of the acid, neutral and alkaline earthworm extract yielded activity primarily in the water layer. The small amount of activity in the chloroform layer was removed by use of a drying agent. Bradford dye-binding tests indicated the presence of protein in the active fractions.

Supported by NIH Grant NS 11713.

EFFECT OF STIMULUS COMPLEXITY ON THE RESPONSE OF MORPHOLOGICALLY-IDENTIFIED CENTRAL OLFACTORY NEURONS. K. A. Hamilton and B. W. Ache, Whitney Marine Laboratory, Univ. of Florida, Rt. 1, Box 121, St. Augustine, FL 32084

Multimodal interneurons descending the circumesophageal connectives from the brain to the lower nervous system in crustaceans respond to stimulation of cephalic sensory structures. The dendritic branching pattern of some of these interneurons has been shown to be correlated with complexity of sensory input (laterality; visual, tactile and proprioceptive modality; Glantz et al., J. Neurobiol. 12: 311, 1981). Using intracellular recording and staining and backfilling techniques, we have examined the dendritic branching pattern and the effect of stimulus complexity on activation of excitatory descending interneurons during antennular (olfactory) stimulation in the spiny lobster.

Variation in the response of excitatory interneurons is attributable to differences between chemostimulants (ANOVA; $n = 31$). Two natural mixtures of amino acids plus betaine at concentrations found in potential foods ("crab" and "urchin") are more excitatory than an "unnatural" mixture (inverse amounts of the crab components) or some single components (e.g., glutamate). Other single components (e.g., taurine), however, are as excitatory as equimolar natural mixtures. Response profiles for single components resemble those for mixtures.

Several morphological types of excitatory interneurons can be identified on the basis of dendritic branching pattern. The extent of branching, which occurs primarily within the antennal and paraolfactory neuropiles of the brain, does not appear to be correlated with chemosensory response. In other crustaceans, high-order descending interneurons like these, which serve premotor function, encode visual and mechanosensory information based on stimulus-dependent impulse coordination within an interacting array, rather than in parallel lines (Wood & Glantz, J. Neurophysiol. 43: 741, 1980). Our results suggest that descending interneurons which are excited by chemostimulation of the antennules may function similarly.

THE ONTOGENY OF THE FLY'S TASTE HAIRS. K. Hansen, E. Hansen-Delkeskamp, G. Waldhorst. University of Regensburg, FRG. The chemosensitive sensilla belong together with cuticular bristles, scales and glands to the so called organules. These organules are favoured objects of developmental biology for studying problems of differentiation, as they contain a rather small and constant number of cells which derive from one stem cell. However, our knowledge of the ontogeny of taste hairs is still incomplete (Peters, Z.Morph.Ökol. 55 1965; de Kramer & van der Molen, Electron Microscopy 2 1980).

We chose as object the tarsal taste hairs of *Protophormia terraenovae*, because the labellar and tarsal taste hairs of this and related species are well studied concerning their structural, electrophysiological and behavioral features (see Dethier's monography "The hungry fly", Harvard Univ.Pr.1976). The adult organs consist of five sensory cells and three sheath cells. The latter surround concentrically the sensory cells and are generally called trichogen trichogen and tormogen cell (see Altner & Prillinger, Int.Rev.Cytol. 67 1980; Hansen & Heumann, Cell Tissue Res. 117 1971). The differential mitoses of the stem cell already happen in the imaginal disc during the last larval instar. The further cellular differentiation occurs during the pupal stage and the imaginal development which together last 11 days at 19°C in our object. The used methods are light as well as scanning and transmission electron microscopy. The most characteristic stage of taste hair development previously not being described is observed 3 days after formation of the white prepupae: the ciliary dendrites protrude over the hypodermal cell layer by half of the final length of the hair shaft; the distal 30 % of the 3 - 5 dendrites are floating freely in the fluid of the space between the hypodermal cell surface and the pupal cuticle, the proximal 70% of the dendrites are enclosed by the thin-walled and tubelike dendritic sheath, at its basis the latter is enveloped by a conical projection of the trichogen cell. This process grows out during the next days and reaches the outer dimensions of the adult hair shaft. At the 6th day it starts the cuticle deposition around the more or less axially oriented sheath as well as on its outer surface. By this manner the two lumina of the adult hair shaft are easily explained. Furthermore the ciliary dendrites seem to play a fundamental role as axial element. This type of taste hair development is neither the same as that of olfactory hairs (Ernst, Cell Tiss.Res.129 1972) nor that of solely mechanosensitive hairs (E.Hansen-Delkeskamp, in prep.) and differs as well from the moulting processes of taste hairs in hemimetabolous insects (Gnatzy & Schmidt, Cell Tiss.Res.126 1972) where in no stage free dendrites occur.

We expect that the study of taste hair development leads to valuable insights into the complex functional organisation of taste sensilla. (Supported by Deutsche Forschungsgemeinschaft SFB 4/C1)

TASTE RESPONSES TO AMINO ACIDS IN RAINBOW TROUT: EVIDENCE FOR MULTIPLE RECEPTOR SITES FROM KINETIC ANALYSIS AND CROSS-ADAPTATION. Toshiaki J. Hara and *Takayuki Marui. Freshwater Institute, Winnipeg, Canada and *Department of Oral Physiology, Kagoshima University School of Dentistry, Kagoshima, Japan.

The importance of amino acids as olfactory and taste stimuli for fishes has generally been recognized. However, the relative significance of their effectiveness between two systems has not been fully understood. This is primarily due to the lack of systematic investigations into both chemoreceptor functions in the same species under controlled experimental conditions. Electrophysiological and biochemical studies of the olfactory system have established a definite structure-activity relationship among stimulatory amino acids, and led to a postulate that a multiplicity of olfactory receptor types exists in rainbow trout, *Salmo gairdneri*.

In the present study, the stimulating effects of amino acids and related chemicals on the trout taste receptors were studied by recording electrical responses from the palatine nerve. Among chemicals tested at 1 mM or lower, only L-Pro, L-Hyp, L-Ala, L-Leu, L-Phe, L-α-amino-β-guanidinopropionic acid (L-AGPA), L-argininic acid, betaine and some of their derivatives were effective at natural pHs (7.5-7.7). The threshold concentration for L-Pro, the most effective amino acid tested, ranged between 0.01 and 0.01 μM. L-Arg, one of the most stimulatory amino acids previously reported, and related compounds including D-Arg were effective only at basic pHs (>8.5). The dose-response relation for most of the stimulatory chemicals, plotted semi-logarithmically, were of sigmoid nature, saturating at 1 mM or higher. Scatchard plots of the saturation curve for each chemical were generally non-linear, indicating interactions with more than one type of receptor site. These data along with cross-adaptation experiments suggest at least three different receptor types exist: 1) Pro-receptor, 2) Leu-/Phe-receptor, and 3) AGPA-receptor. A survey of proline analogues further revealed the specific structural requirements for its effective taste stimulation.

These data demonstrate that the palatal taste receptor of trout is highly specific, responding to a more narrowly-tuned spectrum of chemicals than does the olfactory system.

BILE ACIDS AS OLFACTORY AND TASTE STIMULI FOR RAINBOW TROUT. Toshiaki J. Hara, *Takayuki Marui and Robert E. Evans. Freshwater Institute, Winnipeg, Manitoba, Canada, and *Department of Oral Physiology, Kagoshima University School of Dentistry, Kagoshima, Japan.

Bile acids, when introduced into the nares, elicit electrical responses in the olfactory bulb of grayling and Arctic char, with mean thresholds ranging between 1 and 10 nM (Döving et al., Acta Physiol. Scand., 108, 123, 1980). High sensitivity of the olfactory receptor to bile acids is implicated in their role as specific chemical signals in homing migration.

We report here that bile acids are not only potent olfactory stimuli, but also highly specific taste stimuli for rainbow trout, *Salmo gairdneri*. Integrated electrical activity recorded from the palatine nerve in response to taurocholic acid, the most effective bile acid tested, increased linearly with logarithmic increase in concentration; the threshold ranged between 1 and 10 pM. (The threshold concentration for L-Pro, the most potent taste stimulant among amino acid, ranged between 10 and 100 nM under the same experimental conditions). The response magnitude at 5 nM far exceeded that of 10 mM L-Pro. This suggests the existence of a large number of taste receptor sites with an extremely high affinity for this chemical. Taurocholic acid, taurothiodoxycholic acid, and cholic acid were also effective.

The EOG responses were recorded using Ag-AgCl electrodes via Ringer-gelatin-filled capillary pipettes positioned directly on the surface of an olfactory lamella. All four bile acids tested above were equally or more stimulatory than L-Ser, one of the most potent olfactory stimulants reported for rainbow trout. The threshold concentration for taurocholic acid was estimated at 1 nM or lower, which is comparable to those for amino acids but almost 1000 times higher than bile acid thresholds for trout taste receptors. Specificity of the olfactory response to bile acids was shown by its loss in axotomized fish. The responses to bile acids during cross-adaptation to 10 mM L-Ser remained unaffected, suggesting the existence of separate receptor sites for these two groups of chemicals.

ELECTRIC TASTE STIMULATION: NEURAL EVIDENCE. M. Scott Herness, Dept. Biological Science, Florida State Univ., Tallahassee, FLA. 32306.

The use of a weak electric current to stimulate taste receptors is an inherently useful tool. It not only affords a square wave stimulus to be presented, but it also separates cations and anions allowing them to be studied independently. Up to the present electric taste has been used infrequently primarily because the underlying mechanism has not been elucidated. Classically, two schools of thought have evolved concerning the mechanism of electric taste. They maintain that electric taste is the result of either 1) adequate stimulation of taste receptors by iontophoresis of extracellular ions and/or electrolytic byproducts, or 2) the direct action of the current of either taste cells or taste nerves. More recently, Kurihara et al. (Olfact. & Taste VII) and DeSimone et al. (Sci. 214, 1981) have proposed other schemes by which electric taste might be explained. The former involves alteration of an intra-extracellular depolarizing current, the latter an alteration of a transepithelial potential induced by active transport.

Previous work (Herness, AChems III, Neurosci. Abstr. VII) has demonstrated the marked similarity between electrical and chemical responses in the rat. The present work addresses the question of the role of direct neural stimulation in electric taste. Electrophysiological recordings from gustatory (chorda tympani) and non-gustatory (lingual) nerves from the rat's tongue demonstrate that taste nerves respond to an electric current at much lower current densities (6 - 66 μamps/cm²) than do non-gustatory nerves (133 - 666 μamps/cm²). Since non-gustatory nerves are stimulated directly this evidence indicates that direct neural stimulation is not a factor in the mechanism of electric taste. Moreover, inactivation of taste receptors (e.g. 5% Iodoacetic acid) results in response curves from gustatory nerves which are relatively identical to non-gustatory nerves. It thus seems that electric taste operates at the level of the taste cells rather than at the taste nerves. Stimulation is most likely the result of iontophoresis of extracellular ions to the taste cells.

DEVELOPMENTAL CHANGES IN TASTE RESPONSES FROM RAT SOLITARY NUCLEUS. D.L. Hill and C.M. Mistretta, Center for Human Growth & Devel. and Dept. Oral Biol., Sch. Dent., and R.M. Bradley, Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

Developmental changes in peripheral taste nerve responses have been observed in the rat. For example, response frequencies to NaCl and LiCl increase substantially from 20 days to adulthood and frequencies to citric acid decrease. To determine whether similar changes occur in central nervous system neurons, electrophysiological recordings were made from chemosensitive units in the nucleus of the solitary tract (NST) in developing rats. Twenty-nine single units were studied in rats aged 14-20 days, 26 units in rats aged 25-35 days, and 31 units in adult rats. In addition, multiunit taste responses were recorded from the NST in 5 rats aged 5-7 days. Chemical stimuli applied to the anterior tongue were 0.1M and 0.5M NH_4Cl , NaCl, LiCl and KCl, and 0.1M citric acid. Neural activity was measured for the first 5 sec after stimulation of the tongue; a comparable period of prestimulus spontaneous activity was subtracted to yield response frequencies.

NST neurons in rats aged 7 days are characterized by a general absence of responses to 0.1M salts and to 0.5M NaCl and LiCl. However, 0.5M NH_4Cl and 0.1M citric acid repeatedly elicited responses in all recordings. In contrast, all single units in older rats responded to each stimulus. Therefore, changes occurred in central taste responses between 7 and 14 days. Further changes in central taste responses were apparent later in development. Response frequencies to NaCl, LiCl, and KCl in rats aged 14-20 days and 25-35 days were significantly lower than those in adults ($p < 0.01$). Responses to some chemical stimuli, NH_4Cl and citric acid, did not change after 14 days of age, however ($p > 0.20$).

We conclude that major changes occur in chemosensitive responses from NST neurons between ages 7 to 14 days; furthermore, changes also occur between 35 days and adulthood. As in peripheral taste neurons, striking differences are found in responses of central neurons to NaCl and LiCl. Major response frequency changes to NaCl and LiCl in peripheral fibers, however, occur between ages 20 and 35 days. Therefore, maturation of taste responses may be progressively delayed in more central neural structures. Since central taste responses alter during development, differences in behavioral responses to taste stimuli would be predicted throughout the rat's preadult life. (Supported by NIH Grant NS17404 to D.L.H., NSF Grant BNS80-15737 to R.M.B. and C.M.M., and Res. Career Dev. Award, NIDR, DE-00066 to C.M.M.)

PTC: CONSIDERATIONS IN THRESHOLD DETERMINATION OF TASTE STATUS. Janet E. Hooper, Janneane F. Gent, and Linda M. Bartoshuk. John B. Pierce Foundation Lab., 290 Congress Ave., New Haven, CT 06519.

Taste thresholds for PTC, PROP, and chemically related compounds containing the HNCS group are bimodally distributed. This distribution has been used to classify people into two groups commonly labeled sensitive or "tasters" and insensitive or "nontasters." Earlier research in this laboratory found the thresholds for caffeine which is a non-HNCS group compound were bimodally distributed. The Harris-Kalmus method of threshold detection was used in this early study. The present study examines the distribution of caffeine thresholds with a different method, the Up-Down procedure. Thresholds were evaluated for 50 (14 male and 36 female) randomly selected subjects on five compounds: PTC, PROP, caffeine, urea, and NaCl.

Only the threshold distributions for PTC and PROP were bimodal. The fact that the caffeine distribution from the earlier study was not reproduced led us to examine the differences between the present study and threshold studies in the literature. This investigation yielded two major points for consideration:

1) PTC and PROP were highly correlated ($r = .69$, $p < .000001$). However, PTC thresholds were consistently lower than PROP taster thresholds. They were not identical. This supports recent work suggesting that the assumed interchangeability of PTC and PROP as equal threshold determiners should be re-examined.

2) The PTC and PROP threshold distributions that we determined using the Up-Down method are similar in shape to those found using the Harris-Kalmus method. However, they are consistently displaced lower by 1/4 to 1/2 log step. In addition, threshold distributions we found for caffeine, urea, and NaCl using the Up-Down method were similarly displaced. Certain "cut off" points along the PTC and PROP threshold continuum have come to be accepted as determining the point at which a "taster" becomes a "nontaster." Although the shapes of the threshold distributions for PTC and PROP appear to be independent of threshold determination method, the location of the criterion threshold is not. Thus, the determination of taster status is dependent on the method used to determine the threshold.

Classification of tasters and nontasters of the PTC chemical group has become increasingly important in taste research. These findings suggest further research in threshold procedures is needed to clarify taster/nontaster grouping.

RECEPTOR CELL CONTRIBUTION TO OLFACTORY MUCOSAL ODORANT UPTAKE. D.E. Hornung, Biology Department, St. Lawrence University, Canton, NY 13617, S.L. Youngentob and M.M. Mozell, Physiology Department, Upstate Medical Center, Syracuse, NY 13210.

Our previous work determining the air/mucosa partitioning of odorants suggested that a multicompartiment model with several partitions is required to describe the interaction of odorant molecules with the olfactory mucosa. Since at least one of these compartments likely involves the receptor cells, the present study evaluates the contribution of these cells to the total mucosal odorant uptake. Using four radioactively labeled odorants, we compared partition coefficients for normal olfactory mucosa to the partition coefficients from mucosa in which the receptor cells had been removed. The receptor cells were removed by severing one of the bullfrog's olfactory nerves midway between the bulb and the sac and then allowing two weeks for complete degeneration to occur. The loss of receptor cells was confirmed histologically. With each animal serving as its own control, the air/mucosa partition coefficients for the cut and uncut sides were compared. These comparisons were done by a paired T-test on at least 14 animals per chemical.

For the more water soluble odorants, butanol and isobutyric acid, the cut and uncut sides sorbed the same amount of odorant. This agrees with previous work suggesting that for these chemicals uptake by the mucosa reflects mostly uptake by the water in the mucosa. On the other hand, for the less water soluble odorants, octane and amyl acetate, the uncut side did sorb significantly more odorant than the cut side. Thus it can be demonstrated that the receptor cells are one of the compartments of the several compartment model needed to describe the interactions of these odorants with the mucosa.

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EARLY IXth NERVE REMOVAL PREVENTS DEVELOPMENT OF RAT VALLATE TASTE BUDS

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It is known from previous work with adult rats that bilateral IXth nerve denervation results in a 100% loss of vallate taste buds, whereas unilateral denervation results in only a 10-12% loss of taste buds, apparently because of bilateral innervation. We have found that when infant rats are subjected to unilateral IXth nerve removal, large numbers of vallate taste buds never develop.

The right IXth nerve was removed at 3 days postpartum (pp) and vallate taste buds counted in experimental and control rats at 5, 10, 15, 21, 33, 45, 60 and 90 days pp. By age 90d, control rats have 625 ± 83 SD vallate taste buds. In contrast, experimental rats, with one IXth nerve removed at 3d pp, developed only 37% of the normal number of taste buds --- an average of 230 ± 36 buds at 90d. These taste buds were distributed throughout the vallate papilla, indicating that the deficiency did not result from an inability of the remaining IXth nerve to reach the areas of the taste bud precursor cells. Unilateral denervation must be carried out at an early age to produce a marked reduction in the number of taste buds that eventually form, as indicated by the decreasing effectiveness of unilateral denervation performed from 0d through 20d. No vallate taste buds form after permanent bilateral denervation. Taste buds will form by 90d if, in addition to removal of the right IXth nerve at 3d, the left IXth nerve remains intact until crushed at 10d (197 ± 74 vallate taste buds) or crushed at 10d and again at 17d (158 ± 96 vallate taste buds). We suggest that a critical interaction must take place in early development between the taste fibers and taste bud precursors to establish the normal number of adult taste buds. Prevention of this interaction during an early sensitive period results in a permanent failure of taste buds to form.

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FURTHER EVIDENCE FOR OLFACTORY BEHAVIOR IN PROCELLARIIFORM BIRDS. Larry V. Hutchison, Bernice M. Wenzel, and Kenneth E. Stager, Department of Physiology, School of Medicine, University of California, Los Angeles, Los Angeles, CA 90024

Our earlier work showed that certain procellariiform species are selectively attracted to various food-related odors at sea from downwind. One of these odors is cod liver oil. The present work explored the attractiveness of a volatile fraction of cod liver oil extracted in heptane as solvent. Other stimuli presented were homogenates of squid and of krill, and seawater and heptane as controls. All birds in the study area were identified precisely. Data are analyzed here for the Northern Fulmar, Sooty Shearwater, Western Gull, Common Loon, and Common Murre. The cod liver oil fraction proved to be a more effective attractant than whole oil in terms of earlier arrivals from downwind and closer approaches by fulmars and shearwaters. The shearwaters also flew upwind to squid and krill but were rarely sighted when seawater or heptane was used. Nonprocellariids, on the other hand, showed no differential behavior with respect to stimulus or wind direction. These data were collected throughout a range of climatic and weather conditions using carefully controlled procedures and a systematic protocol of experimental and control stimuli. In tests with control substances, virtually no close approaches were made to any stimulus by birds of any species, nor was any group more likely to fly by from the downwind rather than the upwind direction. Appropriate control tests were always made on the same cruise as the experimental tests so that the same general conditions of bird populations, sea conditions, prey availability, and weather prevailed during both types of tests. Results support the concept of responsiveness of certain procellariiform species to olfactory stimulation by odors related to their natural prey. The study is concerned with the credibility of this basic phenomenon and does not address the question of how such cues might operate in nature.

USE OF THE SIGNAL DETECTION PARADIGM IN THE STUDY OF CHEMORECEPTION. David W. Ingersoll, Department of Psychology, Fordham University, Bronx N.Y. 10458.

Within the activities of the neurosciences, intranasal chemoreception may currently receive the broadest interdisciplinary interest. Lacking, however, are studies providing behavioral assessments of odorant detection capabilities and chemoreceptive physiological processes in animals. A major limitation in using animals as "observers" is the implementation of an adequate behavioral testing procedure. The Signal Detection Paradigm has recently been modified for the testing of animals. Since under this paradigm sensory sensitivity and response criterion can be independently manipulated, it was used to investigate various biological mechanisms of odorant detection. Using mice, three experiments were performed to exemplify the utility of the Signal Detection Paradigm in physiological psychophysics. Specifically, the contribution of a) the vomeronasal organ, b) estrus cycle, and c) testosterone were examined with regard to n-butanol detection. Of particular interest, are the findings regarding the role of hormones in odorant detection. It appears that the presence of some hormones contribute to the maintenance of a stable decision rule or response criterion. For example, the castration of male mice has the effect of increasing response criterion variance and subsequent testosterone therapy reduces fluctuations in the decision rule. The statistical effect of increasing the response criterion variance is to decrease sensory sensitivity, d' . This finding could only have been obtained by using a Signal Detection task.

ROLE OF AFFERENT SYNAPSES IN CODING SENSORY MESSAGES IN MULTIMODAL NEURAL PATHWAYS. B. Jahan-Parvar, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

We have been studying the principles of information processing in the neural pathways which are activated by the sensory qualities of a behaviorally significant stimulus, food, in *Aplysia*. Much of the food related behaviors of *Aplysia* are mediated and modulated by the chemoreceptors and the mechanoreceptors on its tentacles. The tentacular chemoreceptors are essential for the chemolocation of food in the environment. The tentacular mechanoreceptors also play a major role in food location. Tactile stimulation of a tentacle in a food aroused *Aplysia* will evoke appetitive responses such as orienting and mouth opening. An identical stimulation of an unaroused *Aplysia* will elicit a different response, a tentacle withdrawal reflex. Having delineated the neuronal circuits involved in mediating these responses, we are in a position to investigate the following important questions: 1) how are the chemosensory and mechanosensory qualities of food represented in the nervous system?; 2) how does a change in the motivational state of the animal result in the sensory response modification? Results suggest that the chemosensory and the mechanosensory qualities of food activate different input (first-order) neurons. These converge on common 2nd-order neurons, the identifiable cerebral B neurons. B neurons can discriminate chemosensory and mechanosensory information by the transfer properties of the 1st-order synapses. The mechanosensory neurons to B neuron synapses are of the fast habituating type. As a result, the tactile input produces a phasic response in the B neurons. In contrast, the chemosensory neuron to B neuron synapses are of the slowly- or non-habituating type. As a result, the chemosensory input produces a tonic response. These differential B neuron responses to sensory stimuli, if combined with earlier data on other B neuron functions, can provide an explanation for the tactile response modification in the food aroused *Aplysia*. B neurons are multi-action neurons and can elicit different behavioral responses at different levels of activation. B neurons are motor neurons for the tentacles. Together with the 1st-order mechano-afferent cells, they form monosynaptic reflex pathways which are responsible for the touch-induced tentacle withdrawal reflex in the unaroused *Aplysia* when food chemosensory input is absent. B neurons also make connections with the higher (3rd and 4th) order cerebral neurons which are known to have food related motor functions. Tonic activation of the B neurons with the chemosensory qualities of food can activate and cause a reverberating state of excitation in this neural network via positive feedback connections from the 4th order neurons. It follows thus that tactile input to B neurons in a food aroused state where the B neurons are being continuously activated, can have food related behavioral consequences. The basic principles which emerge from these experiments are: 1) that different sensory modalities can share the same afferent pathway; 2) that in such pathways, different sensory information can be discriminated by the transfer properties of the afferent synapses; 3) that activation of reverberating neural circuits may be responsible for the increased responsiveness of animals in the aroused state; and 4) that the presence of multi-action neurons in the nervous system may be responsible for the modification of sensory perception in animals showing altered motivational state (Supported by Grants NS 16022-01 and NS 16022-01).

INHIBITION OF THE GERBIL'S ELECTROPHYSIOLOGICAL SWEETENER TASTE RESPONSE BY METHYL 4,6-DICHLORO, 4,6-DIDEOXY α -D-GALACTOPYRANOSIDE. By Dr. William Jakinovich, Herbert H. Lehman College Bronx, New York.

The gerbil's whole nerve electrophysiological response to sweeteners is suppressed by methyl 4,6-dichloro, 4,6-dideoxy α -D-galactopyranoside (DCL-gal). In our experiments, we prepared mixtures of 0.1 M DCL-gal and various sweeteners, sodium chloride and hydrochloric acid. These solutions were applied to the tongues of anesthetized gerbils from which chorda tympani electrophysiological responses were obtained. The following were observed: (1) 0.1 M DCL-gal either does not or barely stimulates the gerbil's taste nerve; (2) the taste responses produced by sodium chloride or hydrochloric acid were unaffected by the presence of DCL-gal; (3) the taste responses produced by sugars are inhibited in a competitive manner. These included sucrose, methyl α -D-galactopyranoside, methyl α -D-glucopyranoside, and chlorosucrose; (4) the taste responses produced by saccharin are inhibited in a non-competitive manner; (5) the inhibition occurs only when DCL-gal is mixed with the sweeteners, is short-lived, and ceases when the mixtures are rinsed from the gerbil's tongue.

This type of differential inhibition of the sweetener taste response by DCL-gal suggests that there are two major types of sweetener receptor sites, a sugar site and a non-sugar site.

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EFFECT OF CHEMICAL AND PHYSICAL MODIFICATIONS ON THE ASSAY OF AN OYSTER DRILL CHEMOTACTIC RESPONSE. D. Kieber, D. Rittschof, and R. Shepherd, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Oyster drills (*Urosalpinx cinerea*) chemotactically locate prey in the marine environment. One particularly potent oyster drill phagoattractant, released by balanoid barnacles, has been concentrated, and partially purified and characterized. We have used this partially purified phagoattractant to test effects of possible future purification steps on the oyster drill bioassay. Tested were: heat, methanol, distilled water, ammonium acetate, acetic acid, and variations in pH.

Boiling the phagoattractant for 90 minutes did not significantly affect its bioactivity. Methanol used both to elute XAD-7 bound phagoattractant, and as a solvent in high performance liquid chromatographic analyses reduced the bioactivity of the chemoattractant at concentrations greater than 50mM (1.6 ppt). Deionized, distilled water diluted from 1:100 to 1:1000 in seawater had no effect on the bioassay although changes in salinity greater than 3 ppt resulted in snail detachment. At concentrations greater than 2mM for ammonium acetate and 17mM (1.0 ppt) for acetic acid, snail response to standard barnacle stimulus was completely inhibited. We believe that this is a pH effect. The snail response is inhibited below a pH of 6.0 and above a pH of 9.0. A 2mM solution of either ammonium acetate or acetic acid lowers the pH of 32‰ seawater below 5.0.

The assay is relatively insensitive to the common reagents tested with inhibition occurring at ppt levels. In contrast, hydrocarbons such as naphthalene (see Merrill, this conference) affect the assay at ppb levels.

We stress the need to determine the effect of purification procedures on the bioactivity of a chemical cue. Lower response to a chemoattractant may be thought to be due to loss of some of the attractant during a purification step when, in fact, lower responses may be caused by chemical inhibition. The snail bioassay is a sensitive analytical tool for determining behavioral effects of reagents intended for use in subsequent purification of bioactive phagoattractants.

CHEMOSENSE DISTURBANCE IN MELANCHOLIA: PRELIMINARY RESULTS OF A QUESTIONNAIRE STUDY. LOUIS DOUGLASS KING, M.D., NAOMI LHOR, Ph.D. UNIVERSITY OF MICHIGAN MEDICAL CENTER, ANN ARBOR, MICHIGAN, 48109.

Depressed patients often have symptoms which are thought to reflect limbic-hypothalamic dysfunction. These symptoms include loss of hedonic tone and loss of appetite. Some patients also complain of loss of taste for food.

Using a 14 item questionnaire to assess self-report of appetite, food craving and chemosensory function, we found that inpatients with a diagnosis of endogenous depression (melancholia) complained of decreases in olfactory and gustatory sensitivity. Psychiatric patients without melancholia (heterogeneous group) did not complain of loss of smell or taste.

A 100 mm visual analog scale was utilized to follow changes in self-report of taste sensitivity during hospitalization. The data indicate that self-reported gustatory deficits resolve with resolution of depression suggesting that loss of gustatory sensitivity is a "state" related phenomenon of melancholia.

Melancholic patients did not differ from other patients in terms of percent reported appetite change or specific cravings. Melancholic patients more often reported a "dull" taste in their mouth independent of food intake.

GUSTATORY NEURAL ACTIVITY IN ADRENALECTOMIZED RATS. Therese Kooten & Robert J. Contreras. Yale University, New Haven, CT 06520.

Sodium deficiency in rats is associated with heightened taste preference for NaCl solutions. With deficiency caused by dietary restriction, the sodium-retaining mechanisms are intact. Removing the adrenal glands, a major component of the mechanism that maintains stable sodium levels, also produces a sodium deficiency and leads to an enhanced consumption of salt solutions in rats.

The neural mechanisms underlying this altered taste preference were thought to be due to an increased sensitivity of the taste receptors.¹ Electrophysiological evidence did not support this, but suggested a central mechanism was responsible for the preference change.² More recently, decreased peripheral neural responsiveness to NaCl solutions was recorded in dietary restricted rats.³

In the present study, whole nerve chorda tympani responses were recorded from six adrenalectomized and six control rats. The adrenalectomized rats were maintained on .3 M NaCl solution. Some recordings were made from adrenalectomized rats that were deprived of salt solutions for 12 hrs before surgery. The taste stimuli included eight concentrations of NaCl solutions and two concentrations each of LiCl, KCl, HCl, and quinine. A rinse of deionized water was presented after each stimulus. Analyses of neural activity were based on a measure of initial peak neural discharge and a measure of later tonic neural discharge.

Preliminary results suggest that neural responses to suprathreshold NaCl solutions are lowered in adrenalectomized rats. The slope of neural activity across concentrations is flatter for the NaCl solutions, but not for the other taste stimuli. Thus, adrenalectomy is similar to sodium deprivation in its effects on salt intake and in changes in taste sensitivity.

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²Pfaffmann, C. & Bare J.K. *J. Comp. Physiol. Psychol.* 43, 320-324, 1950.

³Contreras, R.J. & Frank, M. *J. Gen. Physiol.* 73, 569-594, 1979.

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TITLE: Application of generalized Procrustes analysis to sensory profile data

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In sensory profile experiments generalized Procrustes analysis can be used to rationalise the variability in judge responses. The relationships between the transformations carried out in the Procrustes analysis and how a judge responds to a sample will be discussed. Examples taken from work on apples illustrate the advantages of taking the transformed data when relating to external or experimental factors.

Misadventures in Physiologizing: Adaptation and Taste Mixology. Harry Lawless, Monell Chemical Senses Center, Philadelphia, PA 19104.

An important characteristic of mixtures of different tasting substances is that the perceived intensities of the components appear weaker in the mixture than in equimolar unmixed solutions. This effect is called mixture suppression. A series of experiments sought to establish the relative central-peripheral positions of neural mechanisms underlying suppression and adaptation by comparing the effects of suppression on adaptation and vice-versa. The following effects were noted in quinine-sucrose mixtures: 1) When the taste of one component of a mixture is adapted, the other taste is released from suppression, i.e. returns to the level it would be perceived at when unmixed. This release is consistent with mechanisms of suppression central to the sites of adaptation. 2) A mixture fails to cross-adapt its components to the same degree as self-adaptation to those components (equimolar, unmixed). This incomplete adaptation is consistent with sites of suppressive mechanisms at or peripheral to the mechanisms giving rise to gustatory neural adaptation. A simple sequential model for the relative central-peripheral positions of mechanisms of suppression and adaptation is inadequate to reconcile these effects.

In contrast to quinine-sucrose mixtures, previous reports indicated that NaCl-sucrose mixtures did cross-adapt their components to the same degree as self-adaptation. The present results indicated that this was true for salt, but not sucrose. The two reports differed in flow rate, temperature and area of stimulation. Manipulating these variables showed that as flow rate and area are decreased, error variance in judgments increased, making results from mixture adaptation and self-adaptation conditions appear similar.

In spite of the difficulty composing a simple physiological model for suppression and adaptation, anterior tongue stimulation with mixtures produces orderly reliable effects, namely release from suppression, and incomplete adaptation to mixtures. These effects may change, however, in mixtures containing electrolytes.

Bitterness as a Potential Deterrent to Accidental Ingestions of Toxic Household Substances by Toddlers

Harry Lawless and Marc Corina, Monell Chemical Senses Center, and Larry Hammer, The Children's Hospital of Philadelphia, Philadelphia, PA 19104

Accidental ingestions of toxic household substances by preschool age children number in the millions annually. Given the wide variation in sensitivity to bitter substances, this study addressed the question of whether individual differences in bitterness perception might predispose some children to such ingestions. A second question of interest was the degree to which bitterness would influence the acceptability of ingestible stimuli and function as a deterrent to ingestion. The oral behaviors of 32 children aged 12 to 41 months were videotaped during 5 - 5 minute test periods with each of five lollipops. Pops were constructed of plexiglass rectangles which were coated with a mixture of sucrose, gum arabic, glycerin, food coloring and the bitter substance, sucrose octaacetate (SOA). Pops were presented in ascending order of SOA concentration, ranging from no SOA (sweet only) to approximately .001 M/l (very bitter). Behaviors measured included mouthing times, oral contacts and latencies to mouthing. 20 children had an episode of accidental ingestion requiring an emergency room visit within the last three months. 12 children were age-matched controls recruited from the general pediatric clinic of the same hospital. No differences were observed between ingesters and controls, except for a higher baseline of mouthing the first pop by controls (no SOA). With increasing SOA concentration, both groups reliably decreased their mouthing times and oral contacts with the pops. These decrements closely paralleled ratings of bitterness and unpleasantness by young adults. These results imply that 1) reactivity to bitterness is not a predisposing factor in ingestion episodes, 2) SOA has potential value as a deterrent to ingestion, and 3) taste perception of bitter substances by two year old children appears to be similar to that of adults.

Intranasal Photography And Its Uses in Clinical Nasal Studies

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The English physicist Hopkins, in the 1950's, developed a rod-lens system which heralded the development of high quality rigid and flexible endoscopes. When coupled with a versatile 35 mm camera, these instruments allow clear, accurate, and reproducible photographs to be made reliable. Measurements from a large cross section of noses are starting to define the previously unknown in vivo intranasal dimensions. Comparisons with data on nasal airflow (rhinomanometry) and olfactory testing show that the position and size of intranasal structures can be used to predict function or dysfunction. It has been clinically suspected that the distance between the anterior middle turbinate and the septum is important to good nasal breathing. Odor access to the olfactory area is also thought to proceed through this portal.

The purpose of this study was to explore the range of equipment and techniques available for intranasal photography, and to map the anterior nasal structures. The results of these studies will be reported, along with correlations to rhinomanometry and olfactory data. The results support the importance of the middle turbinate-septal area.

TOPOGRAPHIC RELATIONS ARE MAINTAINED IN THE PROJECTION FROM THE OLFACTORY EPITHELIUM TO THE BULB IN THE SALAMANDER.

Alan Mackay-Sim and Muriel H. Nathan. Department of Psychology, University of Wyoming and Department of Physiology, University of Pennsylvania.

Consideration of the physiological responses of the salamander olfactory epithelium strongly suggests that odorant quality is coded by differences in the topographic distribution of receptor cell responsivity: different odorants elicit maximal responses from different locations on the epithelium (Mackay-Sim, Shaman and Moulton, 1982), these locations do not vary with odorant concentration (Mackay-Sim and Shaman, 1982) and the locations of peak electrophysiological activity are also the locations of peak metabolic activity induced by odorant stimulation (Nathan and Moulton, 1982). If these topographic patterns of epithelial responsivity do provide a spatial code for odorant quality, then the question arises of whether topographic relations among epithelial regions are maintained in the projection from the epithelium to the bulb. If these relations are not maintained, then it is unlikely that the epithelial response patterns serve a coding function. The present study addresses this question. We injected .1 μ l radioactive leucine (3 H-Leu) into different regions of the dorsal and ventral epithelia of 12 salamanders. In each salamander one injection was made in each epithelium. After 24h the animals were perfused and their heads were embedded in paraffin and coronally sectioned at 10 μ m. Mounted, serial sections were dipped in Kodak NTB2 emulsion, exposed for 2-4wk, developed, and counterstained with cresyl violet. Each epithelial section was traced under a projection microscope (10X) and the injection site was mapped. Each bulbar section was photographed under light- and dark-field illumination (40X) and the region of axonal projection in the glomerular layer was mapped. The results show that topographic relations are maintained in the epithelium-to-bulb projection: the anterior-posterior axis of the epithelium is translated to the ventral-dorsal axis of the bulb and the medial-lateral axis of the epithelium is translated to the anterior-posterior axis of the bulb. The laterally and ventrally located vomeronasal organ projects to a discrete set of glomerulae at the posterior of the bulb. We conclude that topographic patterns of odorant-induced epithelial responsivity would be reflected in topographic patterns of glomerular activity in the olfactory bulbs.

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TOPOGRAPHIC CODING OF ODORANT QUALITY IS MAINTAINED AT DIFFERENT CONCENTRATIONS IN THE SALAMANDER EPITHELIUM. Alan Mackay-Sim and Paul Sheman. Department of Psychology, University of Wyoming and Department of Statistics, University of Pennsylvania.

When electro-olfactograms (EOGs) are recorded from man: sites distributed over the surface of the olfactory epithelium of the salamander, the relative amplitudes of these EOGs can be used to draw a "topographic map" of responses across the epithelial surface. For a particular odorant such a response map shows a peak of highest responsivity, with EOG amplitudes decreasing in all directions away from this peak. Recently it was shown that odorants may elicit unique patterns of responses which may differ both in the location of the peak response and in the slope of the decrement in responses around the peak (Mackay-Sim, Sheman and Moulton, 1982). We proposed that the differing response maps were generated by the distribution of receptor cells with differing response spectra: cells with similar responses are located in similar regions of the epithelium. We concluded that the olfactory epithelium probably conveys a spatial code of odorant quality. If this is so, then not only must different odorants elicit different response patterns, but these patterns should not vary with the concentration of a given odorant. The aim of the present study was to test this proposal. At each of 12 sites on the ventral epithelium of the salamander we recorded at least 3 EOGs to each of 3 concentrations of an odorant. In this way maps of responses were generated for 3 odorants (pinene, amyl acetate, propanol) at 3 concentrations. Each odorant was tested in 4 animals. The odorants, delivered in 1S pulses from an air-dilution olfactometer, were presented through 3 stimulators, one for each concentration, attached to the electrode holder. This arrangement eliminates physical variables in odorant flow which can independently vary the EOG. The results show that the epithelial location of peak responsivity does not vary with concentration. For each odorant, an increase in concentration led to increases in EOG amplitudes over the whole epithelium. This resulted in a decrease in the rate of EOG decrement away from the peak of highest responsivity. We conclude that receptor cells of different response spectra are not mixed randomly throughout the olfactory epithelium. Cells of similar responsivity tend to be grouped together. Consequently, the location of greatest receptor responsivity could a major factor in coding olfactory quality since different odorants elicit maximal responses from different epithelial locations which do not change with odorant concentration.

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TEMPORAL CODING DOES NOT ACCOUNT FOR SALT TASTE QUALITY DISCRIMINATION IN BLOWFLY. Frans W. Maes, Dept. Zool., State Univ. Groningen, Postbus 14, 9750 AA Haren, The Netherlands

Blowflies *Calliphora vicina* are able to discriminate between various chloride salts on the basis of taste quality, as was revealed by classical conditioning experiments [4]. Relative activities across "salt", "water" and "anion" receptors stimulated in the behavioral paradigm yield one possibility for the neural coding of these taste differences [3].

As an alternative, salt taste differences might be encoded in different time courses of the "salt" receptor response. The fine structure (spike interval distribution, serial correlations) of salt cell responses does not seem to depend on the kind of salt in the blowfly *Phormia regina* [1,7]. Therefore we restricted our study to the "global" time course. Some theories expect these to differ between stimuli [e.g. 2,6].

Sixteen to 20 labellar taste hairs of an individual fly were stimulated with a concentration series of one of the alkali chlorides (LiCl, RbCl, CsCl: 2 flies each; NaCl, KCl: 1 fly each), and a reference stimulus (1 M KCl). Times of occurrence of salt receptor spikes were measured with an "electronic ruler" (0.2 ms resolution), which digitized them for computer processing. Variability of individual responses prevented computer curve fitting, therefore a simpler analysis was performed. For each concentration and salt used, successive spike intervals were averaged for salt cells in A and B hairs separately [cf. 5], and from these mean time courses were reconstructed.

The mean time courses of the reference response fitted reasonably well into the arrays of time courses for the five salts. This indicates that the time course of the salt receptor response does not depend on the kind of alkali chloride.

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ASSESSMENT OF CONCENTRATION DISCRIMINATING POTENTIAL IN RAT NTS AND ITS RELEVANCE TO THE LABELED-LINE/CROSS-FIBER PATTERN ISSUE Frans W. Maes* & Robert P. Erickson, Department of Psychology, Duke University, Durham N.C. 27706

Judgement of stimulus intensity presumably is based on the response magnitude of receptors involved. This information should be retained at subsequent levels of neural integration. The differential threshold (DT), then, depends on the sensitivity of taste neurons to concentration changes, and is limited by their intrinsic response variability. Maes & Bijpost (1979, J. comp. Physiol. 133, 53-62) applied this idea to the discrimination of saltiness in the blowfly, using psychophysical signal detection theory to relate neural response with behavior. The DT calculated from salt cell recordings accounted for the discrimination ability found in classical conditioning experiments.

We propose an application of this approach to the rat's discrimination of concentrations of the four classical taste stimuli. This approach includes:

- 1) determination of behavioral DT's by operant conditioning;
- 2) electrophysiological recording from a representative sample of taste neurons involved in the behavioral paradigm;
- 3) estimation of the total number of such neurons present in rat. Surprisingly, data on 1) and 3) are lacking; the feasibility of obtaining these will be briefly discussed. Preliminary data on 2) will be presented. As the behavioral paradigm will stimulate taste receptors on the anterior and posterior tongue as well as on the palate, whole-mouth stimulation is used with the recordings. The recording site is the first neural level integrating these responses: the nucleus of the solitary tract (NTS). Repeated stimulation with concentration series will yield concentration dependence as well as variability of responses. Measures of response quantification will compare phasic and tonic parts of the response, and should fit in with discrimination time in the behavioral paradigm. Taking into account either those neurons responding best to a stimulus or all neurons activated, theoretical lower limits will be calculated for relative DT's under the labeled-line and the across-fiber pattern hypothesis, respectively. Awaiting data on 1) and 3), a comparison of these predictions with available (human) DT values might already favor one of these hypotheses.

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ODOR DETECTION THRESHOLDS IN THE RAT FOR THE VAPORS OF THREE RELATED PERFLUOROCARBONS AND ETHYLENE GLYCOL. David A. Marshall, Richard L. Doty, Daniel P. Lucero, & Burton M. Slotnick. Clinical Smell and Taste Research Center and the Department of Animal Biology, University of Pennsylvania; The Aerospace Corporation, Washington, D.C.; Department of Psychology, American University, Washington, D.C.

As a first step in ascertaining the feasibility of using laboratory rats for explosive detection, we evaluated their sensitivity to vapor-phase concentrations of the explosive ethylene glycol dinitrate (EGDN) and three homologous perfluorocarbons, perfluoromethylcyclohexane (PMCH), perfluorodimethylcyclohexane (PDCH) and perfluorodecalin (PFD). These perfluorocarbons are under evaluation by the Bureau of Alcohol, Tobacco and Firearms of the United States Department of the Treasury as potential "explosive taggants" -- substances added to explosives which identify their source of manufacture or distribution. The mean detection thresholds of these compounds, as determined in an operant discrimination task using water reinforcement, were as follows: EGDN -- .053 ppm (range .045-.060); PDCH -- 1.4 ppm (range .60-6.50); PMCH -- 2.1 ppm (range 1.85-4.65); and PFD -- 1.1 ppm (range .60-1.69). This study provides the first quantitative data on the olfactory sensitivity of rats to the vapors of explosive/taggant compounds and suggests that the detection curves for such odorants are fundamentally similar to those of other stimuli that have been evaluated in previous work.

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SOURCE OF BIOLOGICALLY SIGNIFICANT ODORS IN CAVY (*C. aperea*) PERINEAL GLAND SECRETIONS. I.G. Martin, J.M. Zechman, J.L. Wellington, and G.K. Beauchamp. Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Male wild cavies (*Cavia aperea*) are thought to use perineal gland secretions as a conspecific signal, possibly as a territorial marker. A specific behavior, the perineal drag (mark) serves to place this secretion on a substrate. Dominant animals mark more frequently and have more productive glands than do subordinate animals. We have previously shown that male cavies can distinguish between the perineal secretions of different individuals and that perineal secretions which had been squeezed directly from the gland (and therefore not mixed with urine) are less attractive to conspecifics than the secretions normally found in the perineal sac. We now have evidence that (1) urine and (2) bacterial activity are involved in this difference in attractiveness of the two forms of the secretions.

(1) To evaluate individual differences in odors from urine and perineal gland secretions a variant of a habituation procedure was used. Previous studies have demonstrated that when an animal is habituated to urine from an individual (A) and is then given a choice between urine from individuals A vs B, urine from B is preferred. The same is true if perineal gland secretions are used. Male cavies were habituated to the urine of individual A; they then did not differentiate between perineal secretions of individuals A and B. However, after these cavies had been habituated to the perineal secretion of individual A, they showed a significant preference of the urine of individual B over that of individual A. These results suggest that normal perineal secretions found in the perineal sac are mixed with urine.

(2) Microbiological examinations of accumulated and freshly squeezed perineal gland secretions each revealed approximately 10^6 colony forming units per gram of secretion of both aerobic and anaerobic Gram positive bacteria. The accumulated secretion exhibited a wider variety of bacterial types, however. Freshly squeezed perineal gland secretion which had been incubated 48 hr at 37°C elicited significantly more investigation by male cavies than did freshly squeezed secretions which had been frozen at -60°C for an equal duration. These results indicate that bacteria play an important role in the production of biologically important odors by the male cavy.

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FACTORS INFLUENCING EXPRESSION OF CONDITIONED FLAVOR AVERSIONS IN GROOMING. J. Russell Mason and Russell F. Reidinger, Jr., Monell Chemical Senses Center, Philadelphia, PA 19104. Second author is a Biologist for the U.S. Fish and Wildlife Service.

Rats typically show conditioned flavor aversions to substances associated with illness. Such aversions are observable when the flavor is presented in food or water but allogrooming continues when an aversive material is applied to the animals' flanks. Flavor aversions are formed during allogrooming, however, and are robust and transfer from grooming to other contexts, such as drinking.

Like rats, voles continue to allogroom when presented with an aversive flavor on the fur, even though aversions are expressed toward the flavor in drinking water. Given the stereotypy of grooming in other contexts, one interpretation of such results is that voles are predisposed to groom whenever peripheral irritants are applied. That interpretation can be questioned, however, because important issues remain unaddressed. Such issues include: (a) whether conditioned flavor aversions would be expressed in special contexts (e.g., social or heterogrooming) if not in allogrooming; and (b) whether allogrooming of flavors associated with illness reflects perseverance in behavior as a consequence of arousal rather than an "instinctive" predisposition to groom *per se*. Here, we report experiments in which voles were given conditioned aversions to saccharin while drinking and subsequently presented with saccharin on their own fur or on the fur of a cagemate. Voles expressed conditioned flavor aversions during heterogrooming of a cagemate but not during allogrooming of self. From analyses of trunk blood collected from voles after allo- or heterogrooming, we suggest that the failure to express conditioned flavor aversions during allogrooming is associated with high levels of corticosterone, a hormone implicated in behavioral arousal.

The findings provide a theoretical explanation of the successful use of grooming behavior to increase ingestion of unpalatable toxicants, as when tracking dusts and powders are employed. The findings also indicate that grooming in the presence of an aversive taste may be a robust phenomenon (aversive flavor elicits corticosteroid release, eliciting further grooming) inefficiently exploited with current practices in rodent control.

CONDITIONED ODOR AVERSIONS IN STARLINGS MEDIATED BY THE TRIGEMINAL SYSTEM. J. Russell Mason and Wayne L. Silver, Monell Chemical Senses Center, Philadelphia, PA 19104.

Electrophysiological and behavioral evidence implicate the trigeminal system in odorant detection. However, trigeminal contributions to detection of and discrimination between odorants are usually considered secondary to olfactory contributions. Here, we report three experiments taken to show conditioned aversion learning to 2-phenyl ethanol, apparently mediated by trigeminal rather than olfactory cues. In Experiment 1, starlings were tested for unlearned aversions to 2-phenyl ethanol associated with a food sample in 2-choice preference tests. No aversions (or preferences) were observed. In Experiment 2, starlings were presented food paired with 2-phenyl ethanol followed by an intubation of methiocarb (.2g/kg), a bird repellent. These birds showed conditioned aversions for food paired with 2-phenyl ethanol in 2-choice preference tests. In Experiment 3, the starlings were given conditioned aversions to 2-phenyl ethanol, tested for expression of the aversions and then subjected to bilateral olfactory nerve cuts or sham surgeries. Nerve cuts and sham surgeries had no effect on expression of aversions. However, when the external and internal nares were plugged bilaterally with denture adhesive and cyanoacrylate, the aversions dissipated. Removal of the nares plugs reinstated expression of the aversions.

Because birds have no vomeronasal system, we infer that expression of aversions by birds given olfactory nerve cuts was mediated by the trigeminal system. Moreover, because the nerve cuts had no observable effect on expression of aversions, we suggest that trigeminal, rather than olfactory cues were mainly responsible for the aversion learning by intact birds.

Salt Taste Responsiveness and Preference Among Normotensive, Pre-Hypertensive and Hypertensive Adults. Mattes R.D., Kumanyika S.K., Halpern B.P., Cornell University, Department of Nutritional Sciences, Ithaca, New York, 14853.

The evidence linking salt intake to blood pressure levels, as well as reports of altered salt appetite and taste sensitivity and preference among treated and untreated hypertensives prompted the assessment of salt taste responsiveness and preference as potential predictive markers for hypertension (HT). Based upon blood pressure (BP), family history, relative weight, heart rate and salt intake criteria, 35 normotensive (BP \leq 120/80mmHg & no HT risk factors), 35 pre-hypertensive (positive family history & at least one additional HT risk factor) and 17 essential hypertensive (BP \geq 165/95mmHg) 21-60 year old white male and female hospital employees were recruited. Taste responsiveness was assessed by a magnitude estimation procedure and line scale response form. Five concentrations of model saline solutions (0.65-2.09%Na w/w) and a sweetened cherry flavored beverage (6.89-17.63% sucrose w/w) were sampled by the former procedure while food systems (salted tomato juice (0.24, 0.64, 1.04%Na w/w), salted rice (0.88, 2.60, 4.26%Na w/w) and sweetened cherry flavored beverage (6.89, 12.59, 17.63% sucrose w/w) were employed with the latter method. Measures of taste preference included line scale ratings of the food systems, as well as ad lib salt intake of a low sodium tomato juice sample and ratings for sodium dense foods on a food action rating scale. Sodium intake was determined by a food frequency questionnaire and composite intake score, which included the contribution of food-borne and supplemental (i.e. medication, water softeners, discretionary salt use) sodium sources. Evaluations of sweet responsiveness and preference were included as control measures. After controlling for the effects of relative weight, smoking, sex, age and use of oral contraceptive agents, statistical analyses did not allow the groups to be distinguished based upon their responses to any of the taste or intake measures employed. Validity of the findings is attested to by the observations that: (1) magnitude estimation power functions were consistent with others cited in the literature; (2) food models receiving the highest preference ratings and median ad lib salt intake concentrations were similar to those of commercially marketed products; and (3) the generated composite sodium intake values resembled estimated typical American intake levels. Thus, the taste parameters assessed did not appear to be of predictive value for hypertension in this study population. In addition, correlations among the taste measures in the total sample were generally of a low order of magnitude, indicating that generalizations across parameters are tenuous.

BEHAVIORAL BIOASSAY AS A SENSITIVE INDICATOR OF SUBLETHAL EFFECTS OF MARINE POLLUTANTS, Carl Merrill, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Chemically sensitive receptor organ cells of marine animals are exposed to seawater and to dissolved or suspended pollutants. Anthropogenic input that interferes with chemoreception (eliciting new responses or depressing expected responses) is a very real threat to marine organisms.

I have modified a behavioral bioassay that measures chemoreception by behavioral response of juvenile oyster drills, *Urosalpinx cinerea* (Say), and am studying the potential of this assay for monitoring pollutants. Benefits of the assay include: 1) test of a behavioral response of a complex organism; 2) testing of 50 or more individuals per exposure; 3) a ten minute assay duration; 4) availability of standard stimulus; 5) small volumes of homogeneous solutions for tests; 6) minimal generation of toxic wastes and exposure of test personnel; 7) low cost.

Preliminary tests using the assay have demonstrated sensitivity to naphthalene at ppb concentration and to saturated solutions of anthracene in seawater. This work is supported by a University of Delaware UDRF grant.

FLUORESCENT STUDIES OF THE FROG'S OLFACTORY EPITHELIUM. Daniel B. Michael and Thomas V. Getchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

The cellular uptake and distribution of the fluorescent dye, Procion M-4RAN, has been studied in the olfactory epithelium of control and olfactory nerve sectioned frogs. The general techniques were modified from studies on fish olfactory epithelium (Holl, A., Stain Technology, 56: 67, 1981) and monkey retina (Monasterio *et al.*, Science, 213: 1278, 1981). The frog's internal naris was plugged and the superficial tissue covering the dorsal aspect of the nasal sac was dissected away. Approximately 500 μ l of the solution was injected into the nasal sac and remained in contact with sensory epithelium for 40 minutes. The epithelium was then irrigated with approximately 3 ml of Ringer's solution to remove excess dye. Compound olfactory nerve potentials were elicited in response to electrical stimulation of the olfactory nerve and were recorded from fascicles of receptor cell axons which coursed in the lamina propria of the dorsal olfactory epithelium prior and subsequent to the application of the dye. It appeared that the dye was not toxic to the receptor neurons for they were capable of initiating and transmitting action potentials subsequent to exposure to or uptake of the dye. Following further incubation at 4°C for one hour the frog was perfused transcardially with fixative. The olfactory nasal sacs, nerves and bulbs were removed en block and prepared histologically for fluorescent microscopy. In control tissue, the dye was preferentially taken up by olfactory receptor neurons and to a much lesser extent by sustentacular cells, the cells of the ducts of Bowman's gland and cellular elements in the lamina propria. Typically, the entire receptor neuron was stained. The distribution of Procion-labeled neurons was not uniform throughout the sensory epithelium. But rather, assuming equal access of the dye to all "mature" receptor neurons, the dye was taken up by single neurons or small clusters of neurons. The reason for the apparent selectivity is not known, but may be related to the age of the receptor neuron, its metabolic state or functional activity at the time of exposure. 10 days following olfactory nerve section, the sensory epithelium had undergone marked histological changes. In contrast to control tissue, most of the sustentacular cells were brightly fluorescent suggesting that the properties of these cells had changed as a result of neuronal axotomy. These preliminary results suggest that the use of Procion and other fluorescent dyes may be useful in labeling specific cells in the olfactory epithelium and studying transcellular events associated with neuronal degeneration. Supported by NIH grant NS-16340.

INFLUENCE OF SOLVENT PROPERTIES ON MAGNITUDES OF THE TASTE RESPONSE. Inglis J. Miller, Jr., Dept of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103

The magnitude of taste responses to electrolytes is known to vary with the cation, anion, solute concentration and animal species. Recent studies with deuterium oxide in place of ordinary water show that slight changes in the physical properties of the solvent exert an influence on the taste response. This report examines the effect of water-dioxane mixtures on the electrophysiological response measured from the chorda tympani nerve of the rat to salts. Physical properties of water-dioxane mixtures have been determined by others in the study of solvent effects on the chemical activity of salts. Concentrations of dioxane in water of 1, 3 and 10% (vol:vol) diminish the background response of the nerve, while 30% exerts a slight increase in the response to a water-adapted tongue. Responses to Cl^- salts of Na^+ , K^+ and Ca^{++} are diminished by up to 25% in the range of concentration from .01 to .1 M with dioxane concentrations of 3 and 10%, while the response to 0.3 M salts and 30% dioxane mixtures are variable. The difference in responses between water and water-dioxane mixtures varies with the cations: Na^+ responses are decreased less than Ca^{++} , while the solvent mixtures exert the greatest effect on K^+ . These results and the effect of deuterium oxide lead to a consideration of the state of water in the taste stimulus in terms of its dielectric constant which reflects the degree of polarization in an electric field. The free energy of the stimulus to react with a charged receptor membrane is related to this property of the solvent medium. Dielectric properties of the solvent may differ by as much as 20 fold between the bulk phase and the interface with the receptor due to the presence of hydrophobic molecules in the membrane. Under equivalent conditions the dielectric constant of deuterium oxide is lower than for ordinary water. Solute ions lower the dielectric constant of water by a magnitude which is inversely related to the size of the ion. Examination of the dielectric constant of the solvent offers some insight into such taste phenomena as the water response, the disparate effect of ionic species, and the lack of additivity among responses to stimulus mixtures.

DEVELOPMENTAL CHANGES IN SALT TASTE RESPONSES FROM SHEEP GLOSSOPHARYNGEAL NERVE. C.M. Mistretta and R.M. Bradley. Dept. Oral Biol., S. Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Salt taste responses recorded from the chorda tympani nerve during chemical stimulation of the anterior tongue change developmentally in sheep. To learn whether developmental changes are also observed when taste buds in circumvallate papillae on the posterior tongue are stimulated, we have recorded from the glossopharyngeal nerve. Multifiber responses were recorded during stimulation with 0.5M NH_4Cl , KCl , NaCl and LiCl in five age groups of animals: 7 fetuses at about 110 days of gestation (term = 147 days); 3 fetuses at about 130 days; 8 perinatal animals aged 142 days of gestation to 7 days postnatal; 3 lambs aged 30-40 days postnatal; 6 adult ewes aged 2-4 years. To compare response magnitudes among different animals in various age groups, we calculated a ratio of every response relative to a standard, frequently applied stimulus, 0.5M NH_4Cl . Mean ratios for each salt were then compared among age groups.

Developmental changes, at statistically significant levels ($p < 0.05$), were observed in salt taste responses from the posterior tongue, but the changes are different than those from the anterior tongue. For example, for the anterior tongue there is a marked increase in the relative stimulating effectiveness of NaCl and LiCl throughout pre- and post-natal development. In contrast, glossopharyngeal nerve responses to posterior tongue stimulation with NaCl and LiCl increase only slightly, relative to NH_4Cl . For the anterior tongue, responses to KCl decrease relative to NH_4Cl but the change is comparatively small. KCl responses from the posterior tongue also decrease in magnitude, but the change is much greater than for the anterior tongue. The extent of the changes from the youngest to the oldest age group are summarized in this table:

	RESPONSE RATIOS							
	CHORDA				GLOSSOPHARYNGEAL			
	NH_4	K	Na	Li	NH_4	K	Na	Li
110 day fetus	1.0	0.8	0.2	0.2	1.0	2.0	0.3	0.2
adult	1.0	0.6	1.0	1.0	1.0	0.7	0.4	0.5

Thus, for taste buds in very different anatomical arrangements and locations on the tongue, developmental changes occur in salt taste responses. But our preliminary results indicate that the changes are dissimilar for the anterior and posterior tongue. These results predict that the various membrane components interacting with salts are present in different proportions in fungiform and circumvallate taste buds at different times in development. (Supported by N.S.F. Grant BNS80-15737 and Research Career Development Award, N.I.H., N.I.D.R., DE-00066 to C.M.M.)

THE ULTRASTRUCTURE OF THE NASAL MUCOSA IN MAN: RESPIRATORY AND OLFACTORY EPITHELIUM. David T. Moran, J. Carter Rowley III, and Bruce W. Jafek*, Departments of Anatomy and Otolaryngology*, University of Colorado School of Medicine, Denver, Colorado.

We have designed a special tool that permits safe removal of small biopsies of fresh olfactory mucosa from humans under local anaesthesia and have completed a study of the fine structure of respiratory and olfactory epithelium. Respiratory epithelium, a ciliated pseudostratified columnar epithelium, contains ciliated cells, mucus-secreting goblet cells, and basal cells. Olfactory epithelium, also a pseudo-stratified columnar epithelium, contains ciliated olfactory receptors, supporting cells, basal cells, and microvillar cells. The ciliated olfactory receptors resemble those in other mammals; that is, the axon travels toward the lamina propria, and the dendrite extends toward the epithelial surface. The dendrite terminal is an olfactory vesicle that gives rise to 10-30 radially arranged cilia. The proximal part of olfactory cilia have a "9+2" axoneme that lacks dynein arms. Since dynein arms provide the motive force for the active stroke in motile cilia, it seems likely that human olfactory cilia are immotile. The supporting cells in olfactory epithelium are very different from the goblet cells of respiratory epithelium. They are not specialized for mucus secretion. Their ultrastructure suggests they may participate in uptake and degradation of materials--perhaps including odorant molecules--from the overlying mucus layer. The microvillar cells are of particular interest. Their superficially located flask-shaped cell bodies sport an apical tuft of microvilli that projects into the surface mucus. The basal pole of the cell gives rise to a single long thin, cytoplasmic process that resembles an axon. If the microvillar cells prove to be receptors, as their ultrastructure suggests they may be, we will have to revise our concept of the human olfactory epithelium to include two morphologically distinct populations of olfactory receptor cells. Supported by NIH NS-15203 and NSF BNS 77-03317.

DIFFERENTIAL DIAGNOSIS IN ACUTE RENAL FAILURE: EVALUATION OF A "SNIFF" TEST. Claire Murphy and Jack S. Madowitz, Monell Chemical Senses Center, Philadelphia, PA 19104 and Temple University Hospital, Philadelphia, PA 19140

Rapid deterioration in renal function can be caused by several factors, among them prerenal azotemia and acute tubular necrosis (ATN). With prompt diagnosis and intervention, the former is a potentially reversible condition. Recently it has been reported that urine from patients with ATN is odorless and that a sniff of this urine resembles a sniff of stale water, in contrast to the urine of patients with prerenal azotemia which produces the disagreeable smell of concentrated urinary chemicals. Hence, the use of a "sniff" test has been recommended as a quick, cost-free, readily available, apparently accurate method that should be used in the differential diagnosis of acute renal failure (Najarian, J. S., New Eng. J. Med., 1980, 303: 1128). The following experiment was designed to determine whether or not naive observers could distinguish among urine from normal controls, from patients with ATN, from patients with prerenal azotemia, and deionized water. Urine from 9 adults (3 with ATN, 3 with prerenal azotemia and 3 normal controls) was collected and frozen at -60°C until testing. Samples were defrosted on the day of testing. Twenty ml of each were placed in separate glass specimen jars with caps for equilibration. In separate sessions, each of 10 blindfolded observers sniffed all stimuli twice, in random order, with a 30 second intertrial interval. They judged odor intensity using the method of magnitude estimation with a modulus of 10. The observers were unable to differentiate urine samples from donors with ATN, from those with prerenal azotemia or from those with normal renal function, on the basis of odor intensity alone. The Friedman ANOVA showed no difference in odor intensity among the three categories of urine samples ($\chi^2=3.05$, n.s.). However, the observers had no difficulty distinguishing urine samples from water solely on the basis of odor intensity. A second ANOVA demonstrated a significant difference when the odor intensities of the 3 categories of urine samples were compared with the odor magnitude of deionized water ($\chi^2=19.83$, $p<.001$). It can be concluded that a "sniff" test of intensity is insufficient to distinguish urine excreted from patients with ATN from urine excreted by patients with prerenal azotemia, and that including such a test would hardly serve to clarify the clinical picture.

A COMPUTERIZED APPARATUS FOR DETERMINING THE OLFACTORY PROWESS OF SMALL ANIMALS. O'Connell, Robert J., Walker, James C., Marques, David M. and Parker, Steven. The Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545

One classical technique for determining the normal function of a neural system is to damage it and then to look for deficits in its output. This technique is often used in the olfactory system to determine the role olfaction plays in various social behaviors. It is therefore important that careful, rapid and precise evaluations of olfactory capabilities be routinely applied to individuals both before and after olfactory lesions. We have developed a fully automated apparatus modified after devices originally described by Moulton, Slonick and Eichenbaum, in which mice and hamsters readily learn to discriminate amongst odors. Adjusting odor quality and quantity allows one to devise tests with various degrees of difficulty. The apparatus consists of a small (25 cm on a side) plexiglass box with a horizontal photobeam near the floor in the back of the box and a small guillotine door in the front wall. Just in front of this door is a floor mounted vertical photobeam and the spout of a liquid dispenser. In order to start a trial, water deprived animals are required to break the back photobeam. This activates a valve which delivers the selected stimulus to an odor port located behind the door. After a 3 second delay to allow the odor port to come to equilibrium the door opens allowing the animal access to the odor port. A vacuum purging system prevents odors from leaking out of the port into the box. Once the door is opened the animal is required to break an antidual photobeam in the odor port positioned so that the animal's external nares are located in the odor plume. After a time which insures that a sniff has been taken, the animal signals his decision about the stimulus either by pushing further into the odor port breaking a back response photobeam (R+) or if S- was presented by moving back away from the door far enough to illuminate the vertical photobeam (1.5 cm). All correct responses are rewarded with 0.1M sucrose (0.02 cc for mice, 0.04 cc for hamsters) and signaled by a tone and light flash. A new trial can be initiated immediately after reinforcement. Errors of both types are punished by an enforced time out period during which all lights are turned off and new trials cannot be initiated. The amount of operator time involved in using this apparatus has been minimized by relegating all computational, timing, shaping and contingency parameters to a dedicated microcomputer. Details concerning the training and olfactory capabilities of mice and hamsters will be presented.

CHARACTERIZATION OF OLFACTORY FIBER BUNDLES IN THE LAMINA PROPRIA OF SALAMANDER OLFACTORY EPITHELIUM. Thomas E. O'Hara, Jr., Jose A. Rafols, and Thomas V. Getchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

The axons of olfactory receptor neurons in the salamander (*Ambystoma tigrinum*) penetrate the basal lamina of the olfactory epithelium, then travel in bundles for various distances in the lamina propria before gathering to form the olfactory nerve near the olfactory bulb. The characteristics of the olfactory fiber bundles within the lamina propria were investigated using anatomical and electrophysiological techniques.

Anatomical studies were performed utilizing light and electron microscopy, with light microscopic examination of horseradish peroxidase stained receptor neurons in several of the animals. The olfactory axons were unmyelinated, less than 0.5 microns in diameter, and followed a tortuous, wavy course in the fiber bundles. Large groups of axons were ensheathed by processes of Schwann cells and strands of collagen. The Schwann cell nuclei were located among the fiber bundles and were cylindrical in shape, measuring 32.6 ± 3.9 microns by 4.7 ± 0.7 microns ($n=13$). Schwann cell nuclei were orientated with their long axis parallel to the long axis of the bundle and contained heterochromatin in large clumps along the inner aspect of the nuclear membrane. The axons and Schwann cells were surrounded by a perineurium consisting of collagen and fibroblasts. The nuclei of the fibroblasts were distinguished from those of the Schwann cells by their peripheral location in the fiber bundles, the irregular orientation of their nuclei in relation to the fiber bundles, and the smaller clumps of heterochromatin around the inner nuclear membrane. Cells resembling histiocytes, particularly in the structure of the cytoplasmic granules which contain a dense cylindrical core, are occasionally found in the perineurium. Pigmented cells resembling melanocytes send out large processes which ramify into smaller processes terminating in apposition to the perineurium of the larger nerve bundles in the lamina propria.

Electrophysiological studies of the olfactory fiber bundles in the lamina propria were performed utilizing antidromic stimulation by placing a stimulating electrode on the olfactory nerve near the olfactory bulb and a platinum-black metal filled recording electrode on the fiber bundle. Five nerve bundles in five different animals were studied. Compound olfactory nerve potentials had a triphasic waveform and exhibited a systematic increase in amplitude with increase in shock intensity. Onset latencies ranged from 10 to 16 msec with a mean latency of 14 ± 3 msec. The mean recording distance was 2.4 ± 0.5 mm. The onset latencies remained constant over a wide range of stimulus intensities. The mean conduction velocity was 0.18 ± 0.05 m/sec. Cortical evoked potentials were obtained in the olfactory bulb with stimulation of the nerve bundles. (Supported by NIH Grant NS 16340.)

SIMULTANEOUS RECORDING OF TASTE INTENSITY AND PAROTID FLOW IN RESPONSE TO SALT AND TO ACID STIMULI

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While rinsing the mouth with 10-ml samples of salt or acid solutions, experienced subjects recorded perceived gustatory intensity directly onto a moving chart--the time-intensity method (T-I). Corresponding unilateral parotid saliva was collected via a suction cup and recorded by a gravimetric sialometer.

EXP. I. Ten male and ten female subjects evaluated solutions of 0, 0.1, 0.2, and 0.3 M monosodium glutamate (MSG), sodium chloride (NaCl) and 1:1 mixtures of the two, dispersed in distilled water, and in unsalted chicken and beef broths.

In all media, NaCl >mix> MSG for both T-I and salivary flow. Saltiness decreased quickly upon expectation of samples (at 20 sec), especially for NaCl. T-I patterns were almost identical for NaCl in the three media. MSG, however, was much less intense in chicken than in beef broth. Continuous oral rinsing for 12 min. for all media produced a constant rate of parotid flow with no evidence of adaptation.

EXP. II. Twenty subjects evaluated distilled water solutions of binary mixtures of tartaric, citric, and fumaric acids. In one trial, the dominant anion was varied at a constant pH (3.5), and a constant TiA (4 g/L as tartaric). Maximum sourness intensity correlated with maximum parotid flow: $r = 0.955$, $p < 0.05$, 2 df). Samples with citric acid as the dominant anion were less sour and elicited less saliva than samples with tartaric or fumaric acids as the dominant anion.

In a second trial, either the pH was constant (3.5) with a variable TiA (3.7, 4.0, 4.3, 4.6 g/L) or the TiA was constant (4.0 g/L) with a variable pH (3.0, 3.25, 3.5, 3.75). The dominant anion was constant with tartaric dominating in tartaric-fumaric binary mixtures. Maximum parotid flow correlated with maximum sourness ($r = 0.879$, $p < 0.01$, 6 df). Those stimuli with the lowest pH and the highest TiA had greater sourness and elicited more saliva. Salivary Na⁺ was highly correlated with flow ($r = 0.924$, $p < 0.001$, 6 df).

COLOR MODIFICATION OF ODOR/FLAVOR PERCEPTION IN MICROENCAPSULATED ODORS, AQUEOUS SOLUTIONS, AND MILK DRINKS

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Untrained subjects were asked to (1) identify the odor (or flavor) of food-type odorants; (2) indicate perceived odor/ flavor intensity; and (3) indicate degree of liking using nine-point category scales.

EXP. I. Odor of microencapsulated fragrances (MF). 100 subjects evaluated MF strips with the odors of vanilla, orange, mint, strawberry, grape, chocolate, anise, and a control (diethylphthalate) mounted on 3" X 4" cards with seven background colors: creamy white, orange, red, green, purple, brown, black, plus a blank. There were no significant color-odor interactions for hedonic or identification responses. For intensity, however, vanilla was perceived to be stronger on the blank and on red; mint on the blank; grape on brown, and anise on white. There was a correlation between intensity and hedonic responses ($r = 0.758$, $p < 0.05$, 6 df).

EXP. II. Odor and flavor of colored-flavored solutions. 55 subjects tested solutions of 10% sucrose and 0.1% citric acid flavored with orange, mint, strawberry, grape, chocolate, anise, and unflavored, each colored orange, green, red, purple, brown, black, and uncolored. In general, more correct identifications were obtained when solutions were appropriately-colored. Relative hedonic responses were similar between tasting and sniffing trials across flavorants. Perceived intensity, however, produced a rank order which differed from that when stimuli were evaluated by mouth. Again, hedonic and intensity responses were correlated, especially for the odor tests ($r = 0.806$, $p < 0.05$, 5 df).

EXP. III. Flavor of colored-flavored-sweetened milk drinks. 50 subjects tested sweetened milk flavored with vanilla, orange, mint, banana, strawberry, chocolate, and maple, when samples were uncolored, when miscolored, and when conventionally-colored. There was a significant color X flavor interaction in all measurements. Hedonic responses were highest when samples were correctly colored and lowest when color was masked. Vanilla, chocolate and mint were easiest to identify. Increasing intensity of the correct color in flavored milks did not increase responses.

A 2-DG STUDY OF BEHAVIORAL PLASTICITY IN ODOR DEPENDENT SUCKLING, Pedersen, F.E., Greer, C.A., Stewart, W.B., and Shepherd, G.M., Secs. of Neuroanat., Neurosurg., and Gross Anat., Yale U. School of Medicine, New Haven, Ct. 06510

The 2DG method, used to study the functional development of the olfactory system, has provided evidence that a modified glomerular complex (MGC) at the medial junction of the main and accessory bulb mediates the specific olfactory cue used by neonatal rats to attach to their dam's nipples. Recent experiments have demonstrated that a novel odor can elicit the first nipple attachment by virtue of its exposure to the fetus during the last few days of gestation and the first hour of postnatal stimulation. This apparent behavioral plasticity led us to investigate the hypothesis that alteration of the cue for the first nipple attachment subsequently induces specific patterns of glucose uptake in the same glomerular area of the bulb that has been implicated in suckling in untreated newborns.

Experimental pups received injections of citral into their respective amniotic sacs on Day 20 of gestation and were delivered by caesarean section on Day 22. In order to simulate maternal licking, pups were stroked with an artist's brush for 1 hour in the presence of citral. Control pups were treated identically in the absence of citral. All pups then received an i.p. injection of 14C-2DG (200 μ Ci/kg) prior to being placed in 1 of 3 conditions: (1) controlled exposure to citral or (2) to room air, or (3) placement in contact with nipples of an anesthetized dam cleaned of olfactory cues and scented with citral. Olfactory bulbs from each pup were prepared for analyses with autoradiographic methods.

Extensive areas of increased 2DG uptake occurred in caudomedial regions of the bulb following exposure to citral either in the controlled odor environment or on the dam in the suckling situation regardless of whether pups had prior experience with citral. These areas of activity were not seen in pups exposed to room air. Correlations with histological sections indicated that these areas of localized uptake did not correspond to the MGC, even in those pups that suckled citral-scented nipples. The patterns of activity were, however, different from those previously reported for 0-day pups exposed to amyl acetate. These findings are consistent with the idea that the MGC is associated with the specific suckling odor and that neural processing of novel odors that elicit attachment does not primarily depend on this complex. Studies are presently aimed at identifying possible differences in 2DG patterns in more central olfactory regions in relation to pups' experiences with odors.

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SPECIFIC INHIBITION OF THE EOG OF ISO-VALERIC ACID AND RELATED "SWEATY" ODORANTS IN THE RAT. E. Polak, S. Shirley, and G. Dodd. Dept. of Chemistry University of Warwick, Coventry CV4 7AL, England.

Chemical modification of the olfactory epithelium with group reactive reagents aims at selectively blocking specific receptors. Several reagents used previously with a variety of odorants tended to give effects that were insufficiently specific to be readily exploitable. We have found that treatment of rat turbinates with the lectin Concanavalin A has a selective effect on the EOG response of short chain fatty acids. The treatment abolishes most of the EOG response of iso-valeric acid relative to the EOG's of 8 non-acid odorants tested so far. Four more 3-4 carbon chain acids respond similarly. These acids all share a "sweaty" odor characteristic and specific anosmia for humans. Longer chain acids such as n-valeric and n-hexanoic acids are much less affected by Con A. Lectins are known to bind specifically to certain sugar residues of glyco-proteins or glycolipids found in cell membranes. Hence it is possible that an acid specific receptor cell membrane becomes inactivated by formation of a glyco-molecule Con A complex.

NEURONS IN OLFACTORY CORTEX PROJECT TO TWO PRINCIPAL THALAMIC NUCLEI

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In 1965 Powell, Cowan and Raisman reported that there is an olfactory input to both "mediodorsal and medioventral" thalamic nuclei, via the piriform cortex in the rat. The projection to the mediodorsal nucleus is now well established but there has been relatively little evidence for an olfactory projection into the ventromedial nuclear complex of the thalamus.

In the present study olfactory projections have been identified both to the mediodorsal thalamic nucleus (MD) and to a component of the ventromedial nuclear complex, the submedial nucleus (Sm) in the rat. Injections of 3-12 nl of .25%-5% HRP conjugated to wheat germ agglutinin (HRP-WGA) into these nuclei retrogradely labeled cells scattered in a band deep to layer III of the olfactory cortex. The cells which project to MD are widely distributed deep to the piriform cortex (in the ventral endopiriform nucleus), the olfactory tubercle (in the polymorph zone) and the periamygdaloid cortex. Cells which project to Sm are found primarily in a smaller area deep to the lateral part of the olfactory tubercle and the adjacent medial edge of the piriform cortex. Injections of ³H-leucine into these areas anterogradely label fibers to the medial and central parts of MD and the anteroventral part of Sm, but do not label fiber to other parts of the thalamus.

In parallel physiological experiments we have also found that electrical stimulation of the ipsilateral olfactory bulb evokes short latency (<15msec) unit responses in both the central part of MD and the anteroventral part of Sm. Stimulation of the contralateral olfactory bulb produced longer latency (~20msec) responses. Unit responses to olfactory bulb stimulation were not found lateral or caudal to these parts of MD or Sm.

These results indicate that restricted parts of two thalamic nuclei receive olfactory afferents from the olfactory cortex. Furthermore, analysis of anterograde and retrograde HRP-WGA transport to the neocortex in these experiments confirms previous reports that these parts of MD and Sm are interconnected with separate but adjacent areas of the orbital and anterior insular cortex.

RECOGNITION MEMORY FOR ODORS: THE ROLE OF PERCEPTUAL IDENTITY.

Michael D. Rabin & William S. Cain, John B. Pierce Fndn. Lab. & Yale Univ., 290 Congress Ave., New Haven, CT 06519.

The investigation dealt with recognition memory for odors and with the specific question: Is the ability to recognize the previous occurrence of an odor related to a person's previous knowledge, complete or incomplete, of the identity of the odor? For example, does the knowledge that the identity of a specific odor is pine shavings make the occurrence of the odor more recognizable when it is presented subsequently among a battery of distractors? Intuition would suggest that knowledge of identity should assist simple recognition. Previous experiments imply that knowledge of identity actually plays no such role. Our experiment suggests otherwise and thereby agrees with the intuitively based conclusion that recognition memory does indeed take advantage of prior knowledge.

We tested 45 participants in the following way: A participant smelled 20 everyday products (e.g., banana, pine shavings), rated their familiarity, and sought to name them. The person then sought to recognize the target odors when presented amongst a group of 20 distractor odors. The interval between initial inspection and the memory test was 10 min, 1 day or 1 week. At the end of the recognition task the participant sought to name all of the odors, both the distractors and the test odors. If recognition memory operated independently of an odor's identifiability to the participant, then ability to name the odor initially or to name it consistently in the inspection and recognition periods should have played no role in recognition performance.

Results of the experiment indicated a positive relationship between a) initial label quality and subsequent recognition performance and b) consistent use of labels and subsequent recognition performance. When identified appropriately or consistently, an object proved much easier to recognize than when identified inappropriately or inconsistently. Nevertheless, the advantage gained from appropriate or consistent naming weakened with increases in the retention interval. Throughout the experiment, participants exhibited some ability to recognize the previous occurrence of objects named inappropriately or inconsistently. This prompts the conclusion that recognition memory is not entirely predicated on the complex cognitive processes of naming but is aided substantially by it.

MEASUREMENT OF OLFACTORY SENSITIVITY IN HUMAN BEINGS.

Michael D. Rabin, Ruth Isseroff, & William S. Cain. John B. Pierce Fndn. Lab. & Yale Univ., 290 Congress Ave., New Haven, CT 06519.

The research addressed three issues regarding absolute olfactory sensitivity:

- 1) Do persons exhibit an apparent increase in sensitivity with practice over the course of testing within a day and across days?
- 2) Does any apparent increase in sensitivity generalize from one odorant to another?
- 3) Is relative sensitivity across odorants stable from person to person?

The answer to all three questions appears to be **yes**.

We measured absolute sensitivity to four odorants, n-amyl alcohol (AA), n-butyl alcohol (BA), benzaldehyde (BZ), and pyridine (PY), using a three-alternative forced-choice procedure. Testing took place over three days. Within a day, testing comprised ten blocks of trials, according to the sequence: 1) AA, 2) BZ, 3) BA, 4) PY, 5) BA, 6) PY, 7) BA, 8) PY, 9) AA, and 10) BZ. Participants made hundreds of judgments throughout the course of an afternoon of testing.

Absolute sensitivity showed an apparent increase over blocks. The largest increase occurred on the first day, but continued to show itself on the second and third days. Apparent sensitivity also increased from day to day and failed to approach an asymptote by the third day. The increase did not depend strictly on the amount of experience with a particular odorant.

Our results implied that absolute sensitivity measured in one session in the laboratory may underestimate the sensitivity of individuals having common experience with an odorant. This could account in part for the common observation that persons who live in areas of episodic odor pollution may complain about odors that seem virtually undetectable by unexposed persons.

A particularly important finding concerned the high interperson reliability of relative sensitivity across the four odorants. The average interperson correlation coefficient equalled 0.87. That is, when one person exhibited, say, an 8 to 1 difference in sensitivity to two odorants, other persons were very likely to show the same difference. In general, the results indicated that large individual differences in apparent olfactory sensitivity may disappear with proper attention to the various physical and psychophysical determinants of sensitivity.

EFFECTS OF GYMNEMIC ACID CONCENTRATION AND TIME SINCE EXPOSURE ON INTENSITY OF SIMPLE TASTES: A TEST OF THE BIPHASIC MODEL FOR THE ACTION OF GYMNEMIC ACID

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The present experiment was expressly designed as a psychophysical test of Kennedy and Halpern's (1980) biphasic model for the action of gymnemic acid. At several intervals following exposure to gymnemic acid, subjects judged the sweetness, bitterness, saltiness, and sourness of simple taste stimuli. The model proposes that gymnemic acids affect taste perceptions first by interacting with the surface of the receptor cell membrane and later by interacting with the membrane's lipid interior. The model predicts that the initial interaction with the membrane surface will produce reductions only in perception of sweet; the second stage of the biphasic process will produce non-selective disruption of taste perception. According to Kennedy and Halpern (1980), most investigations have shown reductions only in sweet taste simply because taste evaluations followed immediately after initial exposure to gymnemic extracts.

The results from the present experiment show dramatic reductions in sweet taste which recover with time but no reductions in bitterness, saltiness, or sourness at any time following exposure to any of a wide range of gymnemic acid concentrations. These results do not rule out the possibility that gymnemic acid affects taste perceptions by penetrating into the lipid interior of the receptor cell membrane but do provide evidence against the notion that the time-dependent biochemical event first has selective, then nonselective effects on perception. A psychophysical characteristic of gymnemic acid demonstrated by the results is that recovery from suppression of sweetness is roughly a constant percentage of the maximum suppression, independent of the concentration of the original gymnemic acid treatment.

PREY ODORS AND PHAGOATTRACTANT THRESHOLDS IN OYSTER DRILLS, D. Rittschof, C. Merrill, D. Kieber, and A. Ducharme, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Newly hatched oyster drills, *Urosalpinx cinerea* (Say) respond congenitally to a peptide phagoattractant associated with living, intact balanoid barnacles. Exposure of developing snail embryos to single prey odors from zygote until hatching results in alteration of the threshold level of response to standard purified and concentrated phagoattractant. Five groups of snails were compared; snails exposed to single species odors from oysters, mussels, and barnacles, and snails exposed in the field to odors from a mixture of species at the outer breakwater of Delaware Bay, and snails from our snail hatchery. Snails exposed to oysters and mussels had the highest sensitivity to barnacle phagoattractant. These snails were 15-30 fold more sensitive to the phagoattractant than were snails exposed to barnacle odor or from the breakwater. Hatchery snails were 2-6 fold less sensitive than the mussel or oyster exposed snails and had thresholds that apparently reflected exposure in the field prior to collection. Field assays of endogenous stimulus levels at the outer breakwater indicated that response thresholds of the breakwater snails could be predicted by endogenous phagoattractant levels. This work was supported by Sea Grant.

SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF THE OLFACTORY ROSETTE OF THE BROWN TROUT. J. Carter Rowley III and David T. Moran, Department of Anatomy, University of Colorado School of Medicine, Denver, Colorado.

Beneath each of the Brown trout's nostrils lies a pigmented structure, the olfactory rosette, which is subdivided into 12-14 lamellae. We have studied the fine structure of the olfactory rosette by scanning and transmission electron microscopy. Each lamella is covered in part by olfactory epithelium that is bathed by water in which the fish swims. The sensory surface of the olfactory epithelium contains three major cell types: (1) cells with many cilia that project vertically from a large, flat cell apex; (2) cells with few cilia that project laterally from a small, rounded cell apex; and (3) cells with microvilli. In the goldfish, which has similar cells, degeneration studies have shown cells types (2) and (3) described above are receptors, whereas type (1) is not.¹ Other studies on Salmonid fishes report the existence of a fourth cell type, often called a "rod cell",² in which many ciliary axonemes are enveloped in a single conical extension of the cell surface. Whereas "rod cells" are commonly assumed to be olfactory receptors, our studies indicate they are not. Instead, they are generated by artefactual alteration of the cell surface of non-receptor ciliated epithelial cells (listed above as type 1). We can make them come and go at will by alteration of the fixation process. In olfactory rosettes, prepared by conventional aldehyde fixation, "rod cells" are abundant. In rosettes briefly exposed to osmium tetroxide prior to aldehyde fixation, "rod cells" are absent. Pretreatment with osmium, it seems, stabilizes cell membranes and increases their permeability, thereby preventing inflation and distortion of the cell surface that can occur during aldehyde fixation.

- (1) Ichikawa and Ueda, 1977; Cell Tiss. Res. 183: 445.
(2) Yamamoto and Ueda, 1977; Bull. Jap. Soc. Sci. Fisheries 10: 1163.

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INTRACELLULAR FILLING OF MITRAL AND TUFTED CELLS WITH HORSE RADISH PEROXIDASE CONFIRMS THEIR IDENTIFICATION BY ANTIDROMIC ACTIVATION. Stephen P. Schneider, John W. Scott and Edward Orona, Department of Anatomy, Emory University, Atlanta, Georgia 30322

We reported recently that mitral and tufted cells of the rat olfactory bulb can be distinguished electrophysiologically by their axonal projections. While both mitral and tufted cells can be antidromically activated by stimulation of the lateral olfactory tract at the level of the olfactory peduncle, stimulation of the posterior piriform cortex antidromically activates predominantly mitral cells. We have studied the responses of mitral and tufted cells to olfactory nerve stimulation and found that tufted cells have lower thresholds, shorter latencies, a tendency to multiple spiking and give excitatory responses to stimulation of a more widespread area of the olfactory nerve layer.

We report here that intracellular injections of horseradish peroxidase (HRP) have been accomplished in some of these neurons. These injections confirm the identification based on antidromic activation. Antidromically identified, HRP filled tufted cells in the superficial external plexiform layer (EPL) were activated only from the lateral olfactory tract and/or olfactory tubercle. Some more superficial tufted cells could not be antidromically activated. The intracellular recordings confirmed that the multiple spike responses seen in extracellular recordings were from single tufted cells. The tufted cells which we observed had basal dendrites which ramified in the superficial part of the EPL. In contrast, the basal dendrites of the few mitral cells we filled were confined to the deep part of the EPL. These results are in agreement with differences in the laminar organization of mitral and tufted cell dendrites illustrated in previous Golgi studies of the olfactory bulb. While we are not yet confident that these observations are based on complete filling of the mitral dendrites, this organization may be related to the differences in response properties.

Our data are consistent with the hypothesis that mitral and tufted cells function differently in the processing of primary olfactory information and that these differences correlate with differences in cellular morphology and axonal projection patterns.

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MATURATION OF OLFACTORY EXPLORATION IN HAMSTERS IS CORRELATED WITH LATE AFFERENTATION OF THE OLFACTORY TUBERCLE. Thomas A. Schoenfeld and James V. Corwin, Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610

Neuroanatomical and behavioral experiments were conducted to further clarify the correlation between the maturation of the olfactory tubercle and the maturation of olfactory orientation seen in the 2nd week of life of hamsters (Leonard, '74, '75; Devor and Schneider, '74). We first looked at the development of spontaneous behaviors (exploration, huddling, grooming, etc.) displayed by litters of 7-8 hamster pups in brief, relatively unrestricted tests hoping to find a common denominator with other odor-guided behaviors, collectively called olfactory orientation (e.g., home orientation), which mature in the 2nd week of life. We found that, following placement in a test cage filled with pine shavings, litters of pups initially displayed a period of locomotor exploration which ended reliably with huddling. Whether tested sequentially every 3rd day from P3 to P18 or in separate age groups across a similar time span, exploration changed dramatically between P9 and P12. The latency to huddle (i.e., the duration of exploration) on a 1st trial with fresh shavings was significantly higher in pups P12 and older than in younger pups. Since the increased exploration in the 1st trial habituated in a 2nd trial with slightly soiled shavings, the older pups were probably responding to the initial novelty of the test environment. To ascertain whether the increased exploration was olfactory-guided, we added a 3rd trial in which lemon extract was mixed with fresh shavings. Pups tested at P5 or P8 did not respond differentially to the three test conditions and explored for relatively short times, whereas pups tested at P12 explored for long durations in both the fresh and lemon shavings. The common denominator between olfactory exploration and other olfactory orienting behaviors may be an overt motor response to either novel or nest odors.

In an anatomical experiment using degeneration techniques, we found that the maturation of olfactory exploration is correlated with the maturation of the afferents of the olfactory tubercle but not those of the piriform cortex. Whereas primary afferents arising in the olfactory bulb invade the tubercle and most of the rest of olfactory cortex at birth (Schwob & Price, '78), association afferents arising in the anterior piriform cortex were found not to invade the olfactory tubercle until P9-P12, even though terminals arising there were found to invade the posterior piriform cortex and adjacent parts of the anterior piriform cortex by P5. Thus, only late afferentation of the tubercle is correlated with the maturation of olfactory exploration.

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GLUCOSE INSENSITIVITY IN RELATIVES OF DIABETICS. R.G. Settle, University of Pennsylvania Clinical Smell and Taste Research Center; Philadelphia VA Medical Center.

It has been proposed that the fundamental abnormality underlying insulin-independent diabetes mellitus (IIDM) is a reduced sensitivity to glucose. Previous studies have found elevated glucose taste thresholds in IIDM. Although poorly understood, diabetes has a strong genetic component. The present study was undertaken to determine if a subpopulation of nondiabetic subjects with a family history of IIDM could be identified which displayed a reduced taste sensitivity to glucose. Nineteen students with first degree relatives with IIDM (group PFH) and eight with no incidence of IIDM in first degree relatives (group NFH) served as subjects. A magnitude estimation procedure was used to scale the perceived intensity of six glucose (0.64 to 1.95M) and six fructose (0.41 to 1.25) concentrations. For each subject, the mean log response to all glucose concentrations was subtracted from the mean log response to all fructose concentrations. The range of values obtained was quartiled. All subjects in group NFH fell into the second and third quartiles, which were collapsed (Q2+3). The mean log response to fructose was used to adjust for differences among subjects in their use of numbers. An ANOVA showed that there were no significant differences among the three groups (Q1, Q2+3 and Q4) in response to fructose. An ANOVA on glucose responses indicated that Q1 responses were significantly higher than Q2+3 and that Q4 was significantly lower than Q2+3. In addition, there was a significant interaction effect attributable to significantly lower responses of Q4 to the lowest 3 concentrations of glucose compared to Q2+3. The shape of the glucose intensity function of Q4 relative to Q2+3 resembled that for recruitment phenomena (i.e., sensitivity to weak stimuli is poor, but is normal to stronger stimuli). There are two implications of the data. It appears possible to identify a subgroup of individuals, who are genetically predisposed to diabetes ("prediabetics"?), who show reduced glucose taste sensitivity. Second, the recruitment phenomenon for glucose demonstrated by this subgroup suggests that there may be a separate receptor site with which glucose interacts but with which fructose does not, a possible "glucoreceptor".

CHEMOSENSORY PROPERTIES OF ACIDS. R.G. Settle, K. Meehan, University of Pennsylvania Clinical Smell and Taste Research Center; Philadelphia VA Medical Center.

Chemosensory properties of acids other than sourness (eg., secondary taste qualities, nasal detectability) may influence the results of clinical testing procedures. Thirteen students participated in a study of seven acids: citric (Cit), hydrochloric (HCl), lactic (Lac), malic (Mal), phosphoric (Pho), sulfuric (Sul) and tartaric (Tar). Magnitude estimation of total perceived intensity with fractionation of taste qualities (sweet, sour, salty, bitter, other taste and other sensation) was performed using 6 concentrations of each acid matched in intensity to 2.5, 4.0, 6.3, 10, 16 and 25mM citric acid. Each stimulus was presented three times and the median was used in the analysis. Duncan's tests revealed: 1) the proportion of sourness (X) of HCl (.65) and Sul (.64) was significantly lower ($p < .05$) than Pho (.78), Tar (.77) and Lac (.74) with Mal (.74) and Cit (.73) intermediate; 2) the proportion of bitterness of Sul (.17) was significantly greater than Mal (.08) and Tar (.06) with Lac (.11), Cit (.10), HCl (.10) and Pho (.09) intermediate; 3) the proportion of saltiness of HCl (.12) was significantly greater than Tar (.06), Cit (.05), Lac (.05) and Pho (.05) with Sul (.09) and Mal (.09) intermediate; and 4) the proportion of sweetness did not differ significantly. These data indicate that HCl and Sul have relatively low proportions of sourness and relatively strong secondary qualities. Tar and Pho have relatively high proportions of sourness and relatively weak secondary qualities.

A forced choice sniff-bottle procedure was used to determine the nasal detectability of the acids. Eight water-acid pairs were presented for each acid at each of two concentrations. One set of acid concentrations was determined by matching the total taste intensity to 8.4mM Cit, the second set by matching to 2.5mM Cit. At the higher concentration, a majority of subjects were able to detect the acid in 7 or more of the 8 pairs ($p < .05$) for all seven acids. At the lower concentration, only lactic was detected by a majority of the subjects. These data indicate that at concentrations corresponding to relatively moderate taste intensity all acids tested can be discriminated by nasal detection. Lactic acid was discriminable even at concentrations corresponding to a relatively low taste intensity.

ISOLATION, PURIFICATION, AND CHARACTERIZATION OF A PEPTIDE MARINE PHAGOATTRACTANT. R. G. Shepherd, K. Mopper, and D. Rittschof, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Last year at AchemS we demonstrated that the predatory marine snail, *Urosalpinx cinerea* (Say) crept up current in response to specific molecules found in association with living intact Balanoid barnacles. We showed that the phago-attractant molecules could be concentrated and desalted by adsorption onto Amberlite XAD-7 resin and were between 5000 and 1000 Daltons.

Here we report our procedures for isolating, purifying, and characterizing this phagoattractant. Concentration and purification procedures are simple and relatively rapid, taking approximately three days and also easily adapted to large scale or micro preparations. Highly purified material is obtained (active maximally at 10^{-6} M) by extraction and desalting of the molecules from seawater with Amberlite XAD-7 resin followed by a heat treatment step and then pressure dialysis. Active material from these steps is further purified by two rounds of micropreparative high-pressure liquid chromatography with microparticulate Amberlite XAD-7 resin as the stationary phase and methanol/ water mixtures as the mobile phase.

The active, partially purified material contains two size classes of peptides, one of approximately 2,000 and one of 4,000 Daltons by Urea SDS gel electrophoresis. Biological activity can be destroyed by treatment with carboxypeptidase and pronase, and amino acids are liberated. At the time of this writing we are preparing to further characterize the peptide by two-dimensional polyacrylamide gel electrophoresis, to analyze for amino acids and carbohydrates, and to perform sequencing of the electrophoretically and chromatographically purified peptides. This work was supported by Sea Grant.

INTERSTRAIN DIFFERENCES IN BITTER TASTE RESPONSES IN MICE. Tomio Shingai and Lloyd M. Beidler, Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Behavioral investigation has revealed that mice of strain, SWR/J showed a strong aversion to drinking water which contained a low concentration (10^{-4} M) of bitter-taste substance sucrose octaacetate (SOA), but the other strains showed no such aversion (Lush (1981) Genet. Res. 38: 93-95). Whitney et al. (Dept. of Psychology, Florida State Univ.) have obtained almost the same results.

In the present experiment, electrophysiological studies were performed in order to obtain a neurophysiological basis of the behavioral difference of drinking. Since bitter taste is considered to be mediated mainly through the glossopharyngeal nerve, neural responses were recorded from the lingual branch of the glossopharyngeal nerve and were represented as integrated responses. Taste solutions were applied on the back of the tongue which was widely exposed by cutting the side of the face. In addition to bitter taste solutions (SOA, quinine hydrochloride, phenylthiourea (PTC)), sucrose, NaCl, KCl, NH_4Cl and HCl solutions were also used as taste stimuli. Animals were adult male mice of SWR/J, LP/J, BDP/J and DBA/2J strains.

The results showed a big difference in the neural response to SOA between SWR/J and the other strains. Solutions of SOA elicited a marked response in SWR/J. The threshold was between 10^{-6} M and 10^{-5} M. The magnitude of the response was a rising function of concentration (10^{-6} M- 10^{-3} M). On the other hand, the solution produced a very small response or almost no response even at the concentration of 10^{-4} M and 10^{-3} M in mice of LP/J, BDP/J and DBA/2J. PTC at the concentration of 10^{-2} M elicited only a small response compared with SOA response in SWR/J mice. There was no appreciable difference in the PTC response among the strains. The results obtained in the present experiment showing high sensitivity of SWR/J mice to SOA agree with the behavioral data of the mice.

CHEMOSENSITIVITY OF RAT TRIGEMINAL RECEPTORS TO NINE ODORANTS COMMONLY USED IN OLFACTORY RESEARCH. Wayne L. Silver and David G. Moulton. Clinical Smell and Taste Research Center and Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Although it has long been known that the free trigeminal nerve endings in the nasal cavity respond to chemical stimuli, the kinds and concentrations of effective stimuli are not well known. This knowledge will contribute to our understanding of the involvement of the trigeminal system to the perception of odors as well as the effect of odors on physiologic function. Stimulation of trigeminal receptors leads to many reflex changes, including cardiovascular, respiratory and hormonal. In addition, trigeminal stimulation can alter access of stimulus molecules to the nasal cavity by reflexly changing mucus secretion, respiratory patterns and nasal patency. The purpose of the present study is to examine the chemosensitivity of rat trigeminal receptors to nine odorants commonly used in olfactory research.

Integrated multiunit responses were recorded from the ethmoid branch of the trigeminal nerve in male Sprague-Dawley rats. Stimuli were presented to the external nares via an air-dilution olfactometer at a flow rate of 2 L/min. A 1 L/min stream of clean air was drawn through the nose via a nasopharyngeal cannula connected to a vacuum. For stimulus presentation, the vacuum was turned on for 30 seconds in the middle of which an odor was delivered for 10 seconds. The interstimulus interval was 3 minutes. Stimuli were presented in an ascending concentration series and threshold was defined as the range between the concentration which first produced a response distinguishable from baseline and the preceding concentration.

Threshold ranges in ppm for the stimuli tested were 260-519, amyl acetate; 164-499, butanol; 45-224, cyclohexanone; 21-137, heptanol; 39-99, propionic acid. However, not all odorants elicited responses in all animals. These included linalool (5/6), 28-198; α -terpineol (4/8), 15-151; benzyl acetate (3/6), 66-186; phenyl ethyl alcohol (2/6), 12-73. There was a striking correlation between the rat whole-nerve electrophysiological response magnitudes of this study and the intensity ratings of human anosmics established by Doty et al. (Physiol. Behav. 20: 175-187, 1978) to the same nine stimuli at vapor saturation. For the anosmics, pleasantness was negatively correlated with intensity, so that the more intense an odorant was perceived, the more unpleasant it was judged. The high correlation between the rat and human data suggests that the degree of irritation or "unpleasantness" for the compounds tested may be similar for both rat and man and that the rat is an excellent model for assessing the stimulatory effectiveness of odorants on human nasal trigeminal receptors. (Supported by NIH grant NO P01 NS 16365 01).

INFORMATION TRANSMISSION IN TARSAL SUGAR RECEPTORS OF THE BLOWFLY. David V. Smith, Elizabeth Bowdan, and Vincent G. Dethier. Dept. Psychology, Univ. Wyoming and Dept. Zoology, Univ. Massachusetts.

The concepts and methods of information theory were applied to the responses of tarsal "sugar receptors" of the blowfly, *Phormia regina*. Responses were recorded with a tip electrode filled with sucrose, ranging from 0.001 to 2.0 M. Recordings were from hairs D₁ through D₅ on seven isolated legs. The stimulus-response functions for these five receptors were logarithmic between 0.01 and 1.5 M sucrose ($R = a \log S + b$). The responses of each receptor were arranged by stimulus concentration in a matrix containing the frequencies of occurrence of responses in 5-impulse categories. From these frequency distributions we calculated the uncertainty of the stimulus, $H(S)$, the response, $H(R)$, and the joint uncertainty of the stimulus-response relation, $H(S,R)$. Uncertainty is defined (e.g., for the stimulus) as: $H(S) = -\sum p_i \log_2 p_i$, where p_i is the probability of occurrence of each stimulus. Generally, the larger the number of choices, the greater the uncertainty. The maximal uncertainty, $H_{max}(S,R)$, of the stimulus-response matrix is given by calculating the joint uncertainty in a reference matrix constructed from the products of the marginal values of R and S in the experimental matrix. This is the amount of uncertainty that would exist if there were no lawful relation between stimulus and response. The difference between maximal uncertainty and the joint uncertainty in the experimental matrix is the contingent uncertainty, $H(S,R) = H_{max}(S,R) - H(S,R)$, which represents the amount by which the total possible uncertainty is reduced by the relation between stimulus and response. This difference is the amount of information transmitted about sucrose intensity.

The information capacity of these sugar receptors, as defined by the foregoing analysis, was 2.22 bits in tarsal hair D₁, 2.29 bits in D₂, 2.20 bits in D₃, 2.24 bits in D₄ and 2.30 bits in D₅. This amount of information capacity means that input from these receptors would allow the fly to make reliable discriminations among 4.60 to 4.93 (2^{2.2} to 2^{2.3}) levels of sucrose intensity across the range of concentrations employed. That is, there are 4-5 discrete levels of concentration discernable in the response continuum of these receptors. Behavioral data suggest that the discrimination capacity of *Phormia* falls in this range, although further behavioral studies are currently underway.

FINE-GRAINED MEASUREMENT OF CANINE CONSUMMATORY BEHAVIOR. James C. Smith, Michael E. Rashotte, Tracey A. Austin, Ross P. Henderson, Graham K. Oliff, Department of Psychology, Florida State University, Tallahassee, FL 32306.

We present a method for obtaining fine-grained measurements of consummatory behavior in large animals such as dogs. Each dog can gain access to any combination of three feeding stations, via a computer controlled door. This arrangement allows presentation of single foods or multiple foods simultaneously in choice procedures. From the dog's point of view, a feeding station is comprised of a standard pan which holds a solid food or a liquid. The dog's access to a pan is detected by an infra-red photobeam which initiates a frequent (160 times/sec) weighing of the pans by means of a calibrated load beam. All measures are sent to a micro-computer which records the duration of consumption and ten (10) pan weights per second. We compare examples of fine-grained measurements of short-term eating and drinking behavior with traditional intake measures in dogs.

RAPID RECOVERY FROM TASTE AVERSION AS THE RESULT OF POSTCONDITIONING FORCED TASTE EXPERIENCE. Alan C. Spector, James C. Smith & Glee R. Hollander, Department of Psychology, Florida State University, Tallahassee, FL 32306.

Taste aversions have been observed in human cancer patients receiving radiotherapy. However, these aversions are not as profound as those commonly observed in laboratory animals. In order to gain a better understanding of the role radiation-induced taste aversions play in the dietary problems associated with radiotherapy patients, it is necessary to examine what factors strengthen or weaken these aversions. The strength of a radiation-induced taste aversion can be attenuated in the laboratory rat by treatments such as preconditioning taste-exposure and low radiation dose levels. The purpose of this study was to determine how much postconditioning taste experience an animal requires under ad lib. conditions in order to totally recover from a radiation-induced taste aversion.

In the first experiment rats given the flavor-cue as their sole fluid source following conditioning recovered within 48 hours, whereas rats given a choice between water and flavor-cue required 33 days before their flavor-cue intake reached levels comparable to controls.

In Experiment 2, on the day after saccharin was paired with either a 100R or sham radiation exposure, rats received a 0-, 3-, 6-, 12-, 24-, or 48-h saccharin presentation followed by a series of 23-h two-bottle preference tests. Generally, faster recovery from the aversion was observed in animals receiving longer postconditioning taste-alone presentations.

In the third experiment a moment by moment, 24 hour, lick analysis was performed, in efforts to determine exactly when during the 24-h saccharin-alone presentation the animals started to drink.

A CHEMICAL CHARACTERIZATION OF THE FRESHWATER ATTRACTANTS OF THE AMERICAN EEL. Peter W. Sorensen, Graduate School of Oceanography, University of Rhode Island, Narragansett, R.I. 02882

Migrating larvae (elvers) of the American eel (*Anguilla rostrata*) possess a strong tendency to swim into flowing fresh-water. As this response is not evoked by distilled water or charcoal-filtered stream water, a chemical attractant(s) is believed responsible. The unconditioned responses of young eels to different waters and their extracts have been studied with a Y-maze. Work to date indicates that the same attractant(s) is recognized by all elvers, regardless of collection point. Decaying leaf detritus is probably an important source of the attractant(s).

The attractant(s) have been chemically characterized by a variety of techniques. Common stream electrolytes are not attractive. The attractant(s) is present in both the dissolved and particulate fractions of stream water. It is nonvolatile. Neither freezing, nor autoclaving, nor exposure to extreme pHs destroy it. Anion exchange resins remove it from solution. Several amines and amino acids have been found to be attractive.

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THE FUNCTIONAL AND HISTOLOGICAL MATURATION OF A MODIFIED GLOMERULAR COMPLEX IN THE NEONATAL RAT OLFACTORY BULB. W.B. Stewart, C.A. Greer, M.H. Teicher, P.E. Pedersen and G.M. Shepherd, Secs. Neuroanatomy, Neurosurgery and Gross Anatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

It was recently reported that a modified glomerular complex (MGC) in the olfactory bulb (OB) of 9-12 day postnatal rats exhibited increased 2-deoxyglucose (2DG) uptake during suckling behavior (Teicher, Stewart, Kauer and Shepherd, *Brain Res.*, 1980, 194: 530). The data suggested that the MGC may preferentially respond to olfactory cues important for directing and initiating suckling in the neonatal rat. In light of these observations, we have studied the functional and histological maturation of the MGC during early postnatal development.

Neonatal rats at 0, 3, 6, 9, 12 and 15 days postnatal were injected with 200uCi/Kg of 14 C-2DG in physiological saline and placed with their anesthetized dam where they suckled for a period of 1 hour. They were immediately sacrificed at the end of suckling and their olfactory bulbs processed for autoradiography. Littermates were perfused with Bouin's solution and processed for histological study of the MGC.

At all ages studied there was increased 2DG uptake in the MGC during suckling. Even in the youngest rats (0-6 days postnatal) the uptake appeared focal and restricted within the MGC. Elsewhere, in the glomerular layer of the main OB, uptake appeared as a diffuse and variable band. This pattern differs from that elicited by amyl acetate as shown in an earlier developmental study of the OB (Greer, Stewart, Teicher and Shepherd, *Neurosci. Abst.*, 1980, 6: 306).

Examination of the histological material established that at parturition the MGC was easily identified as a discrete grouping of demarcated glomeruli in the dorso-caudal OB at the medial border of the accessory OB. The MGC was not continuous with the glomerular layer of either the main or accessory OB.

We conclude that there is a correlation between focal 2DG uptake and histological maturation of the MGC. The focal uptake appears to depend on the development of the MGC as a histological entity. This extends the results of our previous study which showed that focal 2DG uptake elicited by amyl acetate does not appear until around 6 days postnatal when the glomeruli in the main OB first become histologically demarcated.

These observations suggest that the MGC is more functionally and histologically mature at birth than the glomeruli of the main OB. The earlier maturation of the MGC may reflect its importance as a functional unit for processing odors which are behaviorally significant at birth.

THE EFFECT OF INTRANASAL ZINC SULFATE TREATMENT ON SUCKLING BEHAVIOR AND ODOR-INDUCED ACTIVITY IN THE NEONATAL RAT OLFACTORY BULB. William B. Stewart, Charles A. Greer and Martin H. Teicher, Sec. Neuroanatomy, Neurosurgery and Gross Anatomy, Yale Univ. Sch. Med., New Haven, CT 06510

We have correlated the behavioral effects of zinc sulfate ($ZnSO_4$) treatment with odor-induced 2-deoxyglucose (2DG) uptake in the developing rat olfactory bulb.

Eight - nine day old rat pups received intranasal irrigation with either 0.9% NaCl, 1% $ZnSO_4$, or 5% $ZnSO_4$. One and 5 days following treatment the rats were tested for suckling behavior and for vibrational responses to the anesthetized dam and a heating pad. Six days following treatment the pups were injected with 200uCi/kg of 14 C-2DG and placed, in groups of 3, in an odor chamber where they were exposed to a 10^{-1} flow dilution of amyl acetate. The brains were then processed for autoradiography.

At 1 day following treatment none of the $ZnSO_4$ pups attached to the dam's nipple, while 89% of the saline pups attached within 5 min. At 5 days none of the 5% pups attached while 67% and 100% of the 1% $ZnSO_4$ and saline pups, respectively, attached. On the vibrational tests of maternally directed behavior, similar results were obtained. At one day following treatment neither of the $ZnSO_4$ groups could discriminate between the dam's ventrum, dorsum, or a heating pad. At 5 days post treatment, the 1% group had recovered to control levels while the 5% group was still unable to differentiate. On day 13 postnatal, the saline control pups had gained 5.5g, the 1% $ZnSO_4$ pups had gained 3g and the 5% pups had lost 2.7g.

The 2DG results may be summarized as follows. The saline pups had large regions of increased focal 2DG activity in the glomerular layer of the olfactory bulb. In marked contrast, the 5% $ZnSO_4$ pups did not have distinct focal 2DG activity. The 1% pups had 2DG foci in the glomerular layer but to a lesser extent than the saline control pups. The olfactory bulbs of both the 1% and 5% pups were smaller than the saline pups. This was particularly evident in the nerve and glomerular layers.

These results are consistent with those of Singh et al. (*Physiol. & Behav.* 17: 373, 1976) who demonstrated deficits in suckling behavior following 5% $ZnSO_4$. Our results extend these findings demonstrating that 1% treatments produce only temporary deficits. Also, the 2DG results on the functional organization within the olfactory bulb correlate well with the behavioral findings. Overall, the combined use of behavioral and 2DG methods provides a powerful strategy for assessing functional capacity and recovery of function in sensory systems.

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QUALITIES OF TOXIC COMPOUNDS IN THE RODENT TASTE SYSTEM

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The chemical protection of plants and animals from predation by means of endogenously produced toxins is widespread and undoubtedly provides considerable survival advantage to species so endowed. Because humans characterize many naturally occurring toxins, such as nicotine in tobacco plants and other alkaloids and glycosides, as bitter, it is commonly suggested that herbivores avoid poisonous plants based on their bitter tastes. It remains to be demonstrated, however, that such toxic substances are indeed coded as bitter by predator species. Such a demonstration is important since there is recent evidence that rodent species differ in the taste qualities assigned to some substances (Nowlis, et al., 1980).

In our initial investigations, we have attempted to characterize the taste qualities of several rodenticides to *Rattus norvegicus* (laboratory strain), including two compounds that occur naturally in plants (i.e., scilliroside, strychnine). The determination of taste qualities was based on procedures described by Nowlis et al. (1980), and involves pairing the taste of small quantities of the rodenticide with a LiCl (ip)-induced illness, and then testing for generalization of the learned taste aversion to five non-toxic tastants (sucrose, NaCl, HCl, quinine sulfate, and sucrose octaacetate-SOA).

From the data, we suggest that at least one naturally occurring toxin, scilliroside, was coded by the rats as primarily acid-tasting (i.e., learned aversion generalized to HCl), although the compound also appeared to have a bitter component (i.e., learned aversions also generalized somewhat to quinine and SOA). Further, the results suggested that although both strychnine and thiosemicarbazide tasted bitter, their bitter components were qualitatively different. If confirmed in additional tests, the results indicate a more complex taste-coding of some toxins than has been previously assumed. Finally, the approach appears suitable for objectively assessing among rodent species the idea that primary aversions are associated with bitter tastes.

Nowlis, G. H., M. E. Frank., & C. Pfaffman. 1980. Specificity of acquired aversions to taste qualities in hamsters and rats. *J. comp. physiol. Psychol.* 94: 932-942.

RESPONSES OF OLFACTORY BULB UNITS IN CHANNEL CATFISH TO AMINO ACIDS. Hilary Thompson and John Caprio. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, La. 70803.

The response patterns of catfish olfactory bulb units to amino acid stimulation of the olfactory receptors are under investigation. The aim of this study is to determine how second order neural activity correlates with multiunit (EOG and integrated neural) receptor responses in the same species. Access to the pedunculated olfactory bulb in an *in vivo* preparation was accomplished by the removal of overlying tissue and bone immediately behind the olfactory capsule. An isthmus of tissue between the open capsule and the bulb incision maintained their separation. The olfactory tract was left intact in the initial series of experiments. Calomel electrodes with Ringer-agar bridges positioned in the water above the surface of the olfactory mucosa recorded the electroolfactogram (EOG). Dye-filled (fast green) glass micro-electrodes (impedance 1-5 M Ω) were advanced into the olfactory bulb with a hydraulic microdrive to record extracellular unit activity. The single unit activity obtained was recorded simultaneously with the EOG, which indicated the time course and intensity of peripheral input to the bulb. In this study, 13 amino acids (AA), alone and in mixtures, were introduced as stimuli into the continuous water flow (8 ml/min.) bathing the olfactory mucosa. All compounds were tested, where possible, at applied concentrations of 10^{-6} M, 10^{-4} M and 10^{-2} M.

The preliminary results are from units at various depths in the glomerular-mitral cell layers. Cell type identification was not conclusive in the preparation because of the diffuse organization of the fish olfactory bulb in comparison to the more laminate bulbs of other vertebrate groups. Units showed excitation, inhibition, or both inhibitory and excitatory responses to a variety of AA stimuli. The responses of other units were not well characterized as simply excitatory or inhibitory. Spike train parameters are under examination to find adequate descriptors of these complex responses. (Supported in part by NIH Grant NS 14819)

THE PSYCHOLOGICAL EFFECTS ARISING FROM ADMINISTRATION OF THE ODOURS ANDROSTANONE AND ANDROSTENONE IN THE LABORATORY SETTING. C. Van Toller, Department of Psychology, University of Warwick, Coventry, CV4 7AL, England.

A review will be made of a number of experiments carried out by various members of the Warwick Olfaction Research Group (WORG) involving the putative human pheromone 5- α -androster-16-en-3-one and the laboratory synthesized compound 5- α -androstan-3-one.

The techniques used vary from measurements of threshold levels, using psychophysical methods to psychophysiological and behavioural studies. The WORG studies have also measured odour, attitude and mood profiles evoked by these compounds.

The results indicate that compared to males, females are more likely to use these odours as an indication of maleness but complex cognitive factors need to be taken into account to explain the results.

STIMULUS VOLUME AND TASTE DETECTION THRESHOLDS. James M. Weiffenbach and Ronald Taylor, National Institute of Dental Research, Bethesda, MD 20205

The amount of stimulus fluid that different taste threshold measurement procedures make available to subjects varies over a wide range. As might be expected, thresholds obtained with larger volumes are often lower than those obtained with smaller volumes. However, since procedures using different stimulus volumes often differ in other ways as well, the role of stimulus volume in determining threshold is only poorly defined.

Taste detection thresholds were obtained from eight women and eight men for stimulus volumes of .005, .05, .5, and 5 ml. Thresholds for sucrose, sodium chloride, citric acid, and quinine sulfate were obtained from each subject at each volume. Subjects were tested with a different stimulus volume on each of four days. The same forced choice psychophysical procedure was used throughout, each threshold being the geometric mean of the last four of five turn around concentrations obtained by an up down transformed response method.

An analysis of variance revealed significant main effects for quality and volume, but no effect for sex and no interaction effects. For each substance, the threshold decreased by approximately one log step as stimulus volume increased from .005 to .5 ml. In each case the threshold for .5 ml did not differ significantly from that for 5 ml or from those obtained on a larger independent sample tested with the same psychophysical procedure and 10 ml stimuli.

Thresholds for substances representing the four basic taste qualities undergo parallel decreases with increasing stimulus volume to .5 ml, but do not significantly decrease with further increase in volume.

DEVELOPING OLFACTORY CIRCUITS: AChE-CONTAINING CELLS DEMONSTRATED BY OLFACTORY BULBECTOMY. C.R. Wirsig, J. Morasco & C.M. Leonard. Dept. of Neuroscience and Center for Neurobiological Sciences, College of Medicine, University of Florida, Gainesville FL 32610

Olfactory bulbectomy (OBx) causes a striking reactivity in AChE-filled cells in the molecular layer of the anterior olfactory nucleus (AON) and the polymorph layer of the olfactory tubercle (OT) of the 5 day old hamster (Wirsig, Morasco & Leonard, 1981). After OBx cells which normally stain lightly for AChE are filled with dense reaction product and stand out. These cellular changes are accompanied by aberrant thermoregulatory and social behavior possibly as a result of abnormalities in cholinergic function (Leonard, Williamson & Freund, 1981). Since the cells are associated with AChE-filled processes that extend rostrally to the border of the lesion, we initially interpreted their reaction as a retrograde response to axon section. However, cells with this distribution do not project to the OB in the adult. We have therefore examined the possibility that they may still be migrating during the first postnatal week.

The brains of 80 hamster pups were examined 24 hrs after OBx on days 0, 2, 3, 5, 8 and 12. Brains were cut (80 μ) and stained by the Tsuiji method and reactive cells were counted. Prior to day 3 enzyme activity was low and no reactive cells were seen. The position of reactive cells then progressively shifted from the caudal OT on day 3 to the rostral AON on day 12 as would be expected if they were migrating.

To determine whether these cells actually project into the OB, rostral OBx sparing the AON (6) or injections of HRP (6) into the OB were made in 5 day old pups. TMB were used to demonstrate HRP in cells. No reactive cells were seen after lesions sparing the AON and no cells in the molecular layer of the AON or any part of the OT were ever labeled with HRP. In two brains processed to demonstrate both AChE and HRP, no AChE-filled cells were labeled with HRP, although numerous HRP-filled cells were seen in layer II on the AON. It therefore seems unlikely that these cells send efferents to the OB. They may react to the lesion because their leading processes in the rostral AON have been severed.

Haberly and Price (1978) have described cells in the molecular layer of the AON that send projections to the OT and lateral hypothalamus. The trailing processes of the cells we see may remain to form axonal terminals in the OT after their cell bodies have migrated forward. These cells could provide olfactory input to OT cells that modulate thermoregulatory mechanisms integrated in the posterior hypothalamus. Experiments are in progress to examine whether the efferent projection patterns of these cells are altered by OBx. Supported by NS 13516 to CML and MH 15737-02 to CRW.

BIOCHEMICAL STUDIES WITH HETEROCYCLIC ODORANTS. Philip Wood and George Dodd, Warwick Olfaction Research Group, University of Warwick, Coventry, U.K.

Heterocyclic odorants such as the pyrazines and related series have low olfactory thresholds and well-defined structure-odour relationships and are ideal ligands for probing olfactory receptors.

BINDING STUDIES

(³H) 2-isobutyl-3-methoxypyrazine, the impact chemical from green peppers, binds to the supernatant fraction from sheep olfactory mucosa. It exhibited high-affinity ($K_a \sim 10^8 M^{-1}$) saturable binding but the presence of substantial non-specific binding decreased the sensitivity and accuracy of the assay. Only non-specific binding was observed with comparable fractions from liver, brain and respiratory epithelium. The binding is inhibited by other green-pepper-smelling odorants including congeners and homologues but with the exception of (-) carvone the binding is not inhibited by non-congeners.

CHEMICAL MODIFICATION

Reactive brominated derivatives of several heterocyclic odorants, e.g., 3-acetylpyridine, 2-acetyl-3-methylpyrazine, were synthesized as affinity odorants for vapour-phase chemical modification. Each affinity odorant blocked the E.O.G. response both to itself and its non-bromo analogue. Differential reductions in the E.O.G. responses to structurally unrelated odorants were observed.

N.M.R. STUDIES

Binding of green-pepper smelling heterocyclic odorants both to sheep olfactory epithelium supernatant fractions and to the model receptor protein, B.S.A., was studied using FT-(¹H)-NMR. Differential line-broadening was observed and the more polar side chains were seen to be important for the binding.

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INDIVIDUAL DIFFERENCES IN SENSITIVITY TO ANDROSTENONE ARE GENETICALLY DETERMINED. Charles J. Wysocki & Gary K. Beauchamp. Monell Chemical Senses Center, Philadelphia, PA 19104. Cynthia Calloway, Bo Dupont & Marilyn S. Pollack. Sloan-Kettering Institute for Cancer Research, New York, NY 10021.

Odor detection thresholds for the compound 5 α -Androst-16-en-3-one (androstenone) do not exhibit a unimodal distribution. Some people are quite sensitive to the odorant and, at suprathreshold concentrations, find the odor unpleasant. Others exhibit a heightened threshold (hyposmia) and, at suprathreshold concentrations, generally find the odor pleasant. Still others are anosmic to the compound. We have been exploring some of the factors which may contribute to this variability.

Initially, using pyridine as a stimulus, we compared the squeeze bottle technique, developed by John Amoore, with 3 other methods of odor delivery for test-retest reliability and rapidity and ease of testing. Two methods were eliminated because of problems associated with ease of testing and substantial within-subject variability. The third method, which utilized sniff bottles, was less reliable than the squeeze bottle method. Hence, we chose squeeze bottles and tested 55 unrelated people and 45 additional members of their immediate families, ranging in age from 11 to 79 years, for their sensitivities to pyridine and androstenone.

A threshold estimate for each odorant was obtained for each individual by using an ascending concentration series with a forced choice between an odor-containing and a vehicle-containing bottle at each concentration step. The results demonstrated a unimodal distribution for pyridine. However, for androstenone, a trimodal distribution was obtained: 58% of the males and 42% of the females were anosmic, 15% of males and 18% of females were hyposmic and 28% of males and 32% of females were quite sensitive to the odor. Familial patterns generated from this study suggested possible genetic involvement in sensitivity to androstenone.

We next sampled monozygotic and dizygotic twin sets for sensitivity to these two odors to test for genetic contribution to variability in sensitivity to androstenone. Our results suggest a strong genetic component. The threshold sensitivity for one member of an identical twin pair was an excellent predictor of the sensitivity of the other member. This relationship did not hold for fraternal twin sets.

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AN EFFECT OF DIFFERENCES IN MAJOR HISTOCOMPATIBILITY TYPES ON THE INCIDENCE OF PREGNANCY BLOCK IN MICE. Kunio Yamazaki, Gary K. Beauchamp, Charles J. Wysocki and Edward A. Boyse. Monell Chemical Senses Center, Philadelphia, Pa. 19104, and Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

It has been shown that Major Histocompatibility Complex (MHC) types affect the mating choices of mice, and that mice can be trained to distinguish arms of a Y-maze scented by odors from MHC-congenic mice or their urines.

The phylogenetic implications of such a genetically controlled sensory communication system, affecting behavioral and possibly other aspects of reproduction, may be considerable. We wished to determine whether sensory perception of MHC types plays a role in the blocking of pregnancy caused by exposure of fertilized females to strange males.

All females in this study were of the inbred strain BALB/c, whose MHC type is H-2^d. The stud males were either B6 (H-2^b) or congenic B6-H-2^k. The second (test) male, to which the fertilized females were exposed, was either the same stud male, or a male of the same strain as the stud male, or a congenic male (B6 if the stud male was B6-H-2^k, and B6-H-2^k if the stud male was B6).

Females were examined for return of estrus until day 7, (day 0 = discovery of vaginal plug) when the uterus was removed from some females to determine the presence of embryos. Initiation of the estrus cycle during this time was taken to indicate a blocked pregnancy or blocked pseudopregnancy; where there was no evidence of estrus, pregnancy or pseudopregnancy was inferred.

Results indicate that the incidence of pregnancy or pseudo-pregnancy block in BALB/c females is greater when the blocking male differs from the stud male at the MHC (H-2) locus than is the case when the blocking male has the same MHC type as the stud male.

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AN ULTRASTRUCTURAL STUDY OF OLFACTORY RECEPTOR DIFFERENTIATION IN EMBRYONIC RAINBOW TROUT, *SALMO GAIARDNERI*. Barbara Zielinski and Toshiaki J. Hara, Freshwater Institute, Department of Fisheries and Oceans, and Department of Zoology, University of Manitoba, Canada.

The olfactory epithelium of adult rainbow trout contains two morphologically distinct receptor cells, ciliated (CRC) and microvillous (MRC). The significance of this cellular heterogeneity is not known: it may reflect functionally independent cell types or transient stages in the differentiation of a single morphological form. Both the CRC and MRC are identifiable as receptor cells by their ultrastructure and degenerative reaction to olfactory nerve section. In order to clarify the relationship between the CRC and MRC, the development of the olfactory epithelium in embryonic rainbow trout was followed using light and electron microscopy.

During the anlagen stage (at 12°C, 11 days after fertilization), the mitotic stem cells were clustered beneath a microvillar epidermal covering. As the olfactory placode infolded slightly and the epidermis started to degenerate (14 days), young neurons were ultrastructurally distinguishable. Parallel stacks of rough endoplasmic reticulum had begun to form over large ovoid nuclei and axons formed a fasciculus at the base of the placode. The dendritic precursors contained mitochondria, Golgi complexes, smooth and rough endoplasmic reticulum and free ribosomes. The first CRC had differentiated at 18 days, when the olfactory nerve extending to the presumptive olfactory bulb became histologically identifiable. Three days before hatching (29 days), the number of CRC had increased, yet only a few immature MRC were present. In addition to this latency in MRC development, the two neurons formed their dendritic free surface in a different manner. The olfactory knob from CRC initially expanded, followed by ciliogenesis, whereas the free surface of the young MRC was narrow and only after many microvilli had developed did the olfactory knob form.

Throughout this study no transitional forms between the MRC and CRC were observed. These results clearly indicate that the MRC and CRC are independent cell types.

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