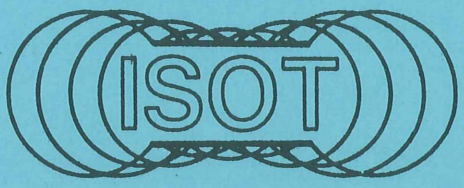


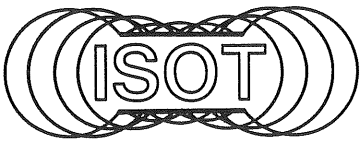
INTERNATIONAL SYMPOSIUM ON OLFACTION AND TASTE

Association for Chemoreception Sciences

July 20-24, 1986

Snowmass Village, Colorado

Abstracts of Papers



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ABSTRACTS

These are abstracts of papers presented at the IX International Symposium on Olfaction and Taste in Snowmass, Colorado, July 20-24, 1986.

The abstracts are listed in order of presentation at the meeting. The abstracts for the slide presentations precede the abstracts for the poster session on each day.

ACKNOWLEDGEMENTS

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Monday, July 21

F1 EVOLUTIONARY PATTERNS IN SENSORY RECEPTORS: AN EXERCISE IN ULTRASTRUCTURAL PALAEONTOLOGY. David T. Moran. (Rocky Mountain Taste & Smell Center, Department of Cellular and Structural Biology, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, CO 80262 USA).

In order to survive and reproduce, living things -- be they amoebae or elephants -- must be able to respond to changes in their immediate surroundings. It is no accident, then, that all things great and small are sensitive beings. Amongst the Protozoa, for example, an amoeba will detect and avoid a region of high pH. Euglena, armed with its photosensitive eye-spot, will move toward the sun. Paramecium has motile cilia that not only act as mechanoreceptors, but also are covered by excitable membranes. Amongst the Metazoa, a bewildering variety of sensory receptors exists that respond to a wide range of environmental stimuli. Investigation of the ultrastructure of Metazoan sensory receptors reveals a pattern of organization common to many of them; many sensory receptors employ cilia, microvilli, or both at the site of stimulus reception. This theme is dramatically played out in the micro-architecture of the sensory receptors of the chemical senses. Recent investigations of the noses of Chondrichthyes reveal that sharks and rays have highly sensitive olfactory receptors that contain microvilli at the site of stimulus reception. Teleost fishes such as the trout, on the other hand, have both microvillar and ciliated olfactory receptors. Mammals, it seems, utilize modified cilia at the transducer sites of their olfactory receptors -- and have microvillar receptors in the vomeronasal organs of their accessory olfactory systems. Some birds, such as the duck, have sensory receptors that contain both cilia and microvilli on the very same receptor cell! It is widely held that the plasma membranes of chemosensory neurons contain specific receptors that participate in the detection of chemical stimulants. Cilia and microvilli, both being long, slender extensions of the plasma membrane, provide tremendous physical amplification of the surface area of the cell available for receptor-stimulant interactions. Since cells, left to passively bow to minimum-energy considerations, would be spheres, it takes special structures to support long, slender, axial extensions of the cell surface. Consequently, both cilia and microvilli are supported -- and probably generated -- by the polymerization of proteinaceous subunits into cytoskeletal elements. Cilia are supported by axonemes; the axonemes center their structure around microtubules; and microtubules are polymers of tubulin, an ancient protein that is at least as old in the evolutionary scale as the mitotic spindle. Microvilli are supported by core filaments made of actin; and actin, too, is an ancient protein, common to the vast majority of cells that can change their shape or move their organelles about within their cytoplasm. Sensory receptors, it seems, have made use of these two ancient proteins -- tubulin and actin -- to generate axial extensions of their cell surface that promote interactions central to sensation.

Supported by NS-20486 (NIH) and BNS-8210327 (NSF).

NOTES

F2 SENSORY TRANSDUCTION IN FLAGELLATE BACTERIA. Judith P. Armitage. (Microbiology Unit, Dept. Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.).

Bacteria swim by rotating semi-rigid helical flagella, composed of monomers of the protein flagellin. The flagella are rotated at their base in the cytoplasmic membrane by the movement of protons down the electrochemical gradient between or near to two protein rings attached to the cytoplasmic membrane. The mechanism by which an electrochemical gradient can be transformed into rotational, mechanical work is unknown.

Bacteria change direction every few seconds, in most bacteria e.g. Escherichia coli this is brought about by briefly changing the flagellar rotation from counterclockwise to clockwise. In the photosynthetic bacterium Rhodobacter sphaeroides it is caused by stopping flagellar rotation for a few seconds, Brownian motion reorienting the cell before the next period of swimming.

When faced with a chemical gradient the direction changing frequency is altered to bias the movement of the bacteria towards a favourable environment. The signal changing the switching frequency of the flagellar motor arises either from the binding of the chemoeffector to specific sensory transducing proteins (MCPs), which span the cytoplasmic membrane, or in the case of PTS sugars, oxygen or light an MCP-independent change in the electrochemical proton gradient (pmf). The majority of bacterial species have both MCP and pmf dependent sensory systems. Photosynthetic bacteria however have only the pmf dependent pathway.

The possible mechanisms involved in motor rotation and the interaction of the different tactic signals with the motor to balance the responses to chemo-effectors, oxygen and light so that bacteria move to, and maintain themselves in the optimum environment, will be discussed.

F3 **EUKARYOTIC UNICELLS: HOW USEFUL IN STUDYING CHEMORECEPTION?** Judith Van Houten and Robin R. Preston. (Department of Zoology, University of Vermont, Burlington, VT 05405)

There are compelling reasons to use unicells in the study of chemoreception, particularly receptor cell function. Their hallmark is versatility and their most important attribute is the availability of useful mutants. In particular, cells can be grown in large homogeneous populations to provide material for biochemistry; cells are large for convenient electrophysiology; and mutant cell lines provide opportunities to apply a genetic dissection to the identification of chemoreceptors and other components of the chemosensory transduction pathway.

Paramecium tetraurelia is an example of one such useful system. Paramecium, like other ciliates, has an excitable membrane; it has been referred to as a swimming neuron. The cells respond to organic stimuli, such as folic acid and cAMP, by binding the stimulus to specific saturable sites on the cell body membrane. These sites can be crosslinked to ligand and response to stimulus is specifically blocked. Binding of ligand is transduced into a characteristic electrical response, a hyperpolarization, which is useful information for the cell. A hyperpolarization will change ciliary beating to produce a long, smooth swimming path and, therefore, gradual movement up the gradient of stimulus, which, in these cases, signifies the presence of food. The membrane potential change may result from an increased cell permeability to calcium, with the cation inducing yet another ion flux (other than K or Na) to produce the hyperpolarization. Fluorescent calcium-sensitive dye experiments support this notion. As do other chemoreception systems, Paramecium adapts, but the molecular basis of this adaptation is as yet unknown. Adaptation (or indeed transduction) may involve a receptor-mediated phospholipid turnover, which liberates inositol phosphates and calcium and activates protein kinase C. Lithium, which should block this turnover pathway, inhibits chemoreception.

Cells harvested in mass provide large quantities of membrane proteins. Affinity chromatography, ^{125}I surface labeling and exo- and photoaffinity-crosslinking of ligand to binding sites is used to enrich for and identify binding proteins of interest. Most importantly, mutants can be generated to provide membranes lacking chemoreceptor binding sites or specific membrane currents. Such mutants can be used to dissect out the chemoreceptors among the other membrane proteins, the ionic basis of transduction, and the molecular basis of adaptation.

(Supported by NSF 12176 and NIH 29045)

F4 IONIC MECHANISM OF GENERATION OF RECEPTOR POTENTIAL IN FROG TASTE CELL. Toshihide Sato, Yukio Okada and Takenori Miyamoto (Dept. Physiology, Nagasaki University School of Dentistry, Nagasaki 852, Japan).

A taste cell membrane consists of the apical taste receptor membrane covered with a superficial fluid (SF) and the basolateral membrane surrounded with an interstitial fluid (ISF). It has been considered that both ionic permeability of the receptor and basolateral membranes and phase boundary potential occurring at the receptor membrane surface may play an important role in generating the receptor potential for taste stimuli. However, an exact understanding of the receptor potential is still lacking.

The purpose of this study is clarify the mechanism underlying the generation of receptor potentials for salt, acid and bitter stimuli. The receptor potentials were recorded intracellularly from taste cells in the tongue of bullfrogs.

Replacement of normal Ringer covering receptor membrane with Ca-free, 1 mM amiloride Ringer resulted in a 50% reduction of receptor potential induced by 0.5 M NaCl. Replacing the normal ISF with Na^+-free Ringer reduced the receptor potential induced by NaCl by 40%. These findings suggest that the NaCl-induced receptor potential is due to a 50% contribution of amiloride-sensitive Na-channel and cation-selective channel at the receptor membrane, a 40% contribution of Na-channel at the basolateral membrane and a 10% contribution of the phase boundary potential.

The receptor potential elicited by 1 mM HCl was independent to ion components of ISF. The HCl-induced receptor potential was reduced to 35% by removing Na^+ and Ca^{2+} in SF. It may be concluded the HCl-induced receptor potential is due to a 65% contribution of Ca-channel at the receptor membrane and a 35% contribution of phase boundary potential.

Removal of Ca^{2+} and Na^+ in SF did not affect the receptor potential induced by quinine-HCl (Q-HCl), while removal of Na^+ or Cl^- in ISF reduced the Q-HCl response by 75%. Furosemide in ISF greatly reduced the Q-HCl response. These findings suggest that 75% of the Q-HCl response is due to a release of Cl^- through the receptor membrane, but the remaining 25% is due to the phase boundary potential.

Supported by a Grant-in-Aid (#60480402), Japan.

F5 TOWARDS A COMPREHENSIVE MOLECULAR ANALYSIS OF OLFACTORY TRANSDUCTION. Doron Lancet, Zehava Chen, Adina Ciobotariu, Ayus Corcia, Judith Heldman, Dov Ophir, Mark Pines and Umberto Pace. (Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel).

Following the demonstration of cyclic AMP (cAMP) as a probable second messenger in olfactory transduction and of the stimulatory GTP binding protein (G-protein) as a signal-coupling element in odor reception, we now concentrate on the identification, isolation and prospective molecular cloning of olfactory receptor proteins, and on the mode of action of cAMP on olfactory ion channels. The effort to isolate and clone odorant receptors proceeds along several routes: a) we have purified polypeptide gp95, the major transmembrane glycoprotein of frog olfactory cilia, and a prominent receptor candidate, in quantities large enough for microsequence analysis. This was done by a combination of Triton X-114 fractionation, wheat germ agglutinin (WGA) affinity chromatography and electroelution from SDS polyacrylamide electrophoretic gels. Proteolytic fragments now being prepared will undergo gas phase microsequence analysis, and serve to instruct oligonucleotide probe synthesis, that can be used to screen olfactory epithelial cDNA libraries; b) monoclonal antibodies, which have been prepared against olfactory cilia and demonstrated to react with gp95, can be used to screen cDNA expression libraries; c) the association of transductory enzymes with receptor candidates is being studied: antibodies to gp95 were found to immunoprecipitate adenylate cyclase activity of olfactory cilia, suggesting an interaction between the two molecular components; d) procedures have been developed to separate detergent soluble ciliary proteins in an attempt to achieve functional reconstitution of odorant stimulation. Since ternary reconstitution of the receptor-G-Protein-cyclase system may be difficult, we developed a GTPγs binding assay that can allow to monitor receptor G-protein interaction directly. Additional information is obtained through the use of polyclonal antibodies against G-protein subunits.

Two alternative scenarios are anticipated for the distal effect of cAMP. The first, that cAMP dependent protein kinase is involved in ion channel modulation. cAMP dependent kinase activity is found to be present in the frog olfactory cilia preparation, and at least two ciliary polypeptides, pp24 and pp26, are specifically phosphorylated in response to cAMP. GTPγs is effective in enhancing ciliary protein phosphorylation, probably through endogenous cAMP generation. cAMP could also act through direct gating of ion channels, similar to cGMP in retinal rods. This possibility is being studied by patch clamp recordings in lipid bilayers reconstituted with ciliary membranes.

NOTES

Monday, July 21

S1 DYNAMIC CONFORMATIONAL CHANGES OF RECEPTOR DOMAINS IN GUSTATORY AND OLFACTORY CELL MEMBRANES. Kenzo Kurihara, Makoto Nakamura and Makoto Kashiwayanagi. (Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan)

Properties of the receptor sites for various stimuli in the gustatory and olfactory cell membranes were examined by measuring dependences of the responses on temperature and divalent cations.
1. The rat taste nerve responses to various stimuli increased with an increase of temperature between 5 and 32 °C and decreased above 32 °C. The slopes of the curves for the response magnitude-temperature relationships greatly varied with species of stimuli. The slopes for sucrose and glycine were much larger than those for NaCl and HCl. The temperature dependence of the response to 10 mM NaCl was very small. The results obtained suggested that the temperature dependence does not come from that of the transmitter release process but comes from that of conformational changes of the receptor domains. The temperature dependence of the frog olfactory responses were also examined. The responses to various odorants were increased with an increase of temperature up to 30 °C. Similar temperature dependence was observed with the depolarization of the neuroblastoma by odorants. These temperature dependences were also explained in terms of conformational changes of the receptor domains.
2. The presence of divalent cations in the adapting solution for the frog tongue led to modification of the magnitude and the time course of the taste nerve responses. Adaptation of the tongue to Ca containing solution (e.g. 7.5 mM) led to an enhancement of the magnitude of the response to HCl, while it did not affect the responses to NaCl and quinine. Adaptation of the tongue to Ba containing solution led to enhancement of the responses to HCl and quinine. The ability of the tongue to produce the enhanced responses to these stimuli held in the absence of Ba after the tongue was adapted to solutions containing high concentrations of Ba.

The frog nerve responses to HCl and quinine declined rapidly to the spontaneous level after application of the stimuli. After the tongue was adapted to a solution containing 7.5 mM Ca or 2.5 mM Ba, long lasting tonic component appeared in the responses to HCl and quinine. Thus the time course of the responses was greatly modified by the divalent cations.

The effects of Ca and Ba were not inhibited by Ca-channel blockers, suggesting that influx of the divalent cations into taste cells is not concerned with the modification of the responses. The divalent cations seem to modify the receptor sites for the stimuli.

NOTES

S2 A DIFFUSION POTENTIAL MODEL OF SALT TASTE RECEPTORS. Harry Wms. Harper. (Eastern Research Center, Stauffer Chemical Co., Dobbs Ferry, NY 10522).

Quantitative measurements were made of response magnitudes in the chorda tympani nerve of the hamster as the tongue was stimulated with solutions which varied in composition and concentration. These measurements eliminated a systematic error which has contaminated previous results based on whole-nerve responses: the mean population firing rate of the nerve fibers is proportional to the averaged square of the amplified nerve potential, not the average rectified value.

For salts, the data obtained are not consistent with a first-order stimulus-receptor adsorption process. The data are well accounted for by a model of receptor function based on diffusion potentials. Two kinds of variable diffusion potentials are involved: resting potentials of taste cell receptor membranes (between the variable taste pore contents and the constant interiors of taste cells); and liquid junction potentials (between taste pore contents and the constant interstitial fluid of taste buds). An excitatory current determined by these potentials passes through the taste cell membranes deep within the taste bud. Two kinds of receptor cells are required: one kind with receptor membranes selectively permeable to sodium-like ions, and another kind selective for potassium-like ions.

Receptor membrane resting potentials can be calculated using the Goldman-Hodgkin-Katz equation. Novel methods make possible accurate calculation of liquid junction potentials at experimental concentrations. The diffusion potential model predicts, and neural data confirm, that responses should vary directly with the liquid junction potential. Observed responses are also consistent with the Nernst potentials expected at the receptor membranes of sodium (or potassium) cells when the tongue is exposed to varying concentrations of sodium (or potassium). Peripherally distinct sodium and potassium receptors are confirmed by the reported effects of amiloride on taste responses.

Research conducted at Rockefeller University.

S3 COMPARISON OF EFFICACY OF STIMULATORS AND INHIBITORS OF THE GERBIL'S SUGAR TASTE RESPONSE. William Jakinovich, Jr. and Vasiliki Vlahopoulos (Dept. of Biological Sciences Herbert H. Lehman College, CUNY, Bronx, N.Y. 10468).

Over the last several years, we have been conducting experiments, using the gerbil's electrophysiological taste responses, to determine the mechanism of sugar taste reception. In pursuit of that mechanism, we have tested derivatives of sugar stimulators as well as inhibitors of the gerbil's sugar taste response. Based on our structure-activity experiments, we have found the following commonalities in responses to the stimulators and to the inhibitors of the animal's sucrose response:

1. With regard to orientation of the substituent group at the C-1 position of the glycopyranoside ring, all the alpha derivatives are more potent than their beta counterparts.

2. We have also discovered that a methyl group at the C-1 position enhances the action of the stimulator as well as that of the inhibitor.

3. In addition, we have found an inverse relationship between the stimulators and inhibitors in that the most potent inhibitors are derivatives of the weakest stimulators.

These discoveries suggest that the stimulators and the inhibitors are all interacting with the same receptor site-THE SUCROSE RECEPTOR SITE.

Supported by Grant H. ROINS 16022 from the NIH and PSC-CUNY.

S4 **ENANTIOMERIC SPECIFICITY OF ALANINE TASTE RECEPTOR SITES IN CATFISH.** Joseph G. Brand[1,2], Bruce P. Bryant[1], Robert H. Cagan[3] and D. Lynn Kalinoski[1]. ([1]Monell Chemical Senses Center and [2]Veterans Administration Medical Center, University of Pennsylvania, Philadelphia, PA 19104 and [3]Colgate-Palmolive Co., Research and Development, Piscataway, NJ 08854).

The mechanisms underlying discrimination of enantiomers by taste receptors are not known. This question has been addressed using an experimental model amenable to a combined biochemical and electrophysiological approach. Specific binding of amino acid taste stimuli is known to occur to a sedimentable fraction (P2) from catfish (Ictalurus punctatus) taste epithelium and to purified plasma membranes derived from that fraction. The binding characteristics of L-alanine, a potent taste stimulus for the catfish, were documented previously. The extent to which the enantiomeric stimuli, L- and D-alanine, interact with the same or different receptor/transduction processes is reported here, using both electrophysiological and biochemical techniques. The electrophysiological assay showed L-alanine to be a more potent stimulus than D-alanine across a concentration range of 10^{-9} to 10^{-3} M, even though their threshold values appeared to be nearly equal (10^{-9} to 10^{-8} M). With most of the nerve bundle preparations studied, L- and D-alanine cross-adapted one another, but this cross-adaptation was not always complete. This suggested that some, but not all, of the responses occur through a common pathway. Experiments in which both L- and D-alanine were present in a 1:1 mixture of equally stimulatory concentrations, suggested the existence of receptor or transduction processes unique to each enantiomer. Biochemical binding studies demonstrated high affinity binding sites for both enantiomers, with values for K_{Dapp} for L-alanine of 1.5 µM and for D-alanine of 25 µM. For both enantiomers additional lower-affinity binding sites were observed. The capacity of the lower affinity sites was particularly great for D-alanine. The enantiomers compete with one another for binding, with L-alanine showing greater competitive ability than D-alanine at low concentrations. Double reciprocal plots of the binding inhibition data suggest a competitive mechanism. The lower affinity sites for D-alanine are less susceptible to L-alanine inhibition than are the high affinity sites for D-alanine. Taken together, these studies suggest that a portion of the responses to L- and D-alanine occurs through a common receptor/transduction process, and also that more than one receptor/transduction process exist for the enantiomeric pair, L- and D-alanine.

Supported in part by NIH Research Grants NS-15740 and NS-22620, by NRSA Fellowship #1 F32 NS07809-01-BNS-1, and the Veterans Administration.

S5 **SPECIFIC L-ARGININE TASTE RECEPTOR SITES: BIOCHEMICAL AND NEUROPHYSIOLOGICAL STUDIES.** D. Lynn Kalinoski[1], Bruce P. Bryant[1], Gadi Shaulsky[1] and Joseph G. Brand[1,2]. ([1]Monell Chemical Senses Center, and [2]Veterans Administration Medical Center, University of Pennsylvania, Philadelphia, PA 19104).

Amino acids are effective taste stimuli for the channel catfish, Ictalurus punctatus. From the results of neurophysiological (Caprio, Chemoreception in Fishes, Elsevier, p. 109, 1982) and behavioral studies (Stewart et al., Biol. Bull. 157: 396, 1979), at least two different classes of taste receptors have been inferred: one responding to alanine and some other neutral amino acids, the other responding to arginine. While the alanine binding site has been extensively characterized (Cagan, Biochemistry of Taste and Olfaction, Academic Press, p. 175, 1981), less is known of the site(s) for L-arginine binding. We report here the initial characterization of the arginine binding site(s). Binding of L-[^{3}H]arginine to the sedimentable Fraction P2 from the taste epithelium was measured by a modification of the method of Krueger and Cagan (J. Biol. Chem. 251: 88, 1976), in which an isotope dilution step was included to reduce nonspecific binding. Time and pH for measuring maximal binding activity were established. At pH 7.8, the rate constant for association at 4°C was 1.1×10^5 M^{-1} min^{-1}. Dissociation was more complex yielding rate constants of 1.4 min^{-1} and 4.1×10^{-2} min^{-1}. From these data there appear to be at least two binding sites for arginine with K_D's of 1.3×10^{-5} M and 3.7×10^{-7} M. The ability of D-arginine, L-lysine, L-α-amino-β-guanidino-propionic acid (L-AGPA) and L-arginine to displace L-[^{3}H]arginine binding was tested under equilibrium conditions. The complex patterns of inhibition by L-arginine and L-lysine support the existence of at least the two different affinity classes of the receptor. L-AGPA and D-arginine inhibited binding of only the lower affinity L-arginine site. Significant inhibition by L-glutamate, glycine and L-alanine occurred only above 10^{-3} M, indicating that the binding site(s) for L-arginine is/are selective. Using multiunit recordings, neurophysiological studies examined the stimulatory effectiveness of a number of guanidinium-containing compounds. Only L-arginine and L-AGPA were effective stimuli. Cross-adaptation experiments using the same preparation examined the ability of 10^{-4} M glycine, L-AGPA, L-lysine, L-glutamate, and L-alanine to inhibit responses to 10^{-6} M L-arginine. Only L-AGPA was an effective cross-adapting stimulus. Taken together, these results indicate that effective agonists of L-arginine receptors must contain a guanidinium group and an unblocked L-α-amino group.

Supported in part by NIH Research Grants #NS-15740 and NS-22620, by NRSA Fellowship 1-F32-NS07809-01-BNS-1, and the Veterans Administration.

S6 **ODORANT- AND GUANINE NUCLEOTIDE-STIMULATED PHOSPHOINOSITIDE TURNOVER IN OLFACTORY CILIA.** Richard C. Bruch and Taufiqul Huque (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104).

A method was developed for the isolation of olfactory cilia from the channel catfish (Ictalarus punctatus) with improved yield. The cilia were detached by calcium shock treatment of the olfactory epithelium in the presence of 300 mM sucrose. Following centrifugation to remove the deciliated epithelium, the cilia were centrifuged at 28,000 x g, resuspended, and layered on a 45% (w/w) sucrose cushion. The gradient was centrifuged at 50,000 x g and the cilia were collected from the top of the sucrose cushion, whereas pigmented material was pelleted at the bottom of the cushion. Under these conditions, the yield of cilia was 165 ± 9 ug protein/g tissue (n = 5 preparations). Using the same conditions, but with no sucrose in the deciliation medium, the yield of cilia was 92 ± 30 ug protein/g tissue (n = 3). The isolated preparations were characterized by transmission electron microscopy, SDS-polyacrylamide gel electrophoresis, and Western blotting to identify individual protein components such as tubulin.

The isolated cilia exhibit phosphatidylinositol-4,5-bisphosphate phosphodiesterase (PIP_2-PDE, E.C.3.1.4.11) activity which is enriched about 4-fold over the activity of the intact epithelium. Electrophysiological studies have demonstrated previously that amino acids are olfactory stimuli for the catfish. The isolated cilia preparations contain receptors for odorant amino acids as demonstrated by tritiated L-amino acid binding measurements. PIP_2-PDE activity was stimulated in the isolated preparations in the presence of odorant amino acids. For example, the enzyme activity was stimulated 57% over basal level in the presence of 5 uM L-alanine. Furthermore, the PIP_2-PDE activity was also stimulated 39% over control levels in the presence of 0.1 uM GTP. The latter observation, in combination with the identification of guanine nucleotide-binding proteins (G-proteins) by immunoblotting, strongly suggest the participation of a G-protein in activation of phosphoinositide turnover. In combination, these results indicate that olfactory receptor occupancy stimulates phosphoinositide turnover by a mechanism that probably depends on the participation of a G-protein.

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NOTES

S7 EFFECTS OF ODORANT MIXTURES ON OLFACTORY RECEPTOR CELLS. Steven Price. (Dept. of Physiology and Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298)

Olfactory neurons respond to odorants by changing the frequency with which they generate action potentials. Hyperpolarization decreases their odorant sensitivities while partial depolarization increases them. Thus, changes in membrane potential caused by any chemical will alter the responses of the cell to other chemicals. The membrane potential of a neuron depends upon its metabolic state and membrane charge density as well as on the composition of the mucus or extracellular fluid. Chemicals can alter the membrane potential via effects on any of these as well as through transduction processes mediated by membrane receptors. Since different chemicals will have different effects, the behavior of a mixture is expected to vary with its composition. Thus, some mixtures may have additive effects on olfactory neuronal firing frequency while others may be synergistic or show evidence of inhibition.

Supported by the U.S. Army Research Office.

NOTES

S8 TRANSDUCTORY PROTEINS OF OLFACTORY RECEPTOR CELLS: IDENTIFICATION OF GUANOSINE NUCLEOTIDE BINDING PROTEINS AND PROTEIN KINASE C. Robert R.H. Anholt[1], Suzanne M. Mumby[2], Doris A. Stoffers[1], Peggy R. Girard[3], J.F. Kuo[3], Alfred G. Gilman[2] and Solomon H. Snyder[1]. ([1]The Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, [2]The Department of Pharmacology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235, and [3]The Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322).

We have analyzed the transductory guanosine nucleotide binding proteins (G-proteins) in the olfactory epithelium of Rana catesbeiana using monospecific antisera. The olfactory epithelium contains the α subunits of three transductory G-proteins, migrating on polyacrylamide gels in SDS with apparent molecular weights of 45,000, 42,000 and 40,000 daltons, corresponding to G_s, G_i and G_o, respectively. A single β subunit with an apparent molecular weight of 36,000 daltons is detected. The olfactory cilia appear to be enriched in G_s relative to G_i and G_o when compared to deciliated epithelial membranes prepared from the olfactory epithelium after detachment of the cilia. G-proteins are not detected in cilia detached from the non-chemosensory respiratory epithelium of the palate. Immunohistochemical studies using antisera that recognize all α and β subunits of G-proteins reveal intense staining of the ciliary surface of the olfactory epithelium. The cell membranes of the olfactory receptor cells and axon bundles in the lamina propria are also stained along with the acinar cells of the Bowman's glands and the deep submucosal glands. In addition to G-proteins, we have identified protein kinase C in olfactory cilia via phorbol ester binding and via a protein kinase C specific antiserum. However, in contrast to the G-proteins, protein kinase C occurs also in cilia isolated from respiratory epithelium. A monospecific antiserum against the α subunit of retinal transducin fails to detect immunoreactive proteins in either olfactory or respiratory cilia. Thus, our observations suggest that signal transduction mechanisms at the chemosensory membrane bear more resemblance to common hormonal transduction mechanisms than to the retinal phototransduction process.

S9 THE ODORANT-SENSITIVE ADENYLATE CYCLASE OF OLFACTORY RECEPTOR CELLS: DIFFERENTIAL STIMULATION BY DISTINCT CLASSES OF ODORANTS. Pamela B. Sklar, Robert R.H. Anholt, and Solomon H. Snyder (The Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205).

We have characterized odorant-stimulated adenylate cyclase activity in isolated chemosensory cilia prepared from frog and rat olfactory epithelium. Cilia from both species exhibit high levels of adenylate cyclase activity. Basal activity was stimulated approximately 2-fold by GTP and approximately 5-fold by $GTP\gamma S$ (guanosine-5'-O-(3-thiotriphosphate) and forskolin. Odorants stimulated enzyme activity 20-65°/° above the basal level in a tissue-specific and GTP-dependent manner. Calcium reduced GTP-stimulated activity with a half-maximal effect at 10 μM.

We have characterized the effect of a large number of odorants on olfactory adenylate cyclase activity. In general, fruity, floral, minty, and herbaceous odorants stimulate the enzyme. Citralva, menthone, D-carvone, L-carvone and 2-isobutyl-3-methoxypyrazine display similar potencies in activating the adenylate cyclase with maximal activity observed at 100 μM. Putrid odorants and chemical solvents such as isovaleric acid, triethylamine and pyridine do not stimulate enzyme activity. In homologous series of pyrazine, thiazole, and pyridine odorants, compounds with the longest hydrocarbon side chains were best able to enhance enzyme activity. Certain odorants fail to stimulate adenylate cyclase activity implying that at least one additional transduction mechanism is involved in olfaction.

S10 LOCALIZATION OF ODORANT BINDING PROTEIN (OBP) TO NASAL GLANDS AND SECRETIONS. Jonathan Pevsner, Pamela B. Sklar and Solomon H. Snyder. (Depts. of Neuroscience, Pharmacology and Experimental Therapeutics, Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205).

We purified an odorant binding protein (OBP) from bovine nasal epithelium (Pevsner et al., P.N.A.S. 82:3050), a finding obtained independently by Bignetti et al. (Eur. J. Pharm., 149:227). Bovine OBP is a soluble homodimeric protein with a subunit molecular weight of 19,000 daltons. It binds the tritiated odorants 2-isobutyl-3-methoxypyrazine, 3,7-dimethyloctan-1-ol, amyl acetate, and methyldihdrojasmonate with micromolar to millimolar affinities (Pevsner J., Sklar P.B. and Snyder S.H., in press, 1986). OBP has been purified from rat olfactory epithelium and has a molecular weight of 21,000 daltons and similar odorant binding activity.

A polyclonal antiserum to bovine OBP was produced. Immunohistochemical localization in olfactory and respiratory epithelium reveals heavy staining in the glands of the lamina propria. Based on this localization to mucus-secreting glands, we have purified OBP from rat mucus. Odorant binding activity was detected in tears but not saliva. OBP purified from rat nasal epithelium, mucus and tears comigrate by reverse-phase HPLC and all have identical molecular weights.

Specific and saturable binding is detected in the mucus of rats exposed in vivo to the odorant [^{3}H]3,7-dimethyloctan-1-ol. OBP may serve to transport hydrophobic odorants within nasal mucosa. The degree of facilitated diffusion of several odorants by OBP is estimated to be comparable to that of oxygen by myoglobin.

S11 MAB RB-8 THAT DISTINGUISHES CHEMICALLY DISTINCT ZONES IN PRIMARY OLFACTORY PROJECTION RECOGNIZES 125 KDA MEMBRANE-ASSOCIATED PROTEIN. James E. Schwob and David I. Gottlieb. (Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110).

We have recently reported the immunohistochemical characterization of a novel mouse monoclonal IgG1, designated RB-8, which identifies two chemically distinct zones in the primary olfactory projection of adult rats (Soc. Neurosci. Abstr., 1985). The axons from the ventrolateral part of the olfactory epithelium and their terminals in the glomeruli of the ventrolateral olfactory bulb are densely stained by RB-8 (we term this part of the projection RB-8 positive), while axons from the dorsomedial part of the epithelium and their terminals in the dorsomedial bulb are unstained or only lightly stained (termed RB-8 negative). RB-8 is nervous system specific and stains other parts of the CNS and PNS nonhomogenously.

We report here the biochemical characterization of the antigen recognized by RB-8 in whole brain and in olfactory nerve from adult rat. As assayed by the binding of ^{125}I-RB-8 IgG, the RB-8 antigen in whole brain homogenates was shown to be membrane associated, since it is sedimented by centrifugation after repeated washing with hypotonic buffer and is solubilized by nonionic detergents. Binding of RB-8 to brain membranes is saturable and high affinity. RB-8 recognizes a distinct antigen since bound ^{125}I-antibody is displaced only by unlabelled RB-8 IgG and is unaffected by other antibodies. This antigen is trypsin-sensitive.

Membrane proteins harvested from the olfactory nerve layer of the bulb and those from forebrain excluding the bulbs were separated by SDS-PAGE and then compared on nitrocellulose blots. Both contain immunoreactive proteins of 125 kDa M_r which comigrate when olfactory nerve and whole brain membranes are electrophoresed together in a single lane. This 125 kDa protein is enriched more than 100-fold when whole brain membrane proteins are solubilized with octyl glucoside detergent and passed over an RB-8 immunoaffinity column.

In conclusion, the RB-8 antigen in whole brain and olfactory axons is a membrane associated protein of 125 kDa M_r. The division of the primary olfactory projection into two zones may be functionally important, given evidence for some spatial organization in olfactory sensory coding and given a similar division of the rabbit olfactory system as revealed by an independently generated monoclonal antibody (Mori et al, 1985, JCN, 242: 214). Further characterization of the RB-8 antigen will prove useful in understanding its functional role and the reason for its differential distribution in the primary olfactory projection.

Supported by NIH grants NS 12867, NS 07076, and NS07057.

S12 LECTIN BINDING SITES IN OLFACTORY SENSILLA OF THE SILKMOTH, ANTHERAEA POLYPHEMUS. Thomas A. Keil (Max-Planck-Institut für Verhaltensphysiologie, Gruppe Kaissling, D-8131 Seewiesen, West Germany).

In single-walled olfactory sensilla of insects, the "pore tubules" are thought to be the pathways by which odor molecules traverse the cuticular hair walls and reach the membranes of the olfactory dendrites (Steinbrecht & Müller, Z. Zellforsch. 117, 1971). Most probably, polyanionic surface coats of pore tubules and dendritic membranes are responsible for the formation of contacts between these structures (Keil, Tissue & Cell 16, 1984). In order to investigate the composition of these coats, sugar-specific lectins, conjugated with ferritin (e-y-laboratories, San Mateo, California), have been applied to apically opened hairs which were then processed for transmission electron microscopy.

The dendritic membranes bound the following lectins (approximately in order of their binding intensity):

Concanavalin A (Con A)
(this is quite unspecific, but is said to have a preference for α-D-mannose and α-D-glucose);

Bauhinia purpurea (BPA)
(N-Ac-D-galactosamin, D-galactose);

Limax flavus (LFA)
(sialic acid);

Glycine max (SBA)
(N-Ac-α/β-D-galactosamin, α-D-galactose);

Griffonia simplicifolia (GS II)
(N-Ac-α/β-D-glucosamin);

Ulex europaeus (UEA I)
(L-fucose).

The inner surface of the hair cuticle showed binding only of Con A, whereas the pore tubules only bound LFA very heavily.

Thus, the surface coats of the dendritic membranes seem to consist of several sugars, whereas that of the pore tubules contains only a high amount of sialic acid. Although these experiments have as yet yielded a way only for discriminating dendritic membranes and pore tubules, they might finally make possible the identification of receptor molecules, which most probably are glycoproteins (Chen & Lancet, Proc. Natl. Acad. Sci. USA 81, 1984).

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S13 IDENTIFICATION OF THE TERMINAL NERVE IN TWO AMPHIBIANS SHOWN BY THE LOCALIZATION OF LHRH-LIKE IMMUNOREACTIVE MATERIAL AND AChE. Celeste R. Wirsig and Thomas V. Getchell. (Dept. Anatomy and Cell Biology, Wayne State University, Detroit MI 48201).

The identification and characterization of the terminal nerve (TN) in the nasal mucosa and forebrain of the tiger salamander and bullfrog were studied using immunocytochemical or histochemical techniques. Antibodies against luteinizing hormone-releasing hormone recognized fusiform neural perikarya and smooth or varicose processes of the TN in the ventral forebrain and within the olfactory, vomeronasal and myelinated trigeminal nerve bundles in the nasal mucosa. Central projections of the LHRH-ir TN consisted of two groups: one terminated in the olfactory bulb whereas the other projected through the forebrain to the region of the lamina terminalis where TN fibers mingled with other LHRH-ir perikarya rostral to the preoptic recess. The peripheral termination pattern of the LHRH-ir fibers is less clear; we have observed only a few fibers apparently terminating in the olfactory or vomeronasal epithelium per se. Absorption of the antiserum with LHRH eliminated TN labeling. Up to four weeks following hypophysectomy, the labeling intensity and number of TN immunoreactive neurons was not altered.

Acetylcholinesterase (AChE) histochemistry in the salamander demonstrated two groups of labeled neurons. A lightly labeled group of fusiform neurons was found within the olfactory nerve proper. The data suggest that these AChE-containing neurons belong to the LHRH-ir population of neurons as previously demonstrated in the hamster. A second group of more heavily labeled neurons was present within AChE-positive fascicles on the ventral surface of the nasal mucosa. Inspection of these fascicles suggests that they are myelinated trigeminal bundles which also contain LHRH-ir fibers. This study has identified the TN in two amphibians and has demonstrated that the TN follows the olfactory, vomeronasal and trigeminal nerve bundles to reach peripheral targets. The central projection to the preoptic region is similar to TN projections in fish.

Supported by NIH-NS-16340.

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S14 IMMUNOFLUORESCENT STUDIES OF THE DEVELOPMENT OF RAT OLFACTORY EPITHELIUM. A. I. Farbman, J. I. Morgan and J. L. Hempstead. (Dept. of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201 and Roche Institute of Molecular Biology, Nutley, New Jersey 07110).

We have used monoclonal antibodies as specific probes to study the phenotypic expression of molecules during development of the olfactory epithelium. Our rationale was to monitor the first appearance of antigens of olfactory epithelial cells during development and possibly to identify those molecules that play significant roles in neuronal function. Antibodies were taken from 1) a panel of monoclonals made from homogenates of adult rat olfactory mucosa (Hempstead & Morgan, J. Neurosci., 5:438, 1985), 2) monoclonals made to crude membrane fractions of adult and neonatal rat olfactory mucosa, and 3) monoclonals made to total homogenates of E13-E14 fetal snouts. Screening was done by immunofluorescence on cryostat sections of adult, neonatal and fetal rat nasal cavities. Various antibodies were found that are specifically immunoreactive to the luminal surface of olfactory epithelium, olfactory supporting cells, Bowman's glands, the luminal surface of respiratory epithelium, and some to both respiratory and olfactory luminal surfaces.

In adult rat olfactory epithelium, Neu-5 antibody was bound primarily to olfactory nerve bundles and only weakly to perikarya. This antibody was first demonstrable in these structures as early as the 15th embryonic day.

In adults, Neu-4 and Neu-9 bound to both perikarya and axons. That these likely bound to different determinants was shown by the fact that Neu-9 was demonstrable on E-15, but Neu-4 was not demonstrable until E-16.

1A-6 antibody bound to the primitive cerebral vesicle and olfactory perikarya and axons as early as E14, but was virtually absent by E20. This antigen seemed to be transiently expressed by developing neurons but not in more mature cells.

Sus-1 bound to sustentacular cells in the main olfactory organ of adult rats. This monoclonal was only faintly reactive at E20.

4A-5 and 3B-3 were reactive with the luminal surfaces of respiratory epithelial cells of adult rats. In fetuses they became reactive at the time when ciliogenesis first occurs in these cells, i.e., sporadically at E18 and E19, and more in E20.

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S15 **MARKED POSTNATAL INCREASE IN THE TOTAL NUMBER OF OLFACTORY NEURONS AND SURFACE AREA OF THE MUCOSA IN THE RABBIT.** Esmail Meisami, Janice Leu, Robyn Hudson* and Hans Distel*. (Dept. Physiol.-Anat., Univ. California, Berkeley CA 94720, and *Inst. Med. Psychol., Univ. Munich, D-8000 München 2, FRG).

Recent behavioral studies indicate that the newborn rabbit is a particularly suitable animal for the study of olfactory development. At birth the animal's main olfactory system appears to be functional, as evident from its responsiveness to nipple-search releasing odors (Hudson & Distel, 1983; Distel & Hudson, 1984).

To determine the anatomical status of the newborn rabbit olfactory system, the olfactory mucosa and bulb were studied by means of light microscopic numerical and morphometric methods. The results were compared to the 30-day-old rabbit in which brain development is essentially completed. It was found that the olfactory mucosa (OM) possesses a mature-like histological organization in the newborn. However, between birth to day 30 the surface area of OM increases 3x (from 1.06 to 3.23 cm /side) and the mean thickness of the OM by 60% (from 60 to 82 u). Analysis of the distribution of surfaces revealed the increase in thickness and area to occur uniformly along the entire mucosa, suggesting a global expansion. Counts of nuclei of olfactory neurons (ON) at the two ages revealed no change in the volume density but a 50% increase in the surface density (from 65000 to 98000/mm). Total counts of ON per side increased 5x (from 6.1 to 31.4 million) during this period while the mean number of supporting cells increased 2.5x (from 3.3 to 8.4 million) and of basal cells 3.7x (from 1.0 to 3.7 million).

The postnatal expansion of OM is presumably a continuation of fetal growth and is accomplished by de novo formation of basal and supporting cells which are added laterally whereas the postnatal olfactory neurons are added vertically both by the new and pre-existing basal cells.

In addition to the possibility of endowing the growing animal with new olfactory capacities, the added population of ONs may provide a neural basis for the observed postnatal increase in olfactory sensitivity (Alberts & May, 1980), and as well for the maturational improvement in reactivity to odors releasing nipple-search behavior (Hudson, 1986). Supporting these contentions is the fact that the number of glomeruli in the bulb increases 2x while the number of mitral cells remains unchanged, during the same age period which would presumably lead to a marked increase in the degree of convergence of ONs onto the mitral cells.

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S16 **EVIDENCE FOR DIFFUSE AND FOCAL PROJECTIONS FROM THE OLFACTORY EPITHELIUM TO THE BULB** *G.D. Adamek, W.T. Nickell and M.T. Shipley.* (Dept. of Anatomy and Cell Biology, University of Cincinnati Medical Center, Cincinnati, OH 45267).

The labeling of glomeruli in olfactory bulbs (OB) of rats following small iontophoretic injections of WGA-HRP into the olfactory epithelium gave several surprising results. It is generally believed that there is a diffuse topological relationship between the primary olfactory neurons (PONs) and the OB. We have explored this relationship by tracing the projection of small, contiguous populations of PONs to OB.

The epithelium on the medial septum was exposed by removing the frontal bone and cauterizing a small hole in the dorsal epithelium. Pipettes with tip diameters of 2-5 μm were filled with 1% WGA-HRP and 0.9% NaCl and were placed ~80 μm below the level of the tight junctional band. Pulsatile (7s on-7s off) currents of 1-5 μA for 5-10 minutes deposited the WGA-HRP.

In all cases, heavily labeled glomeruli were scattered among lightly to faintly labeled glomeruli. In nearly all cases, a faint background of labeling was visible in all glomeruli throughout the OB. Although there were usually larger numbers of heavily labeled glomeruli on the medial side, isolated heavily labeled glomeruli could be found in any sector of OB. Our smallest injections showed a few heavily labeled glomeruli in both the medial and lateral sides of the bulb. In addition, within some heavily labeled glomeruli, reaction product was not uniformly distributed throughout the glomerulus but was predominantly localized within one half. Glomeruli in the contralateral bulb were never labeled. In a few cases, there was uptake of label by neurons outside the area of the injection site. There was never a general labeling of the epithelium.

Based on restricted injections of retrograde tracer in the olfactory bulb, others suggest that inputs come from a large area in the epithelium. However, it is not known whether small regions contribute differentially to the innervation of specific glomeruli. Our results suggest that within any small, contiguous population of PONs there are two sub-populations: one which projects diffusely throughout the bulb and another which projects more focally to a few glomeruli. Since there is continual neuronal turnover in the olfactory epithelium, it is tempting to speculate that immature and relatively non-selective PONs generate the diffuse label and that more odorant selective, mature cells have a dense, focal projection.

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S17 CYTOCHROME OXIDASE STAINING IN THE OLFACTORY EPITHELIUM AND BULB OF NORMAL AND ODOR-DEPRIVED NEONATAL RATS. P.E. Pedersen, G. M. Shepherd and C. A. Greer (Sec. Neuroanatomy & Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510).

Cytochrome oxidase (CO) staining, a putative marker for neuronal activity within the CNS, has been well studied in the visual system (Wong-Riley, 1979). More recently, CO has been applied to the study of functional organization in the mammalian olfactory system (Costanzo et al., 1984;Wysocki et al., 1984). In the course of studying the development of the olfactory system we assessed the suitability of the CO technique for simultaneously indexing neuronal activity in the olfactory epithelium and bulbs of normal and odor-deprived neonatal rats.

Sprague-Dawley rats between 5 and 30 days postnatal were removed from the nest and immediately sacrificed. In addition, littermates had one naris occluded by electrocautery 2 to 30 days prior to sacrifice. The nasal cavities and olfactory bulbs were serially sectioned and processed for CO staining following the procedures established by Wong-Riley.

Consistent with previous studies, variations in density of CO staining among the glomeruli were evident in the bulbs of normal animals. The olfactory epithelium exhibited heterogeneity in the intensity of CO staining. In those regions of the epithelium that had CO staining a bilaminar distribution was evident. Staining was most intense in the dendritic zone of the mucosa and the somata of mature receptor neurons. Pups unilaterally deprived of odor input exhibited marked differences. The epithelium was void of CO staining on the occluded side. Of particular significance, the deprived olfactory bulb did not differ from its control in CO staining although bulb volume was reduced.

The results thus far emphasize the critical importance of identifying the mechanisms of CO staining in the developing olfactory system. Moreover, understanding the persistence of normal staining in the absence of odor input may provide insight into the principles of functional organization in the olfactory system.

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S18 **THE SIZE OF MITRAL CELLS DEPENDS ON THE AGE AT WHICH CONTINUOUS ODOUR EXPOSURE COMMENCES.** H. Panhuber, D.G. Laing and A. Mackay-Sim* (CSIRO Div. of Food Research, North Ryde, Australia 2113. *Dept Physiol. Univ., Adelaide, Australia 5000).

Continuous exposure of rats to an odour for 10 weeks, had different effects on the size of mitral cells when compared with rats reared in a normal laboratory environment (Controls) or rats exposed to deodorized air. Exposure from day 1 postnatal caused the size of some mitral cells to increase compared with Controls while other cells were the same, or significantly smaller; exposure from day 14 postnatal produced cells which were the same or significantly smaller than those of Controls; exposure from 13 weeks of age caused most mitral cells to shrink compared with Controls, many cells being smaller than those of rats exposed to deodorized air. Exposure to deodorized air at all ages caused a general reduction in mitral cell size compared with controls.

The results indicate that adult rats are particularly vulnerable to prolonged odour exposure and show more marked changes than those of neonates. Exposing the neonate to an odour may interfere with the normal development of the inhibitory and centrifugal circuitry of the bulb, as these develop during the first 3 weeks postnatal while the mitral-receptor cell (excitatory) connections develop before birth. The severe cell shrinkage observed in adult rats exposed to an odour could therefore be due to the effect of inhibition on the mitral cells rather than a lack of stimulation; particularly as these rats had many cells smaller than those of rats exposed to deodorized air. Such hypothesis could also explain why exposing rats to an odour from 14 days postnatal produced cell changes intermediate between those found after exposing neonate or adult rats to an odour.

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S19 EARLY-STAGE PROCESSING OF ODOR MIXTURES. Graham A. Bell, David G. Laing and Helmut Panhuber. (CSIRO Division of Food Research, North Ryde, Australia, 2113).

Food aromas and most commonly encountered odors are usually complex mixtures of dozens and sometimes hundreds of odour components. Little is known about how the components interact to produce the perceived quality and intensity of the mixture. We have shown that neural metabolic responses to odours, measured with the radioactive 2-deoxyglucose (2-DG) technique, can be used to test inter-relationships between odor mixture components. Metabolic activity was suppressed in reliably identifiable patches of glomeruli, in the rat main olfactory bulb, when the animals were exposed to a two-component mixture at concentrations which human subjects had perceived masking of one of the components. The same glomerular region was not metabolically inhibited by a mixture in which masking was not perceived, but showed activity consistent with stimulation by the two odor components presented singly. Since the glomeruli contain the interfacing synapses between the nasal receptors and the brain, the processing of odor mixtures begins at an early stage in the system, either at the nasal receptor sheet or within the glomerular neuropil.

We now report on recent experiments using 2-DG and cytochrome oxidase to examine the changes in regional metabolic activity at the olfactory receptor cell layer in the rat nose.

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S20 EARLY OLFACTORY LEARNING: CHARACTERISTICS AND MECHANISMS. Michael Leon, Robert Coopersmith, Regina Sullivan, Donald Wilson and Cynthia Woo. (Department of Psychobiology, University of California, Irvine CA 92717).

Norway rat pups develop an attraction to the odor of their mother or to other odors following postnatal experience. This special behavioral response is accompanied by an increased uptake of 2-DG in specific glomerular areas (Coopersmith and Leon, 1984). This enhanced glomerular response is odor-specific, long-lived and is not due to differential respiration (Coopersmith and Leon, 1984; Coopersmith and Leon, accepted; Coopersmith, Henderson and Leon, accepted).

This neurobehavioral response is due to associative learning. Only those pups experiencing the odor while given reinforcing tactile stimulation to mimic maternal care developed a preference for the odor and an enhanced glomerular response (Sullivan and Leon, submitted). Pups given aversive associations with an odor avoided the odor, but did not have an enhanced glomerular response to it (Coopersmith, Lee and Leon, in press), indicating the learning specificity of the neural response.

To study the mechanism of the neural response, we first determined whether the increased 2-DG uptake reflected an increased firing of local mitral cells. We therefore recorded the neurophysiological response to the odor by single units in the mitral cell layer associated with areas of high 2-DG uptake (Wilson, Sullivan and Leon, 1985). Odor-learning pups had fewer odor-responsive cells and a higher proportion of inhibitory responses to the odor than odor-unfamiliar pups. Odor experience without reinforcement did not affect unit responses.

Since the increase in 2-DG uptake to learned attractive odors is not caused by an increase in mitral activity, the external and/or middle tufted cells may mediate the enhanced glomerular response. Associated with the areas of enhanced 2-DG uptake are supernumerary glomeruli and increased numbers of glomerular layer neurons. Early olfactory learning may save tufted cells in these areas from dying, thereby producing extra tufted cell glomeruli. Odor-learning pups also have increased granule cell activity deep to the active glomeruli that could be provoked by an increase in the number of active tufted cells projecting to these local inhibitory interneurons. The activated granule cells could thereby cause the inhibition of local mitral cells.

This research was supported by grant NS21484 from NIH.

S21 A Quantitative Golgi Analysis of Granule Cell Development in the Ferret Olfactory Bulb under Normal and Experimental Conditions. RAIMUND APFELBACH and ELKE WEILER (Dept. Biology, Univ. Tübingen, 7400 Tübingen, FRG)

In the developing ferret (Mustela putorius f. furo) food odor imprinting occurs during a sensitive phase lasting throughout the third month of postnatal life. As part of a neuroanatomical study examining the ontogeny of local circuits in the external plexiform layer of the olfactory bulb the development of granule cell (GC) dendrites and spines was assessed by Golgi procedures.
The number of dendritic spines increases significantly from day 30 to day 60 by about 60%; the mean number of spines remains high between day 60 and 90 and then decreases again reaching adult levels by 150 days of age. Both the increase and decrease are highly significant.
In order to study the influence of early olfactory experience on the developmental changes in the number of GC spines, the effects of early olfactory deprivation on this parameter was investigated. Olfactory deprivation was induced by rearing litters of ferrets from birth in an artificial environment saturated with geraniol odor. The continuous overexposure to a single odor masks the ability of the animals to experience other odors in the environment bringing about a relative state of olfactory deprivation.
The results of this experimental condition reveal that the relative state of odor deprivation does not impart its effects on the initial phase of growth where spine number is increasing; rather, on the later phase of growth where this parameter is either stable or decreasing. Geraniol overexposure significantly enhances (by an additional 25%) the normal decline in spine number observed in the normal animals. This indicates that not only this later phase of GC development in the olfactory bulb is a plastic and vulnerable phase, but that early olfactory experience may regulate the normal decline in this parameter. It should be noted that the effect of geraniol overexposure, presumably reflecting olfactory deprivation, occurs during the sensitive phase of olfactory imprinting to food and prey odors in this carnivorous species.

Supported by the Deutsche Forschungsgemeinschaft (Ap 14/8-6).

S22 DOPAMINE AND SUBSTANCE P ARE CONTAINED IN DIFFERENT POPULATIONS OF TUFTED CELLS IN THE SYRIAN AND CHINESE HAMSTER MAIN OLFACTORY BULB. Harriet Baker. (Dept. of Neurology, Cornell Univ. Med. Coll. New York, NY 10021).

Neurons synthesizing dopamine and substance P have been localized to a population of juxtaglomerular neurons in the main olfactory bulb of the hamster. Based on size and morphological characteristics these neurons were classified largely as tufted cells. The congruence of both distribution and neuronal morphology suggested that the two transmitters might be co-localized, that is, contained within the same neurons. To investigate this hypothesis a double label immunohistochemical technique was utilized that employed different color chromagens. Diaminobenzidine (a brown chromagen) was applied first followed by 4-Chloronaphthol (blue). Substance P containing neurons were labeled with a specific antibody that reacted only with chemically authentic substance P. Dopamine neurons were identified with a specific antibody to tyrosine hydroxylase. The antibodies were applied sequentially and the results were similar independent of order.

Neurons containing both antigens were not observed in either species of hamster analyzed, Chinese or Syrian. However, perikarya labeled either with TH or substance P antibodies often were found adjacent or even partially overlapping in thick (30 um) vibratome sections. The labeled cells in the glomerular layer were about the same size (12 um). However, in the external plexiform layer larger (up to 20 um) substance P containing neurons were observed. The dendritic processes of TH and substance P labeled neurons ramified in the same olfactory glomeruli. In the Chinese hamster substance P containing processes also could be followed for long distances within the external plexiform layer. Interestingly, in the Syrian hamster TH, but not substance P, containing neurons frequently were observed in the external plexiform layer while the reverse was found in the Chinese hamster. Differences in the distribution of substance P containing centrifugal afferents also were observed between the two species. The internal plexiform layer of the Chinese but not the Syrian hamster contained a dense axonal arborization. These studies demonstrate that dopamine and substance P are not synthesized by the same neurons although they are found in the same regions of the main olfactory bulb and in morphologically similar neurons. In addition, these data suggest that the distribution of neurons and axonal arborizations of specific transmitter types may vary even in similar species.

Supported by NSF grant BNS 8317552.

S24 ULTRASTRUCTURAL OBSERVATIONS OF OLFACTORY GLOMERULI WITHOUT A TARGET. P.P.C. Graziadei, J.A. Heckroth and G.A. Monti Graziadei. (Dept. Biological Science, Florida State University, Tallahassee, FL 32306).

Olfactory pits from E12 rats, transplanted into the anterior chamber of the eye or the lateral ventricle, develop into rudimental nasal organs formed by a complex system of vesicles partly surrounded by lamellar bone. The vesicles are lined by respiratory epithelium and by typical pseudostratified olfactory neuroepithelium. In the latter many mature neurons are present, the axons of which collect in bundles converging into a large plexus located beyond the bony structures. The plexus closely resembles the fiber layer observed on the surface of the olfactory bulb. Within the plexus, the olfactory axons terminate in finely textured globose terminal structures (60-90 um in diameter) which resemble glomeruli.

Ultrastructural observations of these glomerular formations indicate that they are formed by the branching of the olfactory sensory axons establishing reciprocal synapses. The axon terminals have the characteristic dense appearance and are filled with synaptic vesicles. In the glomeruli, dendritic profiles are not present; glial cells are present in the glomerulus but they do not seem to significantly contribute to the architecture of the terminal structures. Immunohistochemical preparations show that the glomeruli are positive for OMP (olfactory marker protein). Our observations indicate that the olfactory axons can arrange in discrete globose terminal structures without the participation of dendritic profiles on which to synapse. The potential for glomerular formation seem to be intrinsic to the olfactory axons.

Supported by NIH, NS 20699.

S23 CONJUGATE INTERNALIZATION OF APPOSED DENDRITIC MEMBRANES DURING SYNAPTIC REORGANIZATION IN THE OLFACTORY BULBS OF ADULT PCD MICE. Charles A. Greer (Sections of Neurosurgery and Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510).

The mutant mouse PCD is one of the few models available for studying principles of local circuit plasticity in the adult. PCD exhibits a complete loss of olfactory bulb (OB) mitral cells between 2 and 6 months postnatal. Following mitral cell loss denervated granule cell (GC) dendrites and spines in the external plexiform layer (EPL) reorganize (Greer, 1985) and establish new reciprocal synapses at available sites on tufted cells (TC) (Greer et al., 1984). During this process an unusual feature has been recognized, the invagination of GC spine plasmalemma membranes (IPMs) into apposed TC dendrites. IPMs are frequently found early in development but decrease significantly after 3-4 weeks postnatal. The present report examines the hypothesis that this may contribute to the plasticity and synaptic reorganization observed in the adult OB.

PCD mice, both mutants and heterozyous littermate controls, and 6 - 12 day postnatal Sprague-Dawley rats were employed. They were perfused with aldehydes for conventional EM or prepared with the gold-toning Golgi/EM procedure of Farien et al. (1977). Micrographs of serial sections were used to assess the morphological features and incidence of IPMs.

The incidence of IPMs was significantly higher in the mutant mice (2.5:1). The typical IPM was composed of double membranes separated by 10-20nm. It was goblet shaped and extended up to 250nm into the apposed dendrite. IPMs measured approximately 200nm in diameter at the widest point and narrowed to 30-60nm at the neck. It was not uncommon to find several small vesicles within the evaginating cytoplasm. Moreover, the IPMs were frequently found adjacent to the reciprocal dendrodendritic synapses characteristic of the OB EPL.

Prior studies suggest that IPMs may participate in the remodelling of cell surfaces during early development (Eckenhoff and Pysh 1983). The current data demonstrate that normally quiescent developmental signals can be reactivated in the adult and that membrane remodelling may be central to the expression of synaptic plasticity.

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S25 MECHANISM OF PHEROMONE ORIENTATION IN FLYING MOTHS. R. Preiss and E. Kramer, Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, West Germany

Male moths, orienting upwind towards a chemically calling, conspecific female, control speed, direction and altitude of flight by watching the apparent movement of ground structures. The visual input is used to proceed upwind with rather constant ground-speed along a zig-zagging flight track supposed to have constant track angles either left or right to the wind. The zig-zag sequence is claimed to be caused by an internal program of 'self-steered counter-turning', activated by the perception of pheromone. Besides this internal program, a complex control system is required which can keep direction and speed of image movement constant.

We have found an alternative explanation for the process of chemical orientation in flying moths for experiments with gypsy moths (Lymantria dispar L.), whose flight behavior was analysed by a new method: only a minimum of animal control suffices to explain the observed peculiarities of flight tracks, when the stochastic nature of the input into the system is taken into account.

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S26 Chemical Signal to Noise Ratios Determine Lobsters' Behavioral and Physiological Responses to Food Mixtures.
L. Handrich and J. Atema., Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543

The differentiation of signals from background "noise" is a problem common to all animals. In chemoreception, relatively little work has been done concerning the signal to noise problem. The chemically noisy marine environment contains fairly high levels of amino acids and ammonium, compounds used as food signals by many marine species. We have used the lobster, Homarus americanus, to investigate ways in which its physiologically narrowly tuned chemoreceptors might aid the animal in locating food in its chemically noisy environment.

Using a behavioral assay described previously (Handrich and Atema, 1985), we tested the effects of altered chemical backgrounds on lobsters' locating and feeding behaviors in response to food odor (a synthetic mixture of amino acids and ammonium based on mussel flesh). Ammonium in the background lowered the threshold for behavior and enhanced responsiveness to the mixture. This increase in the perceived intensity of the stimulus mixture can be explained as a release of narrowly tuned amino acid neurons from inhibition caused by the firing of ammonium sensitive neurons at the CNS level. Since peripheral ammonium neurons become completely adapted to the background level of ammonium, their response to the mixture is reduced in the elevated ammonium backgrounds and so presumably, is their suppressing influence on amino acid sensitive neurons in the CNS. Additional experiments were done in normal background but using a mixture containing less ammonium. Here too, ammonium neurons have little input to the CNS as when adapted to a high background.

Alternatively, increased behavioral sensitivity to the mixture in ammonium backgrounds might be explained by changes in peripheral chemoreceptor neurons. Responses of single taste and smell receptors to their best compound can be suppressed (or sometimes enhanced) when the compound is presented within a stimulus mixture or when the receptors are adapted to a mixture background. To test the importance of peripheral effects on the behavioral phenomenon described above, we made physiological recordings from glutamate specific taste receptors in lobster legs. Glutamate cells were tested with concentration series of glutamate and synthetic food mixture in backgrounds containing elevated concentrations of ammonium or of the synthetic mixture. These experiments have given us valuable information about how marine animals deal with the problems of detecting important chemical signals in their noisy environment.

Supported by the Whitehall Foundation and NSF BNS 85 12585

S27 ANALYSIS OF TASTE MIXTURES BY HAMSTERS. Marion E. Frank and Thomas P. Hettinger (Dept. of Oral Biology, University of Connecticut Health Center, Farmington, CT 06032).

In nature, taste stimuli occur almost invariably as components of complex chemical mixtures with gustatory and other sensory features. Once component effects are established, study of mixtures can discover interactive processing of stimuli by the gustatory system. Components can act interchangeably, if they stimulate the same receptive channel, independently, if they stimulate different receptive channels, or interactively. Gustatory interactions, seen as synthesis, suppression or synergism, may occur at any level from chemoreceptive elements to central nervous system and may function in the detection of gustatory features in chemical mixtures and affect sensation.

Measured innate preferences for mixtures of gustatory, olfactory and general sensory stimuli, which are individually preferred or rejected, address hedonic processing. Measured generalizations of conditioned food aversions for mixtures of sucrose, NaCl, HCl and quinine, which each have distinct features, address processing of gustatory quality. The mixtures do not have unique hedonic or qualitative properties nor is synergism seen. Mixtures of preferred and rejected substances are together less palatable than the preferred but more palatable than the rejected substance alone, be it a taste, smell or irritant stimulus. Mixtures of two highly preferred substances, however, are not necessarily more palatable nor are mixtures of two rejected substances less palatable. An aversion to a mixture generalizes to the components and to other mixtures containing one of the components; an aversion to a component generalizes to mixtures containing the component. The usually lesser amount of generalization to a mixture than to the components may compare to mixture suppression seen in human psychophysics; the reduced effectiveness of components in quinine-NaCl mixtures in both preferences and generalizations may be an example of a specific suppression. However, overall, the effects of individual stimuli predict their effects in mixtures if saturability is considered.

Support by NIH grant NS 16993

S28 MULTIPLE BITTER RECEPTOR SITES IN HAMSTERS. Carl Pfaffmann and M. Scott Herness (Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021)

Previous research (Snyder 1931; Fox, 1932; Blakeslee, 1932) showing that human taste thresholds for bitterness of phenylthiocarbamide (PTC) were bimodally distributed led to the conclusion that a separate PTC receptor site was genetically coded by a single Mendelian recessive gene. Cross-adaptation among different bitter stimuli of human taste (McBurney et al., 1972) suggested the existence of three (or more) bitter receptor sites that did not cross adapt. Electrophysiological and behavioral data in frogs (Sugimoto and Sato, 1981, 1982) in rats (Stewart et al., 1983) and mice (Lush 1981; Harder et al., 1984) have similarly indicated that multiple bitter receptor sites exist in these species.

In this study we present evidence for two major bitter sites or domains based on stimuli which taste bitter to humans and are avoided by hamsters in two bottle preference tests. Taste aversions were formed in hamsters to one of several tastants and subsequently tested for generalization to a variety of tastants by the Conditioned Taste Aversion (CTA) paradigm of Nowlis et al. (1980). One of the putative bitter stimuli, the sodium salt of nitrobenzene sulfonic acid (NaNBSA), which is bitter to humans, was not avoided but preferred in the two bottle test. A conditioned taste aversion to it generalized to sucrose indicative of a species difference between man and hamster for this compound. All the other putative bitter compounds, Na picrate, Na cholate, Na nitrobenzoate (NaNBA) as well as quinine were avoided in the preference test. Na saccharin, as expected, was preferred in mid-concentration 0.001 to 0.03 molar values but avoided at higher concentrations of 0.1 and 0.3 M.

Hamsters conditioned against quinine suppressed drinking of strychnine and L-phenylalanine as well as quinine but did not suppress to urea. Na picrate and Na cholate generalized to NaNBA but not to quinine. Hamsters conditioned to cholate also generalized to urea.

Thus two different major bitter domains or sites may be delineated, (1) quinine, L-phenylalanine, and strychnine, (2) NaNBA, picrate and cholate, but only cholate overlaps with urea.

Supported by NSF Grant BNS 8111816.

S29 OLFACTORY DETECTION OF KETONES AND ALDEHYDES BY TIGER SALAMANDERS. Thomas Hellman Morton[1] and J. Russell Mason[2], [1]Department of Chemistry, University of California, Riverside, CA 92521 and [2]Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104

Tiger salamanders are found to generalize between carbonyl-containing odorants, as determined using a behavioral assay. Using olfactory detection at stimulus concentrations near threshold, animals generalize from one aldehyde to another and from one ketone to another, but not between aldehydes and ketones of comparable molecular shape (e.g. not between hexanal and 2-heptanone). A new synthesis of the sweet-smelling 2-fluoro-1-alkenes, $RCF{=}CH_2$, has been developed, and we find that animals do not generalize between an aldehyde and a fluoroalkene of comparable molecular shape, dipole moment, and dimensions (e.g. not from hexanal to fluoroheptene $C_4H_9CF{=}CH_2$), although the animals are capable of detecting the fluoroheptene.

Olfactory detection in tiger salamanders is relatively insensitive to direct chemical insults to the olfactory epithelium. For instance, lavage of both olfactory sacs with 5 mM iodoacetamide, a sulfhydryl-modification reagent, does not significantly affect an animal's sense of smell. On the other hand, the comparatively mild (but chemically selective) two-step lavage with 0.5 mM acetoacetic ester followed by 50 mM sodium cyanoborohydride selectively impairs olfactory detection of aldehydes and ketones for several days afterwards. Neither step alone has any effect by itself. When animals are trained to respond to hexanal they generalize to unreinforced presentations of heptanal, and their responding to both aldehydes is impaired to equal extents by the two-step procedure. Similarly, animals conditioned to respond to cyclohexanone generalize to cyclopentanone, while animals conditioned to cyclopentanone generalize to cyclohexanone. In either case, the two-step procedure impairs (but does not obliterate) responding to both the odorant to which the animals generalize as well as the odorant on which they were trained. The results are interpreted as implying the existence of carbonyl-binding "generalist" olfactory receptors in addition to other classes of "generalist" receptors that are not affected by the two-step procedure. Generalization is inferred to require overlap in the response profiles of more than one class of receptor.

S30 BEHAVIOR-STEREOTYPES OF FEEDING AND THOSE DISPLAYED IN REJECTION OF AVERSIVE-TASTING FOOD BY THE FRESHWATER PRAWN: Macrobrachium rosenbergii.

Sheenan Harpaz[1] and Jacob E. Steiner[2]. [1] Dept. of Zoology, Life Sciences Institution [2] Dept. of Oral Biology, Hadassah Faculty of Dental Medicine, The Hebrew University of Jerusalem, Israel.

Food search and food intake behavior stereotypes of the freshwater prawn Macrobrachium rosenbergii were studied on a sample of 27 adult animals of both sexes in the intermolt phase of the molt cycle. Food-deprived animals were stimulated with an actual food-item, to which they were accustomed, or with small aliquots of different concentrations of the chemoattractant betaine-HCl, or with food pellets adulterated with quinine-HCl. The behavioral displays induced by all these stimulants were videotaped and recordings were evaluated by two independent viewers. Behavior analysis was based on quantitative assessment of antennular flicks; on that of probing movements by pereiopods as well as pereiopod lifting movements to the labial region.

Results revealed that the food search and food intake behavior sequences induced by actual food can be evoked in an identical manner when only betaine simulated the nutrient. The betaine-induced features show a clear dependence on stimulus intensity.

The quinine-adulterated food sample triggered all the initial sequences of food search and food lifting behaviors apparently due to stimulation of distant chemoreceptors, located on antennae and antennulae. Detection of the aversive taste most probably by other chemoreceptors induced a different set of motion features, resulting in food rejection (dropping or active expulsion) accompanied by typical mouth-cleaning movements.

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S31 ARE FEEDING RESPONSES BY CRUSTACEA TUNED TO THE RELATIVE ENERGY AND NUTRIENT QUALITIES OF ODOR? Richard K. Zimmer-Faust. (Marine Science Institute, University of California, Santa Barbara CA 93106).

Experiments were performed to test whether crustacean feeding is tuned to the energy and nutrient properties of odor. While free amino acids provide utilizable nitrogen abundant in intact prey, ammonia nitrogen is a non-utilizable end product of amino acid catabolism. Interactions between these substances are proposed to signal the relative nutritional (nitrogen) quality of food. Supporting this model are data showing that six bathypelagic and littoral species exhibit probing and searching to amino acids, but not to ammonia. Amino acids cause forward ambulation in some species while ammonia induces only tail-flipping (fleeing) in others. Mixtures combining amino acids with ammonia typically suppress both feeding and fleeing, as predicted by the model, and chemical interactions between these substances are clearly antagonistic. Additional experiments with the spiny lobster, Panulirus interruptus, show that chemical excitation by ATP > ADP > AMP = adenosine. Solutions which are identical in total phosphoadenylates or in ATP initiate searching and feeding according to their ranked order of adenylate energy charge. Results demonstrate that chemosensory responses of crustacea are tuned to substances which enhance energy and nutrient payoffs, and it is suggested that olfactory mediated behavior of spiny lobsters may have evolved more in response to nitrogen than to energy demands.

Supported by a grant from the Whitehall Foundation.

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S32 EFFECT OF DIETARY PROTEIN ON THE TASTE PREFERENCE FOR AMINO ACIDS AND SODIUM CHLORIDE IN RATS. Kunio Torii, Kazunori Mawatari, and Yasumi Yugari.(Central research laboratories, Ajinomoto Co., Inc., 214, Maeda-cho, Totsuka-ku, Yokohama, Japan 244).

Appetite and taste preference is affected by the nutritional state within normal limits or not. The major compartment of dietary protein is L-glutamate (Glu), which has umami taste. The synergistic enhancement of umami taste of Glu by some 5'-ribonucleotides were recognized biochemically (Torii & Cagan, 1980), electrophysiologically and psycophysically. Taste perception of each L-amino acid(AA) in animals may be a marker of dietary protein intake. Changes of taste preference for AA and NaCl in male growing rats under various degrees of protein or some essential AA restriction were examined. Fifteen kinds of AA or preferable taste solutions (sweetness; 500mM Gly, saltiness; 150mM NaCl, umami taste; 150mM Glu Na (MSG) and 4.5mM MSG + 4.5mM 5'-GMP) were offered in choice to rats(N=6, each group). Preference for NaCl with Gly and/or Thr was induced under protein restriction or some essential AA deficiency, reflecting the negative nitrogen balance. Gly and Thr intake caused to spare the endogenous protein. Preference for umami taste solutions was observed only when rat grew normally. The minimal level of dietary protein to induce umami taste preference paralleled the protein requirement, which declined with age. The total Na intake in both normotensive Sprague-Dawley (SD) and spontaneously hypertensive rats(SHR) declined along with dietary protein increase. Preference for NaCl still sustained in SHR but disappeared in SD rats. The quantitative ingestion of Lys, in the choice from 15 kinds of AA solution, was observed after the plasma and brain Lys concentration alone altered under Lys deficiency, and then rats began to display umami taste preference and normal appetite. Intakes of MSG, Gln and Arg occurred only when rats fed completely fortified diet, indicating that preference for umami taste materials and Arg should be related with the operation of protein metabolism under the sufficient dietary protein intake to body need.

These data strongly suggested that taste preference and appetite should be dependent upon the protein nutrition, which was defined by the amount and quality of dietary protein.

S33 ROLE FOR THE LIVER IN THE FORMATION OF FOOD FLAVOR PREFERENCES. Mark I. Friedman and Michael G. Tordoff. (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104).

Various studies have indicated that changes in liver function alter gustatory responses. We have examined this possibility in an analysis of the mechanisms involved in the formation of food preferences induced by ingestion of sweet drinks.

Ad libitum fed rats ate one of two flavored foods during the first 2 hr of their night period for 20 days. On half the days, rats were given 10% glucose to drink in addition to water while they ate one flavored food; on the others, they ate the other flavored food along with only water. Drinking glucose significantly decreased food intake, but caloric intake remained constant. When given a choice between the two flavored foods at the end of the 20 days, rats showed a strong preference for the food paired with the ingestion of glucose.

To determine the contribution of gustatory and hepatic factors in the formation of this food preference, additional experiments were performed. In one experiment, rats with either hepatic-portal or jugular vein catheters were given four infusions each of glucose (2 ml X 1.5 M/2hr) or isosmotic saline (2 ml X 0.75 M/2hr) during the first 2 hr of their night period. Rats ate one flavored food when infused with glucose and the other food when given saline. Portal, but not jugular, infusions of glucose decreased food intake during the test periods. When given a choice between the two flavored foods at the end of testing, rats showed a strong preference for the food paired with portal, but not jugular, glucose infusions.

To control for the metabolic effects of glucose ingestion, the initial experiment was repeated using 0.2% saccharin instead of 10% glucose. Under these conditions, rats increased their intake of the food eaten during the time they drank the saccharin solution, and, when given a choice, preferred the food paired with saccharin ingestion. This effect of saccharin was unrelated to changes in plasma insulin or glucose and was not affected by celiac vagotomy. Hepatic vagotomy, however, eliminated the increase in food intake and food preference produced by saccharin ingestion.

The results demonstrate that food flavor preferences can be induced by the ingestion of simultaneously available nutritive or non-nutritive sweet solutions and suggest that in both cases metabolic and/or neural effects on the liver are involved.

Supported by NIH grants AM-36339 and NS-19616, and a feasibility study grant awarded by St. Luke's-Roosevelt Obesity Core Center.

S34 CONTACT CHEMORECEPTION AND MATE RECOGNITION BY AN ANTARCTIC CRUSTACEAN. William C. Michel, (Dept. of Biology, University of California, Los Angeles, CA 90024).

During the austral winter of 1985 I examined several aspects of the reproductive behavior of the isopod crustacean Serolis polita. Of particular interest was the chemoreceptive basis for the formation of mating pairs and the maintenance of these pairs until mating occurred. Non-gravid females were carried beneath males for several months prior to mating, termed the passive phase of reproduction or amplexus. Females molted the posterior portion of their carapace 6-10 days before the anterior portion and mating occurred within hours after the anterior molt. Amplexus persisted for up to 8 days after mating, which presumably excluded other males from copulation.

Each pair was formed after an active search by a male resulting in a lunge and clasp of a non-gravid female using the modified second periopod. Females did not search for mates but did occasionally fled from males. Male recognition of a suitable mate occurred only after contact. Given the choice of pairing with either an immature male, a gravid female or a non-gravid female, competent males contacted and rejected the immature males and gravid females but not the non-gravid females. After 24 h, 55% of competent males were found to be paired with non-gravid females, but only 11% were paired with immature males or gravid females. When competent males were given a choice of gravid females or immature males, without non-gravid females present, search behavior was unchanged but only 17% of the males were paired after 24 h. No search was initiated by males to aquarium water holding other males, gravid females or non-gravid females. This indicated that a water-borne sex attractant is not produced. The second periopod, supplied with dense setae upon the merus and carpus, is postulated to function in mate recognition as a contact-chemoreceptive organ.

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S35 SEASONAL AND SEX DIFFERENCES IN GARTER SNAKE CHEMICAL CUES. Robert T. Mason, John W. Chinn*, and David Crews. (Departments of Zoology and *Chemistry, University of Texas, Austin TX 78712).

Female red-sided garter snakes (Thamnophis sirtalis parietalis) produce a sex attractant pheromone. This pheromone serves to elicit courtship behavior from conspecific males. The site of production of the pheromone is unknown, however, it is expressed along the dorsal surface of the female's body. The pheromone is unusual in that it is non-volatile at ambient temperatures encountered in the field. Male garter snakes detect and begin to court females when rapid tongue-flicks by the male deliver chemical cues from the female's dorsal surface to the male's vomeronasal system (Kubie, Vagvolgyi, and Halpern, 1978).

We have previously demonstrated that chemical cues can be removed from the female's back by washing with non-polar solvents such as hexanes (Mason and Crews, 1985). These washes elicit courtship behavior from sexually active males in both field and laboratory behavior trials. Washes removed from sexually active animals in the field were stored in Teflon-capped glass vials and frozen at -20 C. Samples of male and female washes were analyzed on a Finnigan-Mat 4023 gas chromatograph/mass spectrometer (GC/MS) using a 12 m BP-5 capillary column. Data were analyzed using the INCOS data system.

Results of these studies demonstrate a distinct sex difference in the traces of male and female garter snakes. In addition, samples taken during the breeding season were qualitatively different from samples obtained during the non-breeding season. This seasonal difference in skin chemical cues may account for the extinction of courtship after the three to four week Spring breeding season.

Supported by a grant from the University of Texas Research Institute and by NIMH NRSA MH09310 to RTM and NICHHD 16687 and NIMH NRSA MH00135 to DC.

S36 VOMERONASAL CHEMORECEPTION MAY ACTIVATE REPRODUCTION IN REFLEX-OVULATING PRAIRIE VOLES. John J. Lepri and Charles J. Wysocki. (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104).

Unlike spontaneously ovulating mammals, the reproductive system of female prairie voles (Microtus ochrogaster) remains inactive in the absence of stimulation from males. Physical contact with a male rapidly initiates ovarian and uterine growth, with copulation and the subsequent induction of ovulation occurring 30-72 hours after pairing (Carter and Getz, 1985). Chemical signals in the urine of male prairie voles promote the growth of the ovaries and uterus when presented as the sole source of stimulation (Carter et al., 1980). A single drop of male urine onto the upper lip of a female causes changes in the concentrations of norepinephrine and luteinizing hormone-releasing hormone in the caudal portion of the olfactory bulbs (Dluzen et al., 1981) which receives the sensory afferents of the vomeronasal system.

We used three types of adult female prairie voles: i) VNX-vomeronasal organ surgically removed, verified by histology; ii) SHAM-surgical manipulation but vomeronasal organs left intact; iii) NORMAL-no surgical manipulation. VNX females were not impaired at finding a piece of apple hidden in cage bedding, suggesting normal function of the main olfactory sytem. Females from each of the 3 treatment groups were either unexposed to males (zero hours), paired with stud males for 12 hours, or paired with stud males for 60 hours. Preliminary results demonstrate that uterine and ovarian weights were equally low in all females in the zero hours group. Mean uterine weight of NORMAL and SHAM females approximately doubled after 12, and tripled after 60 hours of being paired with stud males. However, mean uterine weight of VNX females did not increase substantially, even after 60 hours of being paired. Furthermore, 5 out of 5 NORMAL, and 4 out of 5 SHAM females in the 60 hours group mated (sperm present in the vagina) whereas only 1 out of 4 VNX females in the 60 hours group mated. Behavioral observations recorded during the first 10 minutes of interactions with stud males, including duration of sniffing, were similar for NORMAL, SHAM and VNX females. The sample sizes will be increased. The inhibition of reproductive activation in the VNX females is a striking result of destroying a chemoreception system that has been traditionally labelled as "accessory." Indeed, vomeronasal chemoreception may be primary in its effects on reproduction in female voles.

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S37 EXPERIENTIAL AND ENDOCRINE DEPENDENCE OF GONADOTROPIN RESPONSES IN MALE MICE TO CONSPECIFIC URINE. A. N. Clancy, F. H. Bronson, A. G. Singer, W. C. Agosta and F. Macrides. (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, University of Texas, Austin, TX 78712, and The Rockefeller University, New York, NY 10021).

Previous research has shown that a urinary pheromone of female mice acts via the vomeronasal organ to elicit rapid release of luteinizing hormone (LH) in conspecific males. Several experiments were conducted to examine the importance of sexual experience for gonadotropin responses in male mice to female urine, male urine, saline, or mixtures of these stimuli, presented as an aerosol spray onto the snout. Both sexually-naive and sexually-experienced male mice had significantly higher plasma LH levels following presentations of female urine as compared to their plasma LH levels after presentations of male urine. However, only experienced males had significantly elevated plasma LH levels in response to female urine when compared to their LH levels after presentations of saline, and in naive males the LH levels tended to be lower following presentations of male urine as compared to their LH levels after saline presentations. Subsequent experiments with sexually-experienced subjects demonstrated that male mouse urine produces a powerful suppression of LH release in other males, a novel pheromonal effect. Specifically, female mouse urine mixed with male urine failed to elicit LH responses in male subjects, whereas female urine mixed with saline was highly effective. Urine obtained from castrated male donors was as potent as urine from intact males in suppressing the gonadotropin response to female urine. The suppressive pheromone in male mouse urine thus does not appear to be critically dependent on gonadal hormones, as has also been found for the stimulatory pheromone in female mouse urine. The pattern of results indicates that sexual experience is not necessary for the differential effects of female versus male urinary pheromones on gonadotropin secretion in male mice, but that sexual experience does enhance the potency of the female urinary pheromone relative to a sexually-neutral component of urine such as sodium chloride.

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Monday, July 21

P1 MULTIPLE RECEPTORS MEDIATING THE FEEDING RESPONSE OF HYDRA AND MONOCLONAL ANTIBODIES AGAINST ONE OF THE RECEPTORS. Kazumitsu Hanai, Masahiko Sakaguchi, Sachiko Matsuhashi*, Katsuji Hori*, Hiromichi Morita. (Dept. Biology, Faculty of Science, Kyushu Universtiy 33, Fukuoka 812, JAPAN and *Dept. Biochemistry, Saga Medical School, Saga 840-01, JAPAN).

Reduced glutathione evokes the feeding response in Hydra. The response is quantitatively observed with the duration of the tentacle ball formation, a behavior associated with the feeding in Hydra japonica (Hanai 1981). The response is mediated by at least 3 types of receptors with different affinities to S-methylglutathione (GSM); a high affinity type (below 0.1 μM), a medium affinity type (from 0.1 to 1 μM), and a low affinty type (above 1 μM). Purified human platelet-derived growth factor (PDGF) is a potent inhibitor of the response mediated by the medium affinity type receptors (Hanai et al. 1986).

The galactose-binding proteins obtained from the membrane fraction of tentacles have been proposed to be candidates for these receptors from the photoaffinity labeling studies in vivo (Hanai & Kitajima 1985) and in vitro (Kitajima & Hanai 1986). Monoclonal antibodies were raised against these proteins. The culture supernatant of the hybridoma was first examined with the antigen-antibody reaction by ELISA, and then with the behavioral response to 0.1 μM GSM. Among hybridomas obtained, the culture supernatant from a hybridoma A3C3-4.8 (class IgM antibody) depressed the response significantly. In contrast, that of myeloma P3U1, which was used for fusion, or a hybridoma raised against antigens unrelated to the galactose-binding proteins, did not. The IgM purified from ascite fluid depressed the response to 50 % of control at concentrations below 0.1 μM of GSM and reduced by 10 % at concentrations above 0.2 μM. This suggests that the IgM interacts with the high affinity glutathione receptor.

We thank to Dr. E.W. Raines & Prof. R. Ross, (Dept. of Pathology, University of Washington, Seattle, USA) for their kind gift of PDGF. Supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

P2 **PROGRESS ON THE IDENTIFICATION OF THE FOLATE CHEMORECEPTOR OF PARAMECIUM.** Stephanie Schulz and Judith Van Houten. (Department of Zoology, University of Vermont, Burlington, VT 05405)

The ciliated protozoan Paramecium tetraurelia is attracted to K_2folate in behavioral assays in which KCl is the control solution. Folate is an essential vitamin, and may serve as a food cue for paramecia, which feed on bacteria. A variety of biochemical approaches have been employed in attempting to identify a membrane protein which serves as a chemoreceptor for folate, and transduces the binding of folate to the hyperpolarization that changes ciliary beating and consequently results in the observed chemoresponse. We knew from ^{3}H-folate binding assays and electrophysiology of deciliated cells that the specific, saturable binding sites involved in chemoreception were on the cell body membrane (Van Houten et al., 1983, J. Cell Biol. 97: 1797; Schulz et al., 1984, J. Comp. Physiol. 155: 113).

Folate-Sepharose chromatography was used to affinity purify folate-binding proteins from Triton X-100 solubilized cell body membranes. Ten groups of proteins eluted specifically with folate, but not with glutamate or PABA-glutamate. Methotrexate, a folate analog which competes only weakly with folate in behavioral assays eluted these proteins with varying degrees of efficiency. Vectorial labelling of whole cells with ^{125}I identified nine individual proteins within these groups as being surface exposed. The folate-binding proteins were further characterized by Con A-Sepharose chromatography; five proteins eluted specifically with α methyl-D-mannoside, identifying them as glycoproteins. Four proteins were both labelled with ^{125}I and identified as glycoproteins; two of these proteins did not elute with methotrexate from the folate-Sepharose affinity column. These proteins, molecular weights 80,000 and 58,000 daltons, are candidates for the folate chemoreceptor of Paramecium. The identity will be verified by crosslinking folate to whole cells, which we know specifically blocks folate chemoresponse, and identifying the crosslinked proteins by immunodection.

(Supported by NSF-12176 and NIH-29045)

P3 STEREOSPECIFICITY OF THE ALKYL SITE FOR OPTICAL ISOMERS OF DIPEPTIDES IN THE LABELLAR SUGAR RECEPTOR OF THE FLESHFLY. Ichiro Shimada, Yuji Maki* and Hiroshi Sugiyama**. (Department of Biological Science, Tohoku University, Kawauchi, Sendai 980, *Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990 and **Chemical Research Institute of Non-aqueous Solution, Tohoku University, Katahira, Sendai 980).

There are four receptor sites in a single labellar sugar receptor of the fleshfly. They are the pyranose (P) site, the furanose site, the alkyl (R) site and the aryl (Ar) site. Most of stimulative dipeptides react with the R site. L-Glu-L-Val is remarkably stimulative of them. We have already proposed the specific accessory site for the glutamyl moiety of Glu-Val (L-L), very close to the R site, based on the analysis of stimulating effectiveness of its analogues (Shimada et al., 1983).

We synthesized optical isomers of Glu-Val, Glu-norVal, Glu-Leu, and Glu-norLeu and examined their stimulating effectiveness. In case of Glu-Val, the order of effectiveness is L-L>D-L>D-D>L-D. Glu-Val (D-D) is clearly effective while Glu-Val (L-D) is almost ineffective, which is contrastive to the ineffectiveness of D-amino acids. This can be explained by considering the flexibility of the conformation of dipeptides and the strong interaction between the glutamyl moiety and the accessory site.

In other cases of dipeptides, the order of effectiveness of less stimulative L-D series is Glu-Val(L-D)<Glu-norVal(L-D)<Glu-Leu (L-D)<Glu-norLeu(L-D). This may be due to the length of the side chain of C-terminal amino acids of dipeptides. Glu-norLeu (L-D), for example, is as stimulative as other optical isomers of Glu-norLeu. The side chain of the C-terminal amino acid can bind with the subsite A of the R site as it becomes longer. The interpretation must be supported by further analysis of other dipeptides.

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P4 CHANGES IN MEMBRANE POTENTIAL AND MEMBRANE FLUIDITY IN RESPONSE TO VARIOUS ODORANTS IN CELL PREPARATIONS ISOLATED FROM PORCINE OLFACTORY MUCOSA. Makoto Kashiwayanagi, Kimie Sai and Kenzo Kurihara. (Fac. Pharmaceut. Science, Hokkaido University, Sapporo 060 Japan)

A number of studies have been attempted to isolate olfactory cells but none of them succeeded to isolate olfactory cells which have the ability to respond to odorants. In the present study, we have succeeded to isolate olfactory cell preparations from porcine olfactory mucosa which respond to various odorants. The cell preparations were used to examine mechanisms of an odor-induced depolarization and an odor discrimination. The results obtained are as follows. 1) The membrane potential of the olfactory cell preparations, which was monitored with rhodamine 6G, was depolarized by an increase in outer K-concentration. 2) Various odorants depolarized the cell preparations dose-dependently. The amplitude of depolarization induced by odorants were practically unchanged by elimination of Na^+, Ca^{2+} and Cl^- in the external solution, suggesting that changes in the permeabilities of specific ions are not concerned with depolarization in response to odorants. 3) Various odorants induced changes in the membrane fluidity at different sites of the membrane which were monitored with various fluorescence probes (ANS, 7-AS, 12-AS, 12-AO and DPH). Odorants having different odors brought about different profiles of the membrane fluidity changes, suggesting that the odorants having different odors are adsorbed on the different sites in the membrane.

In a previous paper (Brain Res. 1985), we showed that neuroblastoma cells (N-18 clone) were depolarized by various odorants. The characteristic profiles of membrane fluidity changes in the olfactory cell preparations were similar to those in the N-18 cells which must not have specific receptor proteins for odorants. The present results together with those obtained with the N-18 cells suggest that specific receptor proteins unique to olfactory cells are not concerned with odorant reception. Biomembranes are composed of various lipids and proteins and hence there exist various sites having different shapes, sizes and affinities to odorants in the membrane. Each odorant seems to be adsorbed at a site which the odorant fits. Odorants can be discriminated if a membrane composition of individual olfactory cell is different from one to another.

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P5 THE BIOCHEMISTRY OF THE OLFACTORY PURINERGIC SYSTEM. Henry G. Trapido-Rosenthal, William E. S. Carr, Scott M. Lambert, and Marsha L. Milstead. (C. V. Whitney Laboratory and Dept. of Zoology, University of Florida).

The olfactory system of the spiny lobster has purinergic receptors that are excited by the purine nucleotide, adenosine 5'-monophosphate (AMP) (Derby, Carr and Ache, 1984). Both biochemical and physiological studies are in progress to characterize the purinergic receptor cells and the factors affecting their activity. Radiolabeled substances have been used to discover that olfactory sensilla (aesthetasc sensilla) excised from the antennule of the lobster dephosphorylate the olfactory stimulant AMP and internalize the dephosphorylated product, adenosine. These findings followed an earlier discovery that ^{3}H-AMP, or some tritiated product of ^{3}H-AMP, was rapidly internalized by a sensillar uptake system shown to be concentration dependent. Evidence that AMP is dephosphorylated prior to uptake was obtained with a double label experiment and by employing an inhibitor of 5'-nucleotidase. In the double label experiment, the rate of uptake of ^{3}H from ^{3}H-AMP (labeled in the purine ring) was about 10-times greater than that of ^{32}P from ^{32}P-AMP, thereby indicating that dephosphorylation precedes uptake. The 5'-nucleotidase inhibitor, AMPCP, inhibited the uptake of ^{3}H-AMP but not of ^{3}H-adenosine, again indicating that the excitatory nucleotide is dephosphorylated prior to uptake. Dephosphorylation occurs very rapidly because the uptake rate for ^{3}H from ^{3}H-AMP and ^{3}H-adenosine are the same. The uptake process for adenosine has a K_m of 5.8 μM and a V_{max} of 3.8 fmole/sensillum/min. Our biochemical studies show that marked similarities exist between the fate of an excitatory nucleotide in an olfactory system, and in the well-known internal purinergic cells of vertebrates. In both cases, excitatory nucleotides are inactivated by dephosphorylation and the product, adenosine, is internalized by an uptake process.

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P6 CHEMICAL ANALYSES OF HODULCIN, THE SWEETNESS-SUPPRESSING PRINCIPLE FROM Hovenia dulcis LEAVES. Richard Sefecka and Linda M. Kennedy. (IBM Instruments, Inc., Danbury, CT 06810 and Department of Biology, Clark University, Worcester, MA 01610)

The chemistry of hodulcin, the selective sweetness-suppressing principle from Hovenia dulcis (Saul, et al, 1985) is not known. However, there are known similarities and differences between compounds from H. dulcis and 1) ziziphins, the selective sweetness-suppressing compounds from Ziziphus jujuba (Meiselman, et al, 1976) and b) gymnemic acids, the selective sweetness-suppressing compounds from Hymema sylvestre (Warren & Pfaffmann, 1959): Gymnemic acids are triterpene saponins, with a known genin structure (Stocklin, 1967). Ziziphins are saponins of unknown structures, probably also triterpenes, yet not the same compounds as the gynmemic acids (Kennedy & Halpern, 1980). H. dulcis also contains some saponins, which have the same genin structure as some saponins from Z. jujuba (Kimura, et al, 1981), but taste-activity of those structurally-identified H. dulcis and Z. jujuba saponins has not been tested. To elucidate the chemistry of taste-active hodulcin and ziziphins, we analyzed a partially-purified preparation of hodulcin and compared it with ziziphins and gynmemic acids. Ziziphins the gymnemic acids were prepared and found active as in Kennedy, et al (1975) and Kennedy & Halpern (1980). The hodulcin was prepared similarly and found to be active.

The identically extracted samples were analyzed by gradient elution HPLC. (Reversed Phase C18) Samples were evaluated chromatographically by comparing their elution profiles and analyzing their similarities and differences under identical conditions. Semi-preperative HPLC was employed using an automatic valve switching technique designed for an IBM Instruments HPLC Automated System, to isolate an active major component from H. dulcis. Structure identifications were performed by NMR. Both chromatographic and NMR data suggest structural similarities for the genins of H. dulcin and Z. Jujuba but different for structure proposed for Genemic acids.

P7 VARIATION IN OLFACTORY PROTEINS: A CONCEPTUAL POSTER ON THE EVOLUTION OF BEHAVIOR. Richard G. Vogt. (Dept. Chemistry, SUNY, Stony Brook NY 11794).

The pheromone sensitive sensory hairs of the silk moth Antheraea polyphemus contain a pheromone binding protein and a pheromone degrading esterase (Vogt & Riddiford, 1981). Both proteins are uniquely situated in the extracellular lymph surrounding the sensory dendrites. Both proteins have been purified and theri properties characterized (Vogt, Riddiford & Prestwich, 1985; Vogt & Riddiford, 1986). Our data suggests that the binding protein functions as a pheromone carrier by phase partitioning the lipophillic pheromone molecules into solution, allowing them to move through the otherwise aqueous lymph space to the presumed membrane bound receptor proteins. The esterase degrades pheromone rapidly (less than 15 msec half-life estimated in situ), suggesting that it functions to maintain a low signal noise level within the hair. Together, the kinetic properties of these two proteins, along with those of the receptor, determine the effeciency of pheromone following during precopulatory flight. Thus, a part of this animal's precopulatory behavior is encoded in gene-coded kinetic properties of these proteins.

I have electrophoretically examined the pattern and activity of these two proteins from individual animals. The esterase appears as a closely spaced quartet to octet of bands, the binding protein appears as either a singlet or a doublet. While all males possess multiple esterase bands, the presence of particular identifiable bands varies from one individual to the next. Activity varies between different bands from one individual, and the same band from different individuals. Variation may be due to a combination of allellic differences, sequential glycosilation or polygenic expression. The binding protein appears as either a doublet or a single fast or slow band.

This pattern of variation suggests a model for the molecular basis of behavior and focuses on the evolvable elements of behavior. The temporal aspects of behavior are encoded in the kinetic properties of neural proteins. Behavioral variability can, in part, be ascribed to the allellic differences in these proteins. Evolution screens for "appropriate" behavior and selects for neural allelles with "appropriate" properties. The silk moth's sensory hair proteins provide a unique oppertunity to study the molecular basis of sexual selection. Unlike the neural proteins of the CNS, these peripheral proteins have no shared function elsewhere in the animal to compromise their evolved design.

P8 NEUROPHYSIOLOGICAL RESPONSES TO PHEROMONE BLEND COMPONENTS IN THE SOYBEAN LOOPER MOTH, PSEUDOPLUSIA INCLUDENS (WALKER). Alan J. Grant, Robert J. O'Connell (The Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545) and Abner M. Hammond, Jr. (Department of Entomology, Louisiana State University, Baton Rouge, LA 70803).

Reproductive isolation among sympatric species of insects is known to depend, in part, on the composition and release of pheromones from each insect. To investigate how pheromones are differentially processed by the olfactory system, thereby providing the sensory limb required for this isolation process, neurophysiological responses were recorded from single antennal sensilla on the male soybean looper moth to stimulation with known amounts of the individual components of its pheromone blend and the individual components of the blend produced by a sympatric species, Trichoplusia ni (Hübner), the cabbage looper moth. These two Noctuid moths have overlapping geographic and seasonal distributions. Additionally, they have similar temporal patterns of activity and share some of the same host plants. Females of both insects release complex blends of 12-14 carbon esters that are used to attract males for mating. Both species share (Z)7-dodecenyl acetate (Z-7,12:AC) as the major component of their respective blends; however, the composition of their minor components differ.

Similar to the previously reported chemosensory system in the cabbage looper, the soybean looper possesses two classes of morphologically distinct antennal sensilla, each containing two chemosensitive olfactory receptor neurons. In both species, one class of sensilla contains a receptor neuron sensitive to Z-7,12:AC. The neurophysiological characteristics of the receptor neurons in this class of sensilla, including their unstimulated spontaneous activity, sensitivity to pheromone, and response spectra to other compounds appear identical in both species. The second class of sensilla, in each species, contains a receptor neuron sensitive to one of the minor components of the pheromone blend. However, the effective compound is different in each of the two species. Differences in the response characteristics of the receptor neurons in this second class of sensilla are thus thought to play a role in the reproductive isolation that exists between these two species of moth.

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P9 **SEX PHEROMONES OF THE SEA LAMPREY (PETROMYZON MARINUS): STEROID STUDIES.** Michael A. Adams, John H. Teeter, Yair Katz and Peter B. Johnsen. (Monell Chemical Senses Center, University of Pennsylvania, 3500 Market Street, Philadelphia, PA 19104).

Preferences of spawning-run landlocked sea lampreys (Petromyzon marinus) for substances released by sexually mature conspecifics of the opposite sex indicate that pheromones may play a role in the reproductive behavior of this species. Pheromone release in sea lampreys coincides with the appearance of secondary sex characteristics. At this time, male sea lampreys release in their urine a pheromone that is attractive to female conspecifics. The possiblility that this pheromone could be steroidal in nature led us to assay both pheromone-containing (behaviorally-active) and pheromone-devoid (behaviorally-inactive) male urine for its content of several metabolically important steroids.

Samples of male sea lamprey urine were analyzed for the concentrations of nine steroids [dehydroepiandrosterone (DHEA), testosterone (T), dihydrotestosterone (DHT), progesterone (P), androstenedione (A), estrone (E_1), estradiol (E_2), corticosterone (B) and cortisol (F)] by radioimmunoassay (RIA). Samples analyzed included native urine and urine that had been enzymatically hydrolyzed with mixed β-glucuronidase/sulfatase. Values of the analyses were used to prepare solutions of the individual steroids for bioassay at concentrations which bracketed the urinary concentrations. Results show that only testosterone elicited a preference response in spawning-run female sea lampreys, and in concentrations three to four orders of magnitude greater than those found in behaviorally active, unhydrolyzed male urine.

It is possible that testosterone, or a closely related structural derivative, functions as a pheromone in sea lamprey when present at the appropriate concentration. This would occur when one or more males release urine in close proximity to a female. At such close range dilution would be minimal and it is possible that the critical concentration might be reached. Even if this is the case and testosterone is functioning as a short-range attractant, our general bioassay results indicate that there is an additional substance which attracts on females at much lower concentrations.

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P10 A LINKAGE BETWEEN CODING OF QUANTITY AND QUALITY OF PHEROMONE GLAND COMPONENTS BY RECEPTOR CELLS OF TRICHOPLUSIA NI. M. S. Mayer and R. W. Mankin (USDA, ARS; Insect Attractants, Behavior, and Basic Biology Research Laboratory; P.O. Box 14565; Gainesville, Fla. 32604).

The responses elicited in two specialized pheromone receptor cells of T. ni by six pheromone gland components link the coding of pheromone quality in the central nervous system (CNS) inextricably with the coding of pheromone quantity.

In a study of quality and quantity coding of T. ni sex pheromone components, we have used a combination of various methods, including GLC, electroantennogram, and radiolabeling, to measure the emission rates of the pheromone gland components from glass and rubber septum dispensers. A newly developed stimulus delivery system controlled the stimulus concentration and duration. Neuronal responses were recorded from a tungsten electrode inserted at the base of a sensillum that contained the receptor neurons. In T. ni there are two morphologically distinct sensilla, HS and LS, that contain two or more neurons, designated (a) and (b), whose responses can be distinguished by spike amplitude.

We found that the HS(a) neuron responded most sensitively to Z-7:12AC, with action potentials elicited at concentrations lower than 1×10^{-10} μMole/cm^3. The next most stimulatory gland component for this neuron was 12AC. Another neuron in this sensillum, HS(b), responded only to Z-7:12OH at or above such concentrations. A neuron in the other sensillum, LS(b), was most sensitive to Z-7:14AC, with a mid-range response at a concentration of 1×10^{-8} μMole/cm^3. The LS(a) neuron was not stimulated by any of the pheromone components. It should be noted that the HS(a) and LS(b) neurons responded to all pheromone gland components except Z7:12OH at stimulus concentrations above about 1×10^{-6} μMole/cm^3. Because HS(a) and LS(b) neurons responded to 6 of the 7 pheromone gland components at these elevated concentrations, it is not clear how the individual components are discriminated by the CNS. We conclude that neither of these neurons can be defined as a specialist cell in the sense defined for the Bombykol receptor neuron in Bombyx mori because of the linkage between quantity and quality. Whether this linkage is unique or general remains to be determined.

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P11 EARTHWORM ALARM PHEROMONE IS A GARTER SNAKE CHEMOATTRACTANT. JEFFREY HALPERN, NANCY SCHULMAN AND MIMI HALPERN. (DEPT. OF ANATOMY AND CELL BIOLOGY, DOWNSTATE MEDICAL CENTER, BROOKLYN, N.Y. 11203).

Earthworms, when irritated, secrete a substance called earthworm alarm pheromone (EAP) that conspecifics avoid on contact (Ressler et al., Science, 1968, 161, 597-599; Ratner & Boice, Psychol. Rec., 1971, 21, 363-371). To produce the EAP 25 to 100 earthworms (Lumbricus terrestris) were placed in a plastic cone between two metal plates and received an instantaneous shock every six seconds for two minutes. The resulting cloudy, viscous, mucus-like secretions were collected in a beaker placed under the stimulation chamber. Earthworms placed on glass plates (8.3x10.2cm) spotted with .5cc of EAP rapidly escaped from the plates whereas earthworms placed on plates spotted with distilled water (dH_2O) did not escape. EAP may be diluted by one-third before it loses its alarm properties, and lyophilization does not alter its aversive properties.

Garter snakes (Thamnophis parietalis) tested with EAP in a two choice discrimination task (Reformato et al., Pharm. Biochem. Behav., 1983, 18, 247-254) spent more time at and tongue flicked more frequently dishes coated with EAP than dishes coated with dH_2O. Earthworm AP lyophilized and chromatographed on an AcA 44 column yielded three peaks as measured by 230 nm absorbance, a high molecular weight peak (F_2 eluting between dextran blue and albumin), an intermediate peak (F_4 eluting with myoglobin) and a lower molecular weight peak (F_6 eluting just in front of DNPalanine). All of the chemoattractant for snakes was found in F_2. Protein content (as measured by Lowry assay) and neutral carbohydrate content (as measured by phenol assay) were greatest in the F_2 peak as compared to the other peaks and troughs. EAP retained its chemoattractant activity at pH2 and pH11 and following dialysis against 50K MW cut off membranes. EAP boiled for 1 hour did not lose its chemoattractant properties but lost these properties when boiled for two hours.

Many of the properties described for EAP are similar to those described previously for earrthworm wash (EWW), obtained by soaking earthworms in 60° H_2O. EWW is not however an aversive stimulus for earthworms and unlike EWW, EAP cannot be produced from dead worms. Both substances appear to be glycoproteins of molecular weight in excess of 50K daltons and are resistant to heat denaturation.

Supported by NIH Grant NS11713.

P12 INFLUENCE OF A SINGLE MUTATION ON THE INCIDENCE OF PREGNANCY BLOCK IN MICE. Kunio Yamazaki[1], Gary K. Beauchamp[1], Osamu Matsuzaki[1], Donna Kupniewski[1], Judy Bard[2], Lewis Thomas[2], and Edward A. Boyse[2] ([1]Monell Chemical Senses Center, Philadelphia, PA 19104; [2]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. More recently mice have been successfully trained in the Y maze to distinguish the scent of mice that differ genetically only by mutation of the H-2K gene, which belongs to the category of MHC genes known as class I, that play a vital part in immunological recognition and response. To investigate the relevance of H-2K genetic variation to reproductive behavior, we have now tested the effect of isolated H-2K genetic variation in the circumstances of pregnancy block.

All female mice in this study were of the inbred strain BALB, whose MHC type is H-2^d. The stud males were either B6/By (H-2^b) or B6.C-H-2^{bm1} differing only by a mutation at the K^b gene. The second (test) male, to which the fertilized females were exposed, was either the same stud male, or a male genetically identical to the stud male (syngeneic male), or a male of the other H-2K type (B6.C-H-2^{bm1} if the stud male was B6/By, and vice versa). Females were monitored for return to estrus by visual inspection of the external genitalia. Return to estrus within 7 days of stud mating was scored by blind testing as a blocked pregnancy or blocked pseudopregnancy.

The results showed that the incidence of pregnancy block was higher when the stud and unfamiliar males differed in the H-2K gene than when the stud and unfamiliar males were genetically identical. Thus, the olfactory distinction of mice differing by a mutation of the H-2K gene can spontaneously influence neuroendocrine communication affecting reproduction.

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P13 EVIDENCE FOR VOLATILE NATURE OF ACTIVE SUBSTANCE(S) NATURALLY OCCURING IN THE URINE OF ADULT LABORATORY MICE AND SUPPRESSING THE SPERMATOGENESIS IN JUVENILES.

Sergej N. Novikov (I.P.Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad 199164, USSR).

Although variety of pheromonal effects on female reproduction has been described in Mammalia, there is a paucity of published works on pheromonal control of testicular function in males (Vandenbergh, 1983).

We report here that 2-hour exposure of 30-days old CBAB6F1 males to olfactory stimuli from the volatile phase of the urine of mature CBA/LacSto males (Sundberg et al., 1982) was sufficient to induce high frequency of autosomal univalents (18 ± 1,1%) and multivalent associations (5,2 ± 0,9%) at the stage diakinesis-metaphase I in spermatocytes (Novikov et al., 1985). These findings correspond to our recent data on the increase in the level of sperm head abnormalities in cauda epididymis in young males 17 days after treatment (Aref'ev et al., 1986). These data strongly suggest that pachitene spermatocytes are the most sensitive to the substance(s)' action. Our results are in agreement with data from other laboratories on pheromone-like inhibition of male reproductive function in Peromyscus maniculatus (Lawton, Whitsett, 1979) and Microcebus marinus (Schilling et al., 1984). The mutagenic action of some chemicals naturally occuring in the urine should be also considered. Taken together these data provide some evidence for possible pheromonal mechanism controlling male reproduction in mammals.

P15 AMP RECEPTORS OF THE SPINY LOBSTER: EXTERNAL RECEPTORS ON THE OLFACTORY ORGANS AND INTERNAL RECEPTORS IN THE BRAIN.

Charles D. Derby (Dept. of Biology, Georgia State University, Atlanta, GA 30303), William E.S. Carr and Barry W. Ache (C.V. Whitney Lab, University of Florida, Rt. 1, Box 121, St. Augustine FL 32086).

Electrophysiological recordings from single olfactory cells in the antennules of the spiny lobster (Panulirus argus) revealed the existence of receptors highly sensitive to the nucleotide, adenosine 5'-monophosphate (AMP). We found that these receptors are most strongly activated by AMP, have a potency sequence of AMP > ADP > ATP > adenosine, are antagonized by theophylline, and have responses that are most affected by changes in the ribose phosphate moiety (Derby, Carr, Ache: J.Comp.Physiol. 155, 341-349, 1984). These olfactory purinoceptors closely resemble P1-type (or R-type) purinoceptors found in internal organs of vertebrates, including the brain.

We have recently obtained electrophysiological evidence for the existence of related purinergic receptors within the brain of the spiny lobster. Addition of AMP into the saline perfusing the brain resulted in marked changes in the spontaneous activity and/or evoked responses of many of the studied brain interneurons. The modulatory effects of AMP were usually depressive, although examples of enhancement were also observed. Adenosine had modulatory effects similar to AMP in some of the neurons.

These results are significant in several respects. (1) This is the first report of purinergic receptors and their modulatory functions in internal organs of invertebrates. (2) These internal purinoceptors in the brains of both lobsters and vertebrates are involved in depressive modulation of neuronal activity. (3) The presence of both internal and external purinoceptors in a single animal suggests that a receptor of a given type may have been conserved through evolution and used for different functions. More detailed comparisons of the physiological and biochemical nature of these receptors will illuminate the extent of similarity.

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P14 ADAPTATION PROCESSES IN INSECT OLFACTORY RECEPTORS: THEIR RELATION TO TRANSDUCTION AND ORIENTATION.

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Receptor potentials and nerve impulses were recorded extracellularly from two olfactory cells in the sensilla trichodea on the antenna of male Antheraea polyphemus and Antheraea pernyi moths; cell type A responds to the sex pheromone component (E)-6, (Z)-11-hexadecadienyl acetate and type B, to (E)-6, (Z)-11-hexadecadienal. Stimulation with the key compound of one receptor cell also cross-adapts the other cell in the same sensillum but cross-adaptation is weaker than auto-adaptation. Local stimulation experiments demonstrate that sections of the olfactory receptor cell can be selectively adapted with respect to the receptor potential response. The mechanism of impulse generation can adapt separately from that generating the receptor potential as indicated by an altered relationship between impulse response and receptor potential after adaptation. These results demonstrate different and distributed adaptation processes in an olfactory bipolar neuron as studied in a time domain of seconds. Cross-adaptation may indicate extracellular alterations caused by excitation of one cell but could also be caused by direct inhibitory action on the unexcited cell.

Adaptation processes in a time domain below one second are studied and discussed with respect to the orientation of flying moths in an odor plume. Odor pulses of 20 ms duration at frequencies up to 10 pulses per s were applied to single hairs. Generally, the number of nerve impulses elicited per odor pulse was reduced for the second pulse of a stimulus if the pulses were separated by less than a few seconds. The average number of nerve impulses per odor stimulus decreased to one or below with higher stimulus rates. Cell type A responded to each odor pulse up to stimulus rates of 2.5 stimuli/s whereas the responses of cell type B remained time locked with stimulus rates up to 10/s. A third cell type (C) responded to E-4, Z-9 tetradecadienyl acetate at stimulus rates at least as high as were followed by cell type B. Therefore, the compounds acting on cell types B and C may play a specific role in the approach to an odor source.

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P16 EVIDENCE FOR PARTICIPATION BY CALCIUM AND CYCLIC AMP IN OLFACTORY TRANSDUCTION. Bruce D. Winegar and Rollie Schafer. (Dept. of Biological Sciences, North Texas State University, Denton, TX 76203-5218).

The role of Ca^{2+} and cyclic AMP in olfactory transduction was explored by aerosol application of inorganic cations, organic calcium channel antagonists, and cyclic nucleotide agonists onto the olfactory epithelium of the frog (Rana pipiens) and tiger salamander (Ambystoma tigrinum) during extracellular recording.

Inorganic cations that block inward calcium currents in other tissues inhibit electroolfactogram (EOG) responses. The rank order of potency of the chloride salts is:
$(La^{3+}) > (Zn^{2+}, Cd^{2+}) > (Al^{3+}, Ca^{2+}, Sr^{2+}) > (Co^{2+})$.
A 2 sec exposure of the olfactory mucosa to La^{3+}, giving a final concentration of 7.5 X 10^{-3}M virtually eradicates EOG responses to isoamyl acetate, with only partial recovery occurring over a period of hours. Unexpectedly, Ca^{2+} itself is significantly inhibitory post-treatment ($p<0.05$) when applied for 2 sec, reaching a concentration of 1.5 X 10^{-4}M ($t=2.065$; $df=9$).

The organic calcium channel antagonists diltiazem and verapamil, applied for 2 sec at 1.5 X 10^{-3}M inhibit over a 33 min period by 35% and 55%, respectively. The effects of diltiazem and verapamil at this concentration are completely reversible within 90 min. Verapamil still produces significant inhibition of EOG's ($p<0.001$) after a 2 sec application at 1.5 X 10^{-5}M ($t=4.597$; $df=9$). In the salamander, 5 X 10^{-3}M verapamil applied as a lavage for 100 sec inhibits > 80% over a 33 min post-treatment period in separate experiments with different odorants, including isoamyl acetate, cyclohexanone, cyclopentanol, and dimethyl disulfide.

Cyclic AMP, cGMP, and their dibutyryl analogs were applied as a 2 sec aerosol spray to give a final concentration of 1.5 X 10^{-3}M. Both cAMP and dibutyryl cAMP inhibit while neither cGMP nor dibutyryl cGMP are inhibitory. Adenosine at the same concentration is also inhibitory. Forskolin, a reversible stimulator of adenylate cyclase, applied in a 2 sec aerosol spray at 1.5 X 10^{-5}M, significantly inhibits olfactory responses ($p<0.0025$) over a 33 min post-treatment period ($t=4.127$; $df=10$).

These data support the hypothesis that Ca^{2+} participates in olfactory transduction. The inorganic cations inhibit EOG's in rank order and concentrations consistent with calcium channel blockade in other tissues. The inhibition by organic calcium channel antagonists also suggests a role for calcium. The inhibitory action of cAMP, dibutyryl cAMP, and forskolin indicates that cAMP may act as a second messenger in olfactory transduction.

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P17 TUNING OF OLFACTORY NEURONS SENSITIVE TO HYDROXY-L-PROLINE IN THE AMERICAN LOBSTER. Bruce R. Johnson, Carl L. Merrill, Roy C. Ogle, and Jelle Atema. (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

A prominent population of olfactory receptors from the american lobster, Homarus americanus, is narrowly tuned to trans-4-hydroxy-L-proline (Hyp), suggesting that Hyp may be an important chemical signal for this organism (Johnson and Atema, Neuroscience Letters, 41: 145-150, 1983). However, Hyp is bound in connective tissue proteins such as collagen and thus may be unavailable as a free amino acid in sufficient quantites to stimulate chemoreceptors. In this study, we tested olfactory receptors sensitive to Hyp with a variety of compounds to determine other adequate stimuli for these cells.

Single olfactory receptors of the lateral antennule sensitive to Hyp (N=77) were identified using extracellular recordings from small nerve bundles. Test compounds included Hyp, Sigma gelatin (SG), proline, kainate, octopamine, serotonin, hydroxylysine, two isomers of Hyp, two ecdysones, and a variety of purified invertebrate and vertebrate collagens and their gelatins. Gelatins are denatured collagens that contain 14 % bound Hyp. Hyp sensitive cells were classified into three groups: those responding best to Hyp (N=50); those responding best to SG (N=20); and those responding best to some other test compound (N=7). Both Hyp best and SG best cells responded consistently only to Hyp and solutions of SG including its greater than 1 kD and greater than 12 kD retentates. These cells rarely responded when tested with the other test compounds.

The effectiveness of the SG solution and its high molecular weight fractions to stimulate Hyp sensitive cells suggests that peptides containing Hyp residues may be the preferred stimuli. In addition, the presence of both Hyp best and SG best olfactory receptor populations suggest that compounds degraded from connective tissues of lobster prey, possibly by bacterial collagenases, may be important signal compounds for lobster feeding behavior.

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P18 ANALYSIS OF OLFACTORY NEURAL RESPONSES BY A METHOD OF SPIKE TRAIN MATCHING. T.A. Harrison & J.W. Scott (Dept. Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322).

Previous attempts to monitor the changes in olfactory unit responses resulting from changes in stimulus concentration have met with difficulty because the responses often change in spike rate and in spike pattern simultaneously. These responses have sometimes been described as nonmonotonic, even though the response patterns seen at concentrations below and above the concentration eliciting peak spike frequency were qualitatively very different from each other. We have developed an index for characterizing differences between spike patterns that overcomes this problem and that shows, for individual olfactory neurons, monotonic increasing curves of difference between responses as the difference between stimulus concentrations increases.

The basic method is one of matching spikes in two response trains on the basis of their latency from the beginning of the spike train. (Two spike trains in which all the latencies were identical would be considered identical while a spike train with all its spikes in the early part of the trace would be very different from one with all its spikes in the later half of the trace.) Since no two trains are perfectly identical, we allow a certain interval (the cutoff value) around each spike for matching to the other train. (For example, a spike might be successfully matched with any single spike in the other train that occurred within 100 msec of the same latency). Matching is without replacement: any spike can be matched only once in the analysis of each pair of traces. The goodness of each match (the latency disparity in msec) is tallied and averaged at the end of each pairing. The final index is based on the number of unmatched spikes and on the mean disparity of the matches. Comparisons of repeated responses to the same stimulus concentration give low index numbers indicating very little disparity in the spike trains. Comparisons of responses to a low stimulus concentration with responses to a high stimulus concentration give high index numbers indicating greater disparity in the spike trains. Statistical significance can be assessed by comparing the index for comparisons of different concentrations with the index for same-concentration comparisons.

It must be noted that the size of the index will depend upon the choice of cutoff value (the value beyond which spikes are called nonmatching). The optimal cutoff value can be chosen nonarbitrarily, by nonlinear regression to an exponential decay curve on axes relating the size of the response difference index to a power function of the ratio of the concentrations for the two stimuli being compared.

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P19 EFFECTS OF CADMIUM ON RAT OLFACTORY SYSTEM. Lloyd Hastings and True-Jenn Sun. (Dept. of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0056.

Clinical studies of workers exposed chronically to cadmium (Cd) suggest that prolonged Cd exposure may result in anosmia (Br. J. Indust. Med. 18:216-222, 1961). Chronic exposure of adult rats to Cd has also been found to result in increased uptake of Cd by the olfactory bulb (Neurotoxicol. 6:109-114, 1985). These two lines of evidence suggest that chronic Cd exposure might impair olfaction.

To investigate the effects of Cd on olfaction, 45 male adult rats were exposed to CdO via inhalation for 5 hrs per day, 5 days a week for a total of 80 days. Exposure values were 250 $\mu g/m^3$ and 500 $\mu g/m^3$ (current Threshold Limit Value for CdO is 50 $\mu g/m^3$). Prior to exposure, olfactory thresholds were obtained using a conditioned suppression technique. Olfactory threshold tests were conducted approximately every 4 weeks during the exposure period.

After 80 days of Cd exposure, there was no evidence of anosmia in any of the rats nor were there any significant changes observed in olfactory thresholds. Failure to produce anosmia may be due to insufficient exposure to Cd, resulting either from the shortness of the exposure period or to the low concentration of Cd used. Analysis of Cd in olfactory tissue as well as histological examination of the olfactory bulb is now in progress.

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P20 OLFACTORY INPUT TO PREFRONTAL CORTEX IN THE RAT. Marie C. Clugnet and J.L. Price. (Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110).

Although previous electrophysiological and anatomical studies from this laboratory have shown that there is an olfactory input to the prefrontal cortex of the rat, these have not provided a comprehensive account of the extent of the olfactory related areas. In particular, very little attempt has been made to define the olfactory input to the medial prefrontal cortex. In this study the olfactory bulb was stimulated with single shocks or brief trains of three shocks (100 µsec, 1.5 mAmp), and entrained multiunit activity was recorded with tungsten microelectrodes. Electrode penetrations were made at close intervals throughout the lateral and medial prefrontal cortex.

Positive responses were found in all of the cortical areas in the dorsal bank of the rhinal sulcus, including the ventral agranular insular area (AIv), lateral orbital area (LO) and ventrolateral orbital area (VLO). Throughout this region one to several peaks of entrained unit activity were recorded, with the mean latency of the first peak 12 to 16 msec. The latency of the response tended to be shorter in the depth of the rhinal sulcus, and to increase laterally and rostrally. Experiments using the retrograde axonal tracer WGA-HRP indicate that the more caudal area in this cortical region (AIv) receives inputs from anterior and posterior parts of the piriform cortex as well as the periamygdaloid cortex. The more rostral areas (LO and VLO) receive inputs only from the anterior piriform cortex.

In the cortical areas on the anteromedial surface of the frontal cortex, including the infralimbic area (IL), prelimbic area (PL) and medial orbital area (MO), very few multiunit responses were obtained to stimulation of the olfactory bulb. However, single units were found that responded to single shock or train stimuli with a constant latency that was considerably longer than the responses in the more lateral areas (22 to 24 msec). Available anatomical experiments indicate that these areas (especially PL and MO) do not receive substantial inputs from the piriform cortex, although they receive fibers from other olfactory cortical areas.

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P21 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF INTERNEURONS IN THE OLFACTORY MIDBRAIN OF THE CRAYFISH. Edmund A. Arbas, Carol J. Humphreys and Barry W. Ache. (C. V. Whitney Lab., Univ. of Florida, St. Augustine, FL 32086.

We have begun to characterize the morphological and physiological properties of interneurons in the olfactory pathway of the crayfish using intracellular recordings from neuropilar processes and dye injection of individual neurons. We have recorded responses from several classes of neurons that are activated by food-related stimuli applied to the antennules (olfactory organs).

i) Neurons whose arbors overlap with the projections of primary sensory axons in the olfactory lobe, and whose total projections are largely confined to the olfactory areas of the brain (i.e., the olfactory and accessory or paraolfactory neuropile areas). These neurons are likely to be primary interneurons and therefore to subserve an integrative function early in the pathway processing olfactory information. We find that they fall into several morphological subtypes based on features of their dendritic arborizations in the olfactory lobe and projections to other brain regions. They are variously excited or inhibited when odorants are applied to the antennules. In some instances, compound responses are obtained where excitation is followed by a delayed wave of inhibition, suggesting that mechanisms may exist for temporally clipping excitatory responses.

ii) Neurons with projections intrinsic to the brain but without branches in the olfactory lobe. These are likely to participate at intermediate levels in the olfactory pathway.

iii) Projection neurons without arborizations in the olfactory neuropil areas and whose axons leave the brain via the circumesophageal connectives to innervate more posterior portions of the CNS. These neurons are usually multimodal and vary in their chemical responsiveness.

We are currently focussing our studies on neurons of the first class with the aim of associating the characteristics of their diverse olfactory lobe projections with the complexity of their response spectra. Furthermore, since earlier work (Derby, Ache and Kennel, Chem. Senses 10: 301, 1985) indicated that central neural events are in part responsible for mixture suppression in the olfactory pathway, we are analyzing these neurons with the aim of identifying central integrative events responsible for suppression.

Supported by NSF Award BNS 85-11256 to BWA and a NERC NATO Research Fellowship to CJH.

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P22 TEMPORAL AND SPATIAL PATTERNS OF RESPONSE TO ODOR IN THE HAMSTER OLFACTORY BULB: SINGLE UNIT RECORDINGS AND COMPUTER SIMULATION. Michael Meredith, Department of Biological Science, Florida State University, Tallahassee, Florida 32306.

Extracellular single unit responses to controlled odor pulses were recorded in 101 cells in the olfactory bulb of anesthetized male hamsters. 41 cells driven by lateral olfactory tract stimulation had latencies less than 2ms and were classified as output cells but their responses were not obviously different from cells with longer latencies. Excitation was more common than suppression, based on the first observable change in firing rate at the beginning of the pulse, but both types of response were seen at all stimulus intensities. Many responses consisted of complex temporal patterns of excitation and suppression, and such complex patterns were more frequent at higher stimulus intensity. Over half of the cells tested had non-monotonic intensity-response functions. Changes from suppression at low concentration to excitation at higher concentration were slightly more common than the reverse. A few cells changed response-type in both directions over different concentration ranges.

Both non-monotonic intensity-response functions and complex temporal patterns of response may be due to lateral inhibitory interactions between regions of the bulb receiving different levels of olfactory input. A computer simulation of the bulb, including lateral inhibitory pathways, suggested that a spatially non-uniform input to the bulb would generate both of these features in a structurally uniform network. The patterns of response to the same stimulus by neurons recorded at known distances apart across the olfactory bulb are consistent with the lateral inhibition hypothesis, as are the sequences of response patterns that neurons develop with increasing stimulus intensity.

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P23 INFORMATION PROCESSING IN THE OLFACTORY BULB: CHANGES IN SINGLE NEURONE RESPONSES DURING REPETITIVE STIMULATION. D.Schild and H.P.Zippel (Physiologisches Institut, Humboldtallee 23, Universität D-3400 Göttingen, FRG)

Mitral cells of the olfactory bulb are known to output patterned discharges. In goldfish these patterns have been described by Meredith and Moulton (1976). We have now found that the characteristics of the discharge patterns often change when the stimulus is repeated. This finding, which has statistical as well as functional (adaptation, plasticity) implications, has been obtained in the following way: The responses of goldfish mitral cells were recorded extracellularly with electrolytically sharpened insolated steel electrodes. With a recently developed computer-controlled device for odour application (Schild, 1985) rectangular pulses of odour (natural Tubifex-food-extracts and synthetic odours diluted in tap water) were applied of at least 30 seconds duration. Thereafter, the olfactory mucosa was washed with tap water for at least 30 s. Each cell was stimulated at least 40 times with the same stimulus in order to investigate changes of the responses in the course of the stimulus repetitions. The most common response to a single stimulus pulse was an initial phasic activity increase or decrease (about 3-5 s) followed by a fairly constant mean activity maintained during the pulse. The activity during the stimulus-free period (at least 30 s) between two stimulus periods usually began with a phasic reaction (about 3-5 s) followed by an interval of fairly constant activity. In 37 of 51 cases the responses to single stimuli were reproducible over all runs of the experiment.
In 14 recordings the single run responses were not reproducible. They rather showed (a) abrupt changes from the first to the second run or (b) slow changes that extended over up to 30 runs. Every pattern of this kind became reproducible after a certain number of runs. The activity changes during the first runs can therefore be attributed to a process that leads to a more pronounced response to a repeated stimulus. With respect to the above data two questions are investigated at present: (i) to describe and to define the above mentioned slow changes by statistical means, and (ii) to determine the interstimulus interval necessary for obtaining identical neuronal responses during repetitve stimulations, i.e., to determine the time after which the effect of a preceding stimulus has ceased.

P24 OLFACTORY RECOGNITION OF CONGENIC STRAINS OF RATS. Richard E. Brown[1], Prim B. Singh[2], and Bruce J. Roser[2]. ([1]Subdepartment of Animal Behaviour, Madingley and [2]Institute of Animal Physiology, Babraham, Cambridge, England).

Recognition of different major histocompatibility (MHC) types of mice is based on urinary chemosignals from congenic strains which differ only in the MHC (Yamazaki, et al, 1979, J. Exp. Med., 150, 755-760). Our experiment provides the first evidence that congenic strains of rats which differ only in the MHC also produce different urinary chemosignals. Urine from adult male PVG and PVG-R1 rats was used in a habituation - dishabituation test (Sundberg, et al, 1982, Behav. Neur. Biol., 34, 113-119) with male PVG-RT1^u rats as subjects.
Urine from PVG males was easily distinguished from that of PVG-R1 males ($p < .001$). Individual PVG males were not distinguished by their urinary odours, but individual PVG-R1 males did produce discriminably different odours ($p < .05$). These results demonstrate that PVG and PVG-R1 males, which differ only in the A region of the MHC, produce different urinary chemosignals and that individual males of the PVG-R1 strain also produce distinguishable "individual" odours. These may be related to individual differences in the quantity of A region classical class 1 antigens in the urine of PVG-R1 rats (Singh and Roser, unpublished observations). We are now using immunological assays to determine how the quality and quantity of class 1 antigens or their fragments in the urine of these rats enables recognition of their substrain and of individuals within a highly inbred substrain.

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P25 OLFACTORY DISCRIMINATION OF PLANT VOLATILES BY THE EUROPEAN STARLING. Larry Clark (1) and J. Russell Mason (1,2) ([1] Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104, and [2] Department of Biology, University of Pennsylvania, Philadelphia, PA 19104).

Passerine species that re-use nest-sites often incorporate fresh green vegetation into their nests. This behavior is consistent with the possibility that some birds may use chemical properties of plants to counteract the selective potential of parasites and pathogens (Clark & Mason, 1985). We tested adult starlings (Sturnus vulgaris) for their physiological capacity and behavioral ability to detect and discriminate among volatiles emitted from plant material. Multiunit electrophysiological recordings from the olfactory nerves of adults indicated that strong responses were reliably elicited by volatiles from six plant species. After pairings of plant volatiles with malaise, birds exhibited conditioned avoidance in behavioral experiments, and made all possible pairwise discriminations between volatiles of the various plant species ($p_s<0.05$). Bilateral olfactory nerve cuts prior to conditioning abolished the ability to acquire avoidance ($p<0.05$), suggesting that olfactory cues mediated responding to at least some plant species. These and previous results (Clark & Mason, 1985) lead us to hypothesize that starlings may use volatile cues to discriminate and choose among plants used in nest construction.

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P26 THE ASSOCIATION BETWEEN ANOSMIA AND ANOREXIA IN THE FELINE SPECIES. Kimberly May, Dr. Lawrence Myers and Dr. Donald Buxton. (Dept. Physiology and Pharmacology, Auburn University, AL 36849).

Anorexia is a common disorder of the feline species but no physiological cause has been described. Anosmia, absence of the sense of smell, has been suggested by current literature to contribute to a specific anorexia effect and for this reason an attempt was made to associate feline olfactory disorders and decrease in food intake.

In this study the olfactory system was treated to induce anosmia. Treatment groups include the following:

1) No treatment (control group)
2) Application of zinc sulfate to the olfactory epithelium.
3) Sham $ZnSO_4$ group (sterile saline replaced $ZnSO_4$)
4) Olfactory bulb transection
5) Olfactory bulb ablation
6) Sham olfactory bulb transection and ablation. The bulb was exposed but no transection or ablation was made.

Each animal's food intake was monitored daily for one month prior to and one month following the treatments. The data was analyzed and differences between before and after treatments were observed to be statistically significant for groups 4 and 5. The above association between anosmia and anorexia was suggested for group 5 but due to an increase in food intake rather than a decrease anorexic conditions did not exist for group 4. Reasons for this increase are being investigated.

In addition, histological preparations of the olfactory bulbs were analyzed. Mass degeneration of axons was observed in the central white matter of each animal in group 4. Group 5 was excluded from these preparations because of the bulbs absence, however preparations of the lateral and medial olfactory strias are being examined for degeneration.

Supported by the Grace Kemper Foundation.

P27 OLFACTORY DISCRIMINATION: BEHAVIORAL ABILITIES OF THE SPINY LOBSTER. Jacqueline Fine and Charles D. Derby. (Department of Biology, Georgia State University, Atlanta GA 30303).

We are attempting to understand how olfactory systems code chemical quality by correlating behavioral and neurophysiological measures of the similarities among a set of behaviorally relevant chemicals. The spiny lobster (Panulirus argus) has proven to be a good experimental system for studying neural mechanisms of olfactory discrimination (e.g., Derby and Ache, 1984, J.Neurophysiol. 51, 906-924). To extend this analysis to the behavioral realm, we have developed an aversive conditioning paradigm that uses electric shock as the aversive stimulus. In experimental tests, behavioral responses to taurine and L-glutamate (at concentrations of 10, 100, and 1000 um) presented discretely to the antennules (the olfactory organ) were compared before, during, and after pairing shock with presentation of taurine. A conditioning regime of 3 pairings per day for 4 days resulted in a decline in responsiveness to both chemicals but the decline to taurine was greater than to glutamate. This differential effect suggests that lobsters are able to discriminate between these two odorants. Control tests, in which shock was presented specifically unpaired with any chemical, showed that a non-specific (non-associative) side effect of the shock itself was to cause a general decline in responsiveness to all chemicals, as was noted in the experimental tests. The difference in response to a chemical in experimental tests versus control tests may be used as a measure of the strength of associative learning. This measure can then be used to rate the degree of perceived similarity between taurine and glutamate. We are presently extending our knowledge of the lobster's behavioral capacities for olfactory discrimination by using a larger set of chemicals.

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P28 MORPHOLOGICAL AND BEHAVIORAL EVIDENCE FOR CHEMORECEPTION BY PREDACEOUS STONEFLY NYMPHS AND THEIR MAYFLY PREY. Lee Anne Martinez. (Dept. of Entomology, Cornell University, Ithaca, New York 14853).

To date, few studies have examined the use of chemosenses by stream-dwelling insects. Scanning electron microscopy reveals that both predatory stonefly nymphs (Plecoptera: Perlodidae) and their mayfly prey (Ephemeroptera: Baetidae, Ephemerellidae, and Heptageniidae) have a complex array of sensilla on their antennae, cerci, and other parts of their anatomy. Stoneflies possess thick- and thin-walled sensilla basiconica, and sheaves of coniform sensilla on their antennae. Mayflies appear to have coeloconic pegs on their antennae, as well as specialized flattened sensilla that may be an adaptation to the aquatic environment. Additionallly, one of the mayfly genera studied (*Baetis* spp.) has antennae that are essentially identical in cuticular fine structure to its cerci. The sensillar complexity exhibited in the nymphs is lost in the non-feeding terrestrial adult stages of these insects.

Behavioral studies with an in-stream olfactometer device show that stonefly nymphs are attracted to upstream wounded prey. This attraction appears to be limited to the immediate vicinity (< 10 cm) of the stimulus source. Moreover, these predaceous stoneflies are attracted to filter paper soaked in an ethanol extract of their prey, while they may avoid paper soaked in an extract of stonefly competitors. Like the stoneflies, mayflies are attracted to an extract of their (algal) food source, while avoiding extracts of predatory stoneflies and fish. Such studies indicate the potential role of chemoreception in prey-detection/predator avoidance in the freshwater environment.

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P29 ORGANIZATION OF AFFERENTS FROM THE NUCLEUS OF THE DIAGONAL BAND TO THE OLFACTORY BULB. *M.T. Shipley, W.T. Nickell, and J. McLean.* (Dept. of Anatomy and Cell Biology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267).

The nucleus of the diagonal band (DB) is the sole source of cholinergic input to the olfactory bulb, and may be the origin of inputs using other transmitters, notably GABA. Activation of DB causes a dramatic synaptic potentiation in the bulb and inhibits the firing of mitral cells (Nickell and Shipley, this meeting). The patterns of termination of DB axons in the bulb are unknown and of importance in elucidating the functional significance of this pathway.

Two tracing methods were used to study the pattern of DB->MOB innervation and the branching characteristics of individual axons. Either wheatgerm agglutinin lectin conjugated to hoseradish peroxidase (WGA-HRP) or phaseolus vulgaris (PHA-L) was injected iontophoretically into DB and the anterograde labelling was visualized by conventional methods.

WGA-HRP labelling of DB fibers corresponded well to the pattern of cholinesterase staining in the bulb: there was heavy labelling in the glomeruli (gl), external plexiform layer (epl), and internal plexiform layer (ipl). Somewhat lighter, but still significant labelling was present in the granule cell layer (gcl). Sagittal sections have been analyzed to define the trajectory of fibers from DB to OB.

PHA-L labelling allowed the branching patterns of isolated fibers in the bulb to be seen. Numerous fibers were visible within and surrounding the glomeruli. In the epl, radial fibers predominate although they give rise to tangentially running collaterals in the deep third and superficial quarter of the layer. Numerous, mostly tangentially oriented, fibers were seen in the ipl and gcl.

These results suggest that the primary synaptic actions of DB fibers are in the glomerular layer, sublaminae of epl, the ipl, and in the superficial half of gcl.

This work was supported by NINCDS-NS-18490 and DAMD17-86-C-6005.

P30 EVIDENCE FOR CHOLINERGIC INVOLVEMENT IN THE SECRETORY RESPONSE OF OLFACTORY GLANDS OF THE SALAMANDER TO PYRAZINE. M.L. Getchell, B. Zielinski and T.V. Getchell. (Dept. Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201).

We previously have described the effects of the odorant 2-isobutyl-3-methoxypyrazine (IBMP) on secretory cells of the olfactory mucosa of tiger salamanders. Four effects of 10^{-4}M IBMP were noted: 1. a 46% reduction (n=30; $P < 0.001$) in secretory granule content of the acinar cells of superficial Bowman's glands (sBG), 2. extensive vacuolation with nuclear compression and apparent swelling of secretory granules in acinar cells of deep olfactory glands (dG), 3. protrusion of the surface of epithelial sustentacular cells (SC) above olfactory receptor cell knobs and 4. vacuolation in SC with small superficial vacuoles and large ones near the nuclei. Because vacuolation in acinar cells of sBG and dG previously was observed after cholinergic stimulation, we examined the effect of a cholinergic antagonist on the response of the mucosa to IBMP. The tissue was treated topically with 9.8 mg/ml scopolamine for 10 min followed by 10^{-4}M IBMP, both in 0.15M NaCl, for 15 min. The secretory granule content of sBG acinar cells was reduced by 22% (n=25; $P<0.01$), significantly different ($P < 0.01$) from the effect of IBMP alone. In dG, there appeared to be less secretory granule swelling, but estimation of the extent of vacuolation was complicated by the partial agonist effects of scopolamine alone. The secretory responses of SC to IBMP did not appear to be affected by pretreatment with scopolamine. The effect of propranolol, a β-adrenergic antagonist, on the secretory response to IBMP was also investigated. An i.p. injection of 42 mg/kg propranolol was given 10 min before the 15-min topical application of 10^{-4}M IBMP. No differences were observed in the secretory responses of tissues pretreated with propranolol compared to those of tissues treated with IBMP alone. These results suggest that IBMP may activate cholinergic mechanisms that regulate secretion from olfactory glands, either by direct action on the acinar cells or indirectly through a secretomotor reflex.

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P31 NUMBER, SIZE AND DENSITY OF MITRAL CELLS IN THE OLFACTORY BULBS OF THE NORTHERN FULMAR AND ROCK DOVE. Bernice M. Wenzel and Esmail Meisami. (Dept. Physiology, University of California, Los Angeles, CA 90024 and Dept. Physiology-Anatomy, University of California, Berkeley, CA 94720).

The great variation in gross size of olfactory bulbs (OB) and conchae across avian species is well known. Detailed measurements, however, have not been provided. Using morphometric and cell counting methods, we have studied the olfactory bulbs of two species, the Northern Fulmar (Fulmarus glacialis) and the Rock Dove (Columba livia). The former represents birds with very large bulbs, the other those with bulbs of average size. The following values were obtained from complete Nissl-stained serial coronal sections : total OB volume, mean diameter of mitral cells (MC), total area of MC layer, density of MC per unit area of MC layer, and the total number of MC per OB. MC counts were based on the presence of a nucleolus. Values given are conservative and approximate, and include corrections for nucleolar overcounts due to binucleolation and transection.

OB volume in the fulmar was 60 mm^3 compared to 3 in the pigeon. In the fulmar, there were ca. 120,000 MC with mean diameter of 19 μm, densely packed (3500/mm^2) in a layer with an area of 35 mm^2; in the pigeon, these values were 20,000 MC, diameter 11 μm, density 4000/mm^2, and layer area 5 mm^2.

The data indicate that the relationship between OB size and number of MC may be more complex than expected. The fulmar's OB is 20 times larger than the pigeon's but has 6 times more MC. The total number of MC in the fulmar is about 2 times higher than the number for rat and rabbit while the pigeon's total is in the range reported for mouse which has a bulb of similar size. The larger size of OB in the fulmar is not only due to its larger MC which are twice the size of those in the pigeon, but to the greater combined thickness of its OB layers (glomerular to granular), about 900 μm, also double the pigeon's.

The impressively high number of MC as well as the very thick cortical sheet of OB (due to well-developed external plexiform and internal granule layers) in the fulmar should stimulate further olfactory research in these animals. Equally interesting is the relatively high number of MC in the pigeon, a bird with known olfactory capacities. The pigeon's total MC number, being in the same range as the small rodents, implies that a minimum number of MC may be a prerequisite for good olfactory function in higher vertebrates. To put the present results in broad functional perspective, further data are needed on the number of primary olfactory neurons and olfactory glomeruli and thus the convergence ratios in the primary afferent relay.

P32 FUNCTIONAL MORPHOLOGY OF THE OLFACTORY EPITHELIUM

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The olfactory epithelium is composed of three types of cells, such as the basal cell, the supporting cell, and the olfactory cell. The olfactory cells are bipolar neurons and are known to fall off and regenerate. So the olfactory cell is regarded as one of the entities of the "Paraneuron".

With the newly developed technique of SEM using back scattered electron image, three dimensional structure and histochemical localization of the crucked surface of the olfactory epithelium were observed. It was found that the nucleus of the olfactory cells and some of the basal cells had affinities to silver. These findings suggest that the basal cells, having affinities to silver, differentiate into the olfactory cells.

The components of the basement membrane, such as fibronectin and laminin, are important to cellular growth and differentiation. The basement membrane of the olfactory epithelium was studied with SEM and immunohistochemistory. On the SEM observation, the basement membrane has many poles and fibrous network. It was revealed that there were the increased amounts of laminin and fibronectin in the basement membrane of the olfactoty epithelium by immunohistochemical observation. These findings suggest that both laminin and fibronectin play an important role in the maintenance of proper tissue organization during olfactory epithelial regeneration.

P33 ELECTRON MICROSCOPY OF OLFACTORY EPITHELIUM IN ZINC DEFICIENCY RATS. Shuntaro Shigihara, Junko Yasukata, Hiroshi Tomita 1, Masaomi Okano 2, (1: Dept. of Otorhinolaryngology, Nihon University, School of Medicine. 2: Dept. of Veterinary Anatomy, College of Agriculture and Veterinary Medicine, Nihon University, Tokyo, Japan.

The role of zinc in the human body is recognized as being important. Also in patients with olfactory disturbance, we found that the zinc concentration in their serum was lower than normal, and to administer zinc relieved those symptoms. The purpose of this study is to prove the relationship between zinc deficiency and olfactory disturbance.

First of all zinc deficiency was in rats, and juvenile and senile rats (Wister strain) were used. The rats were fed zinc deficiency foods (content zinc 1.96ppm) or normal foods (content zinc 48.4ppm) for 7-17weeks. The olfactory mucosa of each rats was removed and after fixation, embedding and thinsection, studied with TEM.

Changes in the olfactory membrane broadly observed and were more apparent in juvenile rats than senile ones. Degeneration was recognized in both receptor cells and supporting cells. In receptor cells the nucleus became electron dense and smaller, and the cytoplasma contained dark vesicles with characteristics of lysosome and multivesiculer bodies.

The conclusions are as follows:

1) Zinc deficiency caused degeneration of olfactory epithelium.
2) The changes existed in both receptor cells and supporting cells.
3) The changes were more apparent in juvenile rats.

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P34 **IMMUNOCYTOCHEMICAL LOCALIZATION OF NERVE GROWTH FACTOR (NGF) AND NGF RECEPTOR IN THE RAT OLFACTORY BULB.** Joe E. Springer, Sookyong Koh, Mark W. Tayrien and Rebekah Loy. (Dept. Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, New York, 14642).

The olfactory bulb contains one of the highest levels of NGF in the rat brain. We have used a monoclonal antibody to NGF receptor (192-IgG, characterized and kindly provided by E. Johnson) and an affinity purified polyclonal antibody to mouse NGF (provided by E. Johnson) to localize cells containing NGF and NGF receptor. The major cells in the olfactory bulb immunoreactive for NGF are the mitral cells, the middle tufted cells of the external plexiform layer, and the external granule cells. The glomeruli exhibit no immunoreactivity for NGF, although the adjacent axons of the olfactory nerve appear to contain NGF. The glomeruli show the densest immunoreactivity for the NGF receptor. Lesser staining for NGF receptor occurs in the axons of the olfactory nerve and the internal granule cells. In support of a sensitivity of the internal granule cells to NGF, Steward and co-workers demonstrated that these cells are especially sensitive to colchicine, which could produce cell death by blocking neuroplasmic transport of NGF. The intense immunoreactivity for NGF receptor in the glomeruli and olfactory nerve indicates that the olfactory receptor cells may also be sensitive to NGF. Thus, NGF may be a critical trophic element in maintaining mucosal cells, which undergo retrograde degeneration following bulbectomy. The source of NGF in the olfactory bulb is not clear. The dense staining in the mitral, middle tufted and external granule cells suggests that these neurons may be source cells. In support of this, the mitral and tufted cells are major targets of both the olfactory nerve axons and the internal granule cells. A possible alternate source of NGF in the olfactory bulb may be ependymal cells and astrocytes which line the olfactory and lateral ventricles and the obliterated pathway connecting these, and which stain for both NGF and NGF receptor.

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P35 **ELEVATED LEVELS OF IMMUNOREACTIVE BETA-ENDORPHIN IN ROSTRAL AND CAUDAL SECTIONS OF OLFACTORY BULBS FROM MALE GUINEA PIGS EXPOSED TO ODORS OF CONSPECIFIC FEMALES.** Jay B. Labov[1,2], Yair Katz[1], Charles J. Wysocki[1], Gary K. Beauchamp[1], and Linda M. Wysocki[1]. ([1]Monell Chemical Senses Center, Philadelphia, PA 19104, and [2]Dept. Biology, Colby College, Waterville, ME 04901).

Recent evidence demonstrates that the vomeronasal (accessory olfactory) system selectively accumulates and binds ^{3}H-endogenous opioids. The present study investigated whether titers of beta-endorphin (BE) would be altered in the rostral and caudal portions of the olfactory bulbs of male domestic guinea pigs after brief exposure to female-generated chemical cues.

Subjects were permitted one minute contacts with each of ten glass plates that had been smeared with either saline (controls) or with secretions from the perineal region of female conspecifics (experimentals). Subjects were observed during each trial for the number and duration of headbobbing bouts (rostro-caudal movement of the head which may facilitate access of molecules to the vomeronasal organ) and for the number and length of time that the nose or mouth was in physical contact with the plate. Twelve minutes after the conclusion of odor presentations, subjects were killed with CO_2, their brains were rapidly removed, frozen in liquid freon, and stored at -80°C. After warming to -20°C, brains were placed on the cold stage of a dissecting microscope and the left olfactory bulb was cut into antero-medial (main olfactory) and latero-caudal (includes accessory olfactory) sections. Each section was macerated and extracted in 0.2N HCl. BE in the rostral and caudal olfactory bulbs was measured using double antibody radioimmunoassay (Immuno Nuclear Corp., Stillwater, MN).

Concentrations of immunoreactive BE (pg/mg tissue) were significantly greater in the olfactory bulbs of experimentals than in controls. Latero-caudal sections contained significantly higher titers of BE than did antero-medial sections. A significant inverse correlation between opioid concentration and tissue weight was observed for latero-caudal sections from both controls and experimentals and from medio-rostral sections from controls, suggesting that BE may be concentrated in localized portions of the bulbs. BE levels in sections containing the accessory olfactory bulb were significantly correlated with the mean duration of headbobbing bouts for experimental, but not for control subjects. There was no relationship between BE concentrations and oro-nasal contact with the glass plate in either experimentals or controls.

These results suggest that following exposure to female odors, BE titers increase in regions of the olfactory bulb of males which receive sensory input from the vomeronasal system.

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P36 PUTATIVE GLUTAMERGIC AND ASPARTERGIC CELLS IN THE OLFACTORY BULB OF THE RAT. J.L. Price and T.A. Fuller. (Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110).

Glutamate and aspartate are thought to be widely used as neurotransmitters, but earlier suggestions that one or both of them are used by the fibers of the lateral olfactory tract (LOT) have recently been questioned. To investigate this, ^{3}H-d-aspartate (d-Asp) has been used as a "neurotransmitter-specific" retrograde axonal tracer to identify cells in the olfactory system whose axons possess a high-affinity uptake mechanism for glutamate and/or aspartate, and therefore may be considered to be putatively glutamergic or aspartergic (glu/asp).

Injections of d-Asp were made into layer I of the anterior piriform cortex, deep to the lateral edge of the LOT, and the brains prepared for autoradiography. Heavily labeled cell somata were found in other parts of the piriform cortex and the anterior olfactory nucleus, indicating that many, at least, of the intracortical associational projections are glu/asp. Many of the mitral cells of the accessory olfactory bulb were also labeled, probably due to involvement of the accessory olfactory tract or its bed nucleus at the lateral edge of the LOT. However, very few mitral or tufted cells were labeled in the main olfactory bulb. Occasional cells were labeled in the mitral cell layer, and among the tufted cells in the external plexiform layer, which had similar staining characteristics to mitral and tufted cells. But these tended to have smaller somata than the mitral or tufted cells as a whole and appear to form a distinct subpopulation.

Injections of d-Asp directly into the olfactory bulb also failed to label the mitral and tufted cells. However, a few cells were labeled in the periglomerular region superficial to the injection site. These are smaller than mitral and tufted cells but generally larger than periglomerular cells, and probably are superficial short axon cells.

Supported by NIH Grants #NS09518 (JLP) and RSD MH00330 (TAF).

F6 COMPUTATIONAL MAPS IN THE NERVOUS SYSTEM. Eric I. Knudsen. (Dept. of Neurobiology, Stanford University, Stanford, CA 94305.)

The brain derives information about the environment through computation. Some of these computations occur in maps - arrays of neurons in which the tuning of neighboring neurons for a particular parameter value varies systematically. Computational maps transform the representation of information into a place-coded probability distribution which represents the computed values of parameters by sites of maximum relative activity. Numerous computational maps have been discovered, including visual maps of edge orientation and direction of motion, auditory maps of amplitude spectrum and time interval, and motor maps of orienting movements. Of the known maps, the construction of the auditory map of space is the most thoroughly understood. Information about interaural delays and interaural intensity differences is processed in parallel by separate computational maps, and the outputs of these maps feed into a higher order map, which integrates sets of cues corresponding to particular locations and creates a map of auditory space.

Computational maps represent ranges of parameter values that are relevant to the species, and may differentially magnify values that are of particular importance. The tuning of individual neurons for values of the mapped parameter is broad relative to the range of the map. Consequently, neurons throughout a large portion of a computational map are activated by any given stimulus, and precise information about the mapped parameter is coded by the locations of peak activity.

There are a number of advantages of performing computations in maps. First, information is processed rapidly because the computations are preset and are executed in parallel. Second, maps simplify the schemes of connectivity required for deriving and utilizing the information. Third, a common, mapped representation of the results of different kinds of computations allows the nervous system to employ a single strategy for reading the information. Fourth, maps enable several classes of integrative mechanisms to sharpen neuronal tuning, mechanisms that cannot operate on information that is represented in a distributed code.

Only a few independent parameters can be mapped simultaneously and continuously in an array of neurons. Although maps may be nested within maps, such schemes lose some of the key advantages of mapping. Perhaps for this reason, the brain tends to map different dimensions of stimuli in separate areas, and interconnects these areas in networks of parallel and serial processing.

F7 FROM RECEPTOR ACTIVITY TO DESCENDING OUTPUT - A SEARCH FOR THE NEURAL CODE UNDERLYING ODOR GUIDED BEHAVIOR. Jürgen Boeckh. (University of Regensburg, D-8400 Regensburg, FRG).

Modes of coding of quality and quantity of chemical stimuli are well known for the receptor cell level. Input-output relationships and local circuitry at the different levels of central signal processing are beginning to emerge. There, the quantitative physiological investigation of morphologically identified neurons helps to uncover further stages of afferent chemosensory pathways, and their connections to neurons of descending pathways which govern motor outputs.

In some species of insects like moths (cf. also work of J. Hildebrand and his group) and cockroaches, first - and second order projections of chemosensory inputs can be described in terms of neuroanatomy, and specificity of participating neurons and neuropiles (glomeruli). A split of the pathway via collaterals of projection neurons is apparent in the second order relay, where one branch reaches a region which is assumed to play a major role for learning and memory (the corpora pedunculata). Multimodality is introduced at several stages of the pathway. Morphologically and physiologically distinct sub-pathways, specialized for the processing of certain families of stimuli are found side by side with sections which are activated by broad spectra of stimuli. Convergence results in high sensitivity while following divergence leads to distribution of certain inputs over large areas. Descending neurons are connected to the ascending pathway at several levels. Some of them show high sensitivity, graded contralaterality, and steep dosage-response characteristics.

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F8 John W. Scott. ORGANIZATION OF OLFACTORY BULB OUTPUT CELLS AND THEIR LOCAL CIRCUIT RELATIONSHIPS. (Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.)

While a topographic representation of the olfactory receptor sheet on the olfactory bulb has been shown in several species, the axons of many mitral/tufted cells branch widely over the olfactory cortex. This apparent degradation of the spatial organization of afferent input may place limitations on the type of information available for central olfactory coding. In spite of this dispersion of olfactory bulb output, there are restrictions on the distribution of individual axons. There is a clear topographic representation of the olfactory bulb on the pars externa of the anterior olfactory nucleus. More caudally, the axons projecting to anterior piriform cortex, posterior piriform cortex and olfactory tubercle arise preferentially from different sectors of the olfactory bulb.

In addition to the issue of sector-by-sector representation in the bulb output, there is a well known laminar arrangement of output cells and a corresponding arrangement of some of their local circuit interneurons. The output cell axons differ in length, ranging from tufted cells with completely intrabulbar axons to tufted cells with axons reaching rostral olfactory cortex and to mitral cells with projections to posterior piriform cortex, entorhinal cortex and amygdala. Several laboratories have shown that mitral and tufted cells exist in several subtypes. Cells of each subtype extend their basal dendrites into one of three sublaminae of the external plexiform layer that can be distinguished by their distinctive staining pattern with cytochrome oxidase procedures. These basal dendrites are contacted by at least two populations of inhibitory granule cells with preferential distributions of synaptic spines in the three sublaminae. No cells with basal dendrites ramifying in the superficial sublamina project to posterior piriform cortex, but it is not yet clear whether the local circuits of the deep and intermediate sublaminae are associated with projections to different parts of olfactory cortex. Alternatively, the granule cell innervations of the deep and intermediate sublaminae may represent functional parallel processing of information transmitted to the same cortical regions.

Determination of any functional differences between these local circuit and projection systems will require more electrophysiology. Stimulation of olfactory nerve shows that cells projecting only to rostral regions are more responsive than mitral cells with posterior projections.

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E9 CORTICAL ORGANIZATION IN GUSTATORY PERCEPTION. Takashi Yamamoto. (Dept. Oral Physiology, Faculty of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565, Japan).

Taste information arising from the taste buds is sent to a confined area in the cerebral cortex (gustatory cortex, GC) in mammals. The GC is located dorsal to the rhinal sulcus in and/or near the insular cortex. Behavioral studies using the conditioned taste aversion technique suggest that the GC plays an important role in cognitive processes of taste sensation.

The response characteristics of GC neurons to taste stimuli applied to the anterior part of the tongue in anesthetized (sodium thiamylal and urethane, i.p.) rats and hamsters were distinct from those of the chorda tympani (CT) fibers, e.g., 1) the mean response rate is smaller, while the mean spontaneous rate is larger in the GC than in the CT, 2) GC neurons are more broadly tuned to the four classical taste stimuli; 3) about 15-30% of GC neurons decrease their firing rates to taste stimulation; and 4) there is no clear initial phasic response in GC neurons.

A relative "chemotopic organization" was detected in the GC in rats such that sucrose responses were most dominant in the anterodorsal region; quinine responses, in the posterior region, and NaCl responses, in the central and ventral regions, while HCl responses were evenly distributed within the GC. An "across-region response pattern" notion, which assumes that taste quality coding involves differences in both the response magnitude across neurons and the spatial localization of those neurons, explained the behavioral categorization of taste quality most successfully among the hypotheses for taste quality coding.

To examine functional specialization among GC neurons, unit activity in the GC was recorded with chronically implanted fine wires during eating and drinking behavior in rats. GC neurons were classified into the four groups according to their response properties: 1) taste-responsive neurons showed excitatory or inhibitory responses to taste stimuli, and were tentatively divided into "taste" and "taste-hedonic" types; 2) excitatory or inhibitory lick responsive neurons increased or decreased their spontaneous firing rates during licking of liquids regardless of their qualities; 3) anticipation type neurons increased their spontaneous rates before the start of licking; and 4) some neurons responded to startle stimulation such as sound, a flash of light, and body touch. These results have shown that several functionally different types of neurons exist in the GC. GC neurons may act to integrate orolingual sensory inputs and control ingestive behavior as well as to discriminate taste quality and intensity.

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S38 OUTWARD CURRENTS IN ISOLATED TASTE RECEPTOR CELLS OF THE MUDPUPPY. S.C. Kinnamon and S.D. Roper. (Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262 and Dept. of Anatomy, Colorado State University, Fort Collins, CO 80523).

In previous studies we have shown that mudpuppy taste receptor cells generate impulses in response to depolarizing current injection (Roper, 1983) and taste stimulation (Kinnamon et al., 1985). The significance of impulses in taste transduction, however, remains unclear. We have recently developed a procedure for obtaining nearly pure populations of isolated taste receptor cells so that the voltage-gated currents underlying the action potential can be studied using patch-clamp techniques.

Taste buds were isolated from the surrounding non-gustatory epithelium by incubating the entire stripped lingual epithelium in collagenase (type 3; 1 mg/ml) for 30 min to 1 hr; the non-gustatory epithelium was then peeled from the underlying connective tissue, thereby isolating the more adhesive taste buds on prominent papillae. The isolated taste buds were then dissociated by a 20 min incubation in trypsin (.5 mg/ml). Isolated taste cells were removed by gentle suction using a fire-polished glass pipette and plated onto concanavalin A-coated glass coverslips.

Whole-cell currents were recorded from isolated taste cells using the whole-cell configuration of the patch-clamp technique. Patch pipettes were filled with the following solution: 140 mM KCl; 2 mM $MgCl_2$; 1 mM $CaCl_2$; 2 mM EGTA; and 10 mM HEPES, pH 7.2. Recordings revealed a large, outward current which activated at -10 to 0 mV; the current reached a peak at 10 to 30 ms and did not inactivate over a time course of at least 10 sec. The outward current was decreased approximately 80% by bath perfusion with 8 mM tetraethylammonium bromide (TEA) or by replacement of KCl in the pipette with $CsCl_2$. These data indicate that this portion of the outward current is carried by K^+. In order to determine if the outward K^+ current is Ca^{++}- as well as voltage-dependent, we recorded currents in the presence of the Ca^{++} channel blocker $CdCl_2$ (5 mM). In some cells, 30-50% of the TEA-blocked current was also blocked by $CdCl_2$. In other cells, however, there was little or no effect of $CdCl_2$ on the outward K^+ current. These data suggest that there are at least two different types of voltage-dependent K^+ channels in taste cells, and that different cells have different proportions of these channel types.

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S39 QUASI-REGENERATIVE RESPONSES TO CHEMICAL STIMULI IN IN VIVO TASTE CELLS OF THE MUDPUPPY. John Teeter (Monell Chemical Senses Center and Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104).

Taste receptor cells have commonly been considered to be electrically inexcitable. Recently, however, action potentials have been recorded from taste bud cells in isolated pieces of mudpuppy lingual epithelium (Roper, 1983, Science 220: 1311) and regenerative anode-break potentials have been reported in frog taste cells (Kashiwayanagi et al., 1983, Am. J. Physiol. 244: C82). Voltage-dependent Na^+ and Ca^{2+} channels were shown to be involved in both types of response. The role of these channels in taste reception, as well as their occurrence in species other than amphibians, however, remain uncertain. Action potentials have not been recorded from in vivo taste cells in any species and experiments were thus conducted to determine if in vivo taste cells in the mudpuppy generate action potentials in response to taste stimuli.

Recordings were made from cells in taste buds on the surface of the tongue of anesthetized mudpuppies using 3 M KCl-filled microelectrodes (resistances of 60-150 MΩ). Taste stimuli were applied at low flow rates (< 10 ml/min) using a sample injection valve and more rapidly using a repeating dispenser or pressure injection. Acids evoked slow potentials that had faster rise times than those produced by salts, even when the salts were applied at higher flow rates. Taste cell responses to high concentrations of acids (0.005-0.01 N) had a distinct phasic component 1-3 sec in duration, which was not observed in surface epithelial cells. Occasionally, acids evoked slow potentials upon which a faster, apparently regenerative, depolarization was superimposed. Repetitive spontaneous depolarizations (< 10 mv in amplitude and 1-10 sec in duration) were also sometimes observed.

The apparently regenerative component of the responses of in vivo taste cells to acid stimulation is consistent with the conclusion that voltage-sensitive channels are normally involved in the generation of taste cell responses, at least to some stimuli. Action potentials comparable to those reported in in vitro mudpuppy taste cells were not observed in vivo, even in cells having resting potentials of -60 mv and input resistances of over 300 MΩ. The slow depolarizations evoked by salts (or slowly applied acids) may result in inactivation of Na^+ channels (and activation of K^+ channels) to a level where a regenerative response cannot develop. The much faster rising depolarizations evoked by concentrated acids may result in significantly less accommodation and, thus, a partially-regenerative response.

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S40 AMILORIDE PRODUCES ACUTE INHIBITION AND CHRONIC SENSITIZATION OF NEURAL TASTE RESPONSES TO SODIUM CHLORIDE. Thomas P. Hettinger and Marion E. Frank. (Dept. of Oral Biology, University of Connecticut Health Center, Farmington, CT 06032).

The effect of amiloride on neural taste responses to sodium chloride in the hamster chorda tympani depends on the order of application of the two substances to the tongue. The greatest suppression by amiloride is observed in responses to amiloride-salt mixtures immediately following tonic responses to salt alone. This suppresson, which can be greater than 90%, is dependent on the concentrations of both sodium chloride and amiloride. With 0.1 M NaCl, the apparent dissociation constant for amiloride has been estimated to be about 1×10^{-6} M and depends on sodium chloride concentration in a manner suggestive of competitive inhibition. Application of amiloride-sodium chloride mixtures to the tongue following water adaptation produces a phasic response practically the same as for NaCl alone, but the tonic response a few seconds later is nearly as diminished as in the above protocol. When amiloride is applied prior to the mixtures both the phasic and tonic levels are reduced compared to those in the absence of amiloride, but the suppression of the tonic level is not as great as in the other two cases. The longer the exposure to amiloride the less is the observed suppressive effect. Thus, amiloride causes both a rapid inhibitory response that is complete in about 2 seconds and a slower opposing sensitization that develops during 1 minute following application of amiloride. These results are wholly consistent with the idea that salt taste is mediated by receptor stimulation via amiloride-sensitive sodium channels. Inhibition is caused by nearly instantaneous blocking of sodium channels, resulting in decreased Na conductance, while sensitization can result from chronic decreased Na entry into receptor cells, so that the membrane potential is poised at a higher level of sensitivity. The overall neural response to amiloride is usually one of suppression, but depends on opposing effects of conductance and voltage changes in the receptor cells.

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S41 EFFECT OF AMILORIDE ON IONTOPHORETIC AND CHEMICAL STIMULATION IN HAMSTER AND FROG - NEURAL AND INTRACELLULAR EVIDENCE. M. Scott Herness (Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021)

This investigation 1) demonstrates the action of amiloride on taste responses in two new species, the hamster and the frog, and 2) tests the hypothesis that its action on iontophoretic application of taste stimuli parallels its action on bulk flow (referred to as 'chemical') delivery. Studies on the iontophoretic application of taste stimuli to gustatory receptors, so called "electric taste", have implied commonality of transduction processes to normal bulk flow delivery (Pfaffmann & Pritchard, 1980; Herness, 1985). Recently, the diuretic amiloride has been shown to inhibit a transduction pathway specific to sodium and lithium stimuli in rats.

Amiloride treatment (4 minutes of 0.0001 M) of the hamster's tongue effectively inhibited NaCl and LiCl chorda tympani responses. Although chemical (0.1 M) application of NaCl produced a larger response than iontophoretic application (+7 uA through 0.001 M NaCl), immediately after amiloride, both responses fell to the same residual response magnitude. Recovery then proceeded along two distinct curves asymptotically returning to pre-treatment response levels. Amiloride inhibition of LiCl by iontophoretic and chemical stimulations behaved identically to NaCl. KCl responses were only slightly inhibited for either method of stimulus delivery and recovered to enhanced response levels. No decrement in response level was observed for sucrose (0.5 M) or saccharin (-9 uA through 0.001 M NaSaccharin). HCl responses (0.01 M or +8 uA through 0.00063 M HCl) gave small and very transient inhibitions. Potassium picrate (0.01 M or -9 uA through 0.001 M) showed no inhibition following amiloride. In every instance the action of amiloride on iontophoretic stimulation parallelled its action on chemical stimulation.

Amiloride treatment of the frog tongue, unlike the hamster, showed little sodium specificity. Chemical or iontophoretic application of NaCl or KCl were inhibited equally in glossopharnygeal nerve recordings. Intracellular records indicate that amiloride treatment reduces the input resistance of the taste receptor cell. Receptor potentials to 0.4 M NaCl and 0.4 M KCl were both smaller after treatment but still accompanied by a conductance increase. Amiloride itself hyperpolarizes the cell and increases its input resistance.

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S42 EFFECT OF INJECTION OF CALCIUM, EGTA AND CYCLIC NUCLEOTIDES INTO THE TASTE CELL OF MOUSE. Keiichi Tonosaki and Masaya Funakoshi. (Dept. of Oral Physiology, Sch. of Dentistry, Asahi University, Gifu 501-02 Japan)

Previous our studies (Tonosaki & Funakoshi, 1984,1985) have shown that the sucrose depolarization response is not simply generated by the membrane depolarization and/or the response does not depend entirely on the membrane resistance change. The sequence of events that couples stimulus-receptor interactions at the apical membrane of the taste cell must controll the amplitude of receptor potential and the modulation of neurotransmitter release at synapses on the baso-lateral membrane of the taste cell. It is suggested that there are some complex intracellular taste transduction mechanisms in the taste cell. Recent experiments have implicated intracellular messengers in controlling ion channels in membranes of a variety of cells. Calcium ions and cyclic nucleotides can all act on the inner surface of the membrane to alter ion channel activity. On these basis, calcium and cyclic nucleotides might be postulated as a candidate for an intracellular transmitter in taste cell. In order to examine the possibility what chemical is an internal transmitter in the process of taste transduction, we attempted to alter the internal ion concentration of taste cell by intracellular injections of some chemicals. Calcium ion, EGTA, c-AMP, c-GMP, sodium ion or potassium ion was iontophoretically injected into the mouse taste cell from one barrel of a double-barreled microelectrode, while the other barrel was monitoring membrane potential and resistance changes. The experiments presented in this paper descrived the evidence about H-type taste cells. Intracellular injected calcium ions induced hyperpolarization accompanied by decrease of membrane resistance. While, intracellular injected EGTA, c-AMP and c-GMP induced depolarization accompanied by increase of membrane resistance. The depolarization caused by c-AMP injection was always smaller than that by c-GMP. The effect of potassium or sodium ions was not clear. The results suggest that calcium ions and c-GMP are involved in the taste transduction process in the mouse taste cells.

S43 IMMUNOREACTIVITY TO NEURONAL GROWTH-DEPENDENT MEMBRANE GLYCOPROTEIN OCCURS IN A SUBSET OF TASTE RECEPTOR CELLS IN RAT TASTE BUDS. Thomas E. Finger[1], Hema Sridhar[1], Mary Womble[1], Var L. St. Jeor[2] and John C. Kinnamon[2]. ([1]Dept. of Anatomy, Univ. Colorado Medical School, Denver, CO 80262 and [2]Dept. Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder CO 80309).

The monoclonal antibody 5B4 (Ellis, et al., 1985; J Cell Biol: 101) is directed against a neuronal cell adhesion molecule (N-CAM)- like membrane glycoprotein which is expressed preferentially in developing neuronal systems. This monoclonal antibody reacts little with the mature nervous system except for the glomeruli of the olfactory bulb which contain growing olfactory nerve elements. Since taste buds comprise neuron-like epithelial cells that undergo continual histogenesis, the 5B4 antibody was applied to an immunocytochemical analysis of this system.

Rats were fixed by means of perfusion with a solution of 2% paraformaldehyde and 0.05% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) to which was added an additional 15% v/v of saturated picric acid solution. The tongue was removed and placed in fresh fixative for an additional 3 hours. The tissue then was washed in pH 7.2 buffer and cryprotected. After sectioning at 40-80 um on a sliding freezing microtome, the tissue was treated for 30 min. in a solution of 1% sodium borohydride in phosphate buffer. Following several changes of buffer, the tissue was transferred to 1% normal goat serum in phosphate buffer for 1 hour at room temperature. The tissue sections then were exposed overnight at 4° C to a 1:10 dilution of antibody 5B4 in phosphate buffer. Following several rinses in buffer, the antibody was localized with standard peroxidase-antiperoxidase (PAP), or with avidin-biotin complex (ABC) methods. For control tissue, the 5B4 monoclonal antibody was replaced by normal mouse serum, or by a different monoclonal antibody, this directed against enkephalin. Neither control preparation exhibited the specific immunoreactivity described below.

Taste buds from all three lingual taste fields exhibit a similar pattern of positive immunostaining to antibody 5B4. Virtually all taste buds contain some immunoreactive taste cells. Different cells within a given taste bud, however, show varying degrees of immunoreactivity. The entire surface of each positive cell is immunoreactive, from the apical pore to the basal region. The 5B4 immunoreactivity is clearly membrane associated; the staining appears to surround a relatively nonreactive cytoplasm. Preliminary electron microscopic observations confirm the membrane localization of the antigen and indicate that the immunoreactive population includes intermediate and light cells.

(Supported by NIH grants to T.E.F.)

S44 The Interaction of Generator Current and Voltage Gated Currents in the Olfactory Receptor Response. Stuart Firestein and Frank Werblin, Neurobiology Group, University of California, Berkeley, California, 94720.

We have attempted to measure and analyze the interaction between the generator potential and the initiation of spike activity in olfactory receptor cells. Currents were measured under whole cell patch clamp in enzymatically isolated cells.

Chemical stimuli consisting of a mixture of known odorants were delivered through a nearby 2 micron pipette by pressure injection. Odorants eliciting a generator current of only 6 pA depolarized the cell from the resting level of -65 mV to threshold near -50 mV where spiking was initiated. The response current increased more than 30 times to 200 pA with higher doses of odorant. The generator current reversed near +30 mV.

Analysis of the electrical properties of the soma reveals mechanisms which serve to sharpen the generator signal, provide high sensitivity and shape the spike output. These include a voltage gated transient inward current carried by sodium ions, several outward currents carried by potassium ions and gated by both voltage and calcium influx, all operating through a very high input resistance in the 2-3 Gohm range.

Taken together these data show that the high sensitivity and slow adaptation commonly ascribed to olfactory receptors can be understood in terms of the gated conductances in the ciliary and soma membranes.

S45 MEMBRANE CONDUCTANCE MECHANISMS IN DISSOCIATED CELLS FROM THE NECTURUS OLFACTORY EPITHELIUM. Vincent E. Dionne. (Division of Pharmacology, Department of Medicine, University of California, San Diego CA 92093).

Olfactory receptor neurons and associated non-neuronal cells were dissociated from the olfactory epithelium of adult Necturus and studied using whole-cell and patch-clamp methods. Single cells were isolated by incubation at room temperature in zero-calcium amphibian saline with Trypsin (0.5 mg/ml, 30 min) followed by a Ca-containing saline with collagenase (2 mg/ml) and bovine serum albumin (1 mg/ml). Isolated cells retained their morphology, and on this basis six types of cells were distinguished, only one of which was neuronal. Whole-cell membrane currents were recorded from olfactory neurons and from a non-neuronal cell type that was spherical and partially covered with asynchronous, motile cilia ("Medusa" cells); columnar support cells did not survive the dissociation well and were not studied. The recording electrode contained 120 mM KCl and 0.1 μM free Ca buffered with BAPTA; pH was 7.2, HEPES buffer.

Delayed, sustained outward membrane currents (presumably potassium) were elicited from Medusa cells by depolarizing voltage pulses. The currents had the kinetics and voltage dependence of a delayed rectifier. No transient, voltage-activated currents were observed in Medusa cells.

Whole-cell records from olfactory receptor neurons exhibited both inward and outward currents in response to depolarizing voltage steps from a holding potential of -70 mV. The inward current, which had a rapid onset and was transient, appeared to be a Na-current based upon its voltage thresholds for activation and inactivation and its kinetics. The outward current closely resembled the delayed rectifier potassium current seen in Medusa cells. The density of both the inward and outward currents declined with time after establishing the whole-cell configuration. These voltage-activated whole-cell currents did not appear to change in response to the addition to the bath of a cocktail of twelve amino-acids (1-5 μM Arg, Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, Val). Single channel current records from on-cell membrane patches of neuron somas contained at least three different kinds of potassium-selective channels with conductances similar to those seen in murine olfactory neurons [R.A. Maue and V.E. Dionne, Biophys. J. 45 (1984) 266a]. Not all of these channel types were reflected by the whole-cell currents.

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S46 **VOLTAGE-DEPENDENT IONIC CURRENTS IN ISOLATED OLFACTORY RECEPTOR CELLS.** Noriyo Suzuki (Zoological Institute, Faculty of Science, Hokkaido University, Sapporo 060, Japan).

Olfactory receptor cells were isolated from bullfrog olfactory mucosa by enzyme(Dispase I) and EDTA treatments. Their electrical properties were studied using a giga-seal technique(Hamill et al.,1981). Isolated receptor cells had a high morphological integrity and were easily identifiable in the heterogenous mixture of different cell types by their motile cilia on the olfactory vesicles, thin dendrites(12-20 um length; 2-3 um dia.), and oblate sphere-shaped somata(10-12 um of long axis;7-8 um of short axis). Receptor cells, which have a short axon extending from the soma terminal, were occasionally found. In the cell-attached configuration with a patch pipette positioned onto the center of soma, current wave forms due to intracellular spontaneous action potentials could be frequently observed. The amplitude and frequency of these action potentials altered by changing the pipette potential from the bath potental level to a more positive or negative level. After rupture of the patch of membrane (whole-cell recording configuration) the pipette-cell seal remained stable for more than 10 minutes. The resting potental measured at a zero-current level in normal Ringer solution was -33 to -39 mV. The input resistance and input capacitance was around 6 G ohm and 2.5 to 5.0 pF, respectively. The current clamp experiment showed that the receptor cell gave a single action potential in response to a short depolarizing current and a single anode break action potential at the cessation of a short hyperpolarizing current. The voltage clamp experiment in the whole cell recording configuration showed that the membrane currents of receptor cells associated with positive voltage pulses in normal Ringer solution were characterized by an initial inward current followed by a slowly developing outward current. The currents were tentatively identified as inward Na^+ current and outward delayed-rectifier K^+ current. The magnitude of the Na^+ current was quite variable between the receptor cells, and frequently cells had no Na^+ current. This variation of Na^+ current may be due to the difference in maturation of the receptor cells in the olfactory mucosa.

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S47 CHANGES IN EXCITABLE PROPERTIES OF OLFACTORY RECEPTOR NEURONS ASSOCIATED WITH NERVE REGENERATION. Leona Masukawa, Britta Hedlund, and Gordon Shepherd. (Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510).

Action potential generating properties of olfactory receptor neurons in the olfactory epithelium of the salamander, Ambystoma tigrinum, were studied in normal control animals, and two and four weeks after olfactory nerve transection. The threshold for impulse generation in response to injected current was extremely low. It increased significantly after transection, from 74±46 pA in control epithelium to 176±41 pA two weeks and 254±80 pA four weeks after olfactory nerve transection. The discharge frequencies of the receptor neurons were exquisitely sensitive to small increments of injected current. After nerve transection this sensitivity decreased. Some cells showed a secondary range in their frequency response to larger injected currents. The changes following transection appear to be associated with an increased potassium conductance, suggested by prominent membrane rectification and greatly reduced amplitudes of membrane action potentials in the spike trains. The changes may reflect the role of potassium conductances in neural mitogenesis following the nerve transections. The olfactory receptor neuron appears to be a favorable model for exploring these properties.

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S48 CYCLIC-AMP MODULATES THE ELECTRICAL PROPERTIES OF OLFACTORY RECEPTOR SITES FUNCTIONALLY RECONSTITUTED INTO BIMOLECULAR LIPID MEMBRANE (BLM). Vitaly Vodyanoy and Igor Vodyanoy, (Department of Physiology & Biophysics, University of California, Irvine, CA 92717).

Functionally reconstituted chemosensitive ion channels from olfactory epithelium of the rat were used to study the molecular mechanism of the ion transport associated with olfaction. We used two techniques to transfer the native membrane macromolecules into a model system: (1) Chemosensitive membrane fragments were incorporated into BLM. (2) The vesicle which contained chemosensitive membrane fragments were attached to the planar BLM and the proton carrier SF6837 was added to the membrane bathing solutions.

We have analyzed a model system for the initial chemosensory events in the mammalian olfactory epithelium, based on the functional reconstitution of membrane proteins from olfactory bipolar receptor cell cilia into the artificial planar BLM. These artificial membranes demonstrate a chemosensitive response to nanomolar concentrations of the odorant diethyl sulfide, measured in terms of current flow at constant voltage. Other molecules which are less odorous, such as (+) and (-) carvone, also appear to activate a similar current flow across these membranes. This current flow appears to have a single ion channel basis, and we have further demonstrated that the carrier of charge for these chemosensitive channels is the potassium ion (K^+). Chemosensitivity is manifested as a change in the mean open time of single channel events in response to odorants presence in the bathing media.

We have utilized as a control analogous homogenates from respiratory epithelium, which appear to lack such chemosensitive channels, or at least from which they can not be reconstituted. It would appear that these channels are specific to the olfactory epithelium.

We have demonstrated that 3',5'cyclic-AMP initiates a slow electrical response of the BLM modified with rat olfactory epithelium homogenate. This response is dose dependent. We have observed a linear correlation between the stationary level of the membrane conductance by the 3',5'cyclic-AMP and logarithm of its concentration in the range of 2-200 uM. On the other hand 2',3'cyclic-AMP appears to be ineffective in the same concentration range.

We hypothesize that these channels may be involved in olfactory transduction, because of their localization in olfactory epithelium, and their specific responses to odorants and cyclic-AMP.

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S49 THE CODING OF PHEROMONAL INFORMATION BY "OUTPUT" NEURONS OF THE ANTENNAL LOBES OF THE SPHINX MOTH *MANDUCA SEXTA*. Thomas A. Christensen and John G. Hildebrand. (Arizona Research Laboratories, Div. of Neurobiology, University of Arizona, Tucson, AZ 85721).

The primary mechanism by which Lepidoptera such as the sphingiid *Manduca sexta* locate conspecific mates is intersexual attraction by species-specific blends of sex pheromones. The antennae of male *Manduca* bear sexually dimorphic trichoid sensilla, each innervated by a pair of specialized olfactory receptor cells. Each cell is selectively responsive to only one of the major components of the female's pheromone blend (Hildebrand & Kaissling, unpublished). We are now probing the antennal lobes (ALs) in the brain to learn how the primary-afferent information carried by these sensory cells is "encoded" by interneurons associated with the AL -- the first-order olfactory center in the CNS. With intracellular recording and dye-marking techniques, we have discovered that the principal, male-specific AL projection neurons exhibit several different physiological classes whose responses represent important features of the pheromonal stimulus. These "output" neurons have dendritic arborizations confined to the male-specific macroglomerular complex (MGC) in the AL, and their axons project to higher centers in the protocerebrum. We have recorded 3 distinct kinds of responses in these cells, 2 of which have been evoked by stimulation of the antenna with female pheromones. Cells of the first physiological class of output neurons (ONs) are purely excited by the natural pheromone blend an by the component pheromones. About 50% of these cells were selective for only one pheromone and showed a clear dose-dependancy. Several of these neurons also displayed restricted arborizations in the MGC. The other half of the neurons of this class respond equally to both principal pheromones in the female's blend. One function of these neurons may be to signal general pheromone-triggered arousal to higher-order CNS regions. Intracellular staining revealed that the axons of these cells project to the calyces of the mushroom bodies as well as to the lateral protocerebrum. The second physiological class of ONs exhibited more complex poly-synaptic relationships with other AL neurons. These cells were characteristically excited by one pheromone and inhibitied by the other, and they gave a mixed response to the natural pheromone blend. These neurons integrate information from the 2 pheromonal receptor pathways, and their mixed response suggests that these cells function as "blend detectors" at this level of the olfactory pathway. A third physiological class was revealed by electrical stimulation of the olfactory sensory fibers. A single suprathreshold shock to the antennal nerve evoked tonic inhibition in these AL ONs. We are now using simultaneous recording from 2 AL neurons to examine synaptic interactions (particularly the role of inhibition) in the ALs and to learn more about integration of pheromonal information in the AL.

S50 **ODOR INFORMATION PROCESSING IN THE OLFACTORY BULB: EVIDENCE FROM INTRACELLULAR RECORDING AND 2-DEOXYGLUCOSE (2DG) AUTORADIOGRAPHY.** J. S. Kauer and K. A. Hamilton. (Depts. of Neurosurgery, Anatomy and Cell Biology, Tufts-N.E.M.C., Boston MA 02111).

Intracellular recording from individual, salamander mitral/tufted (M/T) cells (see Hamilton and Kauer, Brain Res. 338:181, 1985) has shown that hyperpolarization is the most commonly seen first event in response to square pulse odorant stimulation at moderate concentrations. Categorization of the responses into 'E' and 'S' types previously seen in extracellular recordings, can be made on the basis of differences in latencies of depolarization and impulse activity and on whether excitation precedes or follows hyperpolarization after stimulation at higher odorant concentrations. With high intensity stimulation, 'E' responses are characterized by a short latency (60-90 msec) depolarization and burst of spikes followed by hyperpolarization and 'S' responses are characterized by a longer latency (230-450 msec) depolarization and spike burst preceded by hyperpolarization (see Hamilton and Kauer, this meeting). Pooled extracellular data from many animals show that these M/T response types can be elicited by any of the odorants tested, suggesting that the ensemble activity triggered by a pulse of any one odorant contains all types of response, elicited at the same time.

The coordinated activity of all the M/T cells in the bulb cannot be simultaneously examined using conventional electrophysiological methods. Therefore, to examine the ensemble response to odor stimulation, we have used enhanced resolution 2DG autoradiography. By superimposing the 2DG autoradiograph on its histological section, precise comparisons between functional activity and bulbar cell laminae have been made. Using this method, we have found that, at moderate concentrations, the highest levels of odor-induced 2DG uptake in glomerular foci occur over periglomerular (PG) cell somata. We hypothesize that the activity observed in the PG cell population using 2DG may be the source of the hyperpolarization which we have recorded preceding the onset of spikes in M/T cells.

Supported by PHS grants NS-20003 to JSK and NS-22035 to KAH.

S51 **LATENCIES OF SYNAPTIC POTENTIALS IN ODOR RESPONSES OF SALAMANDER MITRAL/TUFTED CELLS.** K. A. Hamilton and J. S. Kauer. (Depts. of Neurosurgery, Anatomy and Cell Biol., Tufts-New England Med. Ctr., Boston MA 02111).

By intracellularly recording the activity of single mitral (or tufted) cells in the olfactory bulb of the tiger salamander, we have shown that complex synaptic potentials coincide with patterns of spike activity which are observed in responses to odor stimulation (Hamilton and Kauer, Brain Research, 338, 181). In order to examine the timing of excitatory and inhibitory inputs, we have analyzed the latencies of the earliest depolarizing and hyperpolarizing components of the potentials for a group of cells (five to date) that displayed a remarkably similar repertoire of responses to odor stimulation.

In these cells, one extreme of the odor-response repertoire was elicited by low-to-moderate concentrations of odorants. These responses contained hyperpolarizing components which had latencies ranging from 550-920 msec. Any depolarizing components which occurred followed the onset of hyperpolarization by >180 msec. Most of these responses could not be classified as either E- or S-types on the basis of spike patterns. The other extreme of the odor-response repertoire consisted of E-type responses that were elicited by high odorant concentrations. In these responses, a depolarizing component always occurred, with latencies ranging from 60-90 msec, and it preceded the hyperpolarizing component, which had latencies ranging from 120-160 msec. The latencies of depolarizing components were 160-380 msec shorter in E-type responses than in S-type responses to high odorant concentrations, and 50-70 msec shorter in responses to supramaximal electrical stimulation of the olfactory nerve and tract than in E-type responses which were qualitatively similar.

This analysis of latencies, together with data obtained using other methods (see Kauer and Hamilton) provides a means for understanding how different spike patterns are generated through the differential activation of synapses in the olfactory bulb.

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S52 RESPONSES OF OLFACTORY BULB NEURONS TO SPATIALLY-PATTERNED ELECTRICAL STIMULATION OF THE NASAL MUCOSA. Tao Jiang and André Holley. (Lab. Physiol. Neurosensorielle, Université Claude-Bernard, 69622 Villeurbanne cedex, France).

The second order neurons of the olfactory system receive highly convergent projections from receptor cells. This convergence plays an important role in the processing of peripheral information. Whereas many studies have explored time-dependent integrative properties of olfactory bulb (OB) neurons, very few have been made on the spatial aspects of input integration. Our experiments were carried out in order to investigate these aspects. In curarised frogs, a set of 9 stimulation electrodes, regularly arranged, were positioned on the ventral part of the olfactory epithelium (OE). A D-C generator delivered 4-8-sec-pulses, including progressive onset and termination, through one or several electrodes under constant-current or constant-voltage conditions. Positive focal stimulations were shown to evoke phasic-tonic impulse discharges from receptor cells located under the electrodes. We studied the effects of these discharges on neurons recorded in 6 subdivisions of the OB. In a majority of these neurons, the spontaneous activity was affected. Excitation was more frequently observed than inhibition. There was a clear relationship between the impulse frequency and the slope of the stimulation onset. In most cases, responses could be evoked from several of the 9 OE-sites, indicating that OB neurons have relatively large receptor fields. Generally, all electrodes which influenced the activity of a certain OB neuron induced responses of a same category (excitation or inhibition). When several sites were activated in combination, different degrees of summation and suppression of their individual effects were observed. An attempt is made to correlate the integrative properties of OB neurons with their location and morphology using intracellular injection of dye.

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S53 **STIMULATING THE DIAGONAL BAND FOR 10 SECONDS AT 10 HZ CAUSES THE OLFACTORY BULB TO GO CRAZY.** *W.T. Nickell and M.T. Shipley.* (Dept. of Anatomy and Cell Biology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267).

Anatomical studies have demonstrated an extensive, largely cholinergic projection from the nucleus of the horizontal limb of the diagonal band (DB) to the olfactory bulb (OB). We have investigated the functional characteristics of this system by stimulating the region of the DB while recording from the OB in anaesthetized rats.

With the recording electrode in the superficial part of the granule cell layer (GCL), single DB shocks produce a biphasic, positive-negative potential. For small numbers of stimulus repetitions this potential shows moderate facilitation. However, stimulation of DB for 10 or more seconds at 10 Hz causes dramatic changes in OB. After a few seconds of DB stimulation, there is a brief period of inhibition of the potential followed, abruptly, by a dramatic, maintained, increase in amplitude of the DB potential. Although both phases of the potential are increased, the negative phase increases so much in magnitude and time-course as to suggest the initiation of a new process. If DB stimulation is continued for several seconds after onset of the potentiation and then terminated, the bulb is left in a potentiated state in which potentials resembling the negative phase of the DB potential often occur spontaneously. This state persists for 5-10 seconds following termination of stimulation, and can be maintained for longer periods by stimulation at low frequency. The spontaneous activity of mitral cells is completely inhibited during the period of potentiation. This inhibition is mimicked by application of a cholinergic agonist and is probably mediated by interneurons.

These results demonstrate that stimulation of the presumably cholinergic input from DB at physiological frequencies causes profound effects on olfactory bulb circuitry. Such frequencies probably occur during active sniffing. Hence, the DB_e input may significantly modify the response of mitral cells to odors.

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S54 HOW THE OLFACTORY SYSTEM GENERATES ITS INTRINSIC BACKGROUND "SPONTANEOUS" EEG AND UNIT ACTIVITY.

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The basal EEGs of the olfactory bulb (OB), anterior olfactory nucleus (AON) and prepyriform cortex (PC) fluctuate continually and erratically. Their autocorrelation functions approach zero with time, showing their unpredictability. Their power spectra are broad with multiple low peaks. Their amplitude histograms are nearly Gaussian. Trains of units from single cells show interval histograms in the form of a Poisson distribution with a refractory period. Yet the activity of each structure is spatially coherent and has the low dimension of chaos.

A model in nonlinear differential equations of each structure, that suffices to simulate averaged evoked potentials, poststimulus time histograms and odor-induced burst, fails to generate chaotic activity. A model consisting of OB, AON and PC models coupled with negative feedback suffices to do so. Two requirements are for distributed delays corresponding to the conduction velocities and distances of the medial olfactory tract, and an ex-citatory bias of periglomerular neurons auto mitral-tufted cells, that is subject to AON control. While each structure in isolation is stable, when coupled they drift into oscillations. These are augmented by AON feedback to the periglomerular cells and quenched by AON and PC feedback to the bulbar granule cells. The outcome is the steady chaotic unit and EEG activity of all three structures, subject to centrifugal modulation in respect to motivation.

NOTES

S55 CONVERGENCE OF OLFACTORY AND VOMERONASAL PATHWAYS IN THE PMCN OF THE HAMSTER AMYGDALA. Gary Licht and Michael Meredith. (Department of Biological Sciences, Florida State University, Tallahassee, FL 32306).

Chemoreceptor pathways from the vomeronasal organ (VNO) and main olfactory system (OLF) project separately to different areas of the cortical amygdala. The VNO pathway projects to the "vomeronasal amygdala" (posteromedial cortical nucleus, PMCN and medial nucleus, MN). The OLF pathways project to the "olfactory amygdala" [posterolateral cortical nucleus (PLCN), and anterior cortical nucleus (ACN)]. The OLF amygdala has further projections to the VN amygdala so there is anatomical evidence for convergence of the OLF and VNO pathways in the PMCN and MN but it has not been shown previously whether the two systems can activate the same neurons.

To investigate convergence, we have recorded field potentials and single units in the hamster amygdala while electrically stimulating the main olfactory bulbs and vomeronasal organs or VN nerves. Twisted wire bipolar electrodes were placed in each VNO and anterolateral portion of each MOB insuring that the current from stimulation in the VNO/AOB system had no stimulatory effect on the MOB. VN elicited field potentials were recorded to determine the stereotaxic coordinates of the VN active zone in the medial-cortical nuclei. Microelectrodes were then used to record single units in that zone. Out of 351 units recorded, 121 were driven at constant latency by stimulation of one or both systems. 12 units were clearly driven at (different) constant latencies by both systems; 75 units were driven only by VN stimulation; 34 units were driven only by OLF stimulation. In addition, 7 units were apparently inhibited by one of the systems. Dye marks, lesions, and visual reconstruction of the electrode tracks in serial histological sections were used to verify the recording sites. All of the units listed above were recorded from the PMCN (few electrode tracks passed through the MN and no MN units were driven by VN stimulation). We conclude that main olfactory and vomeronasal inputs can converge on single neurons in the PMCN of the hamster amygdala.

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S56 GUSTATORY STIMULUS PROCESSING IN THE SOLITARY NUCLEUS OF THE HAMSTER. Martha McPheeters and Marion E. Frank. (Dept. of Oral Biology, University of Connecticut Health Center, Farmington, CT 06032).

Chorda tympani and trigeminal-lingual afferents, which identify gustatory and general sensory stimuli on the anterior tongue, distribute differentially to the medullary solitary nucleus (NTS), the first site of neural processing in the mammalian gustatory system. Neurophysiological experiments have discovered taste responses in the lateral part of the rostral pole of the NTS. We are studying the response properties of cells in all subdivisions of NTS, which receive anterior lingual afferents, to taste and general sensory stimuli.

Platinum/iridium microelectrodes (0.5-2.0 megohms) are used to isolate single units while the anterior tongue is sequentially stimulated with test solutions that independently activate chorda tympani and lingual-trigeminal neurons when applied through a flow chamber isolating the anterior lingual receptive field. Sucrose (0.1 M), KCl (0.1 M), and NaCl (0.03 M), which neither affect the lingual nerve nor alter chorda tympani sensibilities, optimally activate different chemosensory systems of the chorda tympani. Capsaicin (10 ppm) is a behaviorally detectable trigeminal irritant that does not acutely affect responses of chorda tympani neurons, nor does it affect their subsequent sensibilities. Solutions that are cooler (-10°C) or warmer (+10°C) than the tongue evoke in thermally sensitive trigeminal afferents a dramatic characteristic phasic-tonic response; chorda tympani neurons are not so affected. Such test solutions allow us to identify input to a recorded cell from specified gustatory and general sensory modalities. NTS neural activity from exactly located sites is recorded on a videocassette tape and computer-analyzed. Small electrolytic lesions are made near recording areas; the lesions, histologically verified, are used to make three dimensional reconstructions of sites of neural activity. The recorded location, its cytoarchitecture, neurophysiological activity, and afferent sources allow us to establish structure-function relations.

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S58 GUSTATORY ACTIVITY IN THE NTS OF CHRONIC DECEREBRATE RATS. Gregory P. Mark and Thomas R. Scott. (Dept. of Psychology and Institute of Neuroscience, University of Delaware, Newark, DE 19716, USA).

Decerebrate rats retain certain capacities to regulate consumption and lose others. Since ingestion is largely guided by the sense of taste, it is of interest to determine the neural character of the taste system in these animals. Moreover, cells in the neucleus tractus solitarius (NTS) project reciprocally to diencephalic and telencephalic neurons, and the loss of this communication through decerebration could modify NTS taste responses. We recorded the activity of individual NTS cells in chronic decerebrate rats. Supracollicular decerebrations were performed in two unilateral stages, separated by one week. Animals were then permitted at least one week to recover from acute surgical effects before recording took place. Each subject was anesthetized with barbiturate during surgery, then wound and pressure points were liberally infused with local anesthetic and a neuromuscular blocking agent was administered i.p. A minimum of three hours was permitted for the barbiturate to be cleared before recording began. Micropipettes (Z=3-8 Mohm) were used to isolate a total of 50 single cells in NTS. Stimuli were 12 salts, acids, alkaloids and sugars typically used to evoke taste activity. Mean spontaneous rate was 6.3±5.6 spikes/sec, similar to that recorded from intact rats. Sensitivity was typically broad, with a mean breadth-of-tuning coefficient of 0.80 (intact rat = 0.77). Most neural response profiles could be grouped into four clusters, characterized by sensitivity to NaCl (26%), HCl (18%), NaCl-HCl (20%) and sucrose (22%). The clearest distinction was between sweet and non-sweet oriented profiles, as it is in intact rats. Stimuli, placed in a multidimensional space, assumed a rather typical arrangement with sugars and alkaloids widely separated and salts and acids between them. Sodium-lithium salts, however, were situated closer to sweet stimuli and farther from sour and bitter chemicals than is normally the case in intact rats. Overall, responses of NTS taste neurons in the chronic decerebrate were nearly indistinguishable from those of the normal rat.

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S57 A SYNOPSIS OF THE INFLUENCE OF SATIETY FACTORS ON TASTE ACTIVITY. Thomas R. Scott and Barbara K. Giza. (Dept. of Psychology and Institute of Neuroscience, University of Delaware, Newark, DE 19716, USA).

One mechanism by which factors which modify food intake may operate is by altering taste-evoked activity. Gastric distension, for example, influences taste responses at the receptor level, in peripheral nerves and in the CNS. We have studied the effects of systemically administered chemical factors--glucose, insulin and cholecystokinin--on multiunit taste-evoked activity in the rat's nucleus tractus solitarius (NTS). Intravenous injections of 0.5 g/kg glucose resulted in extreme hyperglycemia and as much as a 43% reduction in neural responsiveness to glucose. Taste activity evoked by NaCl and HCl was affected to a lesser degree and quinine sensitivity was unmodified. The implication of this finding, that glucose should be perceived as less intense when systemic glucose availability is high, was tested in a behavioral experiment. Rats with conditioned taste aversions to 1.0 M glucose were tested for the degree to which acceptance of a wide range of glucose concentrations was suppressed under conditions of normal or of high systemic glucose availability. With hyperglycemia, rats reacted to all glucose concentrations from 0.6-2.0 M as controls did to significantly lower concentrations. Thus in the rat, gustatory evoked activity and the perceptions arising from it may be influenced by glucose availability. Accompanying the administered glucose load is a massive endogenous insulin release. Insulin also induces satiety when injected in physiological doses, perhaps by increasing glucose availability to the body's tissues. Therefore, we next studied its effects on taste activity. Intravenous injections of 0.5 U/kg regular insulin caused mild hypoglycemia, insufficient to induce glucoprivic feeding, and a transient reduction of up to 33% in NTS responsiveness to glucose. Responses to the other basic stimuli were unaffected. We then extended this line of inquiry to include cholecystokinin. An intravenous load of 2.0 µg/kg, sufficient to induce a 50% reduction in feeding, had no discernible effect on taste-evoked activity in the NTS. Therefore some (glucose, insulin), but not all (CCK) satiety factors may operate partly by decreasing sensitivity to the appetitive components of foods, thereby reducing the hedonic value of feeding and promoting termination of a meal.

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S59 OFF RESPONSES TO GUSTATORY STIMULI IN THE PARABRACHIAL PONS OF DECEREBRATE RATS. Patricia M. DiLorenzo. (Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901).

Single unit responses to gustatory stimuli in the parabrachial nucleus of the pons (PbN) were recorded in decerebrate rats under flaxedil. Sapid solutions of NaCl (.1M), HCl (.01M), Sucrose (.5M), NaSaccharin (.004M) and Quinine HCl (.01M) were individually bathed over the tongue followed by a 20 sec rinse of distilled water. Gustatory responses from 32 parabrachial units in 13 decerebrate rats were recorded.

The most striking result of this study was the observation of OFF responses in a subset of taste-responsive parabrachial units. These responses took the form of a brief (1-2 sec) increase in the firing rate that occurred immediately following the cessation of the stimulus presentation. This increase most often exceeded the magnitude of the initial transient portion of the taste response and was superimposed on the steady state firing rate that occurred during the stimulus presentation. Six out of 21 OFF responses occurred in the absence of a response to the stimulus. OFF responses were recorded in 12 (38%) units to at least one stimulus. All 4 taste qualities were capable of producing OFF responses in these units: NaCl produced OFF responses in 5 units, HCl in 7 units, Sucrose and NaSaccharin in 6 units and QHCl in 3 units. However, within a given unit, OFF responses were most often selective to a subset of taste qualities: 6 units showed OFF response to only 1 taste quality, 4 units to 2 taste qualities, 1 unit to 3 taste qualities and 1 unit to all 4 taste qualities. No relationship was found between the presence of OFF responses and the best-stimulus categorization of a particular unit.

In other respects, taste units in the PbN of decerbrate rats were similar to those in intact rats. Analysis of response profiles across stimuli showed that PbN units in the decerbrate rat were broadly responsive. Comparison of PbN taste units in decerebrate vs. intact rats suggested that these units may be more narrowly tuned in decerebrate rats.

One possible implication of these results is that the suppression of OFF responses to taste stimuli in the intact animal by the forebrain may be important for the acquisition of conditioned taste aversions, particularly when the CS-US interval is long. In effect the absence of a signal for the offset of a gustatory stimulus may enable the animal to form associations that bridge relatively long intervals of time. Conversely, the presence of OFF responses in the PbN of decerebrate rats may represent an explanation for the fact that decerebrate rats do not acquire conditioned taste aversions.

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S60 RESPONSES OF NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT TO LINGUAL AND PALATAL STIMULATION WITH PREFERRED CHEMICALS. Susan P. Travers and Ralph Norgren. (Dept. Behavioral Science, Pennsylvania State University, Hershey, PA 17033).

Sucrose is a highly preferred taste stimulus for the rat. Some other chemicals preferred by this species, including both sugar and non-sugar stimuli, exhibit generalization to a conditioned taste aversion to sucrose, suggesting that there is a group of preferred chemicals that are psychophysically similar to one another for rats. Many of these stimuli are described as "sweet" by humans. The rat's behavior toward sucrose and psychophysically similar chemicals has been well-investigated, in contrast to the neural responsiveness of this species to these stimuli. This lack of information is due to an unfortunate coincidence: only the anterior tongue (AT) is usually stimulated in neurophysiological studies but stimulation of this receptor subpopulation with sucrose elicits poor neural responses in the rat. Recent work from this laboratory, however, has demonstrated that neurons in the nucleus of the solitary tract (NST) of the rat respond well to sucrose when applied to a different receptor subpopulation, the nasoincisor ducts (NID). The present study compares NST responses arising from AT stimulation to those arising from NID stimulation with a battery of chemicals that generalize to a sucrose aversion in rats (.3M fructose, .3M glucose, .3M glycine, .02M Na saccharin). Two other chemicals tested, .3M maltose and .1M Polycose, do not generalize to a sucrose aversion, but nevertheless are highly preferred by this species. Responses to .3M sucrose, .3M NaCl, .03M HCl, and .01M quinine HCl were also recorded. Preliminary results (n=31) corroborate our previous finding that sucrose is a much more effective stimulus for NID than for AT stimulation. Likewise Na saccharin, fructose, and glucose are much more stimulatory for the NID, but glycine is only slightly more effective. Glycine is more effective than sucrose for AT stimulation, but less effective than sucrose for NID stimulation. This suggests that sucrose and glycine interact with different types of receptors that are present in different proportions on the AT and NID. Regardless of the receptor subpopulation stimulated, Polycose and maltose were poor stimuli. Across-neuron correlations among the stimuli were calculated for both AT and NID responses and summarized using multidimensional scaling. The stimulus space generated by the two receptor subpopulations varied. Most stikingly, for AT responses, the correlations among the chemicals showing generalization to a sucrose aversion were not higher than the correlations between these chemicals and the other stimuli. In contrast, the correlations generated by the NID responses distinguished between these two stimulus groups.

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S61 NEURAL CODING OF GUSTATORY INFORMATION IN THE THALAMUS OF AN AWAKE PRIMATE. Thomas C. Pritchard, Robert B. Hamilton and Ralph Norgren. (Dept. of Behavioral Science, The Milton S. Hershey Medical Center, Hershey, PA 17033).

The neural coding properties of thalamic gustatory neurons are poorly understood primarily because the animal model used most often for neurophysiological research is the anesthetized rodent. The most formidable problems caused by general anesthesia are spindling activity and response depression, neither of which is encountered when using an unanesthetized preparation. This abstract describes the preliminary analysis of 55 single neurons recorded from the gustatory thalamus of an awake, behaving rhesus monkey. Prior to data collection the animal was anesthetized and the gustatory thalamus located electrophysiologically. A stainless steel well was attached to the skull directly above the taste thalamus. Glass insulated, tungsten microelectrodes were used for transdural recording of single neuron activity in the gustatory and oral somatosensory areas of the posterior thalamus. The chemical sensitivity of each neuron was initialy tested with each of the following stimuli: 1.0 M sucrose, 0.1 M NaCl, 0.003 M HCl, and 0.001 M QHCl. Most gustatory neurons sampled were responsive to only one or two of the four prototypical stimuli, with sucrose-best cells being the most common. Many gustatory neurons in the thalamus received convergent tactile information, often from a non-overlapping area of the oral cavity. Water was an effctive stimulus for approximately 70% of the neurons tested. Inhibitory responses were produced by approximately 15% of all applications of water and sapid stimuli. Other neurons, isolated in areas that also contained gustatory neurons, fired in bursts spontaneously but became quiescent prior to and during fluid stimulation. They showed no differential chemical sensitivity and could not be driven with tactile stimulation.

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S62 Taste Intensity and Reaction Time: Cued Versus Uncued Magnitude Estimates. TERESA PANTZER[1,2], BRUCE P. HALPERN[1,3], and STEVEN T. KELLING[1,4] (1. Dept. of Psychology. 2. Dept. of Chemistry. 3. Section of Neurobiology and Behavior. 4. Field of Physiology) (Cornell University, Ithaca NY 14853, U.S.A)

Cued and uncued magnitude estimations of 2mM NaSaccharin (NaSac) or 214mM monosodium glutamate (MSG), and their reaction times, were obtained from 4 to 5 subjects. The Kelling and Halpern (Chem. Senses, 11, 1986) closed flow delivery apparatus was used. A throat microphone connected to a digital timer detected the onset of each intensity judgment. Sessions began with two 2000 msec duration modulus trials (NaSac or MSG; maximum intensity assigned magnitude of 20) and 2 identified non-change, control trials (distilled water). The modulus was repeated after every fifth trial. Ten stimulus solution and ten control trials were randomized in each session. Exp 1 used 2mM NaSac, Exp 2, 214mM MSG, at 50, 100, 300, 1000, and 2000 msec durations; Exp 3 & 4, the same liquids, but only at a 300 msec pulse duration. In Exp 3 & 4, a tone that began at 730, 960, 1240, 1440, or 1640 msec after stimulus liquid onset cued the intensity judgment. RESULTS: A. MAGNITUDE ESTIMATES (ME): Judged taste intensity increased as stimulus duration increased (Exp 1 & 2). There was no significant change in the intensity ratings across the five judgment cue times (Exp 3 & 4). The ME for a 300 msec pulse in exp 1 & 2 were 10 [1] (NaSac) and 6 [1] (MSG) (Median [S.E.]). For Exp 3 (NaSac), ME were 7-8 [<1] across all judgment cue onset times; Exp 4 (MSG) 6-6.5 [<1], except 8 [1], at 1440 msec. B. REACTION TIMES (RT): Uncued MSG RT for a 300 msec pulse was 1639 msec [79]; for the cue at 730 msec, 1138 msec [15]. For NaSac, the uncued RT was 1217 msec [83]; with the 730 msec cue, 1086 msec [34]. CONCLUSIONS: The trend is for cued and uncued MEs to be similar, but cued RT $\leq$ 89% of uncued (cued at simple RT). The RT of a cued ME may be necessary for processing time for maximum intensity at one duration, or for any duration.

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S63 THE SENSITIVITY OF THE TONGUE TO ETHANOL. Barry G. Green (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104).

Sensations of irritation produced on the tongue by ethyl alcohol were measured psychophysically using the method of magnitude estimation in conjunction with a reaction time paradigm. Two locations on the tongue were tested (the tonguetip and a site 3 cm posterior to the tip on the dorsal surface) to learn if the sensitivity to chemical irritation follows the same spatial pattern (i.e., an anterior-to-posterior decline) as has been found for the somatic senses of warmth, heat-pain and touch. Six concentrations of ethanol (35, 45, 55, 65, 75 and 85%) in solution with deionized water were presented to the tongue on saturated disks of filter paper (0.38 cm^2). Subjects responded with a button press when they first felt irritation and with a magnitude estimate 10 sec later. The results confirmed the presence of a spatial gradient of sensitivity, with the tonguetip yielding perceived magnitudes significantly greater and response latencies significantly briefer than those obtained on the dorsum of the tongue. Perceived magnitudes differed between areas by a factor of about 2 to 1, and response latencies to the 85% solution ranged from about 1 sec on the tip to nearly 10 sec on the dorsal surface. On both sites response latency increased as concentration decreased, climbing to an average for the 35% solution of about 10 sec on the tip and 20 sec on the dorsal surface. The relatively poor sensitivity of the middle of the tongue was further exemplified by the inability of some individuals to detect irritation there at even the highest concentrations of ethanol.

Supported by a grant from the National Institutes of Health, #NS-20616.

S64 TASTING ON LOCALIZED AREAS. Linda Bartoshuk, Salli Desnoyers, Courtney Hudson, Laura Marks, Margaret O'Brien (John B. Pierce Foundation, New Haven, CT 06519), Frank Catalanotto (University of Texas, San Antonio, TX 78284), Janneane Gent, Dori Williams, Karen M. Ostrum (UCONN Health Center, Farmington, CT 06032).

The spatial properties of the tongue play an important role in taste phenomena observed in the clinic. Using a spatial screening test that compares 6 loci (the right and left sides of the front and rear edges of the tongue and the right and left sides of the palate), we have found localized losses of taste function in two etiological groups: head trauma and upper respiratory infection. Even patients with losses over extensive areas of the oral cavity can be unaware of the loss. They may also appear to be nearly normal on a conventional, whole mouth, sip and spit taste test. This occurs because relatively small areas of normal tissue can produce very intense sensations and the location of the taste sensations is not salient to the patient.

We have studied the spatial properties of the tongue in the laboratory by scaling the taste intensities of solutions "painted" on localized areas and by scaling the taste intensities of whole mouth stimulation after anesthetization of localized areas. The Q-tip scaling demonstrated that localized stimulation on the tongue and palate (on areas about 250 mm^2) produce taste intensities roughly 50% and 25% respectively, of those produced by sip and spit tasting (which stimulates the whole oral cavity). The anesthesia results complemented those with Q-tip scaling. Anesthetization of one small area (unilateral chorda tympani block or bilateral palate block) does not cause any reduction of sip and spit tasting. In fact, some stimuli tasted stronger, suggesting a release of inhibition from the anesthetized area.

A test of Q-tips vs filter paper for the application of localized stimuli showed that the two were equivalent on the front but not on the rear of the tongue. On the rear edge, filter paper produced weaker sensations.

In the course of conducting the spatial experiments, we discovered a taste illusion that underlines the importance of the tongue as a spatial organ. If taste solutions are painted from the side of the tongue to the tip, the taste sensation grows as the density of taste papillae increases toward the tip. However, if the solution is painted in the opposite direction, the taste intensity does not drop as rapidly as it rose. The taste system appears to borrow the tactile system for localization much as the thermal system does.

Supported by NIH grants NS 21600 and NS 16993.

NOTES

S65 **TASTE CONCEPTS AND QUADRANOMIAL TASTE DESCRIPTION.** Michael O'Mahony, Rie Ishii, and David Shaw. (Dept. Food Science and Technology, University of California, Davis, CA 95616)

The current model of sensory concept formation (Miller & Johnson-Laird, 1976) envisages a concept, like 'redness', as being formed by the process of abstraction from a set of stimuli (red and non-red stimuli) and generalization: an expansion of the concept to enable further shades of red, hitherto unseen, to be included as 'red'. Sensory descriptions ("red") are labels given to such concepts. It follows that different life experience would lead to experience of different stimuli with consequent differences in abstraction and the precise boundaries of the concepts formed.

The approach can be applied to other senses like taste. Taste description, then, is essentially a procedure for communicating concepts. For precise scientific description, it follows that those communicating must have the same set of concepts and the same labeling scheme. Having the same set of concepts means that subjects must have the same conceptual boundaries, the degree of precision depending on the measurement to be made. Furthermore, any labeling system requires that each concept has a distinct label to avoid confusion.

The current research outlines sorting and naming tasks, which determined the number of taste concepts held by subjects and the labels given to them under different naming procedures. The commonly used quadranomial description scheme, where taste labels are limited to combinations of 'sweet', 'sour', 'salty', 'bitter' (and sometimes 'other'), did not always represent the actual number of taste concepts held by the subjects, as well as showing inconsistencies and lack of reliability. In view of its potential for distortion, the procedure should be reappraised.

Supported by grants from U.M.A.J. and U.C. Nuclear Science Fund.

S66 **CAN THE POWER LAW EXPONENTS BE DERIVED FROM OLFACTORY THRESHOLDS FOR PURE SUBSTANCES ?** Paul Laffort and François Patte (Laboratoire de Physiologie de la Chimioréception, C.N.R.S., F-91190 Gif-sur-Yvette, France).

EAG dose-response curves for 59 pure substances have been obtained in the Honey-bee (Patte et al. 1984, J. Physiol. Paris, 79, 67A). As it is well known, this kind of sigmoïds in semi-log coordinates fit the Hill model, by using three parameters : "the power law exponent" (n), the maximal amplitude of response or plateau (Vm) and the concentration corresponding to the inflexion point (Cx). In addition, a fourth parameter (Co) can be easily derived from these three ones: the concentration corresponding to a response of 0.1 mV, which we consider as analogous to an "electrophysiological threshold". From the above study, a strong correlation appears ($P < 0.001$) between log Co and n ; in other words, the following trend is observed : the lower the threshold, the smaller the power law exponent. This correlation is approaching 1 ($r = 0.94$), if the threshold is expressed in terms of fraction of saturated vapour pressure instead of real concentration.

It is not yet sure that a similar strong correlation can be obtained in Vertebrates. An experimentation is in progress, in cooperation with E.P. Koster and colleagues, from Utrecht, to check it with EOG dose-response curves in Frog. However, from several sets of psychophysical data, the same strong correlation between thresholds and power law exponents was demonstrated by Laffort et al. (1974, Ann. N.Y. Acad. Sci. 237, 193-208). In addition, we have recently observed that the best improvements of these correlations are obtained by introducing the saturated vapour pressure as an additional variable, i.e. we have a situation similar to EAG on Honey-bee. However, in this case, the correlations remain farther from 1 (0.8 in average) ; it is suggested that this could be due to a poorer accuracy in psychophysical measurements than in electrophysiological ones. Presently, the major interest of these results could be a practical one in Psychophysics.

NOTES

S67 **FUZZY SET THEORY APPLIED TO PRODUCT CLASSIFICATION BY A SENSORY PANEL**

R. Keuning, E. Backer, R.P.W. Duin, H.W. Lincklaen Westenberg, S. de Jong.

The classification of products of a given type into different categories by consumers or by trained panels using sensory attributes is of great interest to the food industry. It helps us to discover product attributes primarily responsible for the distinction between various products.

The methods commonly used for classification of products of a given type into different categories by consumers or trained panels are mainly statistically founded. Techniques such as Discriminant Analysis assume that the categories are sharply defined and non-overlapping and that each individual product is a full member of just one of these classes.

In reality, class boundaries are vague and so are the attributes and the attribute scores. A proper model should recognise these elements of fuzziness. Zadeh's theory of fuzzy sets and the rules for fuzzy inferences provide us with the tools to model human classification processes.

To investigate this approach we have set up an experiment involving fat spread products to be classified into a number of fuzzy classes by a trained panel of housewives using a set of fifteen appearance and spreading attributes. A fuzzy questionaire was used with statements such as 'This product is hard' or 'It is product A' which could be marked TRUE, BOUNDARY or FALSE. The panel fractions associated with the three possible answers were used as values of a membership function for the fuzzy class of e.g. 'hard products'. These membership functions for each attribute are related to the membership functions for the product classes via a fuzzy inference procedure. In a series of computer experiments we have evaluated the performance of various alternative fuzzy classification models.
We have found good correspondence between the inferred classification and the directly stated classification, showing, in principle, the feasibility of a fuzzy product classification method. The adventages of the approach are:

(i) it gives clues to the underlying model for the reasoning which leads to the eventual classification
(iv) it gives an indication of the considerations about alternative class possibilities made by the subjects.

S68 CONSISTENCY OF PREFERENCES FOR SALT IN DIFFERENT FOODS. Richard Shepherd and Cynthia A. Farleigh. (AFRC Food Research Institute, Norwich, NR4 7UA, UK).

Whilst there is evidence for preferences for salt concentrations in a particular food being predictive for the total salt intake of an individual (Shepherd, Farleigh and Land, 1984a), the prediction is relatively weak. One reason for this may be that individuals do not have a consistent liking for high or low salt levels across different foods.

Thirty-two subjects (16 males and 16 females) took part in a study where they tasted foods varying in salt content, and rated them on a 100mm graphic relative-to-ideal rating scale. The foods were tomato soup (0.3-12.1mg Na/g), bread (1.4-9.5mg Na/g), boiled potato (0.1-13.3mg Na/g), and meat paté (0.7-17.4mg Na/g). Samples with ten concentrations of salt were available for each of the foods, but in order to minimise range bias the samples were presented in an order determined by the responses of the subjects (Shepherd, Farleigh and Land, 1984b). The first sample presented was always the fifth concentration, and if the subject rated this below ideal then the next stimulus was of a higher concentration, whereas if it was rated above ideal then the next concentration was a lower one. The concentrations were subsequently presented to try to equalise the number of samples rated above and below ideal, and to equalise the average distance that these were rated from ideal. From the plot of the ratings against log(concentration), the individual's most preferred concentration (ideal) of salt was calculated, along with the slope, which gives a measure of how concerned he or she is about deviations from the ideal.

The mean ideal concentrations for the foods were found to be for potato 1.90mg Na/g, soup 2.28mg Na/g, paté 3.78mg Na/g, and for bread 4.94mg Na/g. The subjects preferring a high concentration in one food also tended to prefer it in the others, with the correlations between most preferred concentrations varying from $r=0.60$ to 0.78 ($df=30$, $p<0.001$). In general the reliability between the two determinations was high. The correlations between the slope of the function for the different foods were lower ranging from $r=0.20$ ($df=30$, NS) to $r=0.46$ ($df=30$, $p<0.01$), part of this being accounted for by the lower reliability of this measure over the two sessions. These results demonstrate a consistent liking for high salt levels in some individuals across different types of foods.

S69 BRETYLIUM TOSYLATE ENHANCES SALT TASTE. Susan S. Schiffman, Sidney A. Simon, James M. Gill, and Timothy G. Beeker. (Duke Medical Center, Durham, NC 27710).

Amiloride-sensitive sodium channels mediate some components of taste in both humans and rats. The diuretic amiloride (n-amidino-3,5-diamino-6-chloropyrazine carboxamide) diminishes the taste of NaCl and LiCl as well as sweet-tasting compounds in humans and reduces electrophysiological gustatory responses to NaCl and LiCl in rats and gerbils. Furthermore, amiloride blocks the increases in short-circuit current across canine and rat lingual epithelium induced by NaCl, LiCl, and sugars.

Bretylium tosylate (BT), an antifibrillary drug, has recently been shown to increase sodium transport through amiloride-sensitive sodium channels in frog skin. It therefore seemed logical to determine whether BT might amplify the amiloride-sensitive components of taste.

Results of the experiments to be reported show that BT does indeed potentiate the taste of NaCl and LiCl in humans without affecting responses to other salts such as KCl or $CaCl_2$. Electrophysiological taste responses in rats showed that BT potentiated the activity for NaCl and LiCl. The potentiation of taste induced by BT was completely eliminated by amiloride. Furthermore, in the presence of BT, amiloride was ineffective in inhibiting NaCl gustatory responses. BT had no effect on the short-circuit current in isolated dog lingual epithelium. Possible reasons for these findings are discussed.

S70 PURIFICATION AND CHEMICAL STRUCTURE OF TASTE MODIFIERS: TASTE-MODIFYING PROTEIN AND ZIZIPHIN. Yoshie Kurihara, Kazuyoshi Ookubo and Bruce P. Halpern (Dept. Chem., Facu. Edu., Yokohama National Univ., Yokohama : Facu. Agri., Tohoku Univ., Sendai : Dept. Psychol., Cornell Univ., Ithaca)

1. Taste-modifying protein

A number of methods on isolation of the active principle from miracle fruit (taste-modifying protein) have been reported. These methods have problems that the isolated protein preparation contains impurities and the activity of the protein is reduced during long purification procedures. In previous studies, the active protein, which could not be extracted with water, was extracted with a carbonate buffer of pH 10.5. The extracted solution contained deep colored materials which were difficult to be eliminated. In addition, the extraction with the alkaline solution led to partial loss of the activity. In the present study we found that the active protein can be extracted with neutral salt solution as follows. The pulp of miracle fruit was washed with water and extracted with 0.5 M NaCl. The extracted solution contained no colored materials and exhibited a strong activity. This solution was applied to a Sephadex G-75 column. An active fraction from the column was purified on ion exchange chromatography. The activity of the purified protein was much higher than that of the protein isolated by previous methods. Studies for determination of the amino acid sequence of the purified protein are in progress.

2. Ziziphin

Leaves of Ziziphus jujuba contain ziziphin which has anti-sweet activity. The leaves were washed with hexane and extracted with ethanol-water. The extracted solution was concentrated and the residue obtained was extracted with chloroform-ethanol. The extract obtained was fractionated on a Sephadex LH-20 column.. The active fraction obtained from the column was applied to a HPLC RP-18 column. Elution with a mixture of organic solvents (acetonitrile-propanol-water-acetic acid) gave 23 peaks. Anti-sweet activity was found only in one peak fraction. This fraction was concentrated and purified ziziphin was obtained. Analysis of NMR and IR spectra of the purified ziziphin is in progress. Preliminary analysis showed that ziziphin is a glycoside of triterpene.

NOTES

S71 INDEPENDENCE AND PRIMACY OF UMAMI AS COMPARED WITH THE FOUR BASIC TASTES. Shizuko Yamaguchi and Yasushi Komata. (Central Research Laboratories, Ajinomoto Co., Inc., 1-1, Suzuki-cho, Kawasaki 210, Japan)

Umami is defined as the taste elicited by MSG or 5'-ribonucleotides such as IMP and GMP. A series of psychometric experiments provides several lines of evidence that umami is independent and "basic" as long as the four basic tastes are regarded as basic.

A multidimensional scaling yielded a spatial configuration expressing the qualities of single and mixture solutions of the four basic tastes and umami. All the stimuli were located within a four-dimensional regular polyhedron which has five vertices. The tastes composed of the four basic tastes were located within a three-dimensional tetrahedron, which was a subcomplex of the four-dimensional polyhedron with the four basic tastes located at four vertices. Umami was located at the other vertex, indicating that it constructs another dimension independent of the four basic tastes.

Another multidimensional scaling showed the dominance of umami in the tastes of natural foods. The tastes of broths made from meats (beef, pork, etc.) and fishes fell outside the tetrahedron of the four basic tastes and were located close to umami. Those made from vegetables widely distributed around the five taste areas. However, when a small amount of IMP was added, the tastes approached umami due to the remarkable synergistic effect between IMP and glutamic acid contained naturally in the vegetable stocks. Thus the stocks examined were regarded to have dominant or potential umami, which actualized or developed by a small amount of umami substances.

The hedonic properties of umami were examined in comparison with the four basic tastes. As far as simple aqueous systems were concerned, umami did not cause any pleasant sensation. In the selected flavored solutions or actual foods, umami clearly enhanced the hedonic tone. Umami increased the pleasantness of foods only in a certain range of concentration, and an excess amount of umami caused rather unpleasant sensation by which the concentration of intake became self-limited. These hedonic properties were similar to other basic tastes except for sweetness.

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S72 EFFECT OF THE BITTER TASTE STIMULI NARINGIN AND SUCROSE OCTAACETATE ON SWEET PERSISTENCE AND SWEET QUALITY OF NEOHESPERIDIN DIHYDROCHALCONE. Michael Naim, Emmanual Dukan, Lyat Yaron, Martha Levinson and Uri Zehavi. (Dept. Biochemistry and Human Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel).

The perceived sweet intensity (Ip-max) and the sweet persistence constants (T) of neohesperidin dihydrochalcone (NHD), were significantly reduced in a mixture containing naringin (NAR), a bitter flavone analog of NHD. Sucrose octaacetate (SOA), another bitter stimulus, reduced the Ip-max of NHD in mixtures, but no appreciable decrease in T values was found. Linear regression analyses performed on the Ip-max data of either NHD + NAR or NHD + SOA (log Ip-max vs. log concentration) produced slope values lower than those of NHD alone. Moreover, taste similarity experiments revealed that the mixture of NHD + NAR was located further than NHD from the sugar area in the multidimensional scaling (MDS) map. It is concluded that the reduction in T values of NHD by NAR was apparently related to the reduced Ip-max levels and that such a mixture produces a sweet quality inferior to that of NHD.

S73 SCHEMATIC SWEET AND BITTER RECEPTORS. Hans-Dieter Belitz, Hartmut Rohse, Wolfgang Stempfl, Herbert Wieser. (Institut für Lebensmittelchemie, Technische Universität München and Deutsche Forschungsanstalt für Lebensmittelchemie, D-8046 Garching FR Germany). Johann Gasteiger, Christian Hiller. (Organisch-chemisches Institut, Technische Universität München, D-8046 Garching, FR Germany).

For sweet tasting molecules, two polar groups - the AH/B system (Shallenberger & Acree, 1971), or the electrophilic/nucleophilic system (Belitz, Chen, Jugel, Treleano, Wieser, Gasteiger & Marsili, 1979) - are essential, which may be supplemented by a hydrophobic group (Kier, 1972). In contrast, bitter compounds need only one electrophilic or nucleophilic group and a hydrophobic group. (Belitz, Chen, Jugel, Stempfl, Treleano, Wieser, 1983).

Based on these bipolar-hydrophobic and monopolar-hydrophobic concepts, the dependence of the taste thresholds of compounds from different chemical classes on some physical parameters (partition coefficient, polarizability, charge distribution, molar volume, steric factors) was then investigated. Allowed and forbidden areas for sweet and bitter taste qualities were localized relative to the polar contact groups by superposition of space formulas of the tested compounds with the aid of a special computer program. In this way it is possible to develop general space models for compounds with sweet or/and bitter taste which describe the steric requirements of schematic receptors.

P37 **NO TRIGEMINAL DISCRIMINATION AMONG EQUALLY INTENSE ODORANTS.** Wayne L. Silver*, Adam H. Arzt**, and J. Russell Mason**. (*Dept. Biology, Wake Forest University, Winston-Salem, NC 27109; ** Monell Center, 3500 Market St., Philadelphia, PA 19104).

It is unknown whether trigeminal chemoreceptors discriminate between odorants matched for equal intensity. We addressed this issue in electrophysiological and behavioral experiments with the tiger salamander.

For electrophysiological experiments, integrated multiunit responses were obtained from the trigeminal nerve as it passed through the orbit of the eye. An air-dilution olfactometer (also used in behavioral experiments) delivered stimuli to the animals. Concentration-response curves were obtained for amyl acetate (AA), cyclohexanone (CH), butanol (BU), and d-limonene (LI). The concentration of each compound necessary to produce an equivalent response (150% of CH std.,) was then used as the background (adapting) stimulus in cross-adaptation experiments (AA vs CH; BU vs LI). Only concentrations above that of the background stimulus elicited increases in response magnitude.

For each of two identical behavioral experiments, salamanders were randomly assigned to two groups. In experiment 1, animals were trained to avoid CH (S+) but not AA (S-), or vice versa. In experiment 2, the odorants used were BU and LI. When criterion was achieved, (≥90% S+ responding, ≤20% S- responding), groups were given varied concentrations of their respective S+ odorant. S+ concentrations that elicited ≥80% avoidance were used in subsequent discrimination tests. All groups discriminated S+ from S- odorants (AA vs CH; BU vs LI).

Following discrimination trials, all animals were given olfactory nerve cuts, and additional presentations of varied S+ concentrations. Higher concentrations of odorant were necessary to elicit ≥80% avoidance in nerve cut animals. Discrimination tests between behaviorally equivalent odorant concentrations revealed that animals were unable to discriminate CH from AA, or BU from LI. Additional discrimination trials with concentrations that elicited ≥95% avoidance produced similar results.

On the basis of these electrophysiological and behavioral findings, we conclude that trigeminal chemoreceptors are unable to discriminate between odorants matched for equal intensity. Thus, at least for the odorants above, trigeminal chemoreceptors discriminate odorant quantity, not quality.

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P38 OLFACTORY RECEPTOR CELL FUNCTIONNING AFFECTED BY TRIGEMINAL NERVE ACTIVITY AND SUBSTANCE P. Jean-F. Bouvet, Jean-C. Delaleu and André Holley.

In the frog, the antidromic electrical stimulation of the ophtalmic branch of the trigeminal nerve (NV-ob) was performed with 10 ms-pulses at 30 V, 20 Hz. Electrical volleys of 1-2 s duration delivered to NV-ob evoked slow electrical potentials in the olfactory mucosa, with the surface negative in relation to the indifferent electrode. There was a delay of about 1.5s from the onset of the stimulation to the start of the response. In most preparations, the negative potential was a single wave which lasted for 2s to 15s; in average the amplitude was about 1 mV. In a few preparations, this wave was followed by a second one which lasted for 1-3 min with a peak amplitude of 2-4 mV. During the single-wave potentials, the spontaneous firing rate of olfactory receptor cells was increased or, more rarely, unchanged. During the late phases of the composed potentials, the firing rate of receptor cells was decreased; also, the peak amplitude of the electroolfactogram evoked by isoamylacetate was reduced; the unit responses to this odorant were reduced or suppressed.

A 10 min. exposure of the olfactory mucosa to capsaicin vapours suppressed potentials elicited by NV-ob electrical stimulation. Moreover, substance P modified the spontaneous electrical activity and the responsiveness to odour of the olfactory mucosa. These results suggest that the trigeminal system could modulate the activity of the olfactory receptor cells via a local axon reflex inducing the release of substance P.

P39 **PERCEIVED BURN AND TASTE INTENSITY OF PHYSICAL MIXTURES OF CAPSAICIN AND TASTE STIMULI.** Beverly J. Cowart (Monell Chemical Senses Center, Philadelphia, PA 19104).

Recent studies by Lawless and his colleagues (Physiol. & Beh., 1984, 32, 993-998; Chem. Senses, 1985, 10, 579-589) indicate there may be significant suppression of taste intensity when taste stimuli are interspersed with oral rinses of an irritative stimulus such as capsaicin (CAP). In these studies, tastes and irritants were never presented in physical mixture, as they are typically consumed, and the periodic rinse format resulted in significant declines in perceived irritation during the time that taste sensations were being experienced and rated. We further examined this phenomenon under conditions that seemed to be more ecologically valid and likely to produce a more consistent level of irritant sensation. To that end, both simple and complex taste stimuli were presented in physical mixture with CAP to groups of frequent (F) and infrequent (I) consumers of hot spices (n=12/group). In 2 sessions, subjects used 13-point category scales to generate profiles of the sweetness, sourness, saltiness, bitterness, burn and pleasantness of aqueous and chicken broth solutions containing either no additional tastant, 0.34 M sodium chloride (NaCl), 0.01 M citric acid (CA) or both 0.34 M NaCl and 0.01 M CA. In one session, these stimuli were also rated with 1 ppm CAP added to each; in the other, the stimuli were also rated with 2 ppm CAP added to each. Excepting a tendency among F consumers to give slightly higher ratings of side tastes, especially bitter, F and I consumers did not differ in their ratings of any perceptual attributes of the stimuli without CAP. Burn ratings of stimuli with CAP did differ significantly between groups with I consumers giving higher ratings at both 1 and 2 ppm CAP than did F consumers. For both groups, the burn produced by 2 ppm CAP was greater than that produced by 1 ppm, and at each level of CAP, burn intensity at the time of taste ratings remained constant throughout the test session. Nonetheless, we observed no consistent changes in the taste intensity ratings of either group following the addition of CAP to stimuli. In subsequent studies varying such methodological parameters as rating procedure but retaining a mixture presentation format, we have consistently failed to observe significant suppression of either salty or sour tastes. Using a periodic rinse format, however, we have replicated the original findings of apparent taste suppression. Studies are underway to further validate, and elucidate the basis of, differences between results produced by these two presentation procedures. It must be tentatively concluded, however, that suppressive effects of oral irritation on taste are not robust.

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P40 CONTACT CHEMORECEPTORS ON THE WALKING LEGS OF THE SHORE CRAB, CARCINUS MAENAS. Manfred Schmidt and Werner Gnatzy (Gruppe Sinnesphysiologie, Zoolog. Institut, J.W. Goethe-Universität, Siesmayerstr. 70, 6000 Frankfurt a.M., West Germany)

On the walking legs of the shore crab, Carcinus maenas, lie many small sensilla called funnel-canal organs. These sensilla are innervated by two mechanosensory neurons and 1 - 22 further sensory cells which are most likely chemoreceptive (Schmidt & Gnatzy, 1984; Gnatzy, Schmidt & Römbke, 1984). Since the funnel-canal organs are the only sensilla on the tips of the dactyls they can be stimulated selectively.

Based on this favorable condition electrophysiological recordings from the leg nerve were carried out. The dactyl tip was inserted through a piece of silicone tubing into a small chamber superfused by seawater, which could be replaced by various stimulus solutions in about 200 ms. About 20 different compounds, which excite other chemoreceptors in crustaceans (Ache, 1982), were tested in a concentration range from 10^{-7}M to 10^{-2}M.

As to the specificity of the sensory units they were divided into broadly and narrowly tuned ones. The broadly tuned cells respond to taurine, betaine, and most of the amino acids tested except L-glutamate. Most cells with a narrow reaction spectrum respond to taurine and glycine or L-glutamate and L-glutamine, but some are selective only for taurine, glycine, L-glutamate, or betaine. The threshold sensitivity of the sensory cells is between 10^{-6}M and 10^{-3}M, with a working range of about 2 - 3 decades.

According to these data the funnel-canal organs are contact chemoreceptors most likely involved in food detection.

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P41 THE NERVUS TERMINALIS OF THE SHARK: INFLUENCES ON GANGLION CELL ACTIVITY. Joel White and Michael Meredith (Dept. of Biological Science, Florida State University, Tallahassee FL 32306).

Although studied repeatedly since its discovery, the nervus terminalis remains the only cranial nerve for which there is no known function. Anatomists have shown that the ganglion of the nervus terminalis contains bipolar and multipolar cell bodies, and have suggested that the nerve has sensory and/or autonomic components. Based on this anatomy, the terminalis ganglion could be thought of as merely a group of sensory cell bodies, or perhaps a simple efferent relay point. The data that we present here suggests that the nervus terminalis ganglion may be more complex than either of these two possibilities.

As we reported previously (White & Meredith, 1985), action potentials can be recorded extracellularly in the nervus terminalis of the bonnethead shark (Sphyrna tiburo). These impulses of efferent origin and are eliminated by cutting the nerve central to the main ganglion. However, approximately 0.5 to 0.8 seconds following the cut an increase in multi-unit activity is recorded in the nerve. This activity appears to arise from cells in the ganglion, because cutting the nerve peripheral to the ganglion does not eliminate the it, although the level of activity decreases slightly. Reversibly blocking action potential conduction in the central portion of the intact nerve by cooling also results in an increase in multi-unit activity. Upon warming to ambient temperature, the efferent impulses return and the multi-unit activity decreases, yielding a recording similar to that seen before the nerve was cooled.

After cutting the nerve central to the main ganglion, the resulting multi-unit activity can be suppressed with low intensity electrical stimulation of the distal stump of the nerve. Preliminary experiments indicate that the multi-unit activity can also be suppressed by electrically stimulating the peripheral portion of the nerve. These data suggest that efferent impulses may be modifying afferent information from the olfactory epithelium, or influencing the centrally or peripherally directed output of cells in the ganglion through synaptic inhibition.

Supported by NSF Grant BNS 841 21 41.

P42 UPPER AIRWAY (NASAL?) CHEMORECEPTORS IN A BOID SNAKE: VENTILATORY RESPONSE TO O_2 AND CO_2. E. Lee Coates, Tina M. Caton and Gary O. Ballam. (Bioengineering Research Division, Lovelace Medical Foundation and Department of Physiology, University of New Mexico, Albuquerque, NM 87108).

Coates et al. (Federation Proc. 44:1348,1985), using the tegu lizard, Tupinambis nigropunctatus, report a depression of ventilatory frequency when CO_2 (0.4 to 4%) is delivered to the upper airways. The response originates in the nasal epithelium innervated by the olfactory nerves. The present experiment was performed to compare the ventilatory response to upper airway CO_2 of the Haitian boa, Epicrates striatus striatus, to the CO_2 response reported for the tegu lizard. In addition, the ventilatory response to 2 and 4% CO_2 delivered to the upper airways of the Haitian boa during hypoxia (5% O_2), normoxia (20% O_2), and hyperoxia (95% O_2) were measured.

Fresh air was supplied to lungs throughout the experiment via an endotracheal T-tube inserted into the glottis. This isolated the upper airways (mouth and nasal cavities) and made possible the administration of gas mixtures to the upper airways independent of fresh air, delivered to the lungs. A chamber was secured over the snakes head to direct the gas mixtures to the upper airways. The gases were delivered to the head chamber for 5 minutes, with 5 minute control periods before and after the test period. During control periods a constant fresh air flow (100 cc·min^{-1}) was delivered to the head chamber. To add gases, a solenoid was opened allowing preset concentrations of gas mixtures to enter the chamber at 100 cc·min^{-1}. At the same time, fresh air flow to the chamber was discontinued.

It was found that hypercapnia (2 and 4%) in the head chamber during normoxia and hyperoxia caused no significant changes in ventilatory frequency. The breathing response to hypoxic hypercapnia (2% CO_2) was a 20% decrease in frequency which was significantly ($P<0.005$) less than the frequency response to hypoxic normocapnia. No significant difference was observed between hypoxic hypercapnia at 4% CO_2 and hypoxic normocapnia. There were also no significant changes in steady-state ventilatory frequency during hypoxic normocapnia.

These results indicate an upper airway chemoreceptive ventilatory response in the Haitian boa to hypoxic hypercapnia. Neither stimulus, hypercapnia or hypoxia, were capable of inducing an independent response. Unlike the haitian boa, the ventilatory response to low levels of CO_2 in the upper airways of tegu lizards is not dependent on hypoxia (Ballam, Respir. Physiol., 62:375386, 1985).

Supported in part by NHLBI grant #29342.

P43 GARTER SNAKE RESPONSE TO THE CHEMOATTRACTANT IN EARTHWORM ALARM PHEROMONE IS MEDIATED BY THE VOMERONASAL SYSTEM. NANCY SCHULMAN, EVELYN ERICHSEN AND MIMI HALPERN. (DEPT. OF ANATOMY AND CELL BIOLOGY, DOWNSTATE MEDICAL CENTER, BROOKLYN, N.Y. 11203).

Garter snakes (Thamnophis parietalis) were tested in a two-choice discrimination task (Reformato et al., Biochem. Behav., 1983, 18, 247-254) for differential responses to, 1) earthworm alarm pheromone (EAP) produced by shocking earthworms with electric current, 2) earthworm wash (EWW) produced by bathing earthworms in a 60°C bath for two minutes; and 3) amyl acetate (AA) placed on a cotton plug in a perforated container.

Eighteen snakes were initially trained using EWW and subsequently tested with EAP and AA (9 snakes only). All snakes spent significantly more time and tongue flicked significantly more frequently dishes coated with EWW or EAP as compared to distilled water (dH_2O) controls. No differential responses were observed to containers with AA as compared to containers with dH_2O. After preoperative testing nine snakes were tested for locomotor activity in an open field apparatus.

Snakes were subjected to bilateral vomeronasal (N=6), olfactory (N=6) or sham (N=6) nerve lesions. Snakes with olfactory or sham nerve lesions discriminated EWW and EAP from dH_2O following surgery whereas snakes with vomeronasal nerve lesions discriminated neither EWW nor EAP from dH_2O. Locomotor activity was not differentially affected by surgery.

The heads of all snakes were decalcified, embedded in paraffin and stained by the Bodian method. All lesions were verified microscopically by examining the site of the lesion as well as the corresponding sensory epithelium.

Studies by a number of investigators have previously demonstrated the importance of the garter snake vomeronasal system in the detection of and response to earthworm extract. The present study adds another earthworm product, alarm pheromone, to the substances detected by the garter snake vomeronasal organ. The olfactory system does not appear to be necessary for differential responses to earthworm alarm pheromone.

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P44 **EFFECTS OF VOMERONASAL ORGAN REMOVAL IN LACTATING FEMALE MICE: DISSOCIATION OF MATERNAL AND AGONISTIC BEHAVIORS.** N. Jay Bean (Department of Psychology, Vassar College, Poughkeepsie NY 12601 and Monell Chemical Senses Center, 3500 Market St., Phila. Pa 19104), Charles J. Wysocki (Monell Chemical Senses Center)*

Female mice exhibit characteristic behaviors during lactation which are mediated in part by chemosensory cues, but the routes of this sensory input are not fully understood. The present studies were designed to dissociate the roles of the vomeronasal and main olfactory systems in the expression of maternal behaviors. Vomeronasal organ removal (VNX) prior to mating had little effect on many, but not all maternal behaviors. No differences in pup related behaviors were observed between the VNX and the SHAM females. There were no differences in litter size or survival rates between the two groups. Additionally, no differences were observed in pup retrieval latencies, or the total time to retrieve both an alien and one of the mothers' own pups. However, marked differences between the groups were noted in the responses of the females to an intruder male. Twelve of the 17 SHAM group females were highly aggressive, but none of the VNX females attacked, bit or fought with the intruder male. These females did investigate the males and attempted extensive grooming of them. A second study analyzed whether the loss of aggression resulted from deprivation of important chemosensory cues during mating. In this study, aggression by lactating females that had received VNX surgery after one week of cohabitation with the male was assessed. Consistent with the findings of the previous study, 7 of the 9 SHAM females were highly aggressive toward the intruder males, while the VNX females, again, did not attack,bite, or fight with the intruders. A third study determined whether experience with aggression during a previous lactional period could ameliorate the effects of VNX. All of the SHAM females were aggressive during this second lactational period, but only one of the six VNX females fought with the intruder male. Two additional VNX females attacked the intruder. Although these three females were aggressive during these tests, the levels of aggression were markedly reduced relative to that exhibited by the SHAM females.

These results confirm and extend previous findings suggesting that the vomeronasal system is extremely important for the mediation of aggressive behavior in mice. Furthermore, they demonstrate that specific components of a lactating female's behavior are differentially modulated by the vomeronasal system.

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P45 CLINICAL OLFACTOMETRY: IMPROVED CONVENIENCE IN SQUEEZE-BOTTLE KITS; AND A PORTABLE OLFACTOMETER. John E. Amoore and Robert S. O'Neill (Olfacto-Labs, 1414 - 4th. St., Berkeley, CA 94710).

Since their introduction at the first AChemS meeting in 1979, our Firm's quantitative clinical smell test kits, based on 8-oz flip-top squeeze-bottles containing graded concentrations of pyridine in mineral oil, have been widely used for evaluations, research and survey work. We are now exhibiting for the first time a new line of miniaturized kits, based on the same principles, and re-designed for maximum convenience in test administration.

The new 2-oz bottles contain a much reduced proportion of liquid, which is retained in an absorbent matrix to prevent spillage. Extended shelf-life and service performance are maintained by offering odorants and diluents with very low air/liquid partition coefficients, and plastic bottles having minimal inherent odor and enhanced vapor permeation barrier properties. Simplified instructional materials, and an olfactory sensitivity scale covering the full meaningful range of the normal distribution curve from 1/250 th. to 250 x the average odor detection threshold, make for easy use and clear interpretation.

Odor pollution appraisal, chemical worker safety, and litigation support sometimes require a convenient, portable self-contained olfactometer to generate an adjustable, quantitatively odorized air-stream for reference and/or demonstration purposes. Our prototype instrument on exhibit has the following features:

It is compact and portable for hand-carrying on-site, and available for use with line current or battery operation. Ambient air is drawn into the machine for the main air flow and the odorizing air stream. A series of interchangeable, leak-proof saturator "cartridges" for significant industrial chemicals, and replaceable concentration scales, provide flexibility in application. An electrically operated concentration selector system, and rapid equilibration on changing odorants and/or concentrations, contribute to the instrument's practicality.

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P46 NEUROSURGICAL APPLICATIONS OF CLINICAL OLFACTORY ASSESSMENT. Richard M. Costanzo, Peter G. Heywood, John D. Ward, and Harold F. Young. (Depts. Physiology and Biophysics and Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298).

The neurosurgeon, through routine neurological examination, encounters olfactory deficits in those patients with skull fractures, contusions, lesions in the anterior fossa, and intracranial tumors. We employed the CCCRC test of olfactory function (Cain et al., 1983) including a butanol threshold and odor identification test to compare patients against a control population. A subgroup of "surgical anosmics", patients lacking olfactory connections as a result of surgical procedure, were tested to 1) study the difference between trigeminal and olfactory nasal sensations and 2) to validate test scores used to define anosmia. Findings confirm low scores for surgical anosmics and furthermore suggest differences in identification and threshold function that may assist the physician in distinguishing between central and peripheral lesions. The quantitative assessment of olfactory function is a useful clinical tool for 1) diagnosing and locating olfactory lesions 2) quantifying the extent of the sensory deficit and 3) providing a means for monitoring patient progress.

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P47 GUANINE NUCLEOTIDE-BINDING STIMULATORY PROTEIN (G_s) - A REQUISITE FOR HUMAN ODORANT PERCEPTION. H.N. Wright, R.S. Weinstock, A.M. Spiegel, M.A. Levine, and A.M. Moses (Clinical Olfactory Research Center and Dept. Medicine, SUNY Health Science Center at Syracuse; V.A. Medical Center, Syracuse, NY; Molecular Pathophysiology Section, NIADDK, NIH, Bethesda, MD; Dept. Medicine, Johns Hopkins Univ. School of Medicine, Baltimore, M.D.)

The sense of smell has been shown to be mediated by the G_s adenylate cyclase system in the neuroepithelium of the frog. (Pace et al.: Nature 316:255-258, 1985) Because G_s is ubiquitous in the animal kingdom, we tested the sense of smell in the only known model for the intrinsic occurrence of G_s deficiency in humans, G_s-deficient pseudohypoparathyroid (PHP) patients. Each patient was evaluated by requiring them to repeatedly identify ten common odorants randomly presented at concentrations found to be representative of their counterparts in nature: 3.6015 M ammonia (ammonia), 0.0622 M trans-cinnamaldehyde (cinnamon), 0.0130 M anethole (licorice), 0.3990 L-carvone (mint), 0.0244 M napthalene (mothballs), 0.0121 M D-limonene (orange), 1.0467 M phenethyl alcohol (rose), 0.7425 M isopropyl alcohol (rubbing alcohol), 0.0273 M vanillin (vanilla), and 4.3714 M acetic acid (vinegar). The diluent was odorless propylene glycol. The results were cast into a closed-set Odorant Confusion Matrix (OCM) which permitted analysis not only for the percent correct identifications, but also on the pattern of parosmic substitutions of one odor for another. All PHP G_s-deficient patients (n=5) had impaired olfactory ability when compared to PHP patients with normal G_s activity (n=6) and normal controls (n=25). The pattern of error responses in the OCM for PHP patients with normal G_s activity showed some tendency for parosmic substitutions, but otherwise was similar to that obtained for normal controls. The pattern of error responses in the OCM for the PHP G_s-deficient patients was haphazard with only adequate responses to strong trigeminal stimulants. Such a result is consistent with what has been found in patients with cranial nerve I deficiency, thereby supporting the necessity of G_s for human odorant perception.

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P48 TIME-INTENSITY ANALYSIS OF GUSTATORY STIMULI: PRELIMINARY ASSESSMENT OF A NEW TECHNIQUE. Darlene Burke, Aliki Akontidou & Robert A. Frank. (Dept. of Psychology, University of Cincinnati, Cincinnati, OH 45221)

Much of the previous research in gustatory psychophysics has focused on the ratings of a stimulus made at a single point in time despite the fact that the stimulus may be changing continuously over the time between its detection and complete adaptation. A few experiments have investigated the temporal characteristics of various tastants, but most of this research has focused on adaptation time for sweeteners. This preliminary experiment explored the utility of a new approach for studying changes in taste intensity over time. Ten subjects made intensity judgments for two concentrations of sucrose, sodium chloride, quinine sulfate and citric acid. Whatman #5 filter papers measuring 13 by 20 mm were soaked in the solutions and placed on the dorsal anterior tongue. Placement of the filter paper on the tongue completed a lickometer circuit which triggered a stimulus marker on a polygraph. The subjects were instructed to move a small lever mounted on a response console from a rating position of 0 (no taste) to 10 (very strong taste) in proportion to the stimulus intensity and to rate the taste sensation continuously from the time they could first detect a taste until complete adaptation occurred. The lever was attached to a slide potentiometer whose output was amplified by the polygraph and translated into the movements of one of the chart pens.

Five measures were derived from the curves generated for each stimulus. These included onset or simple reaction time (the time between the placement of the filter paper on the tongue and the rating of the stimulus as greater than 0), stimulus rise time (the time between the first rating greater than 0 and maximum intensity), maximum intensity, decline time (the time between the decline from maximum intensity to complete adaptation) and total rating time (the time between stimulus onset and complete adaptation). These measures were sensitive to differences in both concentration and quality. Higher intensity ratings were associated with faster onset times, faster rise times, slower decline times and longer total response times. Quinine and citric acid were found to be less persistent than sucrose or sodium chloride.

Based on this and other preliminary work that is planned, this technique will be used to explore a number of problems in gustation including the psychophysics of taste mixtures, the relationship between hedonic and intensity ratings of gustatory stimuli over time and the temporal characteristics of taste processing in phenylthiocarbamide (PTC) tasters and non-tasters.

P49 **INDUCTION OF HUMAN PAROTID SALIVARY ALPHA-AMYLASE SECRETION BY ORAL STIMULATION.** Deborah Anne Froehlich and Rose Marie Pangborn. (Depts. Nutrition and Food Science, University of California, Davis, CA 95616).

The effects of oral stimulation on human parotid salivary flow, protein concentration, alpha-amylase activity, and electrolyte concentration were measured, with the possible induction of alpha-amylase secretion being of special interest due to its role in the initiation of starch digestion. Unilateral parotid saliva was collected from ten subjects during oral stimulation with water, potato starch (2.5, 5.0, and 10 %), sucrose (0.1, 0.2, and 0.4 M), sodium chloride (0.075, 0.15, and 0.30 M), and citric acid (0.005, 0.01, and 0.02 M). Salivary flow rate increased with increasing levels of each stimulant, with citric acid being the most powerful flow inducer, followed by sodium chloride, sucrose, and starch, respectively. The protein concentration was higher from starch than from water stimulation, but was independent of the actual starch level. Although not significant, there was a downward trend in protein concentration with citric acid stimulation. Stimulation had no influence on the level of alpha-amylase activity. The values for protein per minute and alpha-amylase activity per minute increased with increasing level of each stimulant (response patterns being similar to flow rate response patterns). Citric acid was the only stimulant to have a significant ($p<0.05$) effect on the electrolyte concentration of saliva: Na^+ and K^+ concentrations increased, Mg^{++} decreased, and Ca^{++} was unaffected by increasing citric acid levels. Interesting differences in response to stimulation were obtained when the subjects were divided into "low flow" and "high flow" groups based on water-stimulated flow rates. Both the protein concentration and alpha-amylase activity were significantly higher in the low-flow group than in the high-flow group, however, the two groups had similar values for protein per minute and alpha-amylase activity per minute. The differences in flow rates allowed for similar amounts of protein and alpha-amylase activity to be delivered to the mouth when considered on a time basis. Except for the Na^+ concentration by citric acid stimulation, the low-flow group had higher electrolyte concentrations than the high-flow group.

P50 **DIFFERENTIAL EFFECTS OF COOLING ON THE INTENSITY OF TASTE.** Sandra P. Frankmann and Barry G. Green. (Monell Chemical Senses Center, Philadelphia, PA 19104).

The research presented here addresses the question of how cooling both the taste solution and the tongue affects the taste intensity of caffeine, citric acid, sodium chloride (NaCl) and sucrose.

Subjects were asked to evaluate the intensity (magnitude estimation) and hedonic value (visual analog scale) of five concentrations of caffeine (0.0032 - 0.032 M), citric acid (0.001-0.01 M), NaCl (0.032- 0.32 M), and sucrose (0.056 - 0.56 M) when the temperature of both the tongue and the solution was 20°, 28°, and 36°C. Mouth temperature was controlled by rinsing repeatedly with water of the appropriate temperature, and a thermocouple was used to monitor the temperature of the tongue tip.

The data revealed that the intensity of sourness (citric acid) and saltiness (NaCl) were not affected by temperature. In contrast, the intensity of bitterness (caffeine) and sweetness (sucrose), were lower at 20° than at 28° or 36°C. The effect of temperature on hedonic ratings paralled that of the intensity ratings. Many subjects did not detect any bitterness in the weaker caffeine solutions when presented in the 20°C condition. In contrast all concentrations were perceived as bitter and were disliked in the 28° and 36°C condition.

This is the first report of a clear effect of the temperature of the tongue on taste perception. The data suggest that transduction mechanisms for citric acid and NaCl are very stable, whereas the transduction mechanisms for caffeine and sucrose are vulnerable to temperature. In addition, the results indicate that the taste of a complex mixture is likely to be affected by temperature of the mouth since the perception of some of the components will vary with temperature.

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P51 **CONTRIBUTIONS OF SMELL AND TASTE TO THE PLEASANTNESS OF FLAVOR.** Melvin P. Enns and David E. Hornung. (Depts. of Psychology and Biology, St. Lawrence University, Canton, NY 13617).

To determine how the pleasantness of smell and taste interact to produce the pleasantness of flavor, we had 20 undergraduate students estimate the degree of liking or disliking of smell, taste, and flavor stimuli. The odorants were three concentrations of ethyl butyrate (0.01, 0.04, 0.16% vol/vol), the tastants were three concentrations of sucrose (5.0, 10.0, 20.0% wt/vol), and the flavor stimuli were all combinations of the odorant/tastant mixtures. The degree of pleasantness was measured using the method of absolute magnitude estimation. Stimuli which were liked were given a positive sign, those disliked were given a negative sign and stimuli which were neither liked nor disliked were given a zero. The odorant stimuli were always presented to the external nares.

When both the smell and taste were given a positive rating, the flavor of the mixture was given a positive rating. Likewise, when both smell and taste were given a negative rating, the mixture was given a negative rating. In the condition in which the subject gave smell a positive rating and taste a negative rating or vice versa, the sign of the rating with the largest absolute hedonic value most often determined the sign of the rating of the flavor stimulus. When the hedonic ratings of smell and taste were subjectively equal but opposite in sign, the flavor mixture was usually given a negative rating. These data suggest that in determining the hedonic value of a flavor stimulus, the sensory component which is negative may receive a greater weight.

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P52 **A PSYCHOPHYSICAL-DECISION MODEL FOR SENSORY DIFFERENCE DETECTION METHODS.** Daniel M. Ennis (Philip Morris Research Center, Richmond, Va. 23261) and Kenneth Mullen (Department of Mathematics and Statistics, University of Guelph, Guelph, Ontario, Canada N1G2W1).

We recently developed multivariate psychological models for difference detection methods (Ennis and Mullen, 1985a and b) and discussed their relevance to sensory research. The mathematical form of the models has also been derived and evaluated (Mullen and Ennis, 1985.) These models were multivariate extensions of the Thurstone-Ura model (Frijters, 1979) for the triangular method.

We have now extended these ideas to include new assumptions about the decision rule invoked by a subject and the relationship between the stimulus continuum and the sensation continuum. Unlike the psychological models previously described in which the decision rule was based on euclidean distance comparisons, the new model also specifies ratio judgments, distance judgments or both. Using Stevens' power law, the assumed relationship between the stimulus continuum and the sensation continuum is specified. Using Monte Carlo simulation, the effect of the model parameters on the probability of a correct response with the triangular method has been determined for ratio and distance decision rules assuming unidimensional stimulus and sensation continua. This model opens up the possibility of obtaining new estimates of the power exponent for different modalities, if the decision rule is known, which could be compared with direct approaches to scaling sensation magnitude such as magnitude estimation or cross-modality matching.

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P53 **RATS EATING TOGETHER PREFER THE TASTE OF THEIR FOOD.** Heather J. Duncan, Audrey Buxbaum, and Michael G. Tordoff. (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104).

One way of assessing the reward potential of a stimulus is to measure preferences for foods paired with that stimulus. The validity of this technique has been shown by shifts in preference for neutral flavors paired with stimuli known to be rewarding in classical methodologies, such as sweet solutions or electrical brain stimulation. Here, we show that this "conditioned taste preference" method can be used to examine the complex stimulus of the presence of another rat, a situation that would be difficult to study by operant techniques.

Sixteen male rats were fed regular chow in their home cages and during the first 90 min of the dark period, fed flavored chow (0.8% chocolate or 0.8% chicken flavor) in training cages. Each rat always received one flavor in the company of another rat and the other flavor when alone. This procedure was conducted for 8 pairs of trials (16 days) according to a counterbalanced design. It was found that rats eating together consumed slightly but significantly less food than they did when eating alone (13% decrease). At the end of training, the rats were given a choice between the two flavors. In a 90-min test, 14 of 16 preferred the food they had previously eaten with a partner, and on average the group ate 232% more of this flavor than the other. Thus, food intake during training and food preference were dissociated.

The preference for food eaten with a partner was apparently a strong one. The rats were given free access to both flavors in their home cages for 11 days and throughout this period they maintained their initial preferences. They then received four pairs (8 days) of reversal trials (pairing the other flavor with a partner rat). In a final two-choice test, 10 of 16 rats reversed their preferences and the difference in intake of the two flavors was abolished.

In a second study using similar methods, 13 of 15 rats preferred the flavor eaten together after only one 90-min exposure to the eating-alone and eating-together conditions.

These results suggest that a) eating in the presence of another rat is a very rewarding and/or salient experience, b) food intake in the presence of a rewarding stimulus is not necessarily a good measure of food preference, and c) flavor preference shifts can provide a sensitive measure of reward in situations that are not easily approached by other means.

P54 Human Gustatory Judgments of Aqueous Square Wave Pulse Trains: Reaction Times and Magnitude Estimations. STEVEN T. KELLING, EDWARD SCHWARZCHILD (Dept. of Psychology) and BRUCE P. HALPERN (Dept. of Psychology and Section of Neurobiology and Behavior) (Cornell University, Ithaca NY 14853 USA).

GENERAL PROCEDURE Magnitude estimates of 2 mM NaSac total taste intensity, and their reaction times, were obtained from 5 subjects. The Kelling and Halpern (Chem.Senses: 11, 1986) closed flow liquid delivery apparatus was used. A throat microphone, connected to a digital timer, recorded the onset of each intensity judgment. Subjects judged taste intensity in proportion to a 2 mM NaSac modulus which was presented twice at the beginning of each session, and after every fifth trial. For each session, 2 control stimulus (distilled water) and 2 stimulus solution trials were run at 200, 600, 1000, and 2000 msec pulse train durations. Subjects participated in at least 1 practice and 5 data collecting sessions for each experiment. The flow rate for EXP 1, 2, and 3 was 10 ml/sec; EXP 4, 5 ml/sec. In EXP 1, the modulus stimulus was a 2000 msec, 5 hz pulse train; in all other experiments, a 2000 msec continuous flow. All stimulus presentations were 5 hz pulse trains, except for EXP 3 which was 10 hz. RESULTS Judged taste intensity increased as pulse train duration increased. Across pulse train durations, judged intensity was lowest in EXP 4 (5ml/sec; continuous modulus); highest in EXP 1 (10 ml/sec; 5 Hz modulus). No difference in judged intensity occurred between EXP 2 (5 Hz; continuous modulus) and EXP 3 (10 Hz; continuous modulus) except at 200 msec pulse trains. Intensity reaction times ranged from 1310 msec to 1845 msec. Reaction times were faster to 200 msec than to 1000 or 2000 msec pulse trains. There were no differences in the intensity reaction times between experiments, except responses to the 2000 msec, 5 ml/sec pulse trains in EXP 4. CONCLUSIONS Processing of gustatory intensity information is cumulative over time, with no major difference between 5 Hz and 10 Hz pulse trains. Pulse trains yield less gustatory intensity than continuous flow. Intensity reaction times for pulse trains are equivalent to reaction times for continuous flow presentations (Chem.Senses: 10; 456, 1986).

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P55 RELATIONSHIPS BETWEEN ATTITUDES AND HEDONIC RESPONSES TO SWEET, SALTY AND FATTY STIMULI. Hely Tuorila-Ollikainen (Dept. Food Chemistry and Technology, University of Helsinki, SF-00710 Helsinki, Finland)

It is generally accepted that a decrease in the sweet, salty and fatty components of most western diets would improve their nutritional quality. However, obstacles to the reduction of these components seem to be presented by their hedonic value to consumers. In the present series of studies, the relationships between hedonic responses and verbal attitudes, as well as some other behavioral parameters, were investigated. Attitudes were measured either by the Likert technique[1] or by using the Fishbein model of reasoned action[2,3] as a frame of reference.

Sweetness[1] Subjects (112 males, 112 females) rated the pleasantness of sweetness in soft drinks containing 9% (normal) and 5% (lowered) sucrose. Sugar attitudes correlated only with hedonic responses to the lowered sweetness (r = 0.23 for males, r = 0.20 for females). Females had more negative attitudes towards sugar but they liked sweet foods more than the males, who were thus more consistent in their hedonic/attitude responses. Saltiness[2] Subjects (N = 61) consumed normal or low (half of the normal) salt breads at home during an eight-week period. Their hedonic responses, attitudes, norms and intentions were measured before and after this period. Hedonic responses to low salt breads varied with the type of bread, being most favorable to sour rye and least favorable to white bread. On the basis of regression analysis, hedonic responses were better predictors of the selections than were the attitudes and norms. Attitudes were correlated with hedonic responses (r = 0.36). Fattiness[3] Subjects (N = 236) who used non-fat (0%), low-fat (1.9%) and regular fat (3.9%) milks as their principal milk sources participated in hedonic tests and in the measurement of attitudes and related parameters. The subjects strongly preferred their own milk type in hedonic tests, in survey ratings of liking and in their attitudes. Attitudes and hedonic responses were correlated (r = 0.60, 0.20, 0.43 for non-fat, low-fat and regular fat milks, respectively).

It is concluded that verbal attitudes are closely related to hedonic responses and that both contribute markedly to actual behavior. However, verbal attitudes represent a general predisposition which does not necessarily become activated when familiar, habitually consumed stimuli are presented. Thus, habit forms a powerful protection against any changes.

[1]Tuorila-Ollikainen H, Mahlamäki-Kultanen S. Appetite 1985,6,115-24. [2]Tuorila-Ollikainen H, Lähteenmäki L, Salovaara H. Appetite (in press). [3]Tuorila-Ollikainen H. Appetite (submitted).

P56 A FULLY AUTOMATIC DEVICE FOR TASTE STIMULATION, DATA ACQUISATION AND DATA PROCESSING.
T. Roberts, C. Hård af Segerstad, G. Hellekant, (Dept. Veterinary Science, University of Wisconsin, Madison WI 53706)

Gustatory stimulation and recording of the results in a clinical setting put demands on the equipment which in the laboratory can be taken less seriously. Thus for example errors such as confusion of one solution with another which in the laboratory may be of minor importance will have a major impact in a setting where time or other constrains prevail. This poster will demonstrate our latest gustatory stimulator which can be set up in a surgery room or any other temporary area within a short time period, can deliver 24 different temperatur controlled stimuli in any order and time period, and interfaces with recorder and IBM computer in such a way that confusion of stimulus or stimulation time is impossible. The poster will also present on-line processing of the nerve activity.

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P57 **RELATIONSHIPS BETWEEN SELECTED MEASURES OF SWEET TASTE PREFERENCE AND DIETARY HABITS.** D.J. Mela & R.D. Mattes (Monell Chemical Senses Center, Philadelphia PA)

Previous studies from this laboratory[1,2] have failed to elucidate any clear relationships between measures of gustatory function (threshold sensitivity, suprathreshold intensity ratings, preference) and dietary intakes. However, measures of taste preference appeared to have the greatest potential in this regard. In the present study, we hypothesized that a profile developed from an array of sweet taste preference procedures, using different rating scales, food systems, and conntexts might increase our ability to detect diet-taste relationships.

Preference measures included 1) questionnaires assessing liking and frequency of consumption of selected sweet foods, 2) "adjustment tasks" wherein subjects modified beverage samples to their optimal preferred sweetness levels, and 3) ratings of prepared oatmeal and coffee samples having a range of sweetener levels. The prepared samples were assessed under several conditions: expectorated and swallowed, rated on a visual scale and a verbal pleasantness scale, and in the context of either a prepared "food" or as an isolated laboratory "sample". Dietary records were collected throughout the week of sensory testing and, in addition to food descriptions and portion estimates, subjects recorded the perceived predominant taste of each food consumed.

Variations in conditions of evaluation of the prepared samples had little effect on preferred levels of sweetness and these were significantly correlated with results from the adjustment tasks. Expectoration of samples was found to generate much more repeatable results than swallowing them.

Correlations between single taste preference measures and intake variables demonstrated no consistent relationships. The various taste preference measures were loaded into discriminant analysis functions to establish whether they could be used to classify subjects into upper and lower tertiles for selected intake measures. Initial analyses demonstrated no particular advantage of any one methodological approach, but from these analyses, "best predictor" discriminant functions were developed. Taste measures yielded significant discriminant functions for percent carbohydrate calories ingested (canonical correlation = .82, 100% of subjects correctly classified), percent sweet calories consumed (.80, 94%), and percent sweet food items (.81, 100%).

These results demonstrate that sweet taste preference profiles may be predictive of dietary habits related to sweet food and carbohydrate intake. Whether such relationships exist for other tastes and intake measures will be the focus of future studies.

[1]Mattes, R.D. Am J Clin Nutr 41:672, 1985.
[2]Mattes, R.D. AChemS VII Abstract #126, 1985.

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P58 PERCEPTUAL AND INTRAORAL pH MEASUREMENTS IN RESPONSE TO ORAL STIMULATION. D. B. Kurtz, J. C. Walker, J. H. Reynolds, and D. L. Roberts (BGTC, R. J. Reynolds Tobacco Company, Winston-Salem, NC 27102) and S. L. Yankell (University of Pennsylvania, School of Dental Medicine, 4001 Spruce Street, Philadelphia, PA 19104 and Integrated Ionics, Inc., 2235 State Route 130, Dayton, NJ 08810).

Intraoral pH has been measured in humans using sensors mounted on Hawley appliances (IADR Abstr. #540, 1985). In the present study we have combined this measurement with the recording of perceptual ratings and the measurement of puffing behavior to study orosensory stimulation during smoking. In addition, the responses to simple aqueous tastant solutions, presented in a "sip and spit" fashion, were also recorded. Three electrodes mounted on a Hawley appliance, positioned on the maxilla, were used to measure changes in salivary pH in response to oral stimulation.

Control experiments demonstrated that the appliance itself did not alter perceptual ratings of either cigarettes or simple taste stimuli or puffing behavior on cigarettes. Each puff caused a rapid increase in pH toward neutrality which we attributed to the evaporative cooling of saliva by air brought into the mouth. The rapid increase in pH was followed by a slower partial return to the pH just prior to the puff. Subsequent puffs brought the salivary pH progressively closer to neutrality. The magnitude of the pH change was inversely related to starting oral pH and directly related to FTC "Tar" content. That is, large pH changes toward neutrality were related to an acid starting condition in the mouth and puffs on a high "Tar" cigarette. Unlit or low "Tar" cigarettes caused only small changes toward neutrality.

Solutions of sodium cloride or sucrose caused little change in oral pH while solutions of acids (phosphoric, acetic and citric) caused large acidic excursions. These excursions peaked at approximately the pH of the acid solution by the end of the 5 second sip. Recovery of oral pH to the pre-acid condition was relatively slow, being only 50% complete after expectoration and a 1 minute wait. A distilled water rinse and an additional 1 minute wait failed to return oral pH to the pre-stimulus condition.

This method of monitoring salivary pH has the benefit that pH can be measured on a continuous basis. In addition, because of its unobtrusiveness, it serves as a valuable complement to psychophysical techniques.

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P59 A CHANGING DENSITY TECHNIQUE TO MEASURE NASAL AIRFLOW PATTERNS. D. Leopold, D. Hornung, R. Richardson, P. Kent, M. Mozell, and S. Youngentob. Depts. of Otolaryngology, Physiology, and Radiology, S.U.N.Y. Health Science Center at Syracuse, N.Y. 13210

To study the relationship between nasal airflow and olfactory function, we have developed a radiation detection technique which quantifies the airflow patterns through an anatomically correct model of the human nasal passageways. A collimated gamma ray source (200 millicuries of iodine-125) is placed on one side of the model and a specially designed collimated detector is placed on the other. Because of the collimation, only a small region of the model is viewed at any one time. The detector output is fed into a 1024 Multi-Channel Scaler such that each succeeding channel totals the output for each succeeding 0.4 msec. interval. Our technique to measure airflow patterns takes advantage of the fact that the number of gamma rays received per second by the detector is dependent upon the density of the gas inside the nasal model. Since xenon gas is much denser than air, it will absorb more gamma rays, and the detector will receive fewer photons. The model is first completely filled with xenon gas. Then, room air is drawn in at a controlled flow rate through the external naris and into the model. As the xenon gas is removed from the model, the absorption of the gamma rays is reduced, and the number of gamma rays received by the detector increases. The rate at which the count rate increases is a measure of the flow rate through the particular portion of the model currently being studied. To obtain an adequate number of counts, the technique is repeated multiple times at each position.

The results show that increasing the flow through the model also increases the flow through each particular nasal region evaluated. However, increasing the model flow beyond 15 1/min does not increase the flow through all nasal areas. One explanation for these observations is that at flows greater than 15 1/min, turbulence may be created in at least some nasal areas. Because of the reproducibility of the data, our technique seems to allow for the accurate measurement of the complex and dynamic nasal airflow events that occur during respiration and sniffing. Also, the technique using different density gases will be more applicable to human studies than the previously described radioactive xenon technique.

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P60 SIGNAL-TO-NOISE RATIOS AND A COMPARISON OF CUMULATIVE SELF-ADAPTATION OF TASTE AND SMELL RECEPTOR CELLS. Rainer Voigt and Jelle Atema. (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

The smell and taste receptor organs of the American lobster, Homarus americanus, are composed of largely narrowly tuned populations of receptor cells. Under natural conditions these receptor cells have to detect varying stimulus concentrations against a noisy background. Cell sensitivities change constantly due to 1) self-adaptation to a constant background 2) cumulative self-adaptation to repeated stimulus pulses, and 3) cross-adaptation due to mixture suppression. We have started to characterize adaptation and disadaptation time courses of receptor cells using series of standard 1s stimulus pulses in different backgrounds, and varying the interpulse intervals from 5s to 20s.

The temporal stimulus profile was calibrated by measuring the change of conductivity of flowing deionized water (20 ml/min) after injection of 50 ul of 1M NaCl solution. The stimulus chambers allowed 5s interpulse intervals without interference of the previous stimulus.

Single cells were identified with low stimulus concentration. A series of five pulses was applied in 5s, 10s or 20s intervals in a low stimulus background (10^{-7}M). After 3min of self-adaptation to an elevated backgound (10^{-5}M) the series was repeated.

While stimulation in intervals longer then 20s caused only slight variability in their responses (both in number of spikes elicited and in duration of the response) shorter interstimulus intervals caused cumulative adaptation. Exposure to higher backgrounds reduced the absolute sensitivity; repetitive stimulation caused no further decrease in responsiveness. At two different background levels the same signal-to-noise ratio resulted in similar responses, including similar cumulative adaptation. The greater signal-to-noise ratio caused stronger responses and showed greater cumulative adaptation while the smaller ratio caused weaker responses and less adaptation.

Thus, signal-to-noise ratios and not absolute stimulus levels were predictive of the responses of receptor cells in different backgrounds.

Supported by NSF grant BNS 8512585

P61 INHIBITORY EFFECTS OF Ca^{2+} ON THE Mg^{2+} RESPONSE OF WATER FIBERS IN THE FROG GLOSSOPHARYNGEAL NERVE. Yasuyuki Kitada. (Dept. Physiology, Okayama University Dental School, Okayama 700, Japan).

In the frog glossopharyngeal nerve, single water fibers excited by distilled water application to the tongue respond to $CaCl_2$ and $MgCl_2$. However, the impluses elicited by a mixture solution of $CaCl_2$ and $MgCl_2$ were much lower in frequency than those elicited by each plain salt. This suggests that Ca^{2+} and Mg^{2+} have antagonistic action on each other. The inhibition of the Ca^{2+} response by Mg^{2+} has been demonstrated (Kitada, 1978; Kitada & Shimada, 1980). In the present study, the inhibition of the Mg^{2+} response by Ca^{2+} was quantitatively studied.

Unitary discharges were recorded from single water fibers of the frog glossopharyngeal nerve during stimulation of the tongue with salt solutions. Threshold concentrations for $CaCl_2$ and $MgCl_2$ were around 0.0001 mM and 10 mM, respectively. The impulse frequency elicited by $MgCl_2$ decreased with increase in concentrations of $CaCl_2$ added to the $MgCl_2$ stimulating solution. For example, the response ratio for the mixture of 100 mM $MgCl_2$ + 5 mM $CaCl_2$ relative to plain 100 mM $MgCl_2$ was around 0.1. Under conditions of low Ca^{2+} and relatively high Mg^{2+}in the stimulating solution, the excitatory effects of Ca^{2+} could not be exerted since Mg^{2+} strongly inhibits the Ca^{2+} response (Kitada, 1978). NaCl and choline-Cl had no effect on the Mg^{2+} response. It is concluded that Ca^{2+} inhibits the Mg^{2+} response.

Dose-response curves for $MgCl_2$ at different $CaCl_2$ concentrations were obtained. To explain the inhibition of the Mg^{2+} response by Ca^{2+}, it is assumed that Mg^{2+} combines with a receptor site (X) responsible for the Mg^{2+} response, and that Ca^{2+} competes with Mg^{2+} for X by forming an inactive Ca-X complex. The assumption of competitive inhibition by Ca^{2+} is supported by a double-reciprocal plot analysis of the data. The apparent dissociation constants for Mg-X and Ca-X were 7.0×10^{-2} M and 6.3×10^{-4} M, respectively.

The mutual antagonism between Ca^{2+} and Mg^{2+} indicates that the receptor site responsible for the Mg^{2+} response differs from that for the Ca^{2+} response.

P62 TASTE RESPONSES OF THE CROSS-REGENERATED GREATER SUPERFICIAL PETROSAL (GSP) AND CHORDA TYMPANI (CT) NERVES OF THE RAT. Mohssen S. Nejad and Lloyd M. Beidler. (Dept. of Biological Science. The Florida State University, Tallahassee, FL 32306).

The rat GSP nerve, contrary to the CT, is highly responsive to sucrose and reverse is true for NaCl. We wanted to know how cross-regeneration would affect the gustatory afferent neural responses of the GSP and CT nerves in the rat. In two groups of male Sprague-Dawley rats cross-union-anastomoses between the GSP and CT nerves in the middle ear were unilaterally made in such manner that: in group I (n=6), GSP nerve grew into the front of the tongue and in group II (n=5), CT nerve grew into the palatal regions of the oral cavity. After 16-24 weeks, integrated neural responses were recorded from the regenerated nerves in responses to several tastants (0.1M chloride salts of Na^+, Li^+, K^+, $NH4^+$, Ca^{++}, 0.5M sucrose, 0.01M quinine-HCl, 0.05M citric acid and 0.02M Na-saccharin). In addition, response-concentration functions for NaCl and sucrose were also measured. The response from the experimental nerves, control nerves (contralateral) and nerves of normal animals were compared. Electrophysiological and histological studies suggested that after cross-union-regeneration of the GSP and CT nerves, the palatal and anterior tongue taste buds (ipsilateral) were reformed and were functional. The integrated neural response profile of the regenerated GSP into the anterior part of the tongue resembled that of the normal CT nerve. The integrated neural response profile of the regenerated CT into the palatal regions resembled that of the normal GSP nerve. These findings are in concordance with the CT and IX nerve cross-regeneration studies by B. Oakley ('67). Afferent neural activities of the cross-regenerated GSP and CT appeared to be influenced by their new peripheral gustatory receptor populations. Apparently, central neural influence did not maintain the original afferent neural profiles of the two contrasting GSP and CT nerves in response to the tested chemicals. Quantitative analysis of the response-concentration functions of sucrose and NaCl showed that in both cross-regenerated GSP and CT nerves, the binding strengths remained the same as control nerves, whereas the maximum response declined. It was inferred that the number of receptor sites in fields of the cross-regenerated nerves were less than those of the normal nerves.

P63 THE EFFECTS OF SESQUITERPENE DIALDEHYDES ON THE STYLOCONIC TASTE CELLS OF THE TOBACCO HORNWORM LARVA. Dr. James L. Frazier. (E.I.du Pont, Wilmington, DE. 19898).

The sesquiterpene dialdehydes are a potent class of insect antifeedants (Koul, 1982). One of the most potent members of this class is warburganal, which was shown by Ma (1977) to reduce the firing of taste cells in the African armyworm larva, and was proposed to involve membrane sulfhydryl groups in its action.

Glucose and inositol sensitive taste cells in both the lateral and medial styloconica of the tobacco hornworm larva are blocked by warburganal treatment in a time and concentration dependent manner. Treatment of the medial inositol sensitive cell with saturating concentrations of inositol prevents the blocking by warburganal, indicating that it is binding at or overlapping with the receptor site for inositol. The sulfhydryl specific reagent PMB produces similar effects, while the amino-specific reagent 2,4,6-trinito benzene sulfonic acid does not. The blocking action of both warburganal and PMB reverses spontaneously for the inositol site, but not for the glucose site. Treatment with cystiene accelerates the recovery from blocking for both PMB and warburganal. These results indicate that membrane sulfhydryl groups, but not amino groups in the immediate vicinity of the receptor site are involved in the action of warburganal.

A separate effect of warburganal is to reduce the size of spikes produced by inositol, glucose, and salt sensitive cells. This action results from warburganal acting at some site distinct from the receptor, but has yet to be determined. Warburganal did not increase the firing of any cell in a dose-dependent manner, but after ca. 2.5 minutes of continuous treatment, irregular bursting was seen from more than one cell in the medial styloconica.

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P64 INTRACELLULAR RECORDING OF THE RECEPTOR POTENTIAL IN PRIMARY CHEMOSENSORY NEURONS. Ingrid Schmiedel-Jakob, Peter A. V. Anderson, and B. W. Ache. (C. V. Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086).

The olfactory organ (antennule) of the spiny lobster consists of tufts of hair-like sensilla (aesthetascs) which are innervated by bipolar sensory neurons. These neurons were exposed in a hemicylindric preparation of the antennule and treated enzymatically with papain and trypsin. Intracellular recordings were obtained from these cells using patch pipettes in the whole cell configuration. The exposed neurons retain both their electrical and chemical excitability. The input impedances for hyperpolarizing current pulses are in the range 160 to 220 Mohm; with depolarizing current steps this falls to 80 Mohm. Depolarizing steps to above -30 to -40 mV evoke fast action potentials which overshoot zero by as much as + 33.3 mV. The rising phase of the action potential has a maximum slope of 108 V/s; its repolarizing phase 81 V/s. The duration of the action potential at half-peak amplitude is 1.37 s. When stimulated chemically by an extract of crab muscle, these cells produce prolonged, transient depolarizations on which are superimposed a train of TTX- sensitive action potentials which do not necessarily overshoot zero mV. Chemically evoked depolarizations are dose-dependent and their amplitude is linearly related to the logarithm of the stimulus concentration. The depolarizations reach their peak amplitude in 96 - 300 ms and decline over 5- 31 s, depending on the strength of the stimulus. The magnitude of the receptor potential, expressed as its duration, is found to be a linear function of the number of evoked spikes. The amplitude of the receptor potential is dependent on the membrane potential of the cell and extrapolates to a mean reversal of -22.3 + 7.3 (SE) mV. All cells tested have similar extrapolated reversal potentials, but actual reversal was never observed, even when these cells were depolarized to +50 mV. While these reversals may not accurately reflect the true reversal potential, they should provide useful information about the ionic basis of the transduction process in chemosensory neurons.

Supported by NSF Award BNS-85-11256 and a grant from the Whitehall Foundation.

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P65 GUSTATORY RESPONSES TO TETRODOTOXIN AND SAXITOXIN IN RAINBOW TROUT AND ARCTIC CHAR: A POSSIBLE BIOLOGICAL DEFENSE MECHANISM. Kunio Yamamori*, Moritaka Nakamura*, and Toshiaki J. Hara. (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Canada R3T 2N6; *Kitasato University School of Fisheries Sciences Sanriku, Iwate 022-01, Japan).

Pufferfish toxin, tetrodotoxin (TTX) is a potent neurotoxin, with its lethal toxicity to humans 300 times that of KCN. TTX exerts its toxic action by specifically blocking the voltage-sensitive sodium channels in nerve and muscle membranes. TTX is widely distributed in tetraodontid fishes, but its distribution seems much wider than originally thought. Although much information is available on the mechanism of its pharmacological action, little is known about its biological significance. Our recent behavioral studies demonstrated that some fish species avoid foods containing TTX, suggesting that fish may be able to detect TTX via the gustatory system. The present study was designed to describe the gustatory sensitivity of rainbow trout and Arctic char by measuring the integrated electrical responses from the palatine nerve innervating the palate and inside the upper lip. A shellfish toxin, saxitoxin (STX), which has similar pharmacological actions, was also tested.

The gustatory receptors of rainbow trout were extremely sensitive to TTX; it had a threshold concentration of 10^{-7}M and at 10^{-5} M evoked a response three times that of 10^{-3}M L-proline, the most effective amino acid for this species. The threshold for STX was lower, 10^{-8}M, but unlike TTX the response magnitude reached a maximum at 10^{-6}M and saturation occurred with further increase in concentration. This situation was totally reversed in Arctic char; lower thresholds for TTX than STX, 10^{-8} and 10^{-7}M, respectively. The response magnitude was generally small, never exceeded that of 10^{-3}M L-proline at all concentrations tested. Cross-adaptation experiments indicated that the receptor(s) for TTX are distinct from those which detect amino acids and bile salts, and that TTX and STX react with further different receptor groups. The integrated response to TTX or STX, a fast-adapting, phasic response, rapidly returned to baseline even with continuous stimulation. Perfusion of the gustatory organs with these chemicals had no toxic effect.

These findings indicate the existence of sensitive, specific gustatory receptor system for TTX and STX in fishes, suggesting a defense mechanism for poisonous preys in the aquatic environment.

P66 TASTE RESPONSIVENESS OF HAMSTER GLOSSOPHARYNGEAL NERVE FIBERS. Takamitsu Hanamori, Inglis J. Miller, Jr. and David V. Smith (Dept. Otolaryngology and Maxillofacial Surgery, University of Cincinnati Medical Center, Cincinnati OH 45267, and Dept. Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103).

Responses were recorded to five concentrations each of four stimuli from 83 taste-sensitive fibers in the hamster glossopharyngeal (GL) nerve. Stimuli were delivered to either the vallate or foliate papillae via a syringe pump at 0.1 ml/sec at a temperature of 37°C. Stimuli, presented in ascending order, were: 0.01 - 1.0 M sucrose, 0.01 - 1.0 M NaCl, 0.0003 - 0.03 M HCl, and 0.0003 - 0.03 M quinine-HCl (QHCl), each in 1/2 log steps of concentration. Responses of each fiber were characterized as the number of impulses in the first 10 sec after stimulus onset, corrected for the immediately preceding rate of spontaneous activity. Concentration-response functions were derived for each fiber and there were no differences noted between those fibers innervating the vallate and foliate papillae. Cells were classified into best-stimulus groups on the basis of the area under the concentration-response function. This resulted in a more stable classification than that based on only a single concentration of each stimulus. Of the 83 fibers, 8 (10%) were sucrose-best, 4 (5%) were NaCl-best, 52 (62%) were HCl-best, and 19 (23%) were QHCl-best. This distribution of sensitivities is quite different than that seen in the chorda tympani (CT) nerve (Frank, 1973), where 25% of the fibers were sucrose-best, 53% were NaCl-best, 21% were HCl-best, and 1% were QHCl-best. At the same midrange concentrations used to classify CT fibers, GL fibers are quite narrowly tuned, although they are much less responsive than CT fibers at these concentrations. The breadth of excitatory responsiveness was calculated using the equation developed by Smith & Travers (1979). The mean breadth of tuning of GL fibers was 0.470, compared to 0.608 in CT fibers stimulated with the same concentrations. However, when the responsiveness of the cells to the entire concentration range is used to measure their breadth of sensitivity, GL fibers are less specifically tuned, showing a mean breadth of tuning of 0.706. The characterization of the response properties of gustatory cells can be improved by obtaining responses over a wide range of stimulus intensities.

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P67 NEURAL AND BEHAVIORAL TASTE RESPONSES TO AMINO ACIDS IN MOUSE AND RAT. Shuitsu Harada, Takayuki Marui and Yasuo Kasahara. (Dept. of Oral Physiology, Kagoshima University Dental School, Usuki-Chyo, Kagoshima 890, Japan)

Gustatory responses to amino acids were examined by using electrophysiological and behavioral methods in the mouse and rat. Electrophysiological experiments have revealed that amino acids are classified into at least three groups according to the characteristics of the responses from chorda tympani in both species; i.e., basic (BA), neutral (NA) and acidic amino acids (AA).

The chorda tympani responses to both L- and D- BA HCl salts (BA-HCl) in each animal were quite similar to those for NaCl, and cross-adapted well with those for monovalent chloride salts but not with NA or sucrose. A few structural analogues of L-Arg and L-Lys were tested, to show that the -amino group is essential for the strong stimulative effectiveness of BA-HCl although the BA of free base form are less stimulatory than the corresponding HCl-salts, larger responses similar to those for NaCl were elicited when the pH of the solutions was lowered. A similar effect of lowering the pH was also observed for NA and other substances which have more than one amino group. These results suggest that the charged amino group in the organic substances plays an important role in their strong stimulatory effect. The relative stimulatory effectiveness of L-NA was larger in mice than in rats, and that for L-isomers was significantly larger than D-isomers in both species. Responses to these NA cross-adapt with sucrose, but not with NaCl or BA. The response characteristics of AA were similar to that for HCl.

In order to clarify the taste sensation for amino acids in these species, one effective method of taste aversion conditioning was employed, and the behavioral ability of discrimination of each amino acid was examined. The results showed that the taste of BA-HCl is generalized to quinine-HCl rather than to NaCl. The behavioral data are consistent with those obtained from humans although some discrepancy exist in the comparative electrophysiological data. It is suggested that NA which produces sweet taste in man may produce a similar sensation as sucrose in mice.

P68 RESPONSE PROPERTIES OF THALAMOCORTICAL RELAY NEURONS RESPONSIVE TO NATURAL STIMULATION OF THE ORAL CAVITY IN RATS. Hisashi Ogawa and Tomokiyo Nomura (Dept. Physiology, Kumamoto University Medical School, Honjo 2-2-1, Kumamoto 860, Japan).

The caudal parabrachial nucleus, the second taste relay nucleus, sends afferents to the parvicellular part of the ventral posteromedial nucleus (VPMpc) and the parafascicular nucleus (PF) in the thalamus (Norgren & Leonard 1974), which in turn send afferents to the cortical taste area (CTA). Since taste neurons have been recorded in these two neurons (Nomura & Ogawa 1985), in the present study we aimed to examine the response properties of thalamocortical relay (TC) neurons responsive to taste and mechanical stimulation of the oral cavity in these two nuclei in rats.

After SD-strain rats were anesthetized with amobarbital Na, they were mounted on a usual stereotaxic instrument. Using glass micropipettes, filled with 2 % Pontamine sky blue in 0.5 M Na acetate, we extracellularly recorded single unit activities from the soma of VPMpc and PF neurons, while applying taste and mechanical stimulation to the whole oral cavity. To identify the TC neurons, 3-10 monopolar metal electrodes were placed ipsilaterally at the CTA and cathodal currents (max. 400 uA; 0.02 ms in duration) were to them. PST histograms were constructed with the aid of a microcomputer.

About one-third of the VPMpc neurons recorded were TC neurons, activated antidromically in a latency of 1-4 ms, and most of them were taste neurons (cf. Ganchrow & Erickson 1972). Another one-third were excited orthodromically in a latency of 2- 18 ms. On the other hand, only a few PF neurons were TC neurons and most of the PF neurons were orthodromically activated from the CTA. Among non-TC neurons in the two nuclei, many tactile neurons were included. After the short latency excitation, both types of the neurons in the VPMpc and PF showed an inhibitory period of 150 to 200 ms, followed by repeated rebound facilitations in the PST histogram. The inhibitory period was longer in the present study than reported previously (Yamamoto et al 1980). The sequence of responses as well as the length of the inhibitory period was similar to those found in other sensory systems (Andersen et al 1963). No neuron were found to produce sustaining discharges during the inhibitory period of the above mentioned neurons. The VPMpc neurons produced rather tonic responses of large magnitudes to taste stimulation in comparison with phasic and small taste responses of the PF neurons.

The present findings revealed that the VPMpc sends to the CTA the static nature of intensity and quality of stimulation, while that the PF sends information on the changes in stimulations. No interneuron was found in both the VPMpc and PF.

P69 GUSTATORY, THERMAL AND MECHANICAL RESPONSES OF CELLS IN THE NUCLEUS TRACTUS SOLITARIUS OF THE FROG. Nobusada Ishiko, Takamitsu Hanamori and David V. Smith (Dept. Physiology, Miyazaki Medical College, Miyazaki, 889-16, Japan, and Dept. Otolaryngology and Maxillofacial Surgery, Univ. Cincinnati Medical Center, Cincinnati Ohio, 45267)

The responses of 216 neurons in the nucleus tractus solitarius (NTS) of the American bullfrog (Rana catesbeiana) were recorded following taste, temperature, and tactile stimulation of the tongue. Taste stimuli were: 0.5 M NaCl, 0.5 M quinine-HCl (QHCl) 0.01 M acetic acid, 0.5 M sucrose, each dissolved in 0.01M NaCl, and deionized H_2O. These were delivered to the tongue via gravity flow, as was 30°C 0.01 M NaCl for temperature stimulation. Tactile stimuli were delivered to the tongue and body surface using a sma-1 brush. Cells were clearly classifiable on the basis of their responses to these stimuli. Neurons showing excitatory responses to 1, 2, 3, or 4 of the 5 kinds of taste stimule were named Type I, II, III, or IV, respectively. Cells whose spontaneous rate was inhibited by taste and/or tactile stimulation of the tongue were termed Type V. Type VI neurons were excited by tactile stimulation alone. Of the 216 cells, 115 were excited or inhibited by taste stimuli (Types I-V), with 35 being Type I, 34 Type II, 40 Type III, 2 Type IV, and 4 Type V. The remaining 101 cells were responsive only to tactile stimulation (Type VI). Of those 111 cells excited by taste stimulation (Types I-IV), 106 (95%) responded to NaCl, 66 (59%) to acetic acid, 44 (40%) to QHCl, 10 (9%) to H_2O, and 9 (8%) to warming. No cells responded to 0.5 M sucrose. Of the 111 cells of Types I-IV, 76 (68%) were also sensitive to mechanical stimulation of the tongue. There was a significant correlation ($p < .001$) across cells among the responses to QHCl, acetic acid and warming and between the responses to NaCl and acetic acid. The responses to NaCl, acetic acid and touch were also significantly correlated ($p < .01$) with one another. There was some differential distribution of these nuron types within the NTS, with more narrowly tuned cells (Type I) being located more dorsally in the nucleus than the more broadly tuned (Type III) neurons ($p < .01$). Cells responding exclusively to touch (Type VI) were also significantly ($p < .01$) more dorsally situated than those responding to two or more taste stimuli (Types II and III).

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P70 TASTE AND TACTILE RESPONSES IN THE SUPERIOR SECONDARY GUSTATORY NUCLEUS OF THE CATFISH. C.F. Lamb IV and J. Caprio. (Dept. Zool. and Physiol., Louisiana State Univ., Baton Rouge LA 70803-1725).

The superior secondary gustatory nucleus (nGS) of teleosts receives second-order neurons from the primary medullary taste centers, the facial (FL) and vagal (VL) lobes (Herrick, 1905). Electrophysiological studies indicate that peripheral input into the FL (Marui and Caprio, 1982) and VL (Kanwal and Caprio, 1984) is represented in a somatotopic and viscerotopic manner, respectively. FL and VL efferents remain segregated in the ascending secondary gustatory tract and terminate in separate regions of the nGS (Finger, 1978; Morita et al., 1980). We studied the electrophysiological responses of nGS neurons to mechanical and chemical stimulation of their peripheral receptive fields (RF) to determine if the nGS maintained a topographical arrangement. A mixture of three amino acids, L-alanine, L-arginine, and L-proline (10^{-4} M each) was used for chemical stimulation.

Units responding phasically to mechanical stimulation of oral and extra-oral RFs were scattered throughout the nGS, but were more concentrated in the rostral half of the nucleus. Generally, these units responded to both oral and extra-oral stimulation. Most units had RFs greater than 100 mm^2, with many covering the whole body surface. Restricted RFs were limited to the ipsilateral maxillary or mandibular barbels. Except for the rostral-most region, most of the nGS appeared to lack a topographical arrangement. Units in the rostromedial nGS had RFs covering the head, lips, and barbels. RFs became more restricted laterally, until they included only the ipsilateral maxillary barbel in the rostrolateral region. Units responding to the amino acid mixture were located in the central portion of the mechanosensitive areas of the nucleus. These units were encountered much less frequently than were mechanosensitive units and also did not show a topographical arrangement. Chemosensitive units were bimodal, with similar RFs for chemical and tactile stimulation. The RFs of bimodal units included either the barbels and head or the oral cavity. These results suggest that the precise topographical segregation of sensory input to the medullary taste centers is not conveyed to the nGS.

Supported by NIH NS14819.

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P71-A MULTIMODAL NEURONS IN THE LAMB SOLITARY NUCLEUS: RESPONSES TO CHEMICAL, TACTILE AND THERMAL STIMULATION OF THE CAUDAL ORAL CAVITY AND EPIGLOTTIS. Robert D. Sweazey and Robert M. Bradley. (Dept. Oral Biology, School of Dentistry, The University of Michigan, Ann Arbor, MI 48109).

Using horseradish peroxidase histochemistry, we have shown previously that the primary afferents innervating receptors in the caudal oral cavity and upper airway terminate in overlapping areas of the lamb nucleus of the solitary tract (NST). We have now recorded responses from neurons in the lamb NST to chemical, tactile and thermal stimulation of the palate, caudal tongue and epiglottis.

Neurons which responded to stimulation of at least one of the receptive fields were located in the NST from 2.5 to 5.0 mm rostral to obex ($\bar{x}$=3.79), 3.0 to 4.5 mm lateral to the midline ($\bar{x}$=3.64) and 1.4 to 4.2 mm ventral to the brainstem surface ($\bar{x}$=2.86). Of a total 26 neurons, 15% received input from more than one of the stimulated areas. Most of these cells received converging information from the palate and caudal tongue: the receptive field on the palate having a directly opposing receptive field on the tongue. Only a few cells received information from both the oral cavity and epiglottis. Converging information from all three receptive fields has been observed in the reticular formation just ventral to the NST, but not in the NST itself.

In general, neurons in the NST responded to more than one stimulus modality. Of the 26 isolated cells, 27% responded to 1, 54% responded to 2 and 19% responded to all 3 of the stimulus modalities. Tactile stimulation was the most effeative stimulus, eliciting responses in 80% of the neurons with receptive fields in the oral cavity and 87% of the neurons with receptive fields on the epiglottis. Chemical stimuli were less effective evoking responses in 65% of the isolated neurons. We have found that the most effective stimulus for a chemosensitive cell in the NST was dependent upon the location of the receptive field. In neurons with tongue receptive fields the order of effectiveness for chemical stimuli was NH_4Cl > KCl > HCl > NaCl. The order of effectiveness seen for cells responding to stimulation of the epiglottis with chemicals was KCl > NH_4Cl > distilled water = HCl. Thermal stimuli were the least effective, eliciting responses in 42% of the NST neurons. Of 11 thermal-responsive neurons, 9 respond to cooling, 2 to warming.

The presence of large numbers of multimodal cells suggests that the region of the NST investigated in the present study is important in the integration of afferent information produced by complex stimuli in the caudal oral cavity and upper airway. Furthermore, the information provided by these neurons may be important in the initiation of reflexes such as swallowing and coughing.

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P71-B DEVELOPMENTAL CHANGES OF CHORDA TYMPANI RESPONSES TO FOUR BASIC TASTE STIMULI IN MICE GIVEN SWEET TASTE. Taeko Yamada, Mari Umezu and Yasuko Fukusima. (Physiological Laboratory, Japan Women's University, Bunkyo-ku, Tokyo, Japan).

We (Yamada et al. 1985) have already observed increased neural gustatory responses to sucrose and NaCl relative to HCl in infant mice(Crj:ICR) which were given sweet taste during the period 8-35 days after birth.

We applied to the tongue of adult mice(Crj:ICR) a filter paper soaked with 40% sucrose solution (test group) or distilled water(control group) from 71 to 98 day-old. Then we recorded the integrated responses of the chorda tympani nerve to 0.1M NaCl, 0.1 to 1M sucrose, 0.003M HCl and 0.02M quinine hydrochloride. There was not significant difference but trend for increase in sucrose relative to HCl between two groups; whereas no difference was observed in ratio NaCl/HCl.

We examined the electrical responses of the chorda tympani nerve fibers to 0.1M NaCl, 0.5M sucrose, 0.003M HCl and 0.02M quinine-HCl in mice aged 32-40 days. In young mice about 40% of fibers responded to three or four kinds of four taste stimuli. On the other hand, in adult mice(Slc:ICR) about 80% of the single fibers responded to one or two of four basic taste stimuli that those concentrations agreed with the above except 0.01M HCl(Ninomiya et al. 1982). Preliminary result showed broader responsiveness of the chorda tympani fibers to four basic taste stimuli in young than in adult mice.

P72 RODENTICIDE FLAVOR PROFILES IDENTIFIED THROUGH GENERALIZATION OF CONDITIONED FLAVOR AVOIDANCE. Russell F. Reidinger (1,2), Charles N. Stewart (3), and J. Russell Mason (1). ([1] Monell Chemical Senses Center and Biology Department, University of Pennsylvania, Phila., PA 19104; [2] U.S. Fish and Wildlife Service, Federal Center, Lakewood, CO., 80225; [3] Psychology Department, Franklin and Marshall College, Lancaster, PA 17604).

Various methods have been used to assess the flavor qualities of materials to non-human species. In the present experiments, we investigated whether generalization of conditioned flavor avoidance (CFA) could be used to profile the components of flavors that we believed to be complex. The experiments were also designed to provide data of practical importance, in that 5 rodenticides (alpha-chlorohydrin, alpha-napthylthiourea [ANTU], calciferol, strychnine, Na warfarin) were used as conditioned stimuli. On treatment days, experimental groups were presented with rodenticide in aqueous solution. Following ingestion, animals were injected with LiCl. Control groups were given water followed by LiCl injections. Generalization of CFA to 4 non-toxic flavors was then assessed. Additional conditioning and generalization trials followed until 24 flavors had been presented. CFA was exhibited toward all rodenticides, and avoidance generalized in every case to a subset of the 24 flavors. Strychnine CFA generalization was exhibited toward 'bitter' flavors (0.2M, 0.04M Na saccharin, 0.41M Na_2SO_4, 0.1M $(NH_4)_2CO_3$, 0.1 M $MgSO_4$, 0.1M l-phenylalanine, 3.0M urea, 0.001 M SOA, $ps<0.05$). Warfarin CFA was relatively weak, but generalization was exhibited toward 'bitter', 'sweet' and 'salty' tastes (0.1M $MgSO_4$, 3.0M urea, 0.1M sucrose, 0.1 M KNO_3, 0.15 M l-phenylalanine, 0.001 M SOA, 0.1M NaCl, $ps<0.05$). Calciferol CFA generalized to 'sweet' and 'bitter' substances (0.1M sucrose, 0.2% quassia, $ps<0.05$), ANTU CFA primarily to 'sour' (0.3M NH_4Cl, 0.01M acetic acid, 0.003M citric acid, 0.1M HCl, $ps<0.05$), and alpha-chlorohydrin CFA to 'sour' and 'bitter' (0.3M NH_4Cl, 0.01M acetic acid, 0.2% quassia, 0.2% gentain, 0.15M l-phenylalanine, $ps<0.05$). These results demonstrate that rats are capable of recognizing the components of complex flavors (i.e., rodenticides). In addition, they may provide information useful for rodent control. We speculate that flavor profiles of control compounds might be used to concoct flavor mixtures that mimic the flavors of rodenticides for incorporation into pre-bait formulations. Rodents ingesting the pre-bait would habituate to the flavor of the toxicant, and, in subsequent encounters with the bait, would be more likely to ingest lethal amounts.

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P74 GENETICS OF THE ABILITY TO PERCEIVE SWEETNESS OF D-PHENYLALANINE IN MICE. Yuzo Ninomiya, Tetsuichiro Higashi, Tsuneyoshi Mizukoshi and Masaya Funakoshi. (Depts. Oral Physiology, Asahi University, School of Dentistry, Gifu 501-02, Japan)

D-phenylalanine is an amino acid that tastes sweet to man and is preferred to water by some mammalian species. In mice, there are prominent strain differences in behavioral responses to this amino acid. In the C57BL/6CrSlc strain a taste aversion conditioned to D-phenylalanine generalizes to sugars and saccharin Na but not L-phenylalanine, whereas the opposite is true for BALB/cCrSlc and C3H/HeSlc strains, suggesting that D-phenylalanine presumably tastes sweet to C57BL mice (sweet-taster) but not to BALB and C3H mice(non-sweet-taster)(Ninomiya et al., 1984b). These strain differences corresponded quite well with those observed for the responses of sucrose-sensitive chorda tympani fibers to D-phenylalanine among the 3 strains of mice (Ninomiya et al., 1984a).

We further studied heredity of the ability to taste D-phenylalanine among strains C57BL/6CrSlc (sweet-taster) and BALB/cCrSlc (non-sweet-taster) and their F_1 and F_2 hybrids by measuring both their behavioral and neural responses. Phenotypic classification was made on the basis of whether a taste aversion conditioned to D-phenylalanine generalized to sucrose (sweet-taster) or not (non-sweet-taster). This criterion produced no overlap between the parent two inbred strains. All 42 F_1 mice responded like C57BL mice, indicating they were sweet-tasters. Ninety-three F_2 mice were classified into 71 sweet- and 22 non-sweet-tasters. The proportion of 71 to 22 was statistically compatible with the expected 3 to 1 simple Mendelian ratio for the single locus model. We tentatively designated the possible gene as dpa which has a major effect on tasting abilities for D-phenylalanine in mice. Linkage tests, performed between the gene dpa, and the 3 coat color genes, non-agouti(a, chromosome 2), brown(b, chr. 4) and albino(c, chr. 7), showed that the gene dpa is probably located at about 11% recombination distance from the coat color gene b(brown), on the chromosome 4. Electrophysiological studies on the single fiber responses of the chorda tympani following proteolytic treatment (Pronase E) suggest that the site of action of the gene is in taste cell membrane.

P73 THE EFFECTS OF BILATERAL SECTIONING OF THE CHORDA TYMPANI, THE GREATER SUPERFICIAL PETROSAL NERVE AND THE SUBMAXILLARY SALIVARY GLANDS ON DAILY EATING AND DRINKING PATTERNS IN RATS. Robin Krimm, Mohssen S. Nejad, James C. Smith & Lloyd M. Beidler, (Depts. Psychology and Biological Sciences, The Florida State University, Tallahassee, Florida 32306)

We (Krimm, Nejad, Smith & Beidler, 1985) recently reported that a rat after bilateral sections of both the chorda tympani (CT) and the greater superficial pertrosal nerves (GSP) showed a significant decrease in food intake and an even more remarkable alteration in the pattern of eating. After the surgical technique for sectioning the GSP alone was developed by Nejad, we are now able to report on the roles of either the GSP or CT on this alteration in eating behavior.

The CT nerve innervates the submaxillary and sublingual salivary glands as well as the anterior tongue. Indeed, Striker (1970) has shown that sectioning of CT alters eating behavior in short feeding tests in a similar manner to removal of the submaxillary and sublingual salivary glands.

In our first experiment, the 23-hour daily eating and drinking patterns were compared in rats that had the CT nerves sectioned with rats that had the salivary glands removed in an effort to see if deinnervation of the salivary glands is sufficient to explain changes that occur with a bilateral CT section. The apparatus (Spector and Smith, 1983) allows for monitoring of eating and drinking by the rat during each 30 sec. bin over a 23-hr. daily period. Bilateral sectioning of CT (N=5) resulted in little change in water intake patterns. In contrast, food intake patterns are markedly changed. For example, average time spent in each food bout markedly increases even though there is no increase in food intake. We cannot distinguish CT rats from desalivated rats (N=5) in terms of the food intake patterns. Furthermore, when CT animals are subsequently desalivated (N=2), no additional changes in eating patterns occur.

In a second experiment we studied the effect of GSP nerve sections on daily intake patterns. After the GSP nerve is sectioned the rats have significantly fewer food and water bouts and consequently consume less food and water. The pattern of water licking is more interrupted than that seen with CT rats. They spend significantly less time in each 30-sec bin eating and drinking. When GSP sections and desalivations are performed in the same rats an intake pattern emerges which is different from that previously seen with CT+GSP sections. Implications of these decrements in ingestive patterns for taste research are discussed.

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P75 EFFECTS OF SHORT-TERM EXPOSURE TO LOWERED pH ON THE BEHAVIORAL RESPONSE OF CRAYFISH TO CHEMICAL STIMULI. Ann Jane Tierney and Jelle Atema. (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

We conducted laboratory experiments to determine how two crayfish species, Orconectes virilis and Procambarus acutus, respond to food-relevant chemicals under low pH conditions. The test stimulus was an equimolar mixture of eight amino acids (L-Alanine, L-Serine, L-Histidine, Hydroxy-L-Proline, Glycine, Taurine, L-Phenylalanine, L-Asparagine). The mixture was injected into test chambers (15 cm x 15 cm x 7 cm high; each chamber contained one crayfish) at three single-compound concentrations: 10^{-2}M, 10^{-3}M and 10^{-4}M. We tested all animals at three different pH levels in the following order: 5.8, 4.5, 3.5, 5.8. At the end of testing at each pH level pellets of Purina Trout Chow were placed directly in front of the crayfish and the amount of food consumed by each animal was recorded.

At pH 5.8 animals spent significantly more time performing feeding movements and walking in response to test stimuli compared to control stimuli (aged tap water). P. acutus also lowered the antennules more in response to the amino acid mixture, compared to the control. Response intensity generally increased with stimulus intensity. After 48 h in acidified water (pH levels 4.5 and 3.5) both species showed a significant reduction in the amount of time spent performing feeding movements; the lower the pH the lower the responsiveness. At pH 3.5, time with antennules lowered was also decreased in P. acutus. When the pH level was restored to 5.8, partial recovery of behavioral responsiveness was observed. O. virilis appeared to be more sensitive to acid exposure than P. acutus. At low pH levels feeding was more severely depressed in O. virilis than in P. acutus and showed less recovery during post acidification trials. General activity and amount of food actually consumed were not affected by acidification in either species. These results indicate that short-term acid exposure may interfere specifically with chemoreceptive processes at pH levels above those which cause failure of other organ systems.

This study was supported by a grant from the United States Environmental Protection Agency (Dr. Clyde Bishop, Program Officer).

P76 TOBACCO HORNWORM CATERPILLAR FEEDING: AN ANALYSIS OF BITING AND OTHER ACTIVITIES DURING FEEDING ON TOMATO LEAF E. Bowdan (Department of Zoology, University of Massachusetts, Amherst, MA 01003).

Feeding is controlled by a complex interaction of extrinsic (odor and taste) and intrinsic (postingestive) factors. These factors are so tightly interwoven that it is difficult to examine one without its effects being confounded by the activity of others. This is especially true when the experimental methods used involve ingestion. An automated cafeteria has been developed (Bowdan, 1984) which makes it possible to document each bite an animal takes. Other activities associated with feeding are also documented. Thus it is possible to examine the effects of, for example, taste on feeding behavior before, during, and after this behavior is affected by postingestive factors. In order to use this device to examine how individual extrinsic and intrinsic factors are concerned in feeding regulation, however, it is first necessary to quantify normal feeding.

When the caterpillar first touches a tomato leaf, it explores the leaf before beginning to bite. After a period of chewing (a chewing bout) the animal stops and rests or explores the leaf again. After a short time it resumes biting. This sequences of activities continues for approximately 3 minutes (a meal) and then ends for approximately 15 minutes (intermeal interval). As the hornworms grow larger, they eat more by increasing both the frequency of their biting and the duration of meals. Chewing bout duration does not increase and nor does meal frequency.

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P77 BEHAVIORAL REACTIONS TO TASTE STIMULI IN HATCHLING CHICKS. A.Braun, J.R.Ganchrow, J.E.Steiner. (The Hebrew University-Hadassah Faculty of Dental Medicine, Dept. Oral Biology,Jerusalem, Israel).

Taste buds in chickens begin to develop about 4 days prior to hatching (Ganchrow and Ganchrow, 1986). Behavioral responses to taste stimuli dissolved in egg fluids have been observed in chick embryos one day prior to hatching (Vince, 1977), but not if water is the solvent. In contrast, adult chickens exhibit oral behaviors to chemical stimuli dissolved in water, but these give the impression of being mainly "aversive responses" (Gentle,1982). The present research investigated spontaneous behavioral displays of 28 chicks within 24 hours after hatching. The free-moving chicks were presented double distilled water and aqueous taste solutions in a random sequence. Responses were videotaped and scored under double blind conditions. Specific movement features were counted for 1 min following the first detectable sampling. When compared to water, fewer drinking contacts were initiated when quinine (0.001 and 0.02 M) or citric acid (0.01 and 0.1 M) were given and increasing occurrence of features such as beak wiping, gapes and prolonged beak clapping bouts were observed. These latter behaviors together with head shaking appeared to aid in removing the "offending" solution from the oral cavity. In contrast, responses to the "sweet" stimuli (0.3 and 1.7 M fructose and 0.005 and 0.02 M sodium saccharin) were much less impressive and barely distinguishable from water reactions. Preliminary analyses indicate a tendency for fructose to elicit slightly more fluid contacts than water. These contacts were often followed by rapid beak opening and closing (clapping). For instance, only 22% of the animals clapped more than 20 times per contact when water was the stimulus, while 62% and 66% responded thusly to 0.3 and 1.7 M fructose, respectively. Saccharin was intermediate (40% and 32% for 0.005 and 0.02 M, respectively). Hedonic estimates confirmed these results wherein the higher concentrations of quinine and acid were rated as clearly unpleasand (about 1.5 on a 10 cm. visual analogue scale) while the "sweet" and water responses hovered around neutrality (the higher concentrations being slightly more positive). It is concluded that hatchling chicks display an adult-like array of reactions to taste stimuli. The minimal positive reaction to the sweet stimuli is perhaps related to preference findings from some laboratories suggesting chickens are indifferent to these substances.

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P78 TASTE DETECTION AND DISCRIMINATION IN ZINC-DEPRIVED RATS. Gary M. Brosvic*, Burton M. Slotnick*, & Robert I. Henkin**. (*The American University, Washington, D.C. and **Georgetown University School of Medicine, Washington DC 20016).

Although zinc-deficiency has been implicated as a cause of loss of taste acuity in humans there have been few experimental studies examining this relationship in animals and none in which sensory thresholds have been assessed. We report the results of a pilot study demonstrating a loss of gustatory sensitivity in rats fed a zinc deficient diet. Rats were tested using operant conditioning (Brosvic & Slotnick, AChemS, 1985) for NaCl and taste-mixture thresholds while maintained on a zinc free diet with zinc supplementation. Three rats were then deprived of zinc and four served as pair-fed controls.

Results

Mean pretreatment NaCl detection thresholds (9.5 mM) increased by more than 13 fold after 17 days of zinc deprivation but remained unchanged in controls. Experimental rats also performed more poorly than controls in the taste-mixture discrimination. Plasma zinc levels decreased during deprivation and were significantly different from the controls (52 vs 89 ug/dl). Twenty-seven days of zinc supplement did not lower detection and discrimination thresholds in the zinc-deficient rats. Experiments are currently in progress to replicate and extend the present findings and to examine the effects of varying levels of zinc deprivation on quantitative and qualitative measures of taste acuity.

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P79 GUSTATORY DEFICITS IN RATS WITH LESIONS OF THE THALAMIC TASTE NUCLEUS. Audrey B. Kauff and Burton M. Slotnick. (The American University, Washington DC 20016).

Prior studies indicate that animals with lesions of the thalamic taste nucleus (the parvocellular part of the ventral posteromedial nucleus (VPMpc)) demonstrate a deficit in gustation. Because the results of these studies are not entirely consistent and because the investigations used simple preference tests, the nature and extent of the deficit have not been established. To examine the effects of thalamic lesions on sensory (as opposed to preference) function we tested rats to detect NaCl prior to and after lesions which included the thalamic taste nucleus. Rats received extensive training on an operant discrimination task (Brosvic, et al., AChemS, 1985) to detect 1% to .3% NaCl and were retested 10 to 14 days after surgery. Sham lesioned and non-operated controls, including one rat with lesions posterior and dorsal to VPMpc and one with a unilateral lesion of VPMpc, had little or no loss in detection. One rat with a large unilateral VPMpc lesion, which extended into the medial aspect of the contralateral nucleus, had no retention but performed at criterion levels after 1000 postoperative trials. Three rats with moderate to large bilateral lesions of VPMpc had a complete loss of retention and performed at chance in more than 1000 postoperative trials. The performance of these rats improved somewhat with additional training. These data indicate that lesions of the thalamic taste nucleus result in a severe deficit in taste detection and suggest that changes in preference demonstrated in prior studies stem from alterations in the animal's sensory capacity to detect tastants.

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P80 AN APPARATUS FOR THE DETAILED ANALYSIS OF SHORT TERM TASTE TESTS IN RATS. Laura S. Wilson, James C. Smith, Ross Henderson, Jeffrey Shaughnessy, Mohssen S. Nejad and Lloyd M. Beidler. Departments of Psychology and Biological Sciences, The Florida State University, Tallahassee, Florida 32306.

We have found that a moment-by-moment analysis of feeding and drinking patterns in rats over 24-hour intake periods yields valuable information pertaining to taste quality and concentration discrimination that is not available from mere analysis of amounts consumed (Spector and Smith, 1983). For example, intakes and total licks to 32% sucrose and to .2% sodium saccharin are identical, but a study of patterns of ingestion of the two solutions reveals profound differences. Sucrose is ingested in infrequent long drinking bouts in contrast to saccharin which is ingested in numerous short bouts. The purpose of the research reported here was to determine if such a detailed analysis of licking behavior in short term taste tests would be equally revealing as rats were tested with different taste qualities and concentrations.

An apparatus was developed to test eight rats simultaneously in single bottle taste tests for time periods up to 45 minutes. Individual licks are measured both by tongue contact on the metal sipper tube and by the tongue breaking an infrared beam. The number of licks each 500ms and a 1.0 ms inter-lick-interval histogram are measured by a microcomputer. The taste solutions can be presented to the rat from the normal graduated drinking tubes or from a reservoir via solenoid valves. With the latter mode of presentation we can accurately control the amount of fluid obtained on each lick. Analysis programs allow for plotting inter-lick-interval histograms and a strip chart, which shows the pattern of drinking over time. Criteria for a burst of licking are selected allowing for analysis of number of licking bursts, burst length and inter-burst-interval (IBI). To evaluate the measurement techniques described above we conducted two experiments:

(1) A comparison was made between licking patterns for sucrose (32%) and sodium saccharin (.2%) in six rats in 30 min. tests.

(2) Six rats were tested on three concentrations of sucrose and two concentrations of NaCl before and after bilateral sections were made on the Greater Superficial Petrosal Nerves (GSP). In both of these experiments is was found that the detailed analysis of the pattern of licking allowed for a more thorough understanding of the effects of the various manipulations on taste perception by the rat. Implications of these findings for taste perception in the rat are discussed.

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P81 TASTE PREFERENCE CHANGES AND ADRENAL RESPONSE IN PYRIDOXINE DEFICIENT RATS. Charles N. Stewart, Johanna Ifft (Franklin & Marshall College, Lancaster PA 17604) & Yair Katz (Monell Chemical Senses Center, Philadelphia PA 19104).

A number of micronutrient deficiencies including zinc, iron, vitamins A, B_1 and B_6 have been reported to produce altered taste preference in rodents. Pyridoxine (vitamin B_6) deficiency induced at weaning increases NaCl and KCl preference when the salts are presented in solutions ranging from 0.15 up to 0.45 M. We have now demonstrated that adult rats fed the deficient diet begin to display a significantly enhanced NaCl and KCL (0.3 M) preference (daily 20 min. tests) within three weeks. Further evidence for enhanced salt appetite is found in the preference shown by deficient rats for a high salt diet (13.3 g NaCl vs. 4.32 g NaCl/Kg Lab Chow) when given a choice over four 24-hr ingestion periods. Preference changes are not, however, limited to NaCl and KCl, because increased 0.2 M sucrose and 0.27 M monosodium glutamate intake occurs in both adult and weanling rats fed the deficient diet.

Measurement of blood corticosterone and aldosterone levels revealed a significant increase in the former in deficient rats of both sexes and no difference on the latter, a somewhat unexpected result in the context of the increased NaCl and KCL preference. Females, whether deficient or control, had significantly higher aldosterone levels than males. When the deficiency was reversed by feeding a normal diet to all animals, the increased NaCl/KCl preference disappeared.

P82 FAILURE OF RATS TO ACQUIRE A REVERSAL LEARNING SET WHEN TRAINED WITH TASTE CUES. Burton M. Slotnick and Gary M. Brosvic. (The American University, Washington DC 20016).

Rats trained with odor cues show excellent interproblem transfer on multiple odor discriminations and quickly acquire a learning set. When tested on a series of discrimination reversal tasks they show positive transfer on the first reversal and demonstrate near-errorless acquisition of succeeding problems. They perform much more poorly when tones or lights are used as discriminative cues (Slotnick, Chem. Sen., 1984). In this study we assessed whether the superior performance obtained with odors might also occur with tastes. Eight rats were trained on an operant task (Brosvic et al., AChemS, 1985) to discriminate 1% NaCl from 0.1% saccharin or 0.75% NaCl from 1% sucrose and then tested on a series of 7 reversals. One stimulus of each pair served as S+ and the other as S-; positive and negative stimuli were counterbalanced across animals.

Results

There were no differences in performance as a function of which stimulus pair was used. Approximately 250 errors were made in original learning and all but one rat showed strong negative transfer on the first reversal (mean errors, 669). Performance improved in succeeding reversals and by reversal 7 the error scores of most rats were near those of original learning. In general, the performance of these rats was similar to those trained on visual or auditory stimuli and was clearly inferior to those trained on odors (Slotnick, Chem. Sen., 1984). These results suggest that the rapid acquisition of a reversal set may be uniquely dependent upon olfactory cues and does not occur with stimuli from another chemical sensory modality.

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F10 BENZALDEHYDE BINDING PROTEIN FROM DOG OLFACTORY EPITHELIUM. Steven Price and Amy Willey. (Dept. of Physiology and Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298)

Olfactory epithelium from mongrel dogs was extracted with 0.1 M TRIS, pH 7.2. The residue was extracted with buffer containing 0.1% sodium dodecyl sulfate. The resulting extract was passed through a column with O-methylphenol groups covalently bound to it. This removed the previously described anisole binding protein from the extracts (1). The extract was then passed through a column to which p-carboxybenzaldehyde was covalently bound through the carboxyl group. After washing with 0.1 M TRIS, pH 7.2, the column was eluted with buffer containing 1 mM p-carboxybenzaldehyde. This displaced constituents of the extract that had been bound to the column by virtue of affinity for benzaldehyde-like moieties. Upon polyacrylamide gel electrophoresis and silver staining a pattern of protein was obtained that was indistinguishable from that of anisole binding protein (major and minor bands of molecular weight 61 x 10^3 and 22.5 x 10^3, respectively). The anisole and benzaldehyde binding proteins cannot be the same molecules since the anisole binding protein had been removed from the extracts before the affinity chromatographic isolation of the benzaldehyde binding protein.

The results are consistent with the hypothesis that olfactory receptor cell membranes contain a class of structurally related proteins with affinities for particular types of odorants (2,3). These may be odorant recognition molecules with important roles in initiating olfactory responses.

1. S. Price, CHEM. SENSES 3: 51 (1978).
2. S.J. Goldberg, J.A. Turpin and S. Price, CHEM. SENSES 4: 207 (1979).
3. S. Price and J.A. Turpin, OLFACTION AND TASTE 7: 65 (1980).

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F11 **CODING OF CHEMOSENSORY STIMULUS MIXTURES.** David. G. Laing. (CSIRO Division of Food Research, PO Box 52, North Ryde, NSW 2113 Australia).

Perception of odors in the environment by humans, animals, or insects, invariably involves complex mixtures that often contain dozens of odorous components. Odor perception, therefore, usually depends on the reception and neural processing of many components.

However, little is known about how and where mixtures are processed by the olfactory system, what factors determine how many odorants will be perceived, whilst the influence of physico-chemical features of the stimuli have yet to be determined.

Perhaps the most important problem to resolve initially, concerns the role of peripheral and central components of the olfactory system in the processing of mixtures. If substantial processing occurs at the odor receptors through competitive or non-competitive binding, an understanding of additivity, masking and synergism, the most common outcomes of mixing odors, is likely to be gained through structure-activity studies. On the other hand, if processing is primarily a central phenomenon, an intimate knowledge of neural circuitry and response properties of cells in different regions of the olfactory system e.g. olfactory bulb, will be required.

This presentation will review data from physiological and behavioral studies with very different species including humans, rodents and crustaceans, that provide information on the role of peripheral and central components of the olfactory system in the coding of odor mixtures.

F12 MIXTURES OF TASTANTS AND MIXTURES OF ODOURANTS. Frijters Jan E.R. Dep Human Nutrition, Agricultural University, 6703BC Wageningen The Netherlands

One of the basic issues in psychophysical chemoreception research is the study of mixtures and mixture interaction phenomena. An important question is how the taste or odour quality of a mixture is related to the qualities of the unmixed components used for mixture composition. Can the original tastes or odours be recognized in the mixture, or does fusion and the arise of a new quality occur ? Another topic is the prediction of a mixture's intensity on the basis of the taste or odour strengths of the components outside the mixture. When the mixture's intensity is less than the sum of the intensities of the unmixed components it is concluded that suppression occurred, when it is equal addition took place and when it is higher one speaks of synergy Various measures and reasonings are used to assess which type of mixture interaction has occurred. Also, a number of models has been developed for the description and prediction of the mixture intensity. These will be thogoughly discussed and the recent literature regarding mixture interaction phenomena in chemoreception psychophysics will be reviewed.

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F13 ELECTROPHYSIOLOGICAL RESPONSES OF INSECT OLFACTORY RECEPTOR NEURONS TO STIMULATION WITH MIXTURES OF INDIVIDUAL PHEROMONE COMPONENTS. Robert J. O'Connell and Alan J. Grant. (The Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545).

Multicomponent pheromone systems are the rule in many commercially important insect species. As our knowledge about the number of different chemical compounds actually released by the pheromone glands of a particular species has increased so to has the level of communicative complexity observed when these same materials are evaluated either singly or in multi-component blends by modern behavioral assay techniques. It is becoming increasingly clear then, that this increase in the chemical and behavioral complexity of a particular communication system must be paralleled by an increase in the efficiency of the physiological mechanisms employed for the neural encoding of behaviorally relevant odor compounds and blends.

Here we describe the electrical activity of olfactory receptor neurons in a subset of the individual pheromone sensitive sensilla on the antennae of male cabbage looper moths (Trichoplusia ni). Electrophysiological responses to single and multiple component stimuli, each drawn from among the 7 known behaviorally active compounds for this insect, were obtained at several different stimulus intensities. Both (Z)7-dodecenyl acetate and (Z)7-dodecenol were effective stimuli for both of the receptor neurons found in one of the two classes of pheromone sensitive sensilla, even at relatively low stimulus intensities (0.0005 µg). Dodecyl acetate, although behaviorally active, did not significantly excite either of these receptor neurons. However, when mixed with either of the unsaturated components, it significantly enhanced the receptor neuron's response to its appropriate parent compound only in the middle range of stimulus intensities. A mixture of all three components did not show this enhancement and at the middle range of intensities actually elicited reduced responses when compared to those elicited by appropriate amounts of any of the one and two component stimuli evaluated. Thus, some blends elicited electrical responses from primary olfactory receptor neurons which were not readily predicted from a knowledge of the receptor neurons response to individual components.

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F14 ODOR/TASTE MIXTURES. David E. Hornung and Melvin P. Enns. (Depts. of Biology and Psychology, St. Lawrence University, Canton, New York 13617).

Two basic lines of investigation have been used to study the interaction of odors and tastes. One line of investigation has examined the effect that smell has on the perception of taste and vice versa. These studies have often included a consideration of smell/taste confusions, that is, situations where a smell sensation is perceived as a taste or a taste sensation is perceived as a smell. A description of the possible peripheral and central mechanisms that might account for these types of smell/taste interactions will be presented. A second line of investigation has focused on the role of smell and taste in the perception of flavor. Questions that have been considered include how the intensities of smell and taste add together in the perception of the intensity of flavor, how smell and taste influence the recognition of flavor stimuli, and how smell and taste influence the acceptability of flavor stimuli. In an attempt to draw from both these lines of study, the presentation will conclude with a discussion of the significance of smell/taste interactions in the identification and acceptability of flavor compounds.

S74 OLFACTORY RECEPTOR CELLS IN INSECTS: REACTION SPECTRA AND THE CONCEPT OF GENERALISTS AND SPECIALISTS IN ANTHERAEA POLYPHEMUS L.

Wolf A Kafka (Max-Planck Institut für Verhaltensphysiologie, D-8131 Seewiesen, FRG).

In the general context of primary olfactory coding, quantitative electrophysiological measurements were made of the responses of 50 olfactory receptor cells to a total of 54 substances. The cells are distributed among 3 morphologically distinguishable sensilla on the antennae of the male silkmoth Antheraea polyphemus: sensillum basiconicum and two types of sensillum trichodeum. The (up to 3) receptor cells in a given sensillum are distinguished by the amplitude of the recorded nerve impulses. On the basis of these two critaria, each receptor is assigned to one of 9 morphological-electrophysiological categories.

The responses of all the receptor cells are compared with regard to reaction spectrum and to a substance-efficacy- index defined, on the basis of dose response curves, as the stimulus intensity (molecules/ccm) required to elicit 25 impulses/s.

By treating these efficacy indices as vector components and applying a vector-correlation procedure, it has been possible to obtain a single quantitative measure of the similarity, one-to-one, of the reaction spectra of the receptor cells in a 50x50 matrix. The resulting degrees of similarity are grouped in a manner consistent with the morphological- electrophysiological categorization. There is no appreciable specialisation to substances of particular chemical classes (alcohols, esters, acids, aldehydes, amines, carbohydrates) nevertheless, and despite considerable overlap among the various reaction spectra, the cells are highly selective.

In view of the grouping of the reaction spectra found here, the traditional concept of a dichomity between generalists and specialists requires modification.

S75 SELF- AND CROSS-ADAPTATION OF SINGLE CHEMORECEPTOR CELLS IN THE TASTE ORGANS OF Homarus americanus. Paola F. Borroni and Jelle Atema. (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

This study provides the first systematic description of the effects of self- and cross-adaptation on primary chemoreceptor cells in any animal. We measured the changes in sensitivity of narrowly tuned NH_4 receptor cells (as extracellularly recorded neural output) resulting from adaptation to backgrounds of different compounds and intensities.

In a preliminary study (Borroni and Atema, 1985), stimulus-response functions (.003 to 30 mM NH_4Cl) were obtained in 3 different self-adapting background concentrations (.0016 to .03 mM NH_4Cl). The present study extends the reported results to cover 5 self-adapting background concentrations, over a range of 3 1/2 log units (up to 3 mM). Self-adaptation results in a proportional decrease of the response of single receptor cells to all stimulus concentrations, i.e. a parallel shift of the stimulus-response functions primarily along the abscissa.

Though narrowly tuned, some NH_4 receptor cells respond weakly to other nitrogenous compounds such as amino acids and amines (Johnson et al., 1984). We tested the tuning spectrum of 15 NH_4 receptors using Asp, Arg, Hyp, Glu, Bet, TMO,and TMA at a concentration of .3mM. Of these compounds, only Bet, Glu and Hyp frequently stimulated NH_4 cells. On average, these compounds elicit 10% or less of the response to NH_4 at equimolar concentration.

Bet, Glu and Hyp were used in cross-adaptation tests. First we searched for a NH_4 cell; then we determined whether Bet, Glu or Hyp was its second-best compound. Finally, we recorded two NH_4 stimulus-response functions: one in artificial seawater (ASW), and one in a background of ASW plus various concentrations of the specific second-best compound. When no second-best compound was found, anyone of the three compounds was used in the background. This protocol allowed us to study the effects of cross-adaptation on receptors with and without the most common second-best compounds.

This research was supported by the Whitehall Foundation and NSF (BNS 8512585).

S76 CROSS ADAPTATION EXPERIMENTS PREDICT OLFACTORY AND GUSTATORY RESPONSES TO STIMULUS MIXTURES. John Caprio and John Dudek. (Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803).

In contrast to most experimental studies, olfactory and gustatory receptor cells rarely encounter sequentially spaced, single chemicals under natural circumstances. The normal chemical world is a mixture of substances varying in concentration and potency. Thus, it is critical to the basic understanding of the processes involved in olfaction and gustation to determine how these senses respond to the simultaneous presentation of compounds comprising, at least initially, simple mixtures. Numerous reports in the recent literature indicate that the response to a mixture cannot generally be predicted from knowledge of the responses to the individual components. This is attributed to the occurrence of both mixture suppression and synergism among different components in the mixture. We report, however, that peripheral olfactory (EOG) and gustatory recordings in the channel catfish to binary and trinary mixtures of amino acids clearly show that responses to these mixtures are predictable with knowledge of the respective relative independence of the binding sites of the component stimuli obtained from cross-adaptation experiments (Caprio & Byrd. J. Gen. Physiol. 84:403-422. 1984). The present results indicate that a mixture of amino acids whose components interact with different binding sites (i.e. show minimal cross-adaptation) produce significantly larger responses than a mixture whose component amino acids stimulate the same site (i.e. show significant reciprocal cross-adaptation). For a mixture whose components show reciprocal cross-adaptation, the magnitude of the response to the mixture is equivalent to that produced by a higher concentration of any of the components (i.e. "stimulus substitution" model based on the dose-response function of the stimuli; Hyman & Frank. J. Gen. Physiol. 76:125-142. 1980; Rifkin & Bartoshuk. Physiol. Behav. 24:1169-1172. 1980). For a mixture of amino acids whose components show minimal cross-adaptation, the magnitude of the response is significantly larger than that predicted by the "stimulus substitution" model and may approach additivity, even though the power function exponent characterizing the dose-response functions of amino acid stimuli is 0.2. Since all stimuli were adjusted in concentration (and in pH 7.8-8.0) to provide approximately equal response magnitude and no mixture suppression was observed, this suggests that some of the previous examples of mixture suppression may be explained by simple competitive binding among stimuli with differing potencies that share the same membrane binding site.

Supported by NIH NS14819.

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S77 ATP-SENSITIVE OLFACTORY RECEPTORS: SIMILARITIES TO P_2-TYPE PURINOCEPTORS. William E. S. Carr, Richard A. Gleeson, Barry W. Ache, and Marsha L. Milstead. (C. V. Whitney Laboratory and Dept. of Zoology, University of Florida).

Purinergic receptors, stimulated by ATP and other adenine nucleotides, are present internally in many tissues of vertebrate animals. In the olfactory system of the spiny lobster, electrophysiological procedures have been used to identify a population of ATP-sensitive cells with the following response characteristics shared in common with the P_2-type purinoceptors found in internal tissues: 1) potency sequence of ATP > ADP >> AMP and adenosine; 2) excited by many nucleotide triphosphates including those with modifications in both the ribose and purine moieties; and 3) excited by metabolically stable analogs of ATP, namely, β,γ-imido ATP, β,γ-methylene ATP, and α,β-methylene ATP. The above structure-activity relationships, together with the type of discharge of the receptor cells, clearly distinguish the P_2-like olfactory cells from the AMP-sensitive, P_1-like, olfactory cells identified in the olfactory organ of the spiny lobster in an earlier study (Derby, Carr and Ache, 1984). In addition to the ATP- and AMP-sensitive cells, we also know that this animal has other olfactory cells that are differentially excited by three other neuroactive agents: taurine, glutamate and glycine. Because of the apparent similarities between these "narrowly-tuned" olfactory receptor cells and the synaptic receptors of high specificity found internally, the olfactory system of the lobster provides an attractive model for comparative studies of the physiology and biochemistry of receptors that are stimulated by certain substances known to be neuroactive agents.

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S78 TEMPORAL ANALYSES OF THE ACTIONS OF NORMAL ALCOHOLS ON TASTE RECEPTOR CELL RESPONSES TO SUCROSE. Marilyn B. Whitney and Linda M. Kennedy. (Dept. Biology, Clark Univer., Worcester MA 01610).

Normal alcohols (n-alcohols) suppress fly behavioral and taste receptor cell action potential responses to sucrose (Dethier & Chadwick,1947;Steinhardt et al,1969). Preliminary data (Peters & Kennedy,1983,unpubl.) suggest that the receptor cell effects are biphasic over time-- firing is first suppressed and then increased and irregular-- as also are effects of the gymnemic acids and ziziphins (Kennedy & Halpern,1979,1980). These similar biphasic effects suggest that mechanisms by which n-alcohols act on receptor cells could be similar to mechanisms of the gymnemic acids and ziziphins. We studied the temporal effects of a series of n-alcohols of increasing hydrocarbon chain lengths (methanol - octanol) in order to 1) confirm similarities with effects of gymnemic acids and ziziphins and 2) test a prediction of the Kennedy & Halpern (1980) biphasic membrane penetration model for the actions of gymnemic acids and ziziphins. The prediction is that the onset of the second phase will occur sooner for nonpolar than for polar taste-altering molecules and does hold for the gymnemic acids and ziziphins according to their relative polarities (Kennedy & Halpern,1981). If n-alcohols act according to the model and by mechanisms similar to those of the gymnemic acids and ziziphins, then the onset of the second phase should vary consistently with n-alcohol chain length (polarity).

Single Phormia regina taste hairs in isolated proboscis preparations were tested in two-trial sequences. In the first trial, hairs were treated with Tris-buffer (15mM, pH 7) for 2 min, and then action potential responses to sucrose (50mM in NaCl 50mM) were continuously recorded for 5 min. In the second trial, hairs were treated with n-alcohols (in Tris) for 2 min and responses to sucrose recorded as before. For each fly, the numbers of spikes in 100 or 500 msec excerpts taken at 1 min intervals from responses in the alcohol trial were expressed as ratios to the numbers of spikes in excerpts taken at the same times from responses in the Tris trial. Biphasic effects occurred: firing was initially supressed (ratios <1) and then increased and irregular (ratios >1). The onset of the second phase varied across a range of $\leq$1 min for octanol to $\geq$5 min for methanol, with intermediate onset times for alcohols of intermediate chain length. These data suggest that n-alcohols act by mechanisms similar to those of gymnemic acids and ziziphins and support the biphasic membrane penetration model.

We thank D.Kolodny & J.Mickle for assistance. Supported by Clark Faculty Development and Biomedical Research Grants to L.M.K.

S79 **THE ELECTROLYTE DISTRIBUTION IN INSECT OLFACTORY SENSILLA AS REVEALED BY X-RAY MICROANALYSIS.** Rudolf Alexander Steinbrecht (Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, W.-Germany)

X-ray microanalysis so far is the only method allowing the quantitative analysis of elemental concentrations in situ with the high lateral resolution of the electron microscope. Thus, despite the complex "Bauplan" of insect sensilla, it is now possible to directly measure the electrolyte distribution in the various cellular and extracellular compartments, such as the receptor neurons with their different dendritic regions, the auxiliary cells, the receptor lymph and haemolymph etc..

Cryofixation and cryoultramicrotomy are essential prerequisites, because any solvent treatment might cause redistribution or even extraction of soluble elements. With silkmoth antennae (Bombyx mori) this has been achieved by quick immersion into liquid propane (at 85K) and cryoembedding into heptane (at 190K; Steinbrecht and Zierold, J. Microsc. 136, 69 (1984)). The ultrastructure of frozen dried cryosections allows the unequivocal identification of all important compartments of the pheromone sensitive sensilla.

A high concentration of potassium prevails throughout the cellular compartments and also in the extracellular compartment of the receptor lymph, due to an electrogenic potassium pump located in the apical membrane folds of the trichogen cell. Haemolymph potassium is low. Calcium, on the other hand, is present in the haemolymph, but is below the detection limit in the cellular compartments and in the receptor lymph. Receptor lymph contains little chlorine, electroneutrality, therefore, most probably is established by organic polyanions, e.g. sulfatized proteoglycans. This is also indicated by its fairly high sulfur content.

Although preliminary, some experiments will be reported, in which the potassium distribution is compared in stimulated and unstimulated antennae. Antennae which received a very strong bombykol stimulus before freezing show a significant rise in receptorlymph potassium. This increase is higher than the expected ionic shift produced by the receptor current. Therefore, stimulation possibly activates other ion movements in addition, e.g. the electrogenic potassium pump of the auxiliary cells.

S80 **NEUROPHYSIOLOGICAL RESPONSES TO PHEROMONE BLEND COMPONENTS IN THE SOYBEAN LOOPER MOTH, PSEUDOPLUSIA INCLUDENS (WALKER).** **Alan J. Grant, Robert J. O'Connell (The Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545) and Abner M. Hammond, Jr. (Department of Entomology, Louisiana State University, Baton Rouge, LA 70803).**

Reproductive isolation among sympatric species of insects is known to depend, in part, on the composition and release of pheromones from each insect. To investigate how pheromones are differentially processed by the olfactory system, thereby providing the sensory limb required for this isolation process, neurophysiological responses were recorded from single antennal sensilla on the male soybean looper moth to stimulation with known amounts of the individual components of its pheromone blend and the individual components of the blend produced by a sympatric species, Trichoplusia ni (Hübner), the cabbage looper moth. These two Noctuid moths have overlapping geographic and seasonal distributions. Additionally, they have similar temporal patterns of activity and share some of the same host plants. Females of both insects release complex blends of 12-14 carbon esters that are used to attract males for mating. Both species share (Z)7-dodecenyl acetate (Z-7,12:AC) as the major component of their respective blends; however, the composition of their minor components differ.

Similar to the previously reported chemosensory system in the cabbage looper, the soybean looper possesses two classes of morphologically distinct antennal sensilla, each containing two chemosensitive olfactory receptor neurons. In both species, one class of sensilla contains a receptor neuron sensitive to Z-7,12:AC. The neurophysiological characteristics of the receptor neurons in this class of sensilla, including their unstimulated spontaneous activity, sensitivity to pheromone, and response spectra to other compounds appear identical in both species. The second class of sensilla, in each species, contains a receptor neuron sensitive to one of the minor components of the pheromone blend. However, the effective compound is different in each of the two species. Differences in the response characteristics of the receptor neurons in this second class of sensilla are thus thought to play a role in the reproductive isolation that exists between these two species of moth.

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S81 EPITHELIAL RESPONSES OF RABBIT TONGUE AND THEIR INVOLVEMENT IN TASTE TRANSDUCTION. Simon, S.A., Robb, R. and Garvin, J. Departments of Physiology and Anesthesiology, Duke University Medical Center, Durham, N.C. 27710

The response of rabbit tongue, placed in a modified Ussing chamber, to salts (KCl, NaCl, NH_4Cl, TEACl), sweeteners (D-glucose, sucrose), acid (HCl) and a bitter tasting molecule (quinine HCl) was investigated for the first time. These experiments were conducted to further explore the new paradigm of taste transduction introduced by DeSimone et al. (J. Gen. Physiol. 83: 633-656, 1984) that correlates changes in the electrical behavior of lingual epithelia with neural responses. In addition, comparisons of rabbit tongue data were made with previously published data on dog and frog tongues.

The increase in short circuit current, Isc, for the rabbit tongue induced by several salts in the hyperosmotic concentration range were: KCl>NH_4Cl>NaCl>TEACl. These results are consistent with previously published integrated chorda tympani responses (ICTR) as well as behavioral studies, both of which showed that rabbits are more sensitive to KCl than NaCl. Pharmacological studies using ouabain and amiloride suggest that K and Na are traversing the rabbit tongue through different pathways. The stimulation of Isc by D-glucose and sucrose were significantly smaller than those measured for KCl and NaCl. The saccharide response was inhibited by amiloride. The rabbit tongue was sensitive to HCl at concentrations less than 1 mM in agreement with ICTR measurements.

In contrast to the rabbit, the dog tongue was more sensitive to Na than K. The response of the open circuit potential, Voc, was in the order, NaCl> KCl> NH_4Cl. Also the dog tongue is more sensitive to sucrose and about equally sensitive to HCl compared to rabbit tongue.

In summary we found that the epithelial responses to the primary tastants are well correlated with neural events and with manifestations of behavior in the rabbit. The behavioral responses are consistant with the diet of these animals since rabbits eat food high in potassium, whereas dogs eat food high in sodium.

This work was supported by Grant NS-20669.

S82 DEVELOPMENT OF AMILORIDE SENSITIVITY IN THE RAT PERIPHERAL GUSTATORY SYSTEM: A SINGLE FIBER ANALYSIS. David L. Hill (Dept. Psych., Univ. Toledo, Toledo, OH 43606).

Response frequencies of rat peripheral and central taste neurons increase dramatically to many chemical stimuli during development. Recent research has shown that the epithelial sodium transport blocker, amiloride, effectively suppresses multifiber, chorda tympani responses to NaCl in adult rats by at least 60%. In contrast, amiloride is ineffective in suppressing the NaCl response in rats aged 12-13 days. Moreover, the NaCl response following amiloride in adults is the same as the response in young rats. Therefore, the developmental increase in sensitivity to NaCl appears to be related to a concomitant increase in amiloride sensitivity.

To explore the underlying peripheral events, neurophysiological taste responses were recorded from single chorda tympani fibers in rats aged 14-20 days and adults (90 days). Responses were recorded to a concentration series (0.05M, 0.1M, 0.5M, 1.0M) of NaCl and NH4Cl before and after lingual application of 500 uM amiloride hydrochloride. In both age groups, amiloride was most effective in suppressing NaCl responses in neurons maximally responsive to NaCl whereas, neurons responding maximally to NH4Cl were relatively unaffected by amiloride. Amiloride had no effect on responses to NH4Cl. This is similar to preliminary results reported in the adult (Ninomiya, et al., 1984). However, it is important to note that amiloride had a similar effect on "Generalists" (i.e., neurons responding equally well to NaCl and NH4Cl) and "NaCl-best" neurons in both age groups. Thus, amiloride does not selectively affect one group of neurons. In fact, amiloride appears to suppress NaCl responses in a linear fashion. Response frequencies of neurons to NaCl before amiloride were highly correlated with the change in frequency due to amiloride (frequency before amiloride - frequency after amiloride; r= +0.87 - +0.99). That is, the greater the response frequency to NaCl before amiloride, the greater the suppression by amiloride. This function occurred for all NaCl concentrations and for both age groups. Moreover, the slopes and the y-intercepts were similar for both ages at each concentration of NaCl. Therefore, the developmental increase in sensitivity to NaCl may result from an increase in the number of functional amiloride sensitive components rather than alterations of neural "groups" as a function of age.

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S83 **THE OLFACTORY SENSITIVITY OF MATURE MALE, FEMALE, IMMATURE AND HYPOPHYSECTOMIZED GOLDFISH TO L-AMINO ACIDS, BILE ACID, AND STEROIDAL COMPOUNDS BY UNDERWATER ELECTRO-OLFACTOGRAM (EOG).** Peter W. Sorensen*, Toshiaki J. Hara, and Norman E. Stacey*. (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba R3T 2N6 Canada; *Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E9 Canada).

This study was designed to describe the olfactory sensitivity of goldfish, to determine whether the sensitivity of their olfactory epithelia is influenced by sex or maturity, and to ascertain whether goldfish smell steroidal compounds thought to be pheromones (Colombo et al. 1982, Stacey et al. 1986). Olfactory thresholds of mature spermiated males, vitellogenic females, and immature males and females to L-serine, L-cysteine, L-glutamic acid, L-arginine, taurocholic acid, and six steroidal compounds were measured by underwater electro-olfactogram (EOG). Steroidal compounds tested were testosterone glucuronide, 17B-estradiol glucuronide, etiocholanolone glucuronide, 17B-estradiol, progesterone, and 17α,20B-dihydroxy-4-pregnene-3-one (17,20P). Responses of five-week hypophysectomized immature fish were measured to L-serine, taurocholic acid, and 17,20P.

The olfactory sensitivity of goldfish to L-amino acids and taurocholic acid was similar to that of other species of fish. Mature males, females, and immature fish possessed similar thresholds and response magnitudes relative to L-serine standard, and there were no significant differences between these groups to any of the odorants. The relative responses of hypophysectomized fish were significantly lower and more variable ($P<0.05$), and thresholds higher, than those of control fish to taurocholic acid and 17,20P. The absolute magnitude of their response to L-serine standard was also significantly lower ($P<0.05$) than the other three groups whose responses did not differ from each other. The pituitary may influence the olfactory epithelia by a mechanism which does not directly involve sex steroids.

17,20P is a highly stimulatory odorant: it had a threshold of 10^{-13}M and at a concentration of 10^{-8}M evoked a response three times that of 10^{-3}M L-serine. Progesterone was slightly less stimulatory than 17,20P. Goldfish did not appear to detect the other steroids. Cross-adaptation experiments indicated that progesterone and 17,20P react with the same receptor(s) and that this receptor(s) is distinct from those which detect bile acids and L-amino acids. These findings correlate with behavioral and endocrinological experiments on the pheromonal activity of 17,20P (in press), and the high circulating levels of this steroidal hormone present in teleost fish during final maturation.

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S84 CHORDA TYMPANI NERVE RESPONSES TO INTRALINGUAL AND SURFACE TASTE STIMULATION IN THE RHESUS MONKEY AND THE RAT. G. Hellekant, J. N. Brouwer, T. Roberts, C. Hård af Segerstad, and H. van der Wel.(Dept. Veterinary Science, University of Wisconsin, Madison WI 53706)

It is well known from clinical studies that compounds injected intravascularly can elicit a taste sensation (Winternitz et al. 1931). During intralingual stimulation the molecular size of the infused compounds plays a decisive role in their ability to pass from the capillaries to the extracapillary space. Compounds with a radius of more than 30 Å have very limited capacity to diffuse through the capillary membrane, while compounds with a radius of 16 Å or less readily diffuse through the membrane (Lanken et al. 1985). Monellin, thaumatin and miraculin, three proteins with strong sweet taste or effects on the sweet taste in catarrhina primates, have a molecular size less than 25 Å which may allow them to pass the capillary membrane and perhaps reach the taste buds from within.

Since it is generally thought that taste is the result of an interaction between a compound and a specilized set or sets of receptors on the microvilli (e.g. van der Heijden et al. 1985a, b) it is of interest to see if intralingual stimulation with these compounds would elicit a taste nerve response. If this is the case it will show that these compounds are able to penetrate the capillary walls, and suggest that their receptors are distributed around the taste cells, not only on the parts that are in contact with the oral cavity.

The effect of intrarterial injection of monellin, thaumatin and miraculin on the activity of the chorda tympani proper nerve was recorded in the monkey and rat. The compounds were injected into the blood stream at the branching of the lingual artery. It was found that monellin and thaumatin elicited a nerve response in the monkey but not in the rat. Miraculin had no effect in either species. It was concluded that the response to intra lingual injection in the monkey was caused by stimulation of monellin and thaumatin of parts of the cells within the taste bud.

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S85 TRANSDUCTION OF REPELLENT ENERGY STATE INTO COCKROACH AVOIDANCE BEHAVIOR. Dale M. Norris. (642 Russell Laboratories, University of Wisconsin, Madison WI 53706).

I (Norris, 1985, 1986) observed a linear relationship between primary input and primary output variables involved in energy transduction and information encoding in 1, 4-naphthoquinone perception by the American cockroach (Periplaneta americana). The primary input variable is the moles of naphthoquinone required to cause greater than (>) 99% avoidance behavior in a standardized behavioral assay (Rozental and Norris, 1975; Norris 1985, 1986). The primary output variable is the maximum millivolt (mV) E 1/2 shift induced in the receptor protein by a saturating concentration of a given naphthoquinone. A linear relationship also exists between the primary output variable and the secondary output variable, the maximum inhibition of a standardized amyl acetate-elicited electroantennogram (EAG) by a given 1, 4-naphthoquinone repellent (Norris and Chu, 1974; Norris, 1985). This secondary output variable also is linearly related to the primary input variable (Norris, 1985, 1986). The correlation coefficient for the linear relationship between the primary and secondary output variables is so high that only one of these two output variables needs to be considered in developing a quantitative expression for the involved energy transduction. Thus, the expression, $\text{Log } Y = 3.40 = 0.112 \text{ (Log } X)$, where X is the primary input variable and Y is either the primary or secondary output variable, describes the observed event in millivolts.

S86 RESPONSE CHARACTERISTICS OF OLFACTORY EVOKED POTENTIALS USING TIME-VARYING FILTERING.
Mitsuo Tonoike. (Osaka Branch, Electrotechnical Laboratory, Amagasaki, 661, Japan).

Olfactory evoked potentials(OEPs) were recorded from the central vertex region (C_z) of the intact human scalp by the same techniques as K.H.Plattig and G. Kobal(Plattig and Kobal,1977). Odorants were given to the subjecct's nose with the duration of 200 milliseconds under the control of the synchronized timing with his respirations. Wave forms of OEPs were digitalized by converting every 8 milliseconds from analog potentials.

For the purpose of the odour discrimination the time-varying filtering(TVF) which J.P.C.M. de Weerd.(1981) developed was applied to the wave forms of OEPs. TVF method is able to handle the transient evoked potentials because it was developed to the time-varying from the time-invariant winer filtering. As OEPs are generally influenced by many factors such as olfactory fatigue effects, arousal levels and other various noises, the effectiveness of TVF was examined. The time-varying filtering function G(f,t) and the time-varying power spectrum $\Phi_{ss}(f,t)$ of signals were calculated for three odorants, such as amyl-acetate, vanillin and dl-camphor by separating of 5 banks of band pass filters. From these results $G_1(f,t)$ function and $\Phi^1_{ss}(f,t)$ power spectrum of the first band pass gave accurate estimations for the characteristic peak of OEPs. These mean that a frequency band till 8 Hz is very important for the estimation of OEPs.

$G_1(f,t)$ and $\Phi^1_{ss}(f,t)$ showed the characteristic pattern for each odorant at each response time. The power spectrum $\Phi_{nn}(f,t)$ of noises was also examined and the characteristic patterns of noises were shown in the spectro-temporal representations. From the analysis of the present experiments it was shown that the characteristic of the frequency for the noise of OEPs was at the highest till about 20 Hz.

Finally, TVF method was compared with the simple averaging(AV) and a posterior winer filtering(APWF) which was the time-invariant classical filtering method. These results showed that estimations of OEPs using TVF method were most exellent. Response characteristics of OEPs for above three odorants were drawn in two-dimensional domain of frequency and response time.

I thank Dr. J.P.C.M. de Weerd, Dr. K.-H. Plattig and Dr. S.F. Takagi for useful advices and many discussions.
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S87 **THE ROLE OF TASTE IN THE FEEDING MECHANISM OF THE CARP (Cyprinidae).** Ferdinand A. Sibbing (Dept. Exp. Animal Morphology and Cell Biology, Agricultural University, Marijkeweg 40, 6709 PG Wageningen, The Netherlands).

In many cyprinids and other fish, food is ingested with a flow of water. In case of bottomfeeders, such as carp and bream, water and waste are expelled while the palatable food is retained. Thus, besides the size-dependent retention by the branchial sieve also a quality-dependent selective mechanism is available, sorting out food from non-food. This paper deals with the sensor and effector side of such a refined "taste and sorting" mechanism in carps.

Histological and SEM techniques showed sensory structures in the oropharyngeal lining and permitted quantification of detailed distribution patterns of taste buds. Their densities vary from 40-50/mm² in the orobuccal lining to almost maximal densities of 820/mm² in the pharyngeal area, which permits very local spot-measurement in gustation. Whether taste buds play also a mechanoreceptive role is not yet clear. Mechanoreceptors monitoring the flow of water and particles are required for the adequate manipulation of flow but have not been identified yet. X-ray movies show that water and particles are slowed down in the gustatory area as the tubular buccal cavity widens into the slit-like pharynx. Taste buds in the pharyngeal roof lie on top of a muscular cushion, the palatal organ, which is active (electromyography) in selection and food transport. Local contractions clamp food particles between roof and floor, whereas waste is flushed with the water through the branchial slits. Slime is less abundant here and of a different type compared with the transport- and mastication area. Closed protrusion movements of the snout manipulate the suspension back- and forwards, until the food is finally purified. The slit-shaped cavity assures a large gustatory area as well as contact between roof and floor, whose gill rakers are almost equally densily packed with taste buds.

Such a mechanism requires a very local adjustment of the effector apparatus and close coordination between roof and floor. These demands appear to be fulfilled anatomically by a viscerotopic mapping of the oropharyngeal lining in the vagal lobe, whose laminar structure and radially organized connections between sensory and motorneurons of both pharyngeal roof and floor (Morita & Finger, 1985) will allow very local reflexes in their opposed areas. The less punctuated mapping of the pharyngeal floor compared with its roof, which these authors found, can be explained by the overlap in working area of the gill rakers. This is due to their interdigitation between subsequent arches and their movement with respect to the palatal organ during feeding.

The integration of five subsequent feeding actions (Sibbing, 1985) and the remarkable plasticity in handling different types of food make us expect a highly organized substrate for perception and steering in feeding.

S88 CELL SPECIALIZATIONS IN THE TASTE BUD OF THE EUROPEAN SILURID FISH, SILURUS GLANIS (TELEOSTEI). Klaus Reutter (Anatomical Institute, University of Tuebingen, Oesterbergstrasse 3, 74 Tuebingen, FRG).

Most of the work concerned with the fish's taste bud ultrastructure is done on American Silurid catfishes belonging to the genera Ameiurus and Ictalurus. But up to now there is no information about the ultrastructure of the taste bud of the European Silurid, Silurus glanis. This is surely caused by the fact that this relatively rare and big fish (up to 2.5 m in length) is not a common laboratory animal. Fortunately, we have got two 60 cm-animals from a local breeder and we were able to investigate the taste buds of the barbels light- and electronmicroscopically. - In Silurus, taste bud morphology follows in great parts the well-known one of other Silurids: The taste bud is composed of light and dark sensory cells, which extend apically to the free surface of the epidermis and form the receptor field there, the basally situated basal cells, and the taste bud nerve fibre plexus which lies between the divided bases of the sensory cells and the basal cells. Among others we regularly found two especially striking cytological specializations, which are occasionally mentioned in literature, but not discussed too extensively: 1. The large receptor villus of a light sensory cell contains longitudinally arranged tubular profiles of about 50 nm in diameter, which are directly fused to the plasmalemma of the villus. The tubules are not identical to the profiles of the smooth endoplasmic reticulum. The tubules extend down to the supranuclear zone of the sensory cell and contain an electron-dense material. As ruthenium-staining reveals, this material seems to be a mucous substance. We interpret this structure as a mucus producing organelle, which also transports the mucus directly to the surface of the receptor villus, where it possibly is of significance in chemoreceptive processes. - 2. In view to the basal cells it is of special interest that these disc-like cells, transversally orientated to the axis of the taste bud, possess spin- or spikes-like processes which penetrate between the structures of the nerve fibre plexus. This morphological detail leads, together with former developmental investigations and cytochemical tests done on Ameiurus (Adv. Anat. Embryol. Cell Biol. 55/1, 1978) to the conclusion that the basal cell of the fish's taste bud is similar in structure and possibly in function to a Merkel cell. Our hypothesis that the basal cells in a Teleost taste bud may have not only coordinative functions in view to chemoreception and neurotransmission, but also a mechanoreceptive one, is supposed by this result.

NOTES

S89 DEVELOPMENT OF RAT FOLIATE TASTE BUDS. Bruce Oakley and Mark A. Hosley. (Department of Biology, University of Michigan, Ann Arbor, MI 48109).

Adult rat foliate taste buds are found in an average of five slits on the lateral margin of the tongue. We observed that the development of this taste bud population is complete by about one month postpartum (121 taste buds) in contrast to 3 months for vallate taste buds. We crushed the IXth nerve at different times to determine whether foliate taste buds might be neurally induced, perhaps during a sensitive period. At 90 days postpartum fewer foliate taste buds were present following crush of the IXth nerve at 3 days than at 75 days postpartum (CR3 = 58 vs CR75 = 87 taste buds, $p < .01$, t test). The implication that foliate taste buds may be neurally induced during an early sensitive period is further supported by the observation that the loss of taste buds with IXth nerve crush at 10 days was no more profound than with crush at 75 days (CR10 = 86 vs CR75 = 87 taste buds). The foliate papillae are innervated by both the chorda tympani and IXth nerves. To study the proliferation of foliate taste buds innervated only by the chorda tympani nerve we avulsed the right IXth nerve in three day old rats and sacrificed sub-groups of these rats at 15, 33, 45, 60 and 90 days postpartum. Taste buds formed by the chorda tympani increased moderately over time ($p < .01$, t test). Surprisingly, the chorda tympani seemed indifferent to the presence of the IXth nerve. Thus, at 90 days postpartum the total number of taste buds maintained by the chorda tympani was the same whether the IXth nerve had been removed at 3 days (39.4 buds) or at 75 days (42.0 buds). In the most posterior of the 5 foliate slits the normal chorda tympani supports no foliate taste buds at 15 days, 2% of its taste buds at 21 days, and about 10% thereafter. The inductive/trophic capability of the chorda tympani fibers in the fifth slit was neither impeded by the presence of the IXth nerve nor enhanced by its absence since at 90 days postpartum the chorda tympani maintained about 4 taste buds (10%) in this slit whether the IXth nerve had been present or absent from 3 to 75 days postpartum. These interesting permissive interactions among taste nerves are under investigation.

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NOTES

S90 INDUCED FORMATION OF DOUBLE TASTE BUDS IN GERBIL FUNGIFORM PAPILLAE. David R. Riddle and Bruce Oakley. Department of Biology, University of Michigan, Ann Arbor, MI 48109).

Double fungiform taste buds are rarely found in the gerbil, on average less than 1 per 300 papillae. One can cross the right chorda-lingual nerve to the contralateral side of the tongue. Thus, the left side of the tongue is innervated by the left and right chorda tympani and right lingual nerves. While such hyperinnervation did not increase the total number of taste buds, we did observe a 10 fold increase in double taste buds (1 per 35 papillae). Group data on the double taste buds indicates they are widespread on the anterior surface of the tongue i.e., the probability of occurrence is proportional to the normal taste bud density. For each taste bud pair we measured the taste bud volume, center to center and edge to edge separation, and the relative orientation of the two buds with respect to the anterior-posterior and medial-lateral axes of the tongue and the long axis of the papilla. The principle finding with respect to orientation was that the taste bud situated toward the base of the papilla was virtually always larger, presumably because it had more favorable access to the stem cells or taste axons arising from below. In 71% of the double taste buds the summed volumes of the pair exceeded the volume of a normal control taste bud located in a comparable position on the tongue. This is evidence that double taste buds usually represent an increase in tissue mass. If double taste buds arise by fission or budding, the process must have gone to completion for the taste buds did not overlap in 96% of the double taste buds and were often separated by 10 micrometers or more. An alternative to fission or budding is that the second taste bud arose from stem cells in the papilla, not from the existing taste bud. If the second taste bud arose from stem cells, then in those pairs oriented along the long axis of the papilla the second taste bud should be deeper, since it is difficult to imagine how the second bud could emanate from a site above the existing bud where there is a taste pore but no stem cells. In a few cases the deeper taste bud had no apparent taste pore, and therefore may not have been functional. Further analysis of these double taste buds, along with comparisons of single taste buds in normal and hyperinnervated tongues, may allow us to distinguish among possible mechanisms of formation of double taste buds.

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S91 RELATION OF RECEPTIVE FIELD SIZE AND SALT TASTE RESPONSES IN CHORDA TYMPANI FIBERS DURING DEVELOPMENT. C. M. Mistretta, T. Nagai and R. M. Bradley. (Oral Biology, Dentistry, and Center for Nursing Research, University of Michigan, Ann Arbor, MI 48109).

In continuing experiments to characterize the neural basis of developing salt taste sensation, we determined the size and chemical response properties of receptive fields for single chorda tympani nerve fibers in sheep fetuses and lambs. Seventy-four fibers were studied in 44 animals from three age groups: fetuses about 130 days of gestation (term = 147 days); perinatal animals (about one week before or after birth); lambs (about one to three months postnatal). A single chorda tympani fiber was dissected and the tongue was stimulated with 0.5M NH_4Cl, NaCl and KCl to record salt responses. Then individual fungiform papillae were stimulated electrically with anodal current from a fine platinum probe to determine number of papillae innervated by the fiber. Analysis of variance was used to compare data across age groups.

Number of papillae innervated by single fibers did not differ as a function of development (means and standard deviations for receptive field sizes = 13 papillae $\pm$ 10 in fetuses; 15 $\pm$ 9 in perinatal animals; 11 $\pm$ 7 in lambs). However, the range of field size was very broad, 1 to 40 papillae across ages, and small fields were encountered more frequently in lambs than in fetuses. For example, 48% of lamb fields contained 8 or fewer papillae compared to 41% of fetal and 24% of perinatal fields. Not only were small fields observed more frequently in fibers in older animals, but also there was an increase in the proportion of fibers that responded with highest frequency to NaCl compared to NH_4Cl ($p = 0.06$). Indeed, receptive field size correlated negatively with the NaCl/NH_4Cl response ratio. That is, smaller receptive fields had higher frequency responses to NaCl than to NH_4Cl ($p = 0.05$). Receptive field size correlated positively with NH_4Cl and KCl response frequencies and with the maximum electrical response frequency from the most sensitive papilla in the field ($p = 0.01$).

These results demonstrate that there is extensive branching of peripheral taste nerve fibers already in fetuses, to provide innervation for large and small receptive fields. However, fibers with small receptive fields are encountered more frequently in older animals, and these tend to be more responsive to NaCl than other salts. This suggests a developmental reorganization of peripheral innervation with possible reduction of synaptic contacts in some large receptive fields, or an addition of fibers that synapse in small, NaCl-responsive receptive fields.

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S92 RECEPTIVE FIELDS OF SECOND ORDER TASTE NEURONS IN SHEEP: CONVERGENCE OF AFFERENT INPUT INCREASES DURING DEVELOPMENT. Mark B. Vogt and Charlotte M. Mistretta. (Dept. Oral Biology, School of Dentistry, and Center for Nursing Research, University of Michigan, Ann Arbor, MI 48109)

The extent of convergence of taste afferents onto second order cells during development can be determined by comparing the size of receptive fields of first and second order neurons in animals of different ages. Therefore, we have recorded from taste neurons in the nucleus of the solitary tract (NST) in sheep fetuses (130 days of gestation; term = 147 days) and lambs (40 days postnatal) to determine the number of fungiform papillae that provide input to each neuron. This information is compared to previously collected data on receptive field sizes for single chorda tympani (CT) nerve fibers in the same age groups.

Single NST neurons were isolated and responses to 0.5M NH4Cl, NaCl and KCl were recorded. Then a fine platinum probe was used to electrically stimulate individual fungiform papillae and determine the number of papillae in the receptive field of the neuron. In initial experiments we have studied 8 single units in fetuses and 11 in lambs.

As presented in the table, the receptive field size of NST neurons increases between fetal and lamb ages ($p = .08$). In addition, for either fetal or lamb groups alone, the receptive fields of NST neurons are larger than those of CT fibers ($p = .08, .001$ respectively).

Number of Papillae in Receptive Field

		Fetus	Lamb
NST Neurons:	$\bar{X}$ (SD)	21 (10)	44 (32)
	N	8	11
	range	8 - 36	2 - 100
CT Fibers:	$\bar{X}$ (SD)	13 (9)	11 (7)
	N	17	19
	range	2 - 40	2 - 29

Although the data on second order cells are preliminary, the larger fields of NST neurons indicate convergence of afferent input at both fetus and lamb ages. Furthermore, because peripheral receptive field sizes do not differ between fetus and lamb ages, whereas central field sizes increase, the degree of convergence is greater in lambs than in fetuses. These data provide a quantitative demonstration of convergence of afferent input onto second order neurons in the taste system, and indicate that the extent of convergence increases during development.

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S94 ODOR DETECTION IN RATS WITH LESIONS OF OLFACTORY BULB AREAS IDENTIFIED WITH 2-DG. S. Graham*, B.M. Slotnick*, D.G. Laing** and G.A. Bell**. (*The American University, Washington DC 20016 and **CSIRO Div. Food Res., Australia.)

Using 2-DG, Laing et al. (AChemS, 1985) reported an odor-specific map of glomerular metabolic activity in the rat olfactory bulb after stimulation with propionic acid. The map was characterized by a number of labelled glomeruli on the medial side of the bulb, and a striking feature was the location of a cluster of glomeruli at approximately 3 mm caudal to the rostral tip of the bulb. To assess the behavioral significance for these metabolic findings we tested sham operated rats (n=3) and those with bilateral lesions of medial (n=3) or lateral (n=2) surface of the rostral half of the olfactory bulb for their ability to detect the vapor of propionic acid and other chemicals. An operant conditioning procedure was used (Slotnick and Schoonover, Chem. Sen., 1984) and for all tests the odorant served as the positive stimulus and clean air as the negative stimulus. There were no differences among groups in the acquisition of detection of propionic acid, butanol, or geraniol, but a large number of errors were recorded for acetic acid vapors. Also, there were no differences among groups for propionic acid threshold (mean thresholds relative to percentage of vapor saturation: Shams, 0.0018%; Medial lesioned group, 0.0001%; Lateral lesioned group, 0.0002%). These results indicate that the area of the olfactory bulb containing a major focus of metabolic activity induced by exposure to propionic acid can be removed without producing a deficit in the detection of that odor.

However, since the behavioral paradigm did not contain a two-odor discrimination test, it remains to be shown whether the recognition qualities characteristic of propionic acid were lost. The glomerular map of propionic acid contains a number of active foci that would not have been removed by the lesions, and these may have been sufficient to result in normal thresholds.

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S93 FUNCTIONAL AND MORPHOLOGICAL REGENERATION AFTER PARTIAL OLFACTORY BULBECTOMY IN THE GOLDFISH. H.P. Zippel and D.L. Meyer (Physiologisches Institut, Humboldtalle 23, Institut für Anatomie, Kreuzbergring 36, Universität D-3400 Göttingen, FRG)

Lower vertebrates such as amphibia and fish are ideal organisms for the study of CNS regeneration. In our initial studies we were able to demonstrate the functional and the morphological regeneration of fibre connections in the olfactory system (Zippel and Westerman, 1970; Zippel, v.Baumgarten and Westerman, 1970). From more recent experiments (Zippel, Breipohl and Schoon, 1981) it is evident that after a total bilateral olfactory bulbectomy the receptors in the olfactory mucosa (MO) regenerate, but no connections were found between the MO and the telencephalon; behaviourally, no return to the preoperative behavioural threshold could be found even after a 12 months survival period.

In the present investigations, after a partial bilateral ablation of the olfactory bulbs (BO), a rapid return to the preoperative behaviour could be recorded at different time intervals post op.: rostral bulbectomized (RBE) goldfish respond positively after a short regeneration period of about 7 to 10 days, whereas caudal bulbectomized (CBE) animals react positively after roughly 6 weeks. From neuroanatomical investigations performed immediately after the behavioural test (RBE: 20 days, CBE: 9weeks post op.) it is evident that shortly after the functional recovery a surprisingly high level of regeneration has been achieved: the spherical shape of the BO has been reestablished nearly completely in both the collectives. In RBE-animals the connections between the olfactory mucosa (MO) and the BO are not essentially different from those in intact animals, whereas in CBE-animals the central connections between the BO and the telencephalon are evidently thinner and much more transparent. Light-microscopic investigations of the MO show no differences between operated and intact animals, and from HRP-studies it is evident that fibre-connections between the MO and the BO exist, albeit in smaller numbers in comparison with intact or long-term regenerated fish. Investigations of de- and regenerative processes in different parts of the olfactory nerves and bilateral total and partial bulbectomy, and the period of functional recovery are in progress.

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S95 ALTERATIONS IN BEHAVIORAL RESPONSES TO TASTES FOLLOWING CHORDA TYMPANI (CT) AND/OR GLOSSOPHARYNGEAL (IX) NERVE SECTION IN RATS. Gary J. Schwartz and Harvey J. Grill. (University of PA, Dept. of Psychology, Philadelphia, PA 19104).

Gustatory nerve signals contribute to the acceptance and rejection of foods and fluids. Surprisingly, previous studies have failed to demonstrate significant changes in ingestive behavior following gustatory nerve section. This study was designed to examine changes in two-bottle intake and taste-reactivity (TR) responses following bilateral CT and/or IX nerve section.

Rats received one of the following manipulations: a bilateral CT section, a bilateral IX section, a bilateral section of both CT and IX nerves (CT+IX), or a sham section. All rats received 1ml, 1 min. intraoral infusions of several concentrations of each of the following tastants: sucrose (S), NaCl, quinine hydrochloride (QHCl), and magnesium chloride (MgCl2). TR responses to these infusions were analyzed to determine the number and types of responses elicited. In addition, each rat received several 24-hr. two-bottle tests comparing tap water intake to each of the tastants above.

The experiments reported here demonstrate significant changes in both responses to intraoral infusions and two-bottle intake of tastants following peripheral gustatory nerve section. Nerve section rats showed fewer aversive TR responses to a variety of normally rejected tastants, and these rats also showed greater preferences for normally avoided tastes. CT, IX, and CT+IX rats all show significantly fewer aversive TR responses to 1.0 M NaCl than controls. In addition, IX rats consumed significantly more NaCl than CT or control rats. Both CT and IX rats showed significantly fewer aversive TR responses to QHCl, and aversive responding to QHCl was absent in CT+IX rats. Neither CT, IX nor CT+IX rats differed from controls in two-bottle intake of QHCl. CT, IX and CT+IX rats all showed significantly fewer aversive responses to MgCl2 infusions, while two-bottle intake of this tastant did not differ from controls. All nerve-sectioned rats failed to differ from controls in both TR responses and two-bottle intake of sucrose.

The results suggest: that gustatory input from both CT and IX each contribute to the production of aversive TR responses, that the absence of CT or IX alone is not sufficient to eliminate aversive TR to QHCl but that the combine nerve section may be, that TR to sucrose is not mediated by gustatory input from CT or IX, and finally, that oro-motor responses to intraoral taste stimuli may provide a better measure of gustatory sensitivity, making it easier to demonstrate the effects of gustatory nerve section.

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S96 THE COMPLEX SWEET TASTE OF ALCOHOL: AVERSION GENERALIZATION DATA FROM NORMAL RATS AND RATS LACKING GUSTATORY NEOCORTEX. Stephen W. Kiefer, Christine W. Metzler, and Nancy S. Morrow. (Dept. of Psychology, Kansas State University, Manhattan, KS 66506).

Previous reports utilizing an aversion generalization technique have suggested that alcohol has a complex sweet taste for rats. Having been trained to avoid a 6% alcohol solution, rats showed significant generalization to a sucrose-quinine solution but not to the sucrose or quinine alone. In the present experiment, the taste quality of alcohol was again examined with normal rats. In addition, rats lacking gustatory neocortex, which have been shown to acquire alcohol aversions normally, were examined for their performance in a generalization paradigm. Although rats lacking gustatory neocortex acquire alcohol aversions normally, they do exhibit deficits in taste aversion learning.

Forty eight naive rats (normal, n=12; control neocortex ablation, n=12; gustatory neocortex ablation, n=24) were placed on a schedule of restricted fluid access. Following adaptation to the schedule, half of each group was trained to avoid a 5% alcohol solution by pairing it with lithium chloride illness. After two training trials, all rats were presented with six solutions, one per day. These solutions were compound tastes made up of pairs of the following stimuli: .1 M sucrose, .1 M sodium chloride, .01 M hydrochloric acid, .0001 M quinine hydrochloride. Rats were then tested with the 5% alcohol solution to test for aversion strength. Finally, the rats were tested with the individual taste stimuli.

Results indicated that control rats (normal control rats and rats with control ablations) and rats lacking gustatory neocortex developed significant and equivalent aversions to the alcohol solution. Control rats also exhibited significant generalization to the sucrose-quinine solution and a weaker but significant generalization to the sucrose-acid solution. Tests with the individual taste components revealed no significant generalization by the control rats. Rats lacking gustatory neocortex failed to exhibit significant generalization to any of the compound tastes or individual tastes. These results confirm that the gustatory component of learned alcohol aversions in rats has a sucrose-like taste component.

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S97 DECREASED SENSITIVITY TO BITTER SOLUTIONS FOLLOWING CHRONIC OPIOID RECEPTOR BLOCKADE. Wesley C. Lynch, Charles M. Paden and Susan Krall. (Depts. of Psychology and Biology, Montana State University, Bozeman, MT 59717).

Chronic blockade of opioid receptors leads to temporary receptor upregulation and a corresponding super-sensitivity to the analgesic effects of opioid drugs (Tempel, A., et al., PNAS, 81: 3893, 1984). In addition, we have found that opioid blockade by chronic subcutaneous infusion of naloxone (NAL) leads to inhibition of intake of sweet (sucrose) and bitter (quinine) solutions (Lynch, W., et al., Soc. for Neurosci Abst, 11, 558, 1985). The purpose of the present experiment was to further examine intake patterns for sucrose and quinine solutions during the period of maximal receptor upregulation, one week following chronic NAL treatment. Ten adult male Spragne-Dawley albino rats were implanted with subcutaneous Alzet minipumps (Model 2002). Pumps contained either 150 mg/ml Naloxone-HCl (n=6) or 0.9% NaCl (n=4). An automated lick-monitoring system was used to measure consumption twice daily during the 2 weeks of treatment and for 1 week following pump removal. At 9 a.m. daily (4 hours after lights on) each animal was offered 3 calibrated bottles containing either sucrose (0.2 M and 0.7 M) or water. For this test animals were non-deprived. At 10 a.m. a 6 hour period of water deprivation was begun and at 4 p.m. each animal was again offered 3 bottles, this time containing either quinine-HCl ($1x10^{-5}$M and $3x10^{-5}$M) or water. Intake data were collected for the following 6 hours and these 3 bottles remained on the cages until 9 a.m. the following day.

Data analyses completed thus far show a dramatic increase in quinine intake among the NAL treated animals lasting for approximately 5 days following pump removal. Prior to pump removal, quinine intake in both groups was generally less than 10% of total intake. However, following NAL pump removal quinine intake increased for 3 days to nearly 50% of total consumption in the 6 hour test. Sucrose intake during this same period was unaffected. Thus, opioid receptor upregulation is associated with a corresponding decrease in gustatory sensitivity to quinine. These results may, therefore, suggest a new functional role for opioid systems in the regulation of taste sensation.

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S98 **MYOPATHIC (BIO 14.6) HAMSTERS FAIL TO DEVELOP SALT APPETITE IN RESPONSE TO DOCA.** Rudy A. Bernard, Timothy W. Priehs and Karen J. Mooney. (Dept. of Physiology, Michigan State University, East Lansing MI 48824).

Sodium replete rats and hamsters develop a large salt appetite in response to deoxycorticosterone acetate (DOCA). This effect is considered a behavioral counterpart of the sodium conserving role of the mineral corticoid hormones and as support for the theory that salt appetite is regulated by the renin-angiotensin-aldosterone (RAA) system. Most of the procedures employed in the study of salt appetite, such as adrenalectomy, dietary sodium restriction, I.P. dialysis, parotid gland fistulation, formalin injection, administration of furosemide and other diuretics, tend to reduce plasma sodium and consequently achieve their effect by activating the RAA system. The resulting increase of sodium intake is a classic example of regulatory behavior.

DOCA-stimulated salt appetite, however, cannot be readily explained by the same mechanism, for it is initiated by positive rather than negative sodium balance, which suppresses the activity of the RAA system, and the resulting salt appetite does not promote sodium homeostasis. We propose instead that the stimulation of salt appetite by DOCA is related to the phenomenon of renal escape, in which the initial sodium retention produced by DOCA is followed within a few days by a return to normal sodium balance in spite of continued hormone administration. Renal escape is now believed to be due to the release of endogenous natriuretic factor(s) through the stimulating effect of plasma volume expansion. It is our hypothesis that DOCA-stimulated salt appetite is due to activation of the same endogenous natriuretic system.

We undertook the experiments reported here after Chimoskey et al. (1984) reported that the atria of BIO 14.6 hamsters were deficient in natriuretic factor. We found that these hamsters failed to escape from the salt and fluid retention effects of DOCA, gaining 56% of their initial body weight in 10 days, whereas the controls gained only 5%. During this time the myopathic hamsters did not increase their salt and water intake significantly, whereas the controls increased their sodium intake by over 80% and their water intake by over 250%. It is clear from these results that the retention effects of DOCA are not linked to increased salt and water intake, and that stimulation of salt appetite occurs only in the animals that can escape retention by activating a natriuretic mechanism. The absence of escape from the high endogenous levels of mineral corticoids produced by sodium deficiency is explained by the lack of volume expansion in this condition.

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S99 A FREEZE-FRACTURE STUDY ON THE PRE-NATAL DEVELOPMENT OF CILIATED SURFACES OF RAT OLFACTORY EPITHELIA. Bert Ph. M. Menco. (Dept. of Neurobiology and Physiology, O.T. Hogan Bldg., Northwestern University, Evanston, IL 60201)

In a previous study we postulated that olfactory receptor cells with primary cilia may differ in their odor responsivity from receptor cells with secondary cilia (Menco and Farbman, 1985). In order to further substantiate this hypothesis we decided to study the membrane morphology of both types of cilia using freeze-fracturing. Membranes of apical structures, i.e., cilia and microvilli, of olfactory epithelia were investigated as a function of pre-natal development, with membranes of similar structures of adjacent respiratory epithelium serving as a material for comparison. We used rat embryos from E14 (E1=day that the dams are sperm-positive) through E19 and young adults. Fracturing of chemically fixed, cryo-protected samples was carried out in a Cressington Freeze-Fracture apparatus at -140°C and at a vacuum better than 10^{-7}mbar. Platinum replication was done at an angle of 20° and the replicas were reenforced with carbon evaporated from overhead. During the evaporation the specimens rotated at a rate of approximately 400 rpm. Tentative results indicate that when the receptor cells bear no external cilia or only primary ones, these cilia and the endings from which they emerge do have lower densities of intramembranous particles than when subsequently formed secondary cilia are present. Moreover, the ciliary necklace, a membrane structure present at the base of all types of cilia which consists of a series of concentric strands of membrane-bound particles, seems to be a convenient marker for whether the cilia are primary or secondary. In contrast to the latter ones, which have an average of eight strands in olfactory epithelia and six strands in respiratory epithelia (Menco, 1980), those of primary cilia never have more than four strands, and most commonly only two. This is true of primary cilia of olfactory receptor and supporting cells, glandular cells and putative ciliated and microvillous respiratory epithelial cells. In this respect primary cilia resemble protozoan cilia (Menco, 1980). From the above it seems that when the receptor cells have not yet cilia or only primary ones, the membrane structure of their apical surfaces and surface processes does indeed differ from that of receptor cells found later on. Finally, the rod-shaped intramembranous particles, frequently found above the tight-junctional belts of olfactory supporting cells, can be discerned at least as early as E16 and even when these cells still have (i.e., did not yet lose) their primary cilia.

Refs.: Menco, B.Ph.M. (1980) Cell Tissue Res., 212: 1-16.
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This work was supported by NIH grant #NS 21555.

S100 **TASTE PERCEPTION OF SALT IN YOUNG, OLD AND VERY OLD ADULTS** J. Chauhan, Z.J. Hawrysh, C. Ko and S. Ko. (Dept. Foods and Nutrition, University of Alberta. T6G 2M8)

This study investigated salt taste perception using aqueous and simple food systems in 180 young and elderly Albertans. Subjects, chosen randomly with extra volunteers, were divided into three age groups: young (Y) (20-29 yrs), old (O) (70-79 yrs) and very old (VO) (80-99 yrs) with 30 women and 30 men/group. Magnitude estimations of the intensity and pleasantness of various concentrations (C) of NaCl (20-640mM) in deionized water (20±3°C) and in a soup 55±5°C) were obtained in triplicate, in separate sessions. Subjects were English-speaking, relatively healthy and free-living. Subject attributes: education, salt use etc. were obtained.

Initial analyses show a significant age effect ($p=.001$) on salt intensity estimates (IE) in soup only. There were significant age x C interactions for both systems ($p=.001$). At high salt C in both systems, the O gave smaller IE ($p<.05$) than the Y and VO. In soup at high salt C, the VO gave larger IE ($p<.05$) than the Y. Very salty water and soups tasted less salty to the O than to the Y and VO, while to the VO salty soups tasted even saltier than to the Y. IE of the Y and O for high C of salt in water were significantly larger than those for soup, but for the VO system had no effect.

For salt in both systems significant pleasantness estimates (PE) for age ($p=.001$) and age x C interaction ($p=.001$) were found. As salt C in water increased, the Y gave decreasing PE, while PE of the O tended to plateau, rise slightly and then drop. In water as salt C increased the VO gave increasing PE up to the middle C, the breakpoint at which PE dropped markedly with increasing salt C. PE for the soup showed no marked age differences. For soup, each age group gave increasing PE with increasing salt C to a breakpoint C, which for the Y and O was between the middle and the next highest C, and for the VO the breakpoint was the middle salt C.

For both systems, salt IE show significant sex effects (water, $p=.03$; soup, $p=.004$) and sex x C interactions ($p=.001$ for each system). At high salt C in both systems, the women gave larger IE than the men. Thus, the very salty water and soups tasted saltier to the women than the men. Salt IE in water also showed a significant age x sex x C interaction ($p=.042$), with the Y and O women giving larger IE at high salt C than the men.

At low salt C in both systems, the frequency of zero estimates for the VO was very high compared to the Y and O. These initial findings suggest deficits in salt perception in the elderly. Further analyses of the influence of age and subject attributes in relation to taste perception are required.

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S101 EFFECTS OF AGE AND BIOCHEMICAL STATUS ON PREFERENCE FOR AMINO ACIDS. Claire Murphy. (Department of Psychology, San Diego State University, San Diego, CA 92182-0350).

A primary reason for interest in chemosensory changes over the life span has been the assumption that such changes are linked to changes in nutritional status in the elderly. To date, the data necessary to support this assumption have been lacking. The following studies provide evidence that demonstrates that both age and nutritional status affect the preference for a nutritionally-significant chemosensory stimulus. In the first study 10 young (18-26 yrs) and 16 elderly (65 or more yrs) subjects rated the pleasantness of various concentrations of casein hydrolysate in an amino acid deficient soup base, using a bipolar line scale. Blood assays were performed to assess total protein, albumin and blood urea nitrogen (BUN). Elderly persons and those whose blood values indicated lower biochemical status preferred higher concentrations than young persons and those with blood values indicative of higher biochemical status.

In a second study 20 young and 20 elderly participants performed the same pleasantness task. To test the possibility that the differences in preference could simply be due to reduced input to the olfactory and taste systems of the elderly, subjects also rated the stimuli for intensity using the method of magnitude matching. Blood was assayed and a single index of biochemical status was produced using measures of protein, albumin and BUN. Although the older subjects did rate the stimuli as less intense, mean intensity alone was not a significant predictor of preference. The best predictors of preference for the amino acids were the biochemical index and age, suggesting the importance of further study of the relationship between nutritional status and chemosensory preference in the elderly.

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S102 INFLUENCE OF AGING ON RECOGNITION MEMORY FOR ODORS AND GRAPHIC STIMULI. William S. Cain (John B. Pierce Foundation, 290 Congress Avenue, New Haven, CT 06519) and Claire L. Murphy (Dept. of Psychology, San Diego State University, San Diego, CA 92182).

An experiment with 16 elderly (av. age=72 yrs) and 16 young (av. age=21 yrs) subjects explored long-term recognition memory for various types of stimuli: common odors, faces of American presidents and vice-presidents, and relatively obscure electronic symbols. Twenty of each type were first presented in intermixed order for inspection and then subsets of ten of each were presented again, along with an equal number of distractors, for recognition 10 min, 2 weeks, and 6 months later. After the last memory test, subjects sought to name, i.e., to identify, all of the stimuli that had ever been presented as either test or distractor stimuli. In the recognition memory test, the young performed at a higher level than the elderly for the symbols and the odors, but at a lower level for the presidents and vice presidents. Nevertheless, the elderly tended to forget all stimuli at a somewhat faster rate. This was most apparent for odor memory, where the initially modest performance of the elderly dropped to chance after two weeks.

A difference in competence at identification accounted for some of the difference in recognition memory between the young and old. The young could identify almost three times as many odors as the old, but only about 60% as many presidents and vice-presidents. Neither group showed any significant ability to identify the symbols, yet both showed good ability to recognize them. This was the task where the young and old came closest in initial memory performance, possibly because both groups were at the same advantage/disadvantage in encoding the stimuli at inspection. The results of the investigation reinforce the notion that encoding, particularly verbal encoding, will determine much of recognition memory performance, even for odors. The elderly are not always at a disadvantage in this regard, to wit, they remembered the presidents and vice-presidents better than the young.

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S103 AGING IMPAIRS THE ABILITY TO PERCEIVE GAS ODOR. Joseph C. Stevens and William S. Cain. (John B. Pierce Foundation Laboratory, 290 Congress Avenue, New Haven, CT 06519).

Aging can elevate olfactory detection threshold, weaken suprathreshold strength, and impair odor discrimination and identification. Two studies explored the implications for the perception of gas leaks. Both examined ethyl mercaptan, the most common warning agent added to propane. In the USA propane annually causes thousands of explosions and fires, injures hundreds, and takes many lives. The first study, conducted in the laboratory, compared 21 elderly (70-85 years) and 21 young (18-25) subjects for (a) detection by a forced-choice procedure, (b) suprathreshold strength by magnitude matching to NaCl solutions, and (c) general ability to identify common odors. For detection the average elderly subject needed ten times greater concentration than the young. In the elderly, suprathreshold strength appeared weakened at all concentrations tested and odor identification impaired; hence even if detected, the warning agent is likely to smell weaker and less likely to be identified. The second study compared 110 persons over 60 (mean=72) in senior citizens' centers with 52 persons under 40 (mean=24) for the ability to discriminate between air and odorized commercial propane diluted to the U.S. Department of Transportation's legal safety standard of one-fifth the lower explosive level. Under these conditions the vapor phase concentration of ethyl mercaptan increased systematically as the tank emptied, from 30 ppb (full tank) to 210 ppb (22% full), well above the nominal minimum acceptable concentration of 14 ppb. As expected, subjects tested at low tank levels, where ethyl mercaptan concentration was higher, detected more reliably. Even so, 45% of the elderly persons failed to detect reliably (four or five correct out of five 3-alternative forced-choice trials). Ten percent of the young subjects failed this test. Old people are clearly at greater peril of propane gas explosions.

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S104 TASTE AND SALIVARY GLAND DYSFUNCTION. James M. Weiffenbach, Philip C. Fox and Bruce J. Baum. (Clinical Investigations Section, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892)

Saliva is normally present during tasting and is thought to be essential for the maintenance of oral structures subserving taste. We have recently described remarkably unimpaired taste perception in the complete and chronic absence of salivary gland function. In the present study, we assess the taste function of individuals with less severe salivary gland dysfunction

In our earlier study we documented chronic and severe salivary gland dysfunction in 8 individuals who had no flow from either parotid or submandibular/sublingual glands under resting or stimulated conditions. Biopsy of their minor glands showed massive distruction. Dental caries and deterioration of oral soft tissues attested to chronic absence of saliva. One individual had no taste complaints, denied any change in taste perception and demonstrated performance well within normal limits on threshold and suprathreshold tasks with stimuli representing each of the four basic taste qualities. Taste impairment is not a necessary consequence of salivary gland dysfunction. As a group these subjects were essentially free from subjective complaints and were not impaired on objective measures of suprathreshold perception. The incidence of impairment on the threshold task was, however, elevated.

The 10 subjects of the present study lack flow at rest but do salivate in response to stimulation and thus have periodic exposure to saliva. On objective measures of taste function they resembled the subjects reported earlier. Impairment of suprathreshold performance, as indicated by scores below the 10th percentile, was no more common then expected by chance. Three individuals showed no impairment for either of two measures of suprathreshold perception for any of these four qualities. Similarly threshold performance in the present study resembled that observed in the earlier one. In each study, the incidence of impaired performance on the threshold task was elevated above that expected from normal subjects. However, as in the earlier case, a single individual with normal thresholds for all four qualities demonstrated that impairment of threshold sensitivity is not inevitable. While the objective findings for these individuals parallel those of the earlier study, the subjective reports obtained in the two studies are strikingly different. Only one of the individuals with complete gland dysfunction reported decreased taste perception, whereas 9 of 10 individuals with some residual salivary gland function reported decreases in their sense of taste.

S105 PSYCHOPHYSICAL EVIDENCE OF TASTE DYSFUNCTION IN BURNING MOUTH SYNDROME (BMS). M. Grushka, B.J. Sessle and T.P. Howley. (Fac. Dentistry, Univ. Toronto, Toronto, Canada M5G 1G6).

BMS is a poorly characterized intraoral pain disorder which may primarily affect post-menopausal females. Although little is known of its aetiology, a strong neurological or psychogenic component is often inferred. Because impaired taste (an alteration in taste perception and/or a dysgeusic taste) is a frequent symptom of BMS, we initiated studies to determine whether there was any objective evidence of taste dysfunction in 47 BMS subjects (mean age $\pm$ S.D., 55.2$\pm$10.4 years); 27 age- and sex-matched normal subjects (52.8$\pm$8.4 years) served as controls. Taste detection thresholds and taste intensity scaling for the four taste qualities were obtained. The range of concentrations, with adjacent concentrations differing by a factor of 1.8 were, in Molar units: 96.8×10^{-3} to 3.7×10^{-6} for sucrose and sodium chloride; 17.6×10^{-4} to 3.7×10^{-7} for citric acid, and 1.7×10^{-4} to 4.6×10^{-9} for quinine hydrochloride. All stimulus fluids were available for threshold determination whereas only the eight to 10 most concentrated fluids were used for intensity scaling.

No statistically significant ($p>0.05$) differences in mean log Molar units between BMS and control subjects were found in thresholds for salt (BMS, -3.164$\pm$1.14; controls, -3.618$\pm$1.26), sour (BMS, -4.648$\pm$1.12; controls -4.702$\pm$1.03) or bitter (BMS, -6.039$\pm$1.15; controls. -6.451$\pm$1.39); however, thresholds for sweet were significantly higher ($p<0.05$) for BMS (-2.224$\pm$0.88) than control subjects (-3.546$\pm$1.50). At suprathreshold concentrations, significant differences were not found between the BMS and control groups for salt and bitter. However, perception intensity was significantly higher for the BMS than control subjects for sweet and for sour at lower suprathreshold concentrations. When the BMS subjects were subsequently divided into those subjects who reported a dysgeusic taste ("taste" group, approximately 75 and 60% of the BMS subjects tested at threshold and suprathreshold levels, respectively) and those without a dysgeusic taste ("no taste" group, approximately 25 and 40%, respectively), it became evident, that at suprathreshold levels, differences in taste function of sweet and sour originated mainly from those BMS subjects with complaints of a dysgeusic taste.

These findings provide psychophysical evidence for taste dysfunction in BMS, and lend support to a neurological aetiology of BMS.

Supported by the Gerontology Research Council of Ontario.

S106 BLOCKING LEARNED FOOD AVERSIONS IN CANCER PATIENTS RECEIVING CHEMOTHERAPY. RICHARD D. MATTES, CATHY ARNOLD and MARCIA BORAAS. (Monell Chemical Senses Center, Philadelphia, PA 19104 and Fox Chase Cancer Center, Fox Chase, PA 19111).

Learned food aversions have been implicated in the anorexia of cancer and may adversely affect the quality of patients' lives. The purpose of this project was to evaluate whether chemotherapy patients surreptitiously exposed to a nutritionally inconsequential "scapegoat" food (fruit flavored beverage) in the one hour period prior to their first course of treatment would direct any newly formed aversion towards the scapegoat, and thereby spare previously acceptable, nutrient-dense items from aversions.

Thus far, 45 patients have been randomly assigned to receive scapegoat exposure while 29 patients now serve as controls. Patients are 25-76 years of age with histologically confirmed cancers primarily of the breast or lung. All are receiving their first course of chemotherapy and none has received radiotherapy during their time of participation. An aversion to the scapegoat is said to have formed when, in a two-choice condition, one-third or less of total beverage ingestion is derived from the scapegoat beverage. Aversions to other foods are monitored by open ended questionnaires and 9-point food action rating scale responses to the foods ingested in the 48-hour period surrounding the first treatment session. These assessments are conducted 1,2,4 and 6 months following the the initiation of treatment.

Following mere exposure to the scapegoat beverage, less than 18% (8/45) of patients reported an aversion to any other food during the follow-up period. The incidence in unexposed patients was significantly greater: 52% (13/25) (p .003). In the subset of patients actually forming an aversion to the scapegoat, the incidence of new aversions was reduced to less than 9% (2/16). Neither the perceived novelty of the scapegoat beverage nor the amount ingested was significantly related to its efficacy in limiting aversions to other foods.

The present findings suggest that exposure to a nutritionally inconsequential food just prior to a course of chemotherapy blocks the formation of aversions to other nutrient-dense foods and may therefore be a valuable therapeutic aid in the management of cancer patients receiving chemotherapy.

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S107 OLFACTORY DYSFUNCTION IN ALZHEIMER'S DISEASE. Richard L. Doty, Patricio Reyes, and Tom Gregor. (Smell and Taste Center, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 and Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107).

Alzheimer's disease is the most common cause of dementia. Of those patients over 65 with dementia, approximately half are due to this disease. The present estimated annual cost of caring for patients with such debility is over 11 billion dollars. Excluding new ways of preventing or treating the underlying problem, it has been predicted that by the year 2030, the annual cost of caring for such patients will be eight times the amount we now spend on all medical and mental health research. Thus, detection of such dementia at its incipient stages would be of considerable value in the development of treatments and therapeutic strategies, as well as in better understanding the nature and course of the disease.

Preliminary reports suggest that olfactory impairment exists in patients with Alzheimer's disease (e.g., Peabody et al., Amer. J. Psychiat., 1985, 142, 524). These reports, along with anatomical studies of histopathological changes in regions known to receive primary and secondary afferents from the olfactory system (e.g., the hippocampal formation; cf. Hyman et al., Science, 1984, 225, 1168), support the notion that major alterations in olfactory function may be present in Alzheimer's disease.

We administered both the University of Pennsylvania Smell Identification Test (UPSIT) and the Picture Identification Test (PIT) to 50 individuals with well documented and staged Alzheimer's disease. Forty of these patients performed satisfactorily on the PIT. The UPSIT scores of the majority of these 40 patients fell below those of sex- and age-matched controls, including individuals with the earliest stages of the disease. Detection threshold testing -- which is currently going on -- suggests that some, but not all, Alzheimer's patients evidence marked inability to detect odorants. Since we previously demonstrated, in demented patients with Korsakoff's psychosis, a high correlation between lumbar CSF levels of a major noradrenergic metabolite (MHPG) and UPSIT test scores (Mair et al., Neuropsychologia, in press), a determination of such a relation is also currently being made in Alzheimer's patients. The results of these tests will be presented in detail.

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S108 DEGENERATION-REGENERATION OF THE OLFACTORY NEUROEPITHELIUM FOLLOWING BULBECTOMY: AN SEM STUDY. Edward E. Morrison*, Pasquale P.C. Graziadei and Richard M. Costanzo* (*Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298 and Dept. of Biological Sciences, Florida State University, Tallahassee, FL 32306).

Bulbectomy results in a retrograde degeneration of mature sensory neurons in the olfactory neuroepithelium. Replacement neurons are generated by dividing basal cells that develop and mature into new neurons. In the present study we have examined the olfactory neuroepithelium following bulbectomy using the scanning electron microscope (SEM). This method allowed us to examine the detailed surface morphology of the degeneration - regeneration process. Adult hamsters (n=37) were unilaterally bulbectomized and examined at recovery periods of 3 to 94 days. Nasal septum and turbinates were processed for SEM. During the initial degeneration period (0-4 days) the epithelium lacked cilia and consisted primarily of supporting cells, basal cells, and degenerating olfactory neurons. The absence of mature neurons during early survival times allowed for the detailed examination of supporting cells. The supporting cells extended to the basal lamina where they terminated in a footlike process. We also observed that adjacent supporting cells were attached by intercellular connections. During early recovery periods (5-25 days) we observed an increasing number of neural elements. Immature neurons lacked well developed dendritic and axonal processes, and were primarily located in the lower epithelium. Occasionally, isolated regions of increased basal cell activity (active zones) were observed. Dendrites of the maturing neurons grew toward the apical surface between and along the edges of supporting cells. In some cases the supporting cells appeared to surround and encase the developing dendrite. Newly developed axons (.1-.4 um) grew through the basal lamina where they fasciculated into larger bundles within the lamina propria. At later recovery periods (35-94 days) the epithelial surface was covered with a dense ciliary blanket. The reconstituted neuroepithelium resembled controls, containing numerous mature olfactory neurons and large axon bundles in the lamina propria.

Our findings confirm the degeneration-regeneration process in which basal cell activty gives rise to the replacement of sensory neurons and the reconstitution of the olfactory neuroepithelium. In addition we have observed for the first time detailed surface morphology of the supporting cells and their intimate relationship with developing neurons and neighboring supporting cells.

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S109 OLFACTION TEST IN THE LARYNGECTOMIZED PATIENTS BY THE ARTIFICIAL AIRWAY TUBE METHOD

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(Head: M. Ohyama, M.D.)

The authors noticed the occurrence of the nasal air current in the laryngectomized patients by connecting a tube between the tracheal stoma and the naris, so named this technique an artificial airway tube method.

By this manner, the olfactory acuities of laryngectomized patients, were evaluated by measuring the detection threshold for the smell of various odors such as beta-phenyl ethyl alcohol, cyclotene and isovaleric acid. At the same time we also measured respectively the onset and duration time for the sense of smell of Thiamine propyl disulfide administered intravenously.

The results obtained are summarized as follows; 1. The mean detection threshold for each of these 3 odors in the laryngectomized patients was nearly equal to that obtained in normal persons. While in the intravenous olfaction tests, more inferior data were seen in the laryngectomized patients than that of normal controls. 2. No significant differences of the olfactory acuities were observed between the groups, one with esophageal speech and the other with artificial voice apparatus. 3. When the laryngectomized patients put on the artificial airway tube, microclimate in the nasal cavity might be similar to that of normal one. 4. Olfactory sensation by swallowing procedure appeared in 40% of the laryngectomized patients in this series. But, the mean detection thresholds of individual odors were greatly higher than those obtained by the artifical airway tube method.

This study showed that the olfaction did not change in these patients. Additionally, the results of animal experiments, which have been studied in dogs to verify the clinical results, were also demonstrated.

S110 STEROID DEPENDENT ANOSMIA. Bruce W. Jafek, David T. Moran, Pam Eller, J. Carter Rowley, III. (Depts. Otolaryngology/Head and Neck Surgery and Anatomy, University of Colorado School of Medicine, Denver, CO 80262)

Steroid dependent anosmia has been recognized as one of the reversible anosmias. Attributed simply to obstruction of the olfactory cleft by polyps, the mechanism of anosmia, reversibility and quantitate deficit have never been defined in detail.

A 47-year-old male presented with anosmia and polyps. Two previous nasal operations were unsuccessful in restoring his sense of smell. He did not smoke.

Smell testing at the University of Connecticut showed "bilateral 0% anosmia." The UPSIT score was 10/40. Taste testing was normal.

The patient underwent olfactory biopsy, followed by ethmoidectomy. Postoperatively, his UPSIT improved to 31/40 and he was able to function in his job running a restaurant.

The preoperative evaluation and postoperative results will be discussed in detail along with the biopsy findings. A mechanism for steroid dependent anosmia and its management will be considered.

Supported in part by NIH #1 PO1 NS 20486-01 (Rocky Mountain Taste and Smell Center).

NOTES

P83 **ODOR/TASTE MIXTURES.** David E. Hornung and Melvin P. Enns. (Depts. of Biology and Psychology, St. Lawrence University, Canton, New York 13617).

Two basic lines of investigation have been used to study the interaction of odors and tastes. One line of investigation has examined the effect that smell has on the perception of taste and vice versa. These studies have often included a consideration of smell/taste confusions, that is, situations where a smell sensation is perceived as a taste or a taste sensation is perceived as a smell. A description of the possible peripheral and central mechanisms that might account for these types of smell/taste interactions will be presented. A second line of investigation has focused on the role of smell and taste in the perception of flavor. Questions that have been considered include how the intensities of smell and taste add together in the perception of the intensity of flavor, how smell and taste influence the recognition of flavor stimuli, and how smell and taste influence the acceptability of flavor stimuli. In an attempt to draw from both these lines of study, the presentation will conclude with a discussion of the significance of smell/taste interactions in the identification and acceptability of flavor compounds.

P84 HETEROGENEOUS TYPES OF INTERACTIONS BETWEEN ODORANTS AT OLFACTORY RECEPTOR CELLS. Barry W. Ache and Richard A. Gleeson. (C. V. Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086).

We earlier reported that mixture suppression in the antennular (olfactory) pathway of the spiny lobster was in part due to odor components interacting at receptor cells (Derby, Ache and Kennel, Chem. Senses 10: 301, 1985). We also reported that pairing one odor component in binary mixture with each of two other odor components resulted in an apparent right shift of the concentration-response function of the cells tested, suggesting that competitive inhibition, i.e., competition for common receptor sites, could in part explain mixture suppression at receptor cells. (Gleeson and Ache, Brain Res. 335: 99, 1985). In the present study we compare in more detail the interaction of two excitatory odor components (taurine, glycine) in binary mixture with two non-excitatory, suppressive odor components (proline, arginine) that occur together naturally in tissue extracts which serve as feeding stimuli for the lobster. We find proline and arginine both suppress responses evoked by taurine and glycine, but do so with different efficacy at different cells. In some receptor cells tested, the suppressive effect can be surmounted by increased concentrations of the excitatory component, but in other cells it cannot; the response saturates at a lower maximum, indicative of a non-competitive process. This suggests that at least two different processes could underlie mixture suppression at these receptor cells. None of the four components can be consistently associated with either one of the two types of interaction when the data are compared across different cells. This suggests that one odor component is not necessarily coupled to the same interactive process in all receptor cells. Preliminary evidence indicates, however, that when proline and arginine act on the same cell, they exhibit the same type of interaction. These findings collectively indicate the difficulty in modeling the phenomenon of mixture suppression, even at this one level of the olfactory pathway, with a single, simple function.

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P85 MIXTURE INTEGRATION IN SUCROSE/SODIUM CHLORIDE AND SUCROSE/CITRIC ACID SOLUTIONS: AN ASSESSMENT OF SUBADDITIVITY FOR TOTAL MIXTURE INTENSITY. Robert A. Frank & Gary Archambo. (Dept. of Psychology, University of Cincinnati, Cincinnati, OH 45221).

Using Anderson's (1981) information integration approach, we recently reported that total intensity judgments for sucrose/sodium chloride mixtures showed increasing subadditivity as the solute concentrations increased (Frank, Burke & Estep, 1985). (Subadditivity refers to the tendency for total mixture intensity to be rated as less than the sum of the unmixed component intensities). In the present series of experiments, the first experiment replicated the earlier work with sucrose/sodium chloride mixtures and extended it to sucrose/citric acid solutions. The subjects rated the total intensity of the mixtures on a 21 point category scale using factorial combinations of three sucrose concentrations (0.1, 0.3 & 1.0 M) and four concentrations of sodium chloride (0.09, 0.21, 0.34 & 1.0 M) or citric acid (1.25, 2.5, 5.0 & 10.0 mM). All the stimuli were rated once per session using the sip and spit method (stimulus volume = 5 ml). A tap water rinse and 30 sec intertrial interval followed the presentation of each stimulus.

The pattern of integration for the two types of mixtures was essentially identical to that found in previous research, exhibiting increasing subadditivity as the concentrations of the solutes increased. The same pattern of integration was observed when magnitude estimates rather than category ratings were used, indicating that ceiling effects were not responsible for the extreme subadditivity noted at the higher solute concentration levels.

In a final experiment, subjects rated the individual sweet and salty or sour components of the mixtures to determine whether suppression of the components could explain the subadditivity of the overall intensity ratings. As was expected, mixture suppression was observed for the component tastes of the solutions, but the magnitude of this effect accounted for only a fraction of the subadditivity observed for total intensity judgments. Therefore, component mixture suppression contributes to the pattern of integration observed for the total mixture intensity ratings, but other factors also play a role. These factors may relate to compression of the psychophysical function at high concentration levels (Bartoshuk, 1975) or the inability of subjects to simultaneously attend to the multiple components of the mixtures (Kuznicki & Ashbaugh, 1982).

P86 CONCENTRATION-INDEPENDENCE OF MIXTURE INTERACTIONS IN THE ANTENNULAR (OLFACTORY) PATHWAY OF THE SPINY LOBSTER. T. J. Herder, B. W. Ache, and W. E. S. Carr. (C. V. Whitney Laboratory, Univ. of Florida, St. Augustine, FL 32086).

Behavioral evidence (Carr and Derby, J. Chem. Ecol., in press) indicates that mixture interactions in shrimp are strongly synergistic at lower concentrations, but tend towards suppression at higher concentrations. We previously reported physiological evidence for mixture interaction in the olfactory pathway of the spiny lobster, in which a high concentration of a mixture showed largely suppressive interaction (Derby and Ache, Chem. Senses 9:201, 1984). The present study extends this initial report by determining if the components identified as interactive at a high concentration change the nature of their interaction as a function of stimulus concentration. Evoked neural activity in high-order (post-convergent) olfactory interneurons was used to quantify antennular (olfactory) chemosensitivity to the following: 31-component artificial crab mixture (ACM), 8 individual components of ACM that were previously defined as interactive, and 8, 30-component mixtures, each lacking one of the 8 interactive components. Interactions were predicted according to both the stimulus substitution and the response summation models. None of the 8 components significantly changed the nature of their interaction over 4 log-step dilutions of the mixture. Using the stimulus substitution model, the more conservative model for predicting suppression, two components exhibited the same type of interaction (1 synergistic, 1 suppressive) across all concentrations; the other 6 exhibited a general trend from being synergistic at higher concentrations towards being suppressive at lower concentrations. The stimulus substitution model, which rigorously tests suppression, tends to overpredict synergism, so the instances of synergism were analyzed further with the response summation model, the more conservative model for predicting synergism. In no instance were synergistic interactions indicated. These results suggest that if mixture synergism is common to the behavior of both shrimp and lobsters, then it must either be introduced at a higher level of the neural pathway than the one selected for testing, or be an emergent property of multimodal chemosensory integration, since chemoreceptors other than those on the antennules are stimulated in freely-behaving animals.

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P87 SENSORY RESPONSES TO SUCROSE AND FAT IN MILK DRINKS. Rose Marie Pangborn, Andrea L. Kaye, and Caroline T. Wang. (Food Science and Technology, University of California, Davis CA 95616).

Vanilla Drinks. Thirty female subjects evaluated 20 samples varying in sucrose (0 - 36%) and milkfat (0 - 36%). Intensity of sweetness increased linearly with sugar level with a slight drop at the highest fat level. The terms "fatness", creaminess, and oral viscosity appeared to measure the same attribute and increased linearly with fat level, with a sifnificant increase attributable to sucrose. Hedonic scaling showed a liking for intermediate and a dislike for extreme levels of both additives. Maximum liking was obtained for the sample with 9% sucrose and 20% fat and did not vary across five test sessions. No relationship was obtained between dietary intakes of fat and sugar and most liked sucrose or fat levels. There was a significant ($p<0.05$) negative correlation between most-preferred sugar level and the "Chance" score from the Nutrition Locus of Control test. Hedonic responses were collected from 20 normal-weight (x=129 lbs; 41 yrs) and 22 obese females (x=222 lbs; 45 yrs) at onset and after 6 mo of diet therapy for the obese. The latter reduced dietary intake of sugar and fat by almost 50%, and lost an average of 21.5 lbs. At onset, the obese rated all but the 36% fat sample and the 0% sucrose sample significantly higher than did the lean. Therapy was successful for 10 and unsucessful for 8 obese subjects, with dramatic differences in hedonic responses between the two. The successful decreased while the unsuccessful slightly increased their liking for fat and for sugar in the milk drinks. Reduced obese subjects became more "internal" on the Locus of Control test.

Chocolate Drinks. Twenty subjects evaluated 20 samples varying in sucrose (0 - 36%) and milkfat (0 - 36%). Sweetness intensity increased curvilinearly with increasing amounts of sucrose, but fat levels had little effect on perceived sweetness. Sweetness of aspartame (at 10% sucrose equivalent) was significantly reduced by increasing fat levels. Subjects estimated the caloric value of 100 ml of each sample compared to unsweetened whole milk (67 Kcal). Low-fat and low-sugar samples were overestimated, e.g., 83 instead of 32 Kcal for the skimmilk, whereas high-fat and high-sugar samples were greatly underestimated, e.g., 144 instead of 500 Kcal for the sample with 36% fat and 36% sugar. Knowledge of the exact fat and sugar contents improved estimation, e.g., 37 for the skimmilk and 350 for the 36%/36% sample. Caloric estimation was more accurate among the U.S.-born than the Taiwanese-born subjects tested.

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P88 ELECTROPHYSIOLOGICAL AND INNATE BEHAVIORAL RESPONSES OF THE DOG TO INTRAVENOUS APPLICATION OF SWEET COMPOUNDS. Lawrence J. Myers, Randy Boddie, and Kimberly May. (Department of Physiology and Pharmacology, Auburn University, AL 36849).

Fifteen mixed breed, mesatocephalic dogs were selected as subjects for examination of electrophysiological and innate behavioral responses to ascending and descending series of concentrations of sucrose, fructose, glucose, and saccharine dissolved in Ringer's solution. Unsedated animals were lightly restrained in lateral recumbency and blindfolded with a catheter placed in the left cephalic vein. Electrodes were placed subcutaneously in the standard veterinary EEG montage and in the splenius muscles. Serial dilutions of each of the selected compound were infused via the cephalic catheter at a constant rate of 5 ml/minute for two minutes per dilution. Behavior, electroencephlographic activity, and electromyographic activity were recorded. Descending series of dilutions were found to cause reactions too violent to allow adequate restraint. The use of ascending series of dilutions yielded relatively stable thresholds for electrophysiological and behavioral response for each compound. That the responses to sucrose, glucose, and fructose were mediated by taste was shown by the absence of response when *Gymnema sylvestra* tea was used to block the response to sweet flavor stimuli.

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P89 SEQUENTIAL INTERACTIONS OF ORAL CHEMICAL IRRITANTS. David A. Stevens and Harry T. Lawless. (Department of Psychology, Clark University, Worcester, MA 01610 and S.C. Johnson & Son, Inc. Racine, WI 53403).

Twenty subjects evaluated the intensity of irritation of 37.5 C samples of 1 ppm capsaicin and 37.5 ppm piperine. There were two general sequences: those in which the samples were the same irritant (e.g., capsaicin followed by capsaicin) and those in which the second irritant was different from the first (e.g., capsaicin followed by piperine). Each sample was held in the mouth for 60 sec and the level of burn was rated by magnitude estimation at the 25th and 55th second. After the first sample was spit out, the second was immediately sipped.

The second presentation of each stimulus compound resulted in increased perceived irritation. The mean ratings were: capsaicin (first) = 103 ± 4.1, capsaicin after capsaicin = 128 ± 8.7, piperine (first) = 116 ± 5.3, piperine after piperine = 148 ± 9.9. (No overall differences in the intensity of capsaicin vs. piperine were observed, i.e. the general intensity levels of the two irritants were not statistically different). When the second irritant was different than the first, an even greater increase was observed - capsaicin after piperine = 181 ± 13.3 and piperine after capsaicin = 198 ± 14.5. This enhanced irritation when compounds were switched suggests recruitment of different receptors for the different irritant, and argues against the notion that oral trigeminal chemoreceptors respond nonspecifically.

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NOTES

P90 Long Lasting Effects of Context on Sweetness Evaluation Teresa Anne Vollmecke (Department of Psychology, University of Pennsylvania, 3815 Walnut Street, Philadelphia, PA 19104)

Recent prior experience exerts a potent influence on current sensory/perceptual evaluations (Helson,1964; Parducci,1965). In the taste realm, the sweetness of beverages (Riskey,1979) and saltiness of soups (Lawless 1983) depend upon other beverages or soups experienced in the same session. This study reports that context continues to affect sweetness evaluations as long as one week later.

Forty individual subjects were tested twice, in sessions (High and Low Contexts) separated by one week. Half of the subjects received High and then Low Context while the other half received Low and then High Context. In each session, subjects tasted seven samples each of three different sucrose beverages presented in counter-balanced blocks. All beverages were identically colored red and differed only in sucrose content;.06, .14 and .33 M for the Low Context and .33, .77, and 1.8 M for the High Context. Subjects evaluated sweetness using a nine-point category rating scale with verbal labels.

An effect of context on sweetness was observed for thirty-seven out of forty subjects. Subjects rated the .33 M beverages as sweeter in the Low Context (X=6.78) than in the High Context (X=3.76). In addition, the order of contexts influenced sweetness. High-Low subjects rated .06, .14, and .33 M sucrose beverages as less sweet (X=1.34, 2.83, 5.62 respectively) than the Low-High subjects (X=2.08, 5.13, 7.97 respectively). The High-Low subjects response indicates that they continue to be affected by the initial High Context experience.

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P91 SELECTIVE ATTENTION FOR THE COMPONENTS OF ODOR MIXTURES. Michael D. Rabin, Amy L. Schwartz, and William S. Cain (Department of Psychology, Yale University, and John B. Pierce Foundation Laboratory, New Haven, CT 06519).

Our experiment used three constrained classification tasks to assess the processing of orthogonal combinations of two odorants, amyl acetate (A) and hexenal (H), at two levels of intensity, moderate (M) and weak (W). The orthogonal combinations resulted in the following odor mixtures: MA+MH, MA+WH, WA+MH, and WA+WH. These odorants were chosen to present a challenging discrimination. Each of eighteen subjects participated in nine experimental sessions.

In the control tasks subjects discriminated between each of the four possible pairwise combinations of the mixtures such that the companion stimulus in the mixture was held constant (e.g. MA+MH vs. WA+MH). In the second discrimination task, the correlated set, subjects discriminated between stimuli having correlated levels of each dimension (e.g. WA+MH vs. MA+WH). This task ascertained whether dimensional redundancy helps or impairs the discrimination. A correlated gain relative to controls implies integral stimuli. The final task, the orthogonal set, asked whether subjects could selectively attend to one stimulus in a mixture when the other stimulus in the mixture varied irrelevantly (e.g. WA+MH & WA+WH vs. MA+MH & MA+WH). Orthogonal interference implies integral stimuli. For both error and reaction time data we found orthogonal interference, relative to the control task, and no differences between the correlated data and control data.

While the data fail to support the idea that our subjects selectively attended to the individual components of the mixtures, a number of factors may mitigate the generality of this outcome. One such factor might be our subjects' extra-experimental experience with the odors of amyl acetate and hexenal. Their natural concurrence in a variety of common fruits might lead to a cognitive blending of identities into a superordinate cognitive category much like we label the many odors comprising apple-odor simply as "apple". Thus, although subjects in this study treated the mixtures more as integral stimuli, our outcome does not preclude the possibility of dimensional separability in olfaction.

P92 **PREFERENCE FOR EXTREMELY HIGH LEVELS OF SALT AMONG YOUNG CHILDREN.** Gary K. Beauchamp and Beverly J. Cowart (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104).

It has been stated without evidence that the human liking for salt (NaCl) is learned (e.g., Dahl, LK (1958), N Engl J Med 285: 1205). Studies of infant and childhood responses to salt are required to evaluate this belief. We have been investigating the development of acceptance of, and preference for, salt in water and in soup in humans between birth and 6 years of age. Previously we reported that whereas infants less than 4 months of age ingested water and moderate concentrations of salt (0.10 - 0.20 M) in equal amounts, infants 4-24 months of age exhibited heightened acceptance of saline solution relative to water. This shift may be accounted for by the postnatal maturation of peripheral and/or central structures underlying NaCl perception. Children 3-6 years of age offered 0, 0.17 and 0.34 M salt in soup or in water exhibited a striking context-specific reaction to the taste of salt. On both intake (acceptance) and paired-comparison (preference) measures, we found children responded positively to salt in soup but rejected salt in water. The rejection of salt in water may reflect the novelty of salty-tasting water. Notably, the most preferred concentration of salt in soup was often 0.34 M, which is approximately twice the concentration in most commercial soups; many data indicate that peak preference for salt in soups among adults is about 0.20 M. To further evaluate the level of salt in soup most preferred by 3-6 year olds, a paired-comparison procedure was employed. Vegetable soup broth was prepared with several different concentrations of salt. Children were given every possible pair of stimuli twice and asked which one of each pair they liked best. Almost all subjects were black. The first series employed 0, 0.18, 0.32, 0.56 and 1.00 M NaCl added to the soup. Five of the 9 subjects tested preferred either the 0.56 or 1.00 M soup. In a second series, 14 subjects were tested with 0, 0.18, 0.32 and 0.56 M salt added to soup. Seven of the subjects preferred the 0.56 M soup (two others choose the 0.32 and 0.56 M equally often). In a third series with 0, 0.25, 0.50 and 1.00 M salt added to soup, 10 of 15 subjects preferred one of the two higher concentrations. Thus, 22 of 38 subjects indicated 0.50 M or above was their most preferred level of salt in soup. These data demonstrate that 3-6 year olds from this population exhibit a preference for remarkably high levels of salt in soup. Since these levels are considerably higher then they would ever be exposed to during home soup consumption, it is unlikely that this preference is determined entirely by dietary exposure.

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P93 CONCENTRATION DEPENDENT RESPONSES OF SODIUM-DEPLETED RATS TO NACL IN FOOD. Mary Bertino, Michael G. Tordoff and John Tluczek (Monell Chemical Senses Center, Philadelphia, PA 19104)

At concentrations near isotonicity, rats will consume more salt water than plain water. However, when given a choice, rats consume either equal amounts of salted and unsalted food or will actually prefer the unsalted food. As salt solution preference is known to increase with sodium-depletion in rats, we tested the effects of furosemide-induced sodium-depletion upon preference for various concentrations of salted food.

Each rat was tested under two conditions. In the experimental condition, rats were placed on a sodium-deficient diet for 48 hours and injected with the natriuretic furosemide (Lasix, 5 mg, SC). Twenty-four hours following the injection, salted food (0.06, 0.12, 0.25 & 0.50% NaCl) preference was tested for four days. Each rat was tested with only one NaCl concentration. In the control condition, rats were only given the sodium-deficient diet 48 hours before the preference test. Using different rats, a second experiment was performed identically except that the tested salt concentrations were 0.50, 1.0, 2.0, 4.0 and 8.0%.

Severe sodium-depletion induced by furosemide increased salt preference in food for all concentrations tested. Increased salted food preference was observed in the first hour of the test for concentrations equal to and above 0.25% NaCl. The duration of the salt preference varied with concentration. The salted food preference lasted only 2 hours in the group which had 8% NaCl whereas the preference lasted 3 days in the group which had 0.06% NaCl available. There was also evidence that the sodium-deficient diet alone (the control condition) increased preference for salted food.

The fact that the rats showed a salted food preference in concentrations as low as 0.06% NaCl which is probably below the taste threshold suggests that post-ingestional factors may have contributed to the salted food preference.

P94 SALT DEPRIVATION-AND AMILORIDE-INDUCED ALTERATIONS IN NEURAL GUSTATORY RESPONSES PREDICT SALT INTAKE IN SHAM DRINKING RATS. Robert J. Contreras, (Yale University, Department of Psychology, Box 11A Yale Station, New Haven, CT 06520).

An important goal of our research is to determine the role of the peripheral gustatory system in mediating changes in salt intake in the rat. Our strategy has been first to assess the plasticity and modifibility of the peripheral gustatory system at the neural level after dietary sodium deprivation (Contreras & Frank, 1979) or after topical application of amiloride on the tongue (Contreras, Farnum & Bird, 1985). Each manipulation alone reduces the chorda tympani responses to suprathreshold concentrations of sodium chloride. If the peripheral gustatory system is indeed important in controlling salt intake, then these changes in the NaCl neural response functions should predict corresponding changes in NaCl intake. Thus, we have begun to examine the effects of dietary sodium deprivation and amiloride on salt intake. Recently we have adopted the sham drinking preparation (Smith & Gibbs , 1979) as a means for isolating the contribution of orosensory cues alone, in the absense of visceral feedback present in long-term and short-term drinking tests, to salt intake.

Each rat (N=5) was surgically implanted with stainless steel fistula in the stomach. The fistula was exteriorized outside the body wall and skin. A collection tube was attached to the fistula and the fluid ingested drained freely out of the stomach and down a collection tube by gravity flow. The rat's intake of water and various molar NaCl concentrations (.03, .1, .2, .3, .4, .5) was measured with the fistula closed (control drinking) and open (sham drinking) in 30-min drinking tests. The open fistula condition provided good sham drinking as both the volume and Na^+ concentration of the fluid collected was similar to that ingested. Under these conditions, the rats drank from 2-4 times more fluid when the fistula was open than when it was closed. Even though sham drinking increased intake, the general shape of the function was unchanged; intake was highest for .03 M NaCl and declined steadily with increasing concentration. Surprisely, the intakes of .4 and .5 M NaCl was the same under both drinking conditions. The data suggest that orosensory stimulation (salt taste receptors) alone is critical for determining salt intake. Experiments are now underway to assess the effects of salt deprivation and amiloride on the sham drinking functions for NaCl.

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NOTES

P95 THE SUPPRESSED RESPONSE OF NaCl FOLLOWING AMILORIDE: A HALOGEN RESPONSE. Bradley K. Formaker and David L. Hill (Dept. Psych., Univ. of Toledo, Toledo, OH 43606).

Numerous investigations have shown that the sodium transport blocker, amiloride, partially suppresses the whole nerve response to NaCl. It has been suggested that the response to NaCl is composed of an amiloride-sensitive sodium component and an amiloride-insensitive sodium component. However, a behavioral investigation from our laboratory suggests that the residual response of NaCl after amiloride may be due to the chloride ion, rather than an amiloride-insenstive sodium channel.

To learn if the residual response of NaCl following amiloride is due to the chloride ion, we recorded multifiber, chorda tympani responses to a concentration series (0.05M, 0.1M, 0.25M, & 0.5M) of NaCl, NaBr, sodium acetate (NaAc), sodium bicarbonate (NaHCO3), choline chloride (ChCl), NH4Cl and ammonium acetate (NH4Ac), before and after lingual application of 500 uM amiloride hydrochloride. We also utilized 0.25M ChCl and 0.25M NaCl in a cross-adaptation paradigm; both solutions were mixed in amiloride. Responses to NaCl and NaBr were suppressed by amiloride 65%-90%. In contrast, amiloride suppressed responses to NaAc and NaHCO3 95%-100%. Responses to NH4Cl and NH4Ac were not affected by amiloride. Responses to 0.5M ChCl were similar to the residual responses of 0.5M NaCl after amiloride and these two chemicals completely cross-adapted when mixed in amiloride.

These results suggest that the residual response of NaCl following amiloride may be due to a halogen-sensitive component and not to an amiloride insensitive sodium component. A model dependent upon two or more selectively sensitive sodium channels would not have predicted the complete suppression of NaAc or NaHCO3, nor would such a model predict complete cross-adaptation of ChCl with the residual NaCl response following amiloride. Therefore, it appears amiloride completely blocks sodium transduction. Moreover, the residual response of NaCl and NaBr following amiloride is due to a completely different, halogen-sensitive component.

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NOTES

P96 **TASTE SIGNAL STRENGTHS OF SALT AND WATER STIMULI PROVIDE A MODEL FOR SENSORY DIFFERENCE TESTS, INVOLVING ADAPTATION, LEARNING, AND VARIATION IN SUPRA- AND SUBADAPTING SENSITIVITY.** Linda Goldstein, Nancy Odbert, Ursula Farrelle and Michael O'Mahony. (Dept. Food Science and Technology, University of California, Davis, CA 95616)

When 3mM NaCl (S) and water (W) are tasted in random order, the two stimuli can be tasted in four possible paired sequences. These sequences gave stimulation of different signal strengths which were in decreasing order: W-S, S-W, W-W, S-S. The signal strengths were measured using a signal detection R-index rating procedure. Hypotheses were generated to explain this order of signal strengths from the following experimental results. The variations in physical stimulus strength were examined by atomic absorption spectrometry of saliva and stimulus samples held in the mouth. Secreted saliva and stimulus residuals were seen to change stimulus concentrations, as well as adaptation state. The changes could be large enough to render 3mM NaCl tasted after water (W-S) as a subadapting stimulus. The effects of familiarity and practice were noted and seen to alter the relative order of signal strengths as subjects learned the range of sensations available from specific stimuli. Using a flow presentation procedure, supra- and subadapting NaCl taste sensitivity was measured, the former providing higher R-indices (greater sensitivity) and shorter latencies. All these factors had importance as potential determinants of the order of relative signal strength.

These signal strengths were used to predict the relative sensitivities of sensory difference tests, each test being merely a collection of tasting sequences (S-W, W-S etc.). Those with a greater proportion of stronger signal strengths provided more sensitive tests. Predictions were confirmed for differences in sensitivity within the three different versions of the triangle test (S-odd, W-odd, both odd) and the duo-trio test (W-standard, S-standard, both standards).

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P97 GENERALIZATION OF CONDITIONED TASTE AVERSION TO NaCl IN FISCHER-344 AND WISTAR RATS. Eleanor E. Midkiff & Ilene L. Bernstein. (Dept. of Psychology, University of Washington, Seattle, WA 98195).

Unlike other strains, Fischer-344 (F-344) rats failed to prefer hypotonic and isotonic NaCl solutions over water. Two possible explanations for this are suggested: F-344 rats may be unable to taste NaCl at low concentrations; or their taste system may have anomalies which cause NaCl to taste qualitatively different than it does to other strains. The present studies investigated whether F-344 rats are able to form aversions to dilute NaCl solutions, and whether NaCl aversions generalize to taste solutions which are qualitatively different. Both F-344 and Wistar rats showed a significantly lower intake of 0.033M NaCl following aversion conditioning with LiCl than did controls; thus, F-344 animals are able to detect NaCl at concentrations as dilute as 0.033M. The same animals were reconditioned to 0.1M NaCl and tested for ingestion of 0.0001M quinine sulfate, and 0.003M citric acid. Neither F-344 nor Wistar rats showed generalization of NaCl aversions to either the bitter or the sour solution. In a second study, generalization to other chloride salts was tested: experimental subjects were given LiCl following ingestion of 0.1M NaCl; controls were saline-injected. Subjects were tested for ingestion of 0.1M potassium chloride and 0.05M ammonium chloride. After conditioning, both F-344 and Wistar rats suppressed their intake of KCl relative to controls; however, there was no significant strain difference. Neither F-344 nor Wistar rats suppressed intake of NH Cl. The aversion to NaCl seen in F-344 rats does not appear to be due to taste system anomalies which cause NaCl to be perceived as similar to sour or bitter stimuli. Additionally, F-344 rats do not appear more likely than Wistars to generalize NaCl aversions to other monochloride salts.

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P98 CONTRIBUTION OF CATIONS TO GENERATION OF SALT-INDUCED RECEPTOR POTENTIAL IN FROG TASTE CELL. Takenori Miyamoto, Yukio Okada and Toshihide Sato. (Dept. Physiology, Nagasaki University School of Dentistry, Nagasaki 852, Japan).

Sato and his colleagues (1982) have reported that the permeability change of the basolateral membrane of a frog taste cell plays an important role in generation of salt-induced receptor potential. We reexamined the effects of various modified salines substituted for either superficial fluid (SF) or interstitial fluid (ISF) on the receptor potential of a taste cell in response to salty taste stimuli.

Adult bullfrogs anesthetized with urethane were used. The tongue surface was usually adapted to normal saline (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.2). Arterial perfusion was performed to modify the ionic composition of ISF. The taste stimuli used were 0.5 M NaCl and 0.5 M KCl.

The amplitude of receptor potential evoked by 0.5 M KCl (V(K)) was usually larger than that evoked by 0.5 M NaCl (V(Na)). Reversal potential of V(Na) was obviously different from that of V(K) in a single taste cell.

After Na^+ and Ca^{2+} in ISF were totally replaced with any of the K^+, Li^+, $choline^+$, $tetramethylammonium^+$ and $tetraethylammonium\ (TEA)^+$, V(Na) and V(K) reduced to 30-70% of those controls. When Na^+ was replaced with Li^+, V(Na) and V(K) reduced to 50%, but membrane resistance during salt-induced depolarization did not change significantly. Replacement of $CaCl_2$ in ISF with $MgCl_2$ significantly reduced only V(K), whereas total replacement of ISF with isotonic $CaCl_2$ or $MgCl_2$ markedly potentiated V(Na).

After adapting tongue surface to Ca-free saline containing 1 mM amiloride, V(Na) reduced to 50% of control. Effect of amiloride in saline containing Ca^{2+} on V(Na) was weaker than that in Ca-free saline. Adaptation to Ca-free saline without amiloride reduced both V(Na) and V(K) significantly. Addition of 20 mM TEA to SF did not affect V(Na) and V(K).

These results suggest that the generation of salt-induced receptor potentials in frog taste cell depends on the presence of Na^+ and Ca^{2+} both in SF and in ISF, and V(Na) and V(K) are evoked by a somewhat different mechanism.

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P99 THE ULTRASTRUCTURE OF APICAL SPECIALIZATIONS OF TASTE CELLS IN THE MUDPUPPY, NECTURUS MACULOSUS. T. A. Cummings, R. J. Delay, and S. D. Roper. (Dept. of Anatomy, Colorado State University, Fort Collins, CO 80521).

The first interaction of tastants is likely to occur on the apical membrane of the taste cells because only that portion is directly exposed to the oral cavity. To gain better insight into this interaction, we examined the pore region of taste buds in Necturus maculosus using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and high voltage electron microscopy (HVEM).

The pear-shaped taste buds, which contain up to 100 taste cells, measure 80-100μ in diameter. Individual taste cells extend 70-100μ from the basal lamina to the taste pore, which averages 20μ in diameter. SEM of the pore reveals a patchwork distribution of two morphologically distinct types of microvilli: long and branched (LB), and short and stubby (SS). Patches of LB microvilli cover approximately two-thirds of the surface area in the taste pore. All methods of observation show SS microvilli to be uniform in size, whereas LB microvilli are variable in size. The SS microvilli measure 1μ in length and .25μ in width. The LB microvilli arise from pedestals which range from 1μ to 2μ in length and from .5μ to 2μ in width. After branching away from the pedestal, LB microvilli range from 1.5μ to 2.5μ in length and are uniformly .15μ wide. As demonstrated in thin and thick sections, LB microvilli are specializations of the Dark cells. In the mudpuppy, Dark cells contain clusters of densely staining granular material in their apical region which clearly distinguishes them from other taste cell types (Farbman & Yonkers, 1971). The apical granular clusters in Dark cells are present even in the LB microvilli. In the same preparation, SS microvilli are the apical specializations of Light cells. In the mudpuppy, Light cells are characterized by their abundant smooth ER (Farbman & Yonkers, 1971). The underlying cytoskeleton of both types of microvilli appears to consist of densely-packed parallel bundles of actin-like filaments. In SEM we occasionally observed what appears to be a single cilium projecting beyond the surrounding microvilli in some taste pores. In HVEM we observed what appears to be a corresponding structure, a kinocilium, extending from a Light cell. The ultrastructural similarities and differences of these taste cells may enhance our understanding of the mechanisms involved in chemosensory transduction.

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P100 **DYE-COUPLING BETWEEN TASTE CELLS IN THE MUDPUPPY, NECTURUS MACULOSUS.** J. Yang and S. D. Roper (Department of Anatomy, Colorado State University, Fort Collins, CO 80523)

The fluorescent dye Lucifer Yellow (LY) crosses gap junctions in a number of epithelial and neural tissues. This dye-coupling implies the existence of low-resistance pathways between cells (electrical coupling). Electrophysiological data suggest that taste cells are electrically coupled in the mudpuppy (West and Bernard, 1978). We have used intracellular staining with LY to investigate the possibility of dye-coupling between taste cells in the mudpuppy. A total of 53 taste cells, in as many taste buds were injected with LY.

LY-stained taste cells had an elongate shape. Cells were an average of 111 microns long, and were 13 microns in diameter at the widest region (nucleus). The basal process usually terminated in a number of finely branched, finger-like projections that extended to the base of the taste bud. The apical process extended to the taste pore. This shape is similar to that revealed by histological and ultrastructural studies on taste cells in the mudpuppy (cf. Farbman & Yonkers, 1971; Delay & Roper, in preparation).

Dye-coupling was unequivocally observed in 10 and possibly in as many as 15 instances. In 7 cases, two cells were stained with LY after a single intracellular injection. In 3 cases, 3 cells were stained. Dye-coupled pairs (or trios) of taste cells often appeared to be equally stained and cells in the pairs (trios) had quite similar shapes. Although the site of coupling could not be determined with certainty, we observed close apposition between cell bodies and/or apical processes.

We also injected LY into surface epithelial cells to determine whether there is dye-coupling between epithelial cells, or between epithelial and taste cells. Thirty-five surface epithelial cells were injected with LY. Sixteen of these were immediately adjacent to taste pores. Only in a single case were epithelial cells dye-coupled: no surface epithelial cells were coupled to taste cells.

Our results with LY suggest that some taste cells are coupled to one or two other taste cells under the conditions of our experiments. Surface epithelial cells were rarely dye-coupled. The functional significance of dye-coupling between taste cells, that is, how putative electrical coupling influences taste transduction, remains to be determined.

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P101 INTERACTIONS BETWEEN TASTE CELLS AND NERVE FIBERS IN MURINE FOLIATE TASTE BUDS. Suzanne M. Royer and John C. Kinnamon. (Dept. of MCD Biology, University of Colorado, Boulder, CO 80309).

We have used high voltage electron microscopy and conventional transmission electron microscopy of serial sections (0.09 - 0.50 μm) to study interactions between taste cells and nerve fibers within foliate taste buds of the mouse. Three types of cytoplasmic specializations were observed in areas of close apposition between taste cells and nerve processes:

1) Taste cells made afferent synapses onto nerve fibers. These synapses were similar to those previously described for murine vallate taste buds and were of both macular and fingerlike types.

2) Narrow subsurface cisternae sometimes occurred immediately inside the taste cell membrane at sites of close apposition with nerve processes. The outer membranes of the cisternae were separated from the cell membrane by a narrow gap ($\approx$ 15 nm) and often closely paralleled the plasmalemma for a distance of 2-3 μm.

3) Atypical mitochondria were frequently observed just inside taste cells adjacent to nerve fibers. The limiting membranes of these mitochondria were separated from the taste cell membrane by a gap, about 20 nm wide, which often contained a discontinuous central line of electron-dense material. Such mitochondria, in contrast to other mitochondria in the taste cell, usually had large, vesicular or tubular cristae.

Sometimes a combination of two (or even all three) of these cytoplasmic specializations appeared in one area of contact between a taste cell and nerve fiber. Taste cells of dark, intermediate and light types synapsed onto nerve fibers; atypical mitochondria most frequently occurred in light or intermediate cells.

The role of subsurface cisternae and atypical mitochondria in taste cell-nerve fiber interactions remains unknown. It has been suggested that subsurface cisternae are involved in efferent modulation of taste cell function. However, there is presently little evidence to support this suggestion. Alternative hypotheses are that subsurface cisternae and atypical mitochondria may participate in establishment of synaptic connections or mediate trophic interactions between taste cells and nerve fibers. Mitochondria similar to our atypical mitochondria have been associated with elevated rates of respiration in other tissues, suggesting that there may be specialized, energy-requiring biochemical processes at certain sites of taste cell-nerve fiber apposition.

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P102 ULTRASTRUCTURE OF MOUSE FUNGIFORM TASTE BUDS. David M. Henzler and John C. Kinnamon. (Dept. of MCD Biology, University of Colorado, Boulder, CO 80309).

As part of a study on the comparative ultrastructure of vertebrate lingual taste buds, we are examining the ultrastructural features of mouse fungiform taste buds. Using the combined techniques of high voltage electron microscopy of serial sections and computer three-dimensional reconstructions from serial electron micrographs, we have generated three-dimensional models of a taste bud, taste cells, and synapses.

We have observed many similarities between mouse fungiform taste buds and mouse foliate and vallate taste buds. Their general ultrastructure is similar, each containing four types of taste cells: basal, dark (Type I), intermediate, light (Type II). In addition, nerve fibers are present, receiving synapses from dark, intermediate, and light cells. Also present are large, atypical mitochondria with tubular cristae, and subsurface cisternae, located adjacent to the cytoplasmic leaflet of the taste cell membrane at loci of close apposition between taste cells and nerve fibers.

We have also observed ultrastructural differences between fungiform taste buds and taste buds from foliate and circumvallate papillae. The shape of the fungiform taste bud resembles a garlic bulb, while in foliate and vallate taste buds the shape is more barrel-shaped or ovoid.

In fungiform papillae the general staining characteristics of dark and light cells are similar, unlike foliate and circumvallate taste buds, where dark cells are more electron-dense than light cells. In the fungiform papilla, the primary distinguishing feature between type I (dark) and type II (light) cells is the presence of dense granules in the apical portions of dark cells. Another distinguishing feature of fungiform taste buds is the large diameter of the nerve fibers present in these taste buds. Although all three types of taste buds have nerves of varying diameters, we have observed that the nerve fiber diameters in fungiform papillae can be much larger than in foliate or vallate taste buds.

We have found a structure in a fungiform taste bud which is suggestive of a neuro-neuronal synapse. Since we have observed such a structure only once, the functional significance of this observation remains to be demonstrated.

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P103 HUMAN FUNGIFORM TASTE BUD DENSITY AND DISTRIBUTION. Inglis J. Miller, Jr. (Dept. Anatomy, Bowman Gray Sch. of Medicine, Wake Forest University, Winston-Salem, NC 27103)

Knowledge of the spatial density, regional distribution and total number of human fungiform taste buds is sparse. An objective of this study is to determine normal values through quantification of human taste buds in order to establish reference points for understanding taste function in normal subjects and patients with taste dysfunction. Even among specimens whose donors were assumed to be in reasonably normal health prior death before succumbing to acute episodes such as traffic accidents, suicide, or heart attack, there are demographic differences in human populations such as age,gender, race and phenotypic variation. Studies are underway on tongues from 57 human cadavers ranging in age from premature infants to 95 years old. Photography is used to document morphological features of the tongue surface. Areas of the tongue measuring about 1 cm sq are sectioned serially with 20 um frozen sections, mounted on slides, stained with H & E, and examined by light microscopy. Taste bud density (per sq cm surface), number of gustatory papillae (with taste buds) and number of taste buds / papilla have been counted for two regions of the anterior portion of the tongue: 1.tip and 2.midregion about 2-3 cm caudal from the tip on the lateral margin. Quantitative data are present for 10 adult males ranging in age from 22 to 80 yrs, and preliminary observations are in progress on 5 adult females aged 26-76 yrs at death. Taste bud density on the tip averages 101±133(sd,N=15)tb/cm sq and 23 ±27 tb/cm sq (midregion) for all subjects. The number of gustatory papillae for males is 24.5±23.5 on the tip and 8.25±8.78 on the midregion. The average number of taste buds per papilla (males) is 3.79+2.24 (range 1-18) on the tip and 2.58+1.52 with a range of 1-9 in the midregion. These data yield the following preliminary conclusions: There are differences between individuals in taste bud density of two log. units (range 3.0- 514 tb/cm sq) on the tip. The distribution of tip taste bud densities among subjects is bimodal with a mode at about 10 tb/cm sq and the other at about 150 tb/cm sq. The tip has about 4 times as many taste buds per unit of area as on the midregion. This difference arises because of an approximate average 3 fold greater number of gustatory papillae per area and about 1.5 more taste buds per gustatory papilla. The differences among individuals are not explicable (after preliminary analysis) by the age,gender, race or cause of death of the donor subject.

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P104 BETA-ADRENERGIC CONTROL OF VON EBNER'S GLANDS IN THE RAT. Suat Gurkan and Robert M. Bradley, (Dept. Oral Biology, School of Dentistry, University of Michigan, Ann Arbor, MI 48109).

The von Ebner's salivary glands of the tongue supply the fluid environment of most of the lingual taste buds, because the secretion of these glands drains into the clefts of the circumvallate and foliate papillae. Little is known about the neural control of von Ebner's glands. Recently we have shown that the source of their parasympathetic innervation is via cells in the inferior salivatory nucleus (Brain Res. 361:154 1985). We have now employed morphometric analyses to characterize the extent of β-adrenergic control of the gland acini.

Rats were starved overnight to cause accumulation of the secretory granules in gland acini. Rats in four groups were injected intraperitoneally with the β-adrenergic agonist isoproterenol dissolved in saline at 7.5, 15, 30, and 60 mg/kg. Control rats were injected with saline. Two hours later the rats were sacrificed by cervical dislocation and the von Ebner glands rapidly removed, fixed, embedded in plastic and sectioned at 1 μM. An additional group of rats had injections of 30 mg/kg isoproterenol, and were sacrificed at 0.5, 1, 1.5, 2, 2.5 and 3 hours. Randomly selected acini were photographed at 100x and the areas of the acinar cell and its granules were measured using computer planimetry. There were two rats in each group and 4-6 acini were measured from each rat.

Isoproterenol produced a depletion in secretory granules that was dependent on dose. At doses of 7.5 and 15 mg/kg there was no reduction in granule content of the cells. The higher doses of 30 and 60 mg/kg produced a highly significant reduction in granules ($F = 13.94$, $df = 4,49$, $P < .0001$). However, even the higher doses did not cause complete depletion. In only a few acini did the depletion approach 90%, the mean value being about 50%. Depletion was maximum at 2 hours after injection. By 3 hours the granule content of the acini was approaching control levels.

These results indicate that granule depletion is either indirectly or directly under β-adrenergic control. However, since granule depletion is never complete, other factors also are involved in the control of granule secretion in von Ebner's glands.

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P105 MONOCLONAL ANTIBODIES DIRECTED AGAINST CATFISH TASTE RECEPTORS: IMMUNOCYTOCHEMISTRY OF CATFISH TASTE BUDS. Joan Yonchek[1], Thomas E. Finger[1], Robert H. Cagan[2] and Bruce P. Bryant[3]. ([1]Dept. of Anatomy, University of Colorado Medical School, Denver, CO, [2]Colgate-Palmolive Co., Piscataway, NJ and [3]Monell Chemical Senses Center, Philadelphia, PA).

Monoclonal antibodies were previously raised against a purified plasma membrane fraction derived from taste epithelium of channel catfish (Goldstein and Cagan, PNAS 79, 7595, 1982). One of these monoclonal antibodies, G-10, which inhibits binding of L-alanine to the sedimentable fraction P2, has been used in the present studies for immunocytochemical localization. Antibody from clone G-10 was purified on a Protein-A column. For immunocytochemistry, channel catfish (I. punctatus) barbels were fixed for 1 hour in 4% paraformaldehyde buffered with 0.1M phosphate, pH 7.2 and then washed in phosphate-buffered sucrose. One group of barbels was sectioned at 30 μm on a cryostat and another group remained intact. The sections and the intact barbel tips were exposed for 48 hours at 4°C to a 1:50 dilution of purified antibody G-10 in phosphate buffer with 0.3% Triton X-100. The tissue was washed in buffer and exposed to goat-anti-mouse antiserum at a dilution of 1:50 for 1 hour at room temperature. The tissue was washed again in the buffer and exposed to mouse peroxidase-antiperoxidase complex at 1:100 dilution for 1 hour at room temperature. Following several rinses in buffer the tissue was reacted with diaminobenzidine to develop the peroxidase product. Control tissue was treated similarly except that normal mouse serum was substituted for the monoclonal antibody. No staining was observed in control tissue.

In tissue treated with antibody G-10, specific staining of taste buds was clearly evident both in the sectioned tissue and on the intact barbels. All taste buds exhibited positive staining, but to varying degrees. Within a taste bud, the apical region of the reactive taste cells was stained more heavily than the basal portion, although the basal portion also exhibited clear immunoreactivity. Furthermore, the cells at the edge of the taste bud tended to be more immunoreactive than those in the core of the bud. These results indicate that antigen reacting with G-10 monoclonal antibody is distributed heterogeneously among taste receptor cells, and that the G-10-reactive material is denser in the apical than in the basal portions of the reactive taste cells. This distribution is compatible with the hypothesis that the antigen that is reactive with antibody G-10 is associated with a taste receptor protein in catfish taste buds.

Supported in part by NIH Grants NS-15740, NS-00772 and NS-20486.

P106 **USE OF MONOCLONAL ANTIBODIES TO CHARACTERIZE AMINO ACID TASTE RECEPTORS IN CATFISH: EFFECTS ON BINDING AND NEURAL RESPONSES.** Bruce P. Bryant[1], Joseph G. Brand[1,2], D. Lynn Kalinoski[1], Richard C. Bruch[1] and Robert H. Cagan[3]. ([1]Monell Chemical Senses Center and [2]Veterans Administration Medical Center, University of Pennsylvania and [3]Colgate-Palmolive Co., Piscataway, NJ).

One approach to investigate the specificity of peripheral taste receptor sites is through the use of specific, site-directed agents. Because antibodies are ideally suited for this, we previously developed monoclonal antibodies that interact with catfish taste epithelial plasma membranes and inhibit alanine binding (Goldstein and Cagan, PNAS, 79: 7595, 1982). The interaction of two of these antibodies with putative amino acid taste receptors has been further characterized with respect to the kinetics and specificity of binding, their effect on neural responses and the identity of the antigen. Monoclonal antibodies from two clones, termed G-7 and G-10, have been used for these studies. Antibodies G-7 and G-10, purified using a Protein-A column, competitively inhibited the binding of L-[^{3}H]alanine by Fraction P2 of catfish taste epithelium at antibody protein concentrations of 0.5 and 4.5 ug/ml. Protein controls, such as bovine serum albumin, did not inhibit alanine binding. In a neurophysiological assay, antibodies G-7 and G-10 exhibited slight excitatory activity when applied to the catfish taste epithelium. Subsequently, variable inhibition of neural responses to both L-alanine and L-arginine was observed. Using the G-10 antibody, immunoblots of taste plasma membrane proteins separated by SDS-PAGE revealed a major stained band in the plasma membrane fraction which was absent in all other tissues tested. The molecular weight of this band was about 110,000 daltons. The band was positive to concanavalin A and wheat germ agglutinin, indicating that it contained glycoprotein. These data therefore support the earlier hypothesis that the monoclonal antibodies interact with peripheral taste receptors, and also indicate that monoclonal antibodies are useful in identifying the receptor macromolecules.

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P107 **NEURAL CONNECTIONS FROM/TO THE FACIAL AND VAGAL LOBES IN THE JAPANESE SEA CATFISH, Plotosus anguillaris.** Takayuki Marui, Yasuo Kasahara, *Jagmeet S. Kanwal, *John Caprio, **Sadao Kiyohara. (Dept Oral Physiol., Kagoshima Univ. Dent. Sch., Kagoshima 890, Japan; *Zool. Physiol., Coll. Basic Sci., LSU, Baton Rouge, LA 70803 USA; **Biol. Inst., Coll. Lib. Arts, Kagoshima Univ., Kagoshima 890, Japan.)

The gustatory sense in fish consists of two major dissociable components, facial and vagal nerve systems. The topology and taste-tactile responsiveness of neurons in the primary gustatory centers (facial and vagal lobes, FL & VL) of these systems have been studied with electrophysiological and anatomical techniques. However, the neural connectivities of these centers are relatively unknown, although cross-modality interactions may play an important role in recognizing and orienting towards biologically important objects. In this study, the Japanese sea catfish showing an extraordinary development of gustatory nuclei is used. To provide useful information for an accurate interpretation of the electrophysiological results, neural tracing studies with horseradish peroxidase (HRP) were performed.

The facial lobe projects bilaterally to the posterior thalamic nucleus, superior secondary gustatory nucleus. The FL has reciprocal connections with the nucleus lobobulbaris, medial reticular formation of the rostral medulla, and descending trigeminal nucleus. Also, the FL receives inputs from the raphe nuclei, pretectal nucleus and perilemniscal neurons located adjacent to the ascending gustatory lemniscal tract. The vagal lobe projects bilaterally to the superior secondary gustatory nuceus, lateral reticular formation and ipsilaterally to the nucleus ambiguus. The VL has reciprocal connections with the ipsilateral lobobulbar nucleus, the medullary reticular formation and perilemniscal neurons. These anatomical findings are similar to the results of the bullhead catfish, Ictalurus nebulosus, and are helpful for a better understanding of how taste quality is processed centrally, which may be essential for interpretation of the electrophysiological data.

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P108 CENTRAL PROJECTIONS OF MAJOR BRANCHES OF THE FACIAL TASTE NERVE IN THE JAPANESE SEA CATFISH.
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The Japanese sea catfish, Plotosus anguillaris("Gonzui") have four pairs of barbels: nasal, maxillary(max), medial mandibular(mand), and lateral mand. These barbels have densely concentrated taste buds which are supplied by facial nerve fibers. External taste buds are also found in the entire skin from the lips to the caudal fin. In conjunction with the high degree of development of the facial gustatory system, specific areas of the medulla (facial lobe=FL) have also become extraordinally developed. The FL in the sea catfish is more differentiated than those of North American ictalurid catfish, which show three less distinct lobules. The FL of Plotosus is subdivided by fascicles of nerve fibers into five distinct lobules constituting five longitudinal columns.

We examined the central projections of major branches of the facial nerve in the Gonzui by using the technique of transganglionic tracing with horseradish peroxidase(HRP). The major branches are the four barbel nerve rami plus max, mand, palatine and recurrent nerve rami. When each ramus except the recurrent was treated with HRP, labeled fibers were observed in the facial sensory root and in the descending trigeminal root. The facial fibers of medial mand, lateral mand, max and nasal barbel rami end in a separate lobule of the first four lobules(medial to lateral). The fifth lobule which develops in the dorsal part of the FL receives fibers exclusively from the recurrent ramus. The facial fibers of mand, palatine, and max project respectively in the medial, ventral and lateral portions of the posterior one-third of the FL. These results coincide well with our electrophysiological findings (Marui et al., 1985) and show a topographical relationship exists between the taste bud groups and their locus of representation in the FL.

P109 THE STRUCTURE OF CHEMOSENSORY CENTERS IN THE BRAIN OF SPINY LOBSTERS AND CRAYFISH. David Blaustein, Arthur C. Beall, and Charles D. Derby. (Department of Biology, Georgia State University, Atlanta, GA 30303).

We are using the olfactory system of crustaceans to study sensory and integrative neural mechanisms. While our knowledge of the physiology of this olfactory system is rapidly increasing, we still know relatively little about the anatomy of this system beyond the following general description of its basic pathway: olfactory receptor cells in the antennules projecting to the olfactory lobes, and interneurons in the olfactory lobes and accessory lobes projecting via the olfactory-globular tract to the hemiellipsoid body and glomeruli centrales of the medulla terminalis. We have therefore undertaken a comparative morphological study of the olfactory lobes, accessory lobes, and medulla terminalis of the spiny lobster (Panulirus argus) and the crayfish (Procambarus clarkii). We used three basic techniques: toluidine blue stain to map out clusters of cell bodies in whole mounts; a modified Holmes-Blest silver stain to define and describe the structure of regions of neuropile and to more precisely localize cell bodies; and horseradish peroxidase stain to describe the connections among these structures.

The neuropiles of the olfactory lobes and accessory lobes have a glomerular organization in both species. However, in both species, the structure of the glomeruli in the olfactory lobes and accessory lobes are different from one another. Also, the arrangement of glomeruli in the accessory lobes is more complex in spiny lobsters than in crayfish. The medulla terminalis of the two species has several striking similarities, including six clusters of cell bodies and seven distinct neuropile regions. Although present in both species, these neuropile regions are more discretely organized and apparent in the spiny lobster.

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P110 **AFFERENT CONNECTIONS TO THE TASTE-RESPONSIVE REGION OF THE MACAQUE ORBITOFRONTAL CORTEX.** Leslie L. Wiggins (Dept. Psychol., Univ. Cincinnati, Cincinnati, OH 45221), Gordon C. Baylis and Edmund T. Rolls (Dept. Exp. Psychol., Oxford Univ., Oxford, OX1 3UD, England).

Recent studies of the neurophysiology of the taste system in the monkey, Macaca fascicularis, have shown several cortical areas concerned with taste. Neurons in the dorsal portion of the opercular frontal cortex were shown to be more specifically tuned to taste stimuli than those in the nucleus tractus solitarius (Scott, Yaxley, Sienkiewicz and Rolls, 1985). Cells with gustatory sensitivity were also localized in the rostral and dorsal portion of the insula and were found to be somewhat more narrowly tuned than those in frontal operculum (Yaxley, Rolls, Sienkiewicz and Scott, 1985). Finally, neurons in the caudal and lateral part of the orbitofrontal cortex were shown to be even more specifically tuned to gustatory stimuli than those at these other levels (Rolls, Yaxley, Sienkiewicz and Scott, 1985).

Given these physiological findings, it is important to understand the anatomical connections among these cortical taste areas. Microinjections of horseradish peroxidase were made into the upper layers of the orbitofrontal cortex in the region responding to gustatory stimulation. Recording sites from previous days and injections sites were matched by overlaying lateral and anterio-posterior X-ray views of the brain. When the previous recording sites (marked by microlesions) and the microsyringe tip corresponded exactly, the injections were made. Afferent inputs to the orbitofrontal cortex were revealed using the Hanker-Yates (1977) method. Injections into the orbitofrontal cortex resulted in heavy retrograde labeling of cell bodies in the frontal opercular taste cortex. Secondly, there was heavy labeling of cells in the insula, both in the dorsal and rostral areas where taste-responsive cells are found and in the more ventral area known to receive visceral input. In addition, labeled cells were found in the basolateral and basomedial nuclei of the amygdala, the substantia innominata, the mammillary body, the rhinal sulcus, the postero-medial orbitofrontal cortex and the inferior prefrontal convexity (somatosensory) cortex. Most of these areas have been previously implicated in gustatory or visceral function.

P111 THE SOLITARY NUCLEUS OF THE HAMSTER: CYTOARCHITECTURE AND PONTINE CONNECTIONS. Mark C. Whitehead and Lawrence D. Savoy (Department of Oral Biology, University of Connecticut Health Center, Farmington, CT 06032).

The solitary nuclear complex consists of a number of subdivisions with different cytoarchitectonic features (Whitehead, AChemS Abstr. 1985). These subdivisions differ also in the amounts of input they receive from lingual afferent axons. Chorda tympani, lingual (trigeminal) and glossopharyngeal (lingual branch) afferent axons synapse heavily throughout the central nucleus, less heavily in the lateral, ventral and ventrolateral nuclei, very lightly in the dorsal and laminar nuclei, and not at all in the medial, magnocellular and dorsolateral nuclei. In the present study we determined which of these subdivisions contain cells that project rostrally to the pons. To reveal the pontine projection neurons horseradish peroxidase injections were made which filled or nearly filled the parabrachial nucleus.

The vast majority of retrogradely labelled cells were, in every case, located in the central nucleus at all rostrocaudal levels. The proportion of labelled to unlabelled cells was greater for rostral than caudal parts of this subdivision. Moderate numbers of labelled cells were also observed in the lateral and ventral nuclei, primarily at rostral levels, in the ventrolateral nucleus, and in the medial nucleus at all rostrocaudal levels. Few pontine projection neurons were seen in the dorsal and laminar nuclei, and virtually none in the magocellular and dorsolateral nuclei. Thus, subdivisions receiving heavy input from the lingual periphery contain many pontine projection neurons, subdivisions receiving less input contain fewer projection cells, areas with no lingual input contain no projection cells (with the exception of the medial nucleus which projects to the pons and which probably receives general visceral sensory inputs) (Gwyn et al., JCN 239: 163, 1985). These results demonstrate that the cytoarchitectonic subdivisions of the solitary nucleus are distinguished by their afferent and efferent connections. Moreover, for each gustatory subdivision, there is a correlation between the density of lingual inputs it receives and the density of pontine projection neurons it contains.

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P112 COMPARATIVE SEM AND HISTOCHEMICAL STUDIES OF LINGUAL PAPILLAE IN SOME ANIMALS.

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The surface ultrastructure of the taste buds in lingual circumvallate papillae in some animals were observed under the scanning electron microscope (SEM).

The distribution of binding sites of lectins with different specificities for glycoconjugates was also studied in monkeys lingual circumvallate papillae by staining methods with lectins labeled horseradish peroxidase.

The following results were obtained; 1) SEM findings revealed that there were a couple of the cilia in polypous appearance in opening of taste bud and typical arrangements of polygonal squamous cells with numerous microplicae surrounded by these micropores were also seen. 2) Binding sites for UEA-1, a lectin specific to terminal α-L-fucose residue, were detected only in one restricted site, that is to say, in mucous cells of the glandular duct in papillar lamina propriae. 3) Binding sites for RCA, a lectin specific to terminal ß-D-galactose residue, were detected in mainly glandular tissue and pickle cell layer in the papillar epithelium. 4) On the other hand, binding sites for WGA, a lectin specific to terminal both ß-(1-4)-D-N-acetyl glucosamine and N-acetyl-neurminic acid residues, were found out in pickle cell layer in the epithelium and faintly in glandular tissue except for duct cells. 5) In contrast to these lectins, binding sites for PNA, a lectin specific to terminal galactose ß(1-3)N-acetyl-galactosamine residue, were distributed more widely, in such as intermediate layer in squamous epithelium but for the taste bud, glandular duct and especially in cytoplasm of ganglion cells.

NOTES

P113 HISTOGENESIS OF PONTINE TASTE AREA NEURONS IN THE ALBINO RAT: Phillip S. Lasiter and David L. Hill (Dept. Psychol., Univ. of Toledo, Toledo, OH 43606).

The ontogeny of gustatory afference within the second-order central gustatory relay, the "pontine taste area" (PTA), occurs in several distinct phases. First, PTA neurons in rats aged 4-7 days respond to many, but not all, chemical stimuli. However, most neurons in rats older than 14 days respond to each of the basic tastes. Specifically, most PTA neurons in rats aged 4-7 days fail to respond to 100 mM KCl and quinine hydrochloride, whereas neurons in rats aged 14 days and older respond to 100 mM and 500 mM solutions of NH_4Cl, NaCl, LiCl and KCl, to citric and hydrochloric acids, to sucrose and sodium saccharin, and to quinine HCl. Second, although the number of stimuli to which PTA neurons respond does not change after 14 days of age, major changes occur with regard to sensitivity: Response frequencies increase to all stimuli between 14-60 days of age. Thus, PTA neurons become responsive to more stimuli between the first and second week life, and thereafter PTA neurons show greater response sensitivity.

We have recently begun descriptive neuroanatomical studies to investigate the neural correlates of developing taste responses. To that end, a variant of the Golgi-Fox procedure was used to examine the histogenesis of PTA neurons in rats aged 16, 22, 35, and 131 days of age. Preliminary concentric ring analyses of 72 fully-impregnated PTA neurons show that extensive dendritic outgrowth and branching occurs in PTA neurons between 16 and 22 days. At 16 days of age maximum distal dendritic boundaries are approximately 75 μm from somata. At 22 days of age maximum distal dendritic boundaries are approximately 150 μm from somata. At 35 days of age maximum distal dendritic boundaries are approximately 200 μm from somata, and at 131 days maximum distal boundaries of dendritic branches are approximately 225 μm. Thus, relatively extensive dendritic outgrowth occurs between 16 and 22 days and thereafter the rate of dendritic growth decreases. Although dendritic outgrowth was observed distal to somata, the number of dendrites originating from somata was similar in each age group, as was mean somatic diameter. These preliminary observations suggest that the developmental frequency change in PTA neural responses may be due, in whole or in part, to the proliferation of additional functional synapses along developing dendrites.

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P114 EMBRYONIC DEVELOPMENT OF TASTE BUDS IN THE CHICKEN. J.R.Ganchrow and D.Ganchrow. (Dept. Oral Biology, The Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel, and Dept. Anatomy and Physical Anthropology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel).

Chickens and rats share a 21 day period in ovo and in utero, respectively. Rats are altricial at birth and while the first immature taste bud occurs at 20 days in utero, the bulk of bud development occurs postnatally (Mistretta, 1972) prior to weaning. In contrast, the present investigation demonstrates that in the precocial chick, dramatic taste bud development occurs during the last quarter in ovo. Oral epithelium containing anterior mandibular gland taste buds was serially sectioned (10 μm), stained with hematoxylin-and-eosin and unilaterally counted in Anak (broiler breed) chickens at 16, 17, 18, 19 and 20 days embryonic age as well as on the day of hatching. While no buds were seen at 16 days, spheroid collections of epithelial cells were observed in the basal epithelial regions in proximity to gland duct openings at 17 days. At 18 days the first signs were seen of fine tubules and pores characteristic of chick buds. At 19 days, the spheroid cell collections had elongated to begin to take on the shape characteristic in hatchlings, and lighter staining cells were apparent in the core of this presumptive bud. The number of taste buds continued to increase throughout these stages, peaking at 19 days ($\bar{X} = 83 \pm 4$, N = 4) and more and more buds opened into the oral cavity. Correspondingly, the buds continued to elongate and the cells became more organized until by hatching they had obtained their characteristic pear shape perpendicular to the horizontal layers of epithelial surround. Thus, although rats and chickens share the same gestational time span, the degree of taste receptor development just prior to emerging from the placental confines, reflects demands the immediate postnatal enviroment will place upon the chicks.

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P115 TASTE BUD DEVELOPMENT IN HAMSTER VALLATE AND FOLIATE PAPILLAE. David V. Smith and Inglis J. Miller, Jr.,(Dept. Otolaryngology and Maxillofacial Surgery, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267, and Dept. Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103)

This project examines whether taste buds (tb) from foliate and vallate papillae in hamsters appear at the same age after birth or proliferate at a similar rate to adult totals. Our objective is to study taste receptors from regions beyond the front of the tongue. Animals are mated, and the birthdate of newborn pups is recorded. They are sacrificed by ether anesthesia and decapitation from the first day until 93 days of age. Single vallate papillae and two bilateral foliate papillae are prepared by serial sections in paraffin for microscopic study. Vallates came from 25 animals and foliates from 33 animals. Two foliates were obtained from 19 animals, and one foliate from 14 hamsters for a total of 52 because of lost or damaged sections. Only taste buds with a patent taste pore are counted. There are no taste buds in the vallate or foliate papillae of the hamster at birth, and the epithelium on the walls of the furrows is smooth and dense.Taste buds appear in both structures on the 2nd postnatal day, but the presence of a pore connecting taste buds with the surface of the furrow wall is not apparent until 3-4 days after birth. Foliate papillae have an average of 36±12 (SD.,N=3) tb at 6 days of age, 76±7 (N=6) tb at 15-19 days, 100±15 tb (N=5) at 30 days. From 35 days of age to our oldest group at 120 days, the average number of taste buds per foliate papilla is 114±24 (N=21) without systematic increases according to age. In animals more than 30 days old, the bilateral differences are as large as the differences between ages. At day 6, two vallate papillae have 32 taste buds each. Animals 10 days of age average 57±23 vallate taste buds (N=4); 19-30 day old hamsters have a mean of 69±11 (N=5) tb; and at 50-60 days of age, they average 108+9 tb/papilla (N= 4). Hamsters 75-95 days old average 148±27 (N=3) vallate taste buds; while 120 day old animals have a mean of 168±27 (N=4)taste buds. Vallate taste buds (Y) increase in number according to a logarithmic function (with x in days) to 120 days of age so that $Y = 39.65 \ln(x) - 37.58$ with a coefficient of determination of .868. Thus,foliate taste buds seem to reach a maximum number within 35 days after birth, while vallate taste buds increase in number up to 120 days. The significance of bilateral differences in foliate taste buds is not understood.

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P116 TROUT OLFACTORY RECEPTORS DEGENERATE IN RESPONSE TO WATER-BORNE IONS: A POTENTIAL BIOASSAY FOR ENVIRONMENTAL NEUROTOXICOLOGY? David T. Moran, J. Carter Rowley, and George Aiken. (Rocky Mountain Taste & Smell Center, Department of Cellular and Structural Biology, University of Colorado School of Medicine, 4200 East 9th Ave., Denver, CO 80262, and U. S. Geological Survey, 5293 Ward Road, Arvada, CO 80002).

During the course of our research on the ultrastructure of the trout olfactory system, we observed that wild Brown trout (Salmo trutta trutta) experienced complete loss of their olfactory receptors after spending two days in a large, 250-gallon aquarium in our aquatic facility. When these same fish were returned to the North Fork of the South Platte River -- their home stream -- their olfactory receptors were found to have regenerated within a period of eight days. When these same fish were re-introduced into our laboratory aquarium, their receptors degenerated, once again, within two days. Comparative chemical analysis of the water from the South Platte river and the laboratory aquarium revealed some striking differences. In the aquarium water, the levels of four ions -- cadmium (Cd), cobalt (Co), copper (Cu), and zinc (Zn) -- were present at significantly higher levels than they were in stream water. Suspecting one or more of these ions might be associated with the loss of trout olfactory receptors, a pilot study was done in which trout were placed in separate glass containers. Each container was filled with stream water plus one of the ions named above set at the concentration levels found in the water from the 250-gallon laboratory aquarium. After trout had lived in the "spiked" containers for three days, biopsies of their olfactory rosettes were taken and investigated by transmission electron microscopy. All fish noses appeared normal with the exception of the experimental animal that had lived in the water "spiked" with copper ion: its nose had lost all of its olfactory receptors. These observations suggest 1) that experimental manipulation of ion content may provide a technique for non-invasive chemical olfactotomy, and 2) that the trout nose may serve as a sensitive bioassay for environmental toxicology -- especially in waters where the biological impact of introduced metal contaminants is in question.

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P117 **COMPARATIVE STUDY OF OLFACTORY RECEPTOR CELLS WHICH AXONES HAVE NOT YET REACHED THE OLFACTORY BULB AND THOSE WHICH AXONES HAVE ALREADY REACHED IT.** M.S. Lidow (Northwestern University). R.C. Gesteland and M.T. Shipley (University of Cincinnati).

This work showed that in order to obtain olfactory epithelia with population of developmentally synchronized receptor cells suitable for electrophysiological studies it is necessary to ablate epithelia with $ZnSO_4$, allow it to regenerate freely for 10 days, and then suppress the generation of new cells in them by continuous treatment with hydroxyurea. Since olfactory epithelia begins to generate new receptor cells only on the 6th day after ablation, it is reasonable to assume that receptor cells in the aforementioned epithelia originated between the 6th and 10th day of free regeneration. The difference in age of these cells would be no more than 5 days. The cells develop normally and in lock-step.

Olfactory epithelia with populations of developmentally synchronized receptor cells were used for a comparative study of "immature" olfactory receptor cells which axones have not yet reached the olfactory bulb and "mature" cells which axones reached and penetrated the bulb.

"Immature" olfactory receptor cells are characterized by perikarya with poorly developed rough endoplasmic reticulum, by dendrites containing an abundance of ribosomes and mitochondria in their shafts and olfactory knobs, and by short motile cilia. "Mature" cells have perikarya with well-developed rough endoplasmic reticulum, their dendrites contain no ribosomes, and no mitochondria were observed in the olfactory knobs. Cilia in these cells were long and immotile.

There were no differences in the polarity or shape of EOGs recorded from olfactory epithelia with immature and mature receptor cells. However, amplitudes of the main negative components in EOGs recorded from epithelia with mature receptor cells were generally higher than those with immature cells.

In this study the majority of immature olfactory receptor cells tended to have low spontaneous activity or none at all. Mature olfactory receptor cells displayed a great variety of frequencies of spontaneous activities which ranged from less than 1 to more than 70 spikes/min.

Extracellular single unit activity recordings showed that, contrary to what had been observed in rat embryos, olfactory receptor cells in adult frogs do not go through a stage in which they respond to all odorants.

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P118 DEVELOPMENTAL PATTERN OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE RAT OLFACTORY BULB. Kate M. Guthrie and Michael Leon. (Department of Psychobiology, University of California, Irvine CA 92717).

Preweanling rats concurrently exposed to an odor and tactile stimulation which mimics maternal care exhibit both a behavioral attraction and an enhanced 2-DG uptake in specific areas of the olfactory bulb glomerular layer upon subsequent presentation of that odor. Pups exposed to the odor without simultaneous tactile stimulation fail to exhibit this enhanced response. A mechanism for this enhanced response has been proposed which implicates the sparing of cells associated with the specific glomerular area from early death in development. Recently, ornithine decarboxylase (ODC; EC 4.1.1.17) has been suggested to mediate differential cell death. Brain ODC activity is depressed in young rats deprived of maternal contact but rebounds to normal levels if the pups receive tactile stimulation which mimics maternal care. ODC catalyzes the rate-limiting step in the biosynthesis of the polyamines, which have been implicated in tissue growth and differentiation. Different organs display specific developmental patterns of ODC activity which are correlated with periods of rapid cell growth and replication. Application of exogenous polyamines has been correlated with larger than normal cell populations, possibly a result of cell sparing.

To explore the possibility that ODC/polyamines may mediate the enhanced olfactory response, we first determined the developmental pattern of ODC activity in the rat olfactory bulb. ODC assays were performed on bulbs obtained from animals aged 1-22 days postpartum. The developmental pattern of ODC activity in the bulb is similar to that seen in other tissues; the highest levels occur near birth, followed by a decline with increasing age. The largest decrease in activity occurs on postnatal days 4-5. This pattern follows that of postnatal neurogenesis in the rat olfactory bulb.

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P119 TRANSPLANTS OF OLFACTORY MUCOSA INTO THE OLFACTORY BULB OF RODENTS. G.A. Monti Graziadei, J.A. Heckroth and P.P.C. Graziadei. (Dept. Biological Science, Florida State University, Tallahassee, FL 32306).

After transplantation of neonatal rat olfactory mucosa into the brain parenchyma of littermates, the mature neurons in the olfactory neuroepithelium rapidly degenerate due to the severance of their axons. New neurons differentiate from the neurogenetic matrix located at the base of the olfactory neuroepithelium; of these neurons, a portion reconstitute the neuronal population of the neuroepithelium, while others freely migrate within the surrounding brain parenchyma. The destiny of the migrating neurons is still to be determined. The neurons in the epithelium reach maturity as demonstrated by the presence of ciliated dendrites and long axons. Moreover, they can express OMP (olfactory marker protein) upon reaching maturity, yet their number is greatly reduced when compared with the normal olfactory epithelium in situ.

In order to test the possibility that the expression of OMP could have been impaired by the absence of the specific target, the olfactory bulb, we have transplanted the olfactory mucosa of neonatal rats into the olfactory bulb of littermates. In this experimental paradigm, the olfactory bulb, partially deafferentiated by the surgical procedure, could have provided the ideal target for the transplanted olfactory neurons.

The transplanted neuroepithelium survived and it was arranged into a series of vesicles. As in previous experiments where the transplants were performed into the parietal and cerebellar cortices, the olfactory neurons migrated into the bulbar parenchyma. Surprisingly, the number of olfactory neurons expressing OMP was highly decreased and equal to 0 in several experimental animals. This result strongly suggests that the olfactory bulb is not determinant in the expression of OMP. The reduced amount of the specific marker in the olfactory bulb transplants points to a possible inhibitory effect of the target on the maturation of the olfactory neurons.

Supported by NIH, NS 20699.

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P120 DEVELOPMENT OF OLFACTORY AND VOMERONASAL SYSTEMS IN THE RED-SIDED GARTER SNAKE, THAMNOPHIS SIRTALIS PARIETALIS. DAVID A. HOLTZMAN AND MIMI HALPERN (PROGRAM IN NEURAL AND BEHAVIORAL SCIENCES, DOWNSTATE MEDICAL CENTER, BROOKLYN, N.Y.)

This study describes the morphology of the olfactory and vomeronasal systems in the garter snake. Embryos from pregnant female snakes, obtained from Dr. David Crews, were surgically removed and classified according to Zehr stages. Heads of embryos and neonates were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned, and stained using the Bodian method.

At Zehr stage 26/27, the vomeronasal epithelium (VNE) is composed of a layer 12-15 cells thick bordered dorsally and posteriorly by small, round clusters 3-4 cells in diameter. The thick layer appears to be the embryonic supporting cell (SC) layer, and the clusters appear to be undifferentiated cells and developing bipolar neurons (UD-BP). At Zehr stage 32, the SC layer (15 cells thick) is approximately equal in thickness to the UD-BP layer. At Zehr stage 36, the UD-BP layer is 17-22 cells thick and has formed tall columns. The SC layer has diminished to 2-5 cells in thickness. 0-2 days after birth, the SC is 2-3 cells thick, and the UD-BP layer is 24 cells thick. At 12 days old, the SC layer is 1-3 cells thick and the UD-BP layer is approximately 30 cells thick.

At Zehr stages 26/27 and 32, the olfactory epithelium (OE) is 15 cells thick and homogeneous in appearance. At Zehr stage 36, the OE is 6-8 cells thick and begins to show evidence of stratification. At 0-2 days and 12 days, the OE is 6-10 cells thick with adult-like organization.

At Zehr stage 26/27, neither the main olfactory bulbs (MOB) nor the accessory olfactoy bulbs (AOB) have glomeruli. Both consist of undifferentiated cells concentrated around the olfactory ventricles. The bulbs can not be distinguished from each other except for the location of entering nerve fibers. The vomeronasal nerve enters the AOB from its dorsomedial aspect. The olfactory fila appear to enter the MOB ventrolaterally. At Zehr stage 32, glomeruli are still absent, but the MOB and AOB have become distinguishable from each other. At Zehr stage 36, glomeruli have started forming medially on both sets of bulbs. Hints of cell layer formation are apparent. At 0-2 and 12 days of age, both sets of bulbs have glomeruli and have distinct cell layers as in the adult.

The areas containing the tertiary neurons associated with these two systems first appear as distinct structures at Zehr stage 36.

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P121 POSTNATAL DEVELOPMENT OF ENZYMES IN THE OLFACTORY BULB OF NORMAL AND HYPOTHYROID RAT. R. Safaii, R. Moussavi and E. Meisami. (Inst. Biochem. Biophys., Univ. Tehran, and Dept. Physiol.-Anat., Univ. Calif., Berkeley, CA 94720, USA).

Activity of several brain enzymes, Na-K-ATPase, Mg-ATPase, acetylcholinesterase (AChE), cholineacetyltransferase (CAT), butyrylcholinesterase (BuChE), succinic dehydrogenase (SDH) were measured in the olfactory bulbs (OB) of postnatal albino rats. Both specific (per mg prot.) & total (per whole OB) acitvities were determined. Only Na-K-ATPase and CAT showed no activity at birth; activity was present at day 5 and showed major surge (specially for Na-K-ATPase) between days 10 and 25. AChE and SDH were present in the neonatal OB but also showed major surges after day 10, reaching adult values by day 25. The specific activity of Mg-ATPase remained constant postnatally but BuChE showed high specific activity at birth, declining postnatally to adult levels (day 15). Total activity for Mg-ATPase and BuChE, however, increased throughout development, albeit far less markedly than for Na-K-ATPase and AChE.

The data indicate: 1) in OB, as in other brain regions, various enzymes show different temporal order of development, reflecting differential localization within different cells or different neuronal compartments; 2) the major period for massive development of synaptic membrane enzymes in OB is after day 10, coincident with surge in OB growth and neuropil development; 3) BuChE is not related to AChE, nor to neuronal and synaptic compartments; 4) O2 requiring oxidative metabolism (SDH) and massive energy utilizing mechanisms (Na-K-ATPase) are poorly developed or absent at birth becoming dominant after day 10; 5) cholinergic transmission is either absent or rudimentary in the early postantal period, becoming significant after day 10; 6) with regard to neurochemical development, OB shows similar trends as forebrain cortical regions.

Growth (weight gain) and protein accretion in OB was markely and significantly reduced in thyroid deficient animals at both ages of 10 and 25 days. Determination of Na-K-ATPase and AChE activity (markers of synaptic membranes) in the OB of 10 and 25-day-old hypothyroid rats revealed highly significant reductions in specific and total activity of Na-K-ATPase at both ages. Specific activity of AChE was reduced but not significantly; total activity of AChE was however markedly and significantly reduced.

The data imply that thyroid hormones markedly stimulate proliferation of synaptic membranes in the postnatally developing OB. However thyroid deficiency has more deleterious effects on development of the enzymes which are more ubiquitously and generally associated with synaptic membranes (e.g.,Na-K-ATPase).

F15 HOW MOTILE BACTERIA SENSE AND RESPOND TO CHEMICALS. Julius Adler. (Departments of Biochemistry and Genetics, University of Wisconsin, Madison, Wisconsin 53706)

Bacteria are attracted by certain chemicals and repelled by others; this is chemotaxis. The work of Wilhelm Pfeffer established this 100 years ago.

How do bacteria sense the chemicals that attract or repel them? How are the flagella told what to do? How do flagella work and how are they coordinated? Can bacteria "learn" to change their chemotactic behavior? We have applied the tools of biochemistry and genetics to provide answers to these questions.

It used to be believed that bacteria sense an attractant by measuring the energy produced during its metabolism. We showed that metabolism of a chemical was neither required nor sufficient for it to be an attractant; instead the bacteria have sensors (or "receptors" or "signallers" or "transducers") that detect the chemical itself, not any product formed from it. By now some 20 different chemosensors are known for various attractants and repellents.

I discovered that methionine is required for chemotaxis, and this led to our discovery of a novel sensor, the methyl-accepting chemotaxis protein. Four distinct methyl-accepting proteins are now known, each serving a different set of attractants and repellents.

In an as yet unknown manner, these methyl-accepting chemotaxis proteins signal to the flagella to tell them whether to rotate counterclockwise, leading to swimming in a straight line and thus movement toward attractants, or whether to rotate clockwise, leading to tumbling and thus avoidance of repellents: This is called "excitation." The mechanism of excitation is totally unknown, and it is one of our major goals today to discover this mechanism.

Then excitation is terminated even though the stimulus is still present; this "adaptation" consists of increased methylation of methyl-accepting chemotaxis protein in the case of attractants or decreased methylation for repellents. By now much is known about the adaptation mechanism, but still a lot remains to be learned.

All of this was discovered by first isolating behavioral mutants that fail in each of the steps indicated, and then the biochemistry missing in each mutant was determined.

It looks like bacteria can "learn" to change their chemotactic behavior. We are currently working out the genetics and biochemistry of "learning" in bacteria.

Reference: The Behavior of Rats, Bacteria, and Man. Julius Adler pp. 367-383 in "Biochemistry of Metabolic Processes" by D.L.F. Lennon, F.W. Stratman, and R.N. Zahlten, editors. Elsevier (1983)

F16 STANDARDIZED OLFACTOMETER IN JAPAN —— A REVIEW OVER TEN YEARS

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To control odor polution and to diagnose and remedy olfactory dysfunctions, a research group of 14 members from 13 universities was organized in 1971. At first they aimed to manufacture a standardized olfactometer. Ten odorants were selected as standard test odors. By repeating tenfold dilution, a series of test solutions from 10^{-1} to 10^{-17} was made for each of the ten odors. Detection and recognition thresholds for these solutions were sought among many healthy men and women 18 to 25 years old at the 13 universities. After analysis of the test data, five odors were selected among the ten. Averages of these thresholds were calculated. Repeating tenfold multiplication or tenfold division of these averages, a new series of odor solutions from 10^{-2} to 10^{5} was made for the five odors. The solutions were bottled and set in a metal frame. This newly manufactured "T & T olfactometer" was endorsed in 1975 by the Otorhinolaryngology Society of Japan. Since then it has been widely used in Japan. In 1983, the Ministry of Welfare in Japan approved it and assigned 420 social welfare reimbursement points (at about 12 Yen per point) for a test with this olfactometer. The use of the T & T olfactometer in several university clinics for over ten years is introduced.

F17 VISUAL APPROACH TO FRAGRANCE DESCRIPTION

Maurice Thiboud (L. Givaudan & Cie, S. A. 1214 Vernier-Geneva, Switzerland)

I. What is a fragrance ?
Some indications about the creation and signification of a perfume.

II. Necessity of a common language
It is absolutely necessary to find a language common to perfumery specialists as well as to people not initiated to perfumery creation techniques.

III. Our present language
Having found that the creation and description of fragrances are being based on an overall olfactive note or on harmonies of scents, we have established a philosophy which permits to group fragrances into these "overall olfactive notes" or "harmonies" and we call these groups : analogies.
a) analogies
These are the following : - basic floral / natural
- aldehydic
- Chypre
- spicy
- oriental
- Tabac
b) galaxies
Starting from these analogies, Givaudan has then set up what we call the "fragrance galaxy", which allows to place a perfume with regard to its note and to the fragrances of the market.

IV. Our future language
It becomes more and more necessary to have a precise and functional language in perfumery, so that we can easily answer to customers wishes and tastes.
Some ideas and suggestions are then presented for discussion.

NOTES

ALPHABETICAL INDEX OF AUTHORS

P = POSTER
S = SLIDE PRESENTATION