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ABSTRACTS

Thirtieth Japanese Symposium on Taste and Smell (JASTS XXX)

Held in Osaka, Japan, October 29–31, 1996

1. Studies on chemical senses in Japan—past, present and future

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On the occasion of the 30th anniversary of the Japanese Symposium on Taste and Smell, eight pairs of researchers presented speeches on their own studies, covering past work, current trends and future prospects. Each pair consisted of a teacher and his or her past student. The topics presented are as follows: brain mechanisms of qualitative analyses and hedonic evaluation of taste of food by Yojiro Kawamura and Takashi Yamamoto; taste quality coding by Masayasu Sato and Hisashi Ogawa; taste reception in insects by Hiromichi Morita and Mamiko Ozaki; chemical senses and flavor of food by Masao Fujimaki and Soichi Arai; aroma of food by Tei Yamanishi and Akio Kobayashi; taste system in fish by Masaya Funakoshi and Takayuki Marui; chemical senses in lower vertebrates by Kazuo Ueda and Takatoshi Nagai; and electrophysiological studies on insect olfactory system by Tatsuaki Shibuya and Ryohei Kanzaki.

2. Recordings of electrical response from taste cells using the patch clamp technique

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We present a method for whole-cell recording from non-dissociated taste cells within mouse taste bud. We obtained the preparation consisting of a taste bud and small piece of the lingual epithelium. The whole-cell configuration was established in a non-dissociated taste cell within the taste bud with a patch pipette containing Lucifer Yellow CH by holding the small piece of epithelium in a holding pipette loaded with continuous negative pressure to maintain the orientation of the taste bud. Taste stimuli or blockers were applied from a third pipette near the taste pore under a continuous flow of bathing solution. Using this procedure, we could simultaneously accomplish patch clamping, visualization of taste cell morphology, localized taste stimulation and maintenance of the microenvironment around the taste organ. Rapid responses to a relatively high concentration of salt stimuli or toxic taste substance such as acid were also obtained.

3. An easy recording technique of neuronal responses to taste stimuli in freely behaving rats

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In order to clarify the neural substrates for processing of taste information, it is essential to know how individual neurons respond to taste stimuli in conscious, behaving animals. However, the techniques developed so far are too difficult or time-consuming to use. We report here an easy and stable recording technique of single or multiple unitary responses to taste stimuli in freely behaving rats for a long period. Under Nembutal anesthesia rats were implanted with bipolar chronic electrodes made from two 80 μm stainless-steel wires in the target area. The electrodes and intraoral cannula were fixed securely to the skull with stainless-steel screws and dental acrylic. The animals were allowed to recover for at least 5 days. Neuron activity was recorded continuously during observation sessions. To eliminate the movement artefact, we amplified neuron activity with a miniature operational amplifier attached to the mating plug of the recording cable. The cable was then connected to a slip-ring connector to allow free movement of the rat within the observation cage. The differential amplification of electrophysiological signals minimized

the electromyographic artefact accompanied by orofacial movements. Single unit activities were isolated from multiunit activities by means of a computer system (Discovery 4.0, DataWave Technologies, CO). We investigated unit activities in the gustatory cortex during the development of taste aversion learning and found two types of units in this area that showed facilitatory change with different time courses.

4. Central mechanism of recognition and learned taste preference for a deficient nutrient in L-lysine-deficient rats by Umami taste stimuli

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Rats that were fed diets deficient in an essential L-amino acid (AA), L-lysine (Lys), showed a quantitative preference for Lys, glycine and NaCl. However, Umami taste preference was elicited whenever their protein nutrition were normal or recovered from deficiency. The recognition site for the deficient nutrient in rat brain was localized in the ventromedial and lateral hypothalamus (VMH and LHA), identified by functional magnetic resonance imaging (4.7 T). Under constant injection of Lys (<1% of the requirement for normal growth) into the LHA bilaterally, Lys-deficient rats neglected Lys deficiency, assayed by 1 h operant-type behavior (50 mg of Lys normal diet/30 times bar-pressing). The single-neuron recording in LHA of these Lys-deficient rats suggested that neural plasticity occurred, specifically responding to Lys, both by iontophoretic application and during ingestion of AA. Other LHA neurons of normal rats also differentially responded to monosodium glutamate. Related neurotrophic factors, e.g. activin/inhibin, induce suppression of bar-pressing behavior similar to the case of Lys injection into the LHA. The present results suggest that LHA and probably VMH may play important roles in recognition responses specifically to particular deficient nutrients for the maintenance of AA homeostasis.

5. From the viewpoint of food and nutrition: why is fat delicious?

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The increase in intracellular Ca^{2+} by fatty acids and the expression of the membrane fatty-acid transporter (FAT) was investigated in rat small intestinal epithelium and rat taste buds. Lenoleic acid and oleic acid markedly increased the influx of $^{45}Ca^{2+}$ into dispersed intestinal cells and the small intestinal epithelial cell line (IEC-6), while octanoic acid, methyl linoleate and linolyl alcohol had no effect. Acidic amino acid and derivatives of linoleic acid inhibited the linoleic acid-induced influx of $^{45}Ca^{2+}$, indicating that activation of the influx by linoleic acid depends on the chain length and is affected by the presence of a carboxyl group. Linoleic acid also increased the intracellular Ca^{2+} level in isolated taste buds. Immunohistochemical studies showed that FAT protein was limited to the apical taste buds and to the brush border of the small intestine. In IEC-6, anti-FAT antibody inhibited the increase in intracellular Ca^{2+} by the fatty acid. Western blot analysis and reverse transcriptase polymerase chain reaction indicated the expression of FAT in circumvallate papillae. These results suggest that enterocytes and taste cells have a common system for recognizing fatty acid, and that the excitation of taste cells by fatty acid is involved in the palatability of fat.

6. Development of taste and odor sensors

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A multichannel taste sensor with global selectivity is composed of several kinds of lipid/polymer membranes for transforming information of substances producing taste into electric signals which are input to a computer. The sensor output shows different patterns for molecules which have different taste qualities such as saltiness and sourness, while it shows similar patterns for molecules with similar taste qualities. The sensor responds to the taste itself, as can be understood from the fact that taste interactions, such as the suppression effect which occurs between sweet and bitter molecules, can be reproduced well. Amino acids can be classified into several groups according to their taste based on sensor outputs. The taste of foodstuffs such as beer, coffee, mineral water and vegetables can be discussed quantitatively using the taste sensor, which provides an objective scale for human sensory expression. A multichannel odor sensor using quartz oscillator arrays with coated lipid or polymer materials has been also developed. These kinds of sensors will open doors to a new era of food science, and contribute to clarification of the reception mechanism in gustatory and olfactory systems.

7. From a view of studies on the mechanisms of gustatory reception

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Analysis of the gustatory reception and transduction mechanisms has advanced greatly through the development of experimental methods including the patch-clamp, intracellular Ca^{2+} measurement and molecular biological techniques. However, multiple transduction mechanisms are proposed for responses to each of four basic stimuli, and the transduction mechanism of gustatory signals is known to be much more diverse and complex than those of visual and olfactory signals. Taste receptor cells are polarized, and the plasma membrane is divided into the apical (receptive) membrane which is exposed in the taste pore, and the basolateral membrane, which is immersed in the interstitial fluid. Taste buds contain three types of elongated cells, type I (dark) , type II (light) and type III (intermediate) cells. Only type III cells are believed to be the taste cell because of their synaptic contact with the

gustatory nerve fibers. However, these critical problems have been barely considered in physiological experiments to date. Relationships between transduction and synaptic transmission may be important because taste qualities are possibly discriminated within a taste bud. Thus, more challenging and more elegant approaches, which overcome those problems, may be necessary for understanding the basic design of the gustatory transduction mechanism.

8. Recent progress in olfactory transduction mechanism

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It is generally considered that binding of chemical stimuli to olfactory G protein-coupled receptors (GCRs) leads to accumulation of cAMP or IP_3 in olfactory neurons and activation of cAMP- or IP_3-gated channels, causing cell depolarization and olfactory nerve responses. This scheme is not, however, fully consistent with electrophysiological data obtained with the whole-cell patch-clamp method and ciliary recording. That is, the results obtained by *in situ* hybridization that a single neuron has only one type of olfactory GCR cannot simply explain the observation that a single olfactory neuron responded to various species of odorants. Existence of multiple odorant receptors in single olfactory neurons was directly demonstrated by cross-adaptation experiments using isolated turtle and bullfrog olfactory neurons. A response of an olfactory neuron to an odorant contains not only a component induced via the cAMP or IP_3 pathway, but also a large component via the second messenger-independent pathway. Thus, the component mediated neither via olfactory GCRs nor the second messenger-dependent pathway greatly contributes to an odor response.

9. Genetic approaches to mammalian gustatory receptor mechanisms

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Genetic variation in mammalian gustatory sensitivity was first found in 1931 as human 'taste blindness' for a bitter substance, PTC (phenylthiocarbamide). This finding provided a new insight into the study of taste reception and a good beginning of the genetic approach to taste receptor mechanisms. Nevertheless, during the following four decades, no such approach had been successful. Starting in the 1970s, steady progress has been made in murine taste genetics. During the past three decades, genetic variants for taste sensitivities to four bitter (SOA, quinine, raffinose acetate, copper glycinate and cycloheximide) and three sweet substances (saccharin, D-phenylalanine and L-proline) have been isolated among inbred strains of mice, and single-locus control for each substance has been established (*Soa*, *Rua*, *Qui*, *Glb* and *Cyx* genes on chromosome 6 and *dpa*, *Sac* and *psr* genes on chromosome 4). Two different congenic strains, where specific taste genes from genetically different strains were transferred, are now available: one for the *Soa* gene, and the other for the *dpa* gene. The ultimate goal of the taste genetic study must be to positionally clone the tasting genes. Recent advances in technology for gene mapping using microsatellite markers and for gene cloning using YAC and BAC libraries have made it possible to identify taste receptor molecules at the DNA level.

10. Development of the gustatory system: what we have learned from early environmental manipulations?

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Impressive morphological, physiological and behavioral changes characterize the postnatal development of the rat gustatory system. While much has been learned by studying normal developmental processes, complementary experiments that use early dietary sodium restriction have been of great value in learning about how the taste system is organized. For example, recent experimental findings indicate that rules governing the dramatic postnatal changes in normal animals may be directed by *pre*natal physiological events that precede the emergence of the gustatory system. The development of peripheral and central gustatory morphologies and function will be explored in normal rats and in rats raised with a history of dietary sodium restriction. Coordinated morphological and neurophysiological studies are used to show how the gustatory system changes during normal development and how these normal processes are affected following early dietary sodium restriction. Thus, the overall goal will be to understand the underlying mechanisms involved in determining the structure and function of peripheral and central taste neurons.

11. An analysis of salt signal transduction in vertebrate taste cells

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An amiloride-insensitive component is known to contribute to the salty taste transduction of the vertebrate gustatory system, and to play a more important role than an amiloride-sensitive component in amphibian and human gustatory systems. The amiloride-insensitive pathways of salty signal transduction in frog and mouse were examined using a combination of conventional intracellular recording methods with the arterial perfusion method and several variations of the patch-clamp technique. The results are as follows: (1) The magnitude of receptor potentials evoked by NaCl and KCl is dependent on the interstitial Na^+ concentration, and (2) the reversal potential of the salt-induced receptor potential is greatly affected by concentration and species of inorganic salt stimuli in the bullfrog taste cells. (3) Fujiyama *et al.* (1995) demonstrated that Ca^{2+}-permeable non-selective cation channels, which are activated by rise in the intracellular Ca^{2+} concentration, exist over the taste cell membrane of the bullfrog. (4) No

voltage-dependent Ca^{2+} channels were detected in the frog taste cells. (5) Both amiloride-sensitive and -insensitive components in single taste cells of mice were observed in single taste cells. These results suggest that the non-selective cation channels observed in the taste cell membrane contribute to both salty signal transduction and transmission.

12. Optical imaging of evoked neural activity of the *in vivo* cockroach antennal lobes

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Optical recordings with high spatial and temporal resolution of transmembrane activities were used to characterize the properties of the electrical signals in the *in vivo* cockroach antennal lobes (ALs) of the adult male with a voltage-sensitive dye. Electrical stimulation of the antennal nerve (AN) in the physiological range evoked a compound action potential in the AN fibers that depending on the intensity, but was followed by hyperpolarization at lower stimulus intensities in the proximal region of the AN. This suggests that the depolarizing component may be generated predominantly in the AN region and contribute to masking the hyperpolarizing component at higher stimulus intensity levels. Optical signals subsequently evoked at the macroglomerular and ordinary glomerular regions consisted of a depolarizing response followed by hyperpolarization. A complex correlation exists between the size and time course of optical responses and the intensity of stimulation. The pharmacological results suggested that the depolarizing responses on the AL consist of both a presynaptic response, representing synchronous compound action potentials from the AN, and a postsynaptic response, representing synchronous compound excitatory postsynaptic potentials and action potentials of the projection neurons and local interneurons (LNs). In contrast, the hyperpolarizing component was probably mediated by inhibitory synapses with the GABAergic LNs in the AL. In addition, the hyperpolarizing component recorded at the proximal region of the AN was possibly due to the GABAergic inhibitory actions from the AL LNs to the antennal afferent fibers. The inhibitory responses of GABAergic LNs in the AN are different in time course from those in the AL.

13. The estimation of the gustatory area of human cerebral cortex with measurement of evoked magnetic fields

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Few experiments have addressed human gustatory-evoked potentials because of difficulties involved in precise control and presentation of gustatory stimuli. We have developed a gustatory apparatus to present the stimulus with a short rise-time (17 ms to 80% level), because MEG requires strict stimulus control in the same way as EEG experiments. We measured magnetic fields (MFs) and confirmed the obtained MFs were really evoked by the gustatory stimuli. We then sought to estimate the area of gustatory activity in the human brain. Magnetic fields from gustatory stimulation with 1 M NaCl and 3 mM saccharin were recorded by using a whole-cortex SQUID system. The averaged onset latency of MFs was 93 ms for NaCl, 172 ms for saccharin and no response was obtained for water. A high correlation coefficient was noted between the difference of onset MF latencies in two tastants and that of behavioral reaction times, and responses to saccharin were delayed or abolished after treatment of a subject's tongue with a sweet-suppressing agent. This finding indicates that the MFs obtained were caused by gustatory stimulation. By plotting the equivalent current dipole via magnetic resonance imaging, we could locate the primary gustatory area at the transition area between the operculum and insula, as reported in macaque monkeys.

14. Desert toads may have salt taste receptors in the ventral skin

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When dehydrated, the desert toad (*Bufo alvarius*) presses its ventral skin onto a surface wetted with pure water to absorb water. This stereotypical behavior was suppressed when the animal was placed on 250 mM NaCl solution. However, adding amiloride to the solution caused this behavior to be resumed. These observations suggest that toads have chemosensory receptors in the skin for detecting salts. Afferent impulses were recorded from the spinal nerves innervating the ventral skin of toads. The nerve responded to NaCl, KCl and citric acid solutions with much longer latency than to mechanical stimulation. However, the latency was reduced in stimulus of higher concentration. Fluorescent dye dil was applied to the spinal nerves of fixed toads to label the fibers and target cells for innervation. Skin tissues were examined with a confocal laser scanning microscope. Brightly fluorescent cells occasionally occurred in a small cluster. Single fibers reached those cells, showing transneuronal diffusion of the dye. No cells directly faced the surface of the skin, but were located in the deeper layer, i.e. the germinativum cell layer, in which the nerve fibers expanded. Therefore, either labeled cells nerve fibers, or both, may be a transducer element of spinal nerves to detect Na^+ flowing into through an amiloride-blockable channel in the toad skin.

15. Enhancing effect of nickel ions on the taste response to magnesium ions of single fibers of the frog glossopharyngeal nerve: inhibition of the nickel-enhanced response to magnesium by calcium ions

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Single fibers of the frog glossopharyngeal nerve respond to $MgCl_2$ (>10 mM) and $CaCl_2$ (>0.001 mM). $NiCl_2$ at 1 mM enhanced both the Mg response and the Ca response. However, in the absence of Ni ions, the effects of Mg ions and the Ca ions are mutually antagonistic. That is, Ca ions antagonize the effect of Mg ions and Mg ions antagonize the effect of Ca ions. Ni ions did not affect the antagonism between Ca and Mg ions. In the present study, the inhibition of the Ni-enhanced response to Mg ions by Ca ions was investigated in detail. Double-reciprocal plots of the experimental data indicate that Ca ions competitively inhibited the Mg response in the presence of 1 mM $NiCl_2$ and Ni ions at 1 mM did not affect the affinity of the magnesium receptor for the respective cations. The results indicate that Ni ions did not affect the competition between Mg and Ca ions in responses generated by Mg ions. This implies that Ni ions secondarily affect the Mg response. It appears that a magnesium receptor interacts with a Ni-binding element that is affected by Ni ions and, thus, Ni ions can induce an enhancement of the Mg response.

16. Responses of single glossopharyngeal taste fibers in the aquatic toad, *Xenopus laevis*, to amino acids and basic taste stimuli

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We examined the evidence for taste fiber types of amino acids and basic taste stimuli. Impulse discharges were recorded from 27 individual glossopharyngeal taste neurons in the aquatic toad, *Xemopus laevis*.

Stimuli were 1 mM concentrations of seven amino acids, and 5 mM HCl, 0.1 mM quinine–HCl, 0.1 M $CaCl_2$ and 1 M galactose as basic taste stimuli. Responses were quantified as the number of impulses evoked at the first 5 s of response time. Spontaneous activity of 27 neurons was almost zero spikes/5 s (0.00 ± 0.00).

Hierarchical cluster analysis of 26 neurons for the basic stimuli identified one large and two small groups of cells. The largest group (n = 19) of neurons was stimulated most by L-proline and HCl. A small group comprised of six of the remaining seven neurons was stimulated most by Q–HCl. The remaining neuron was stimulated by $CaCl_2$.

The clusters identified from 27 neurons for the amino acids included a large group of 23 neurons, a small group of three neurons (most effective stimulus L-arginine) and a neuron responding evenly to all the amino acids respectively. The large group was separated into two subgroups of 17 (L-proline/L-tryptophan/L-tyrosine) neurons and 6 (most effective stimulus L-valine) neurons.

17. Multiple nucleotide receptor sites in the taste receptor cells of the fleshfly *Boettcherisca peregrina*

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Nucleotides applied to the labellar chemosensillum of the fleshfly, *Boettcherisca peregrina*, could induce impulse-discharge of the taste receptor cells. While 5'ADP evoked a large response from the sugar receptor cell (sugar response) , 5'GDP evoked a large response from the salt receptor cell (salt response). On the other hand, the salt response to 5'ADP and the sugar response to 5'GDP were comparatively small. This suggested that the nucleotide receptor site of the sugar receptor cell was different from that of the salt receptor cell. Hill plot analysis of dose–response data showed that the Hill coefficient value of 5'ADP was ~1 and that of 5'GDP was ~2. The nucleotide receptor site of the sugar receptor cell was specific to adenosine nucleotides (5'ADP, 5'AMP, 5'ATP). Ranking of potency was: 5'ADP > 5'AMP ≥ 5'ATP. Not only 5'GDP, but 5'NMPs and cNMPs also evoked the salt response. While the potency ranking of 5'NMPs was 5'GMP > 5'IMP > 5'AMP, the dose–response curves of cGMP, cIMP and cAMP were almost the same.

18. IP_3 mediates the taste response of the fleshfly

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We tested the effects of IP_3, neomycin (an inhibitor of IP_3-transduction cascade) and ruthenium red (a blocker of the IP_3-gated channel) on the labellar taste receptor cell of the fleshfly to investigate whether IP_3 mediates the taste response. To introduce IP_3 into the receptor cells, the tip of a chemosensory hair was treated with IP_3 in 0.03% deoxycholate. Spikes of sugar receptor cell were elicited during treatment with IP_3 + deoxycholate, which were not observed during treatment with deoxycholate alone. After the treatment, the responses of the sugar receptor cell to D-glucose, D-fructose, L-Val and L-Phe were enhanced compared with those before the treatment. After treatment with neomycin in 0.03% deoxycholate, the responses of the sugar receptor cell to the same sugars and amino acids were depressed compared with those after treatment with deoxycholate alone. When mixed with ruthenium red, the responses to the same sugars and amino acids were inhibited. Neomycin and ruthenium red were especially effective on the response to D-glucose. On the other hands, similar effects of IP_3, neomycin and ruthenium red were not observed in the responses of the salt receptor cell to cAMP and NaCl. These results suggested that the response of sugar receptor cell was mediated by IP_3, while the response of salt receptor cell was transduced by some other mechanisms.

19. Ligand-binding activity and intramolecular disulfide bonds of the CRLBP

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Previously, we found a lipophilic ligand-binding protein which commonly functions both in the olfactory and the taste organs of the blowfly, *Phormia regina*. From the amino acid sequence, this protein was concluded to be a novel member of the insect pheromone-binding protein superfamily, but has at most 40% similarity to other members. However, it is remarkable that six cysteine residues are completely conserved in whole members of the superfamily. These cysteine residues contribute to construct a functional conformation of the proteins of this superfamily. In fact, the purified protein in its native form could disperse a lipophilic substance, limonene, into the aquatic solution. When it was treated with β-mercaptoethanol, however, limonene would not dissolve into the aquatic solution containing this deduced protein. To date, we have determined two loci of disulfide bounds in the protein of *Phormia* by mass spectrometry.

20. NORPA is involved in *Drosophila* tarsus sugar sensitivity

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NORPA encodes a phospholipase C which is essential for phototransduction in compound eyes and is also involved in olfactory transduction of the maxillary palps of *Drosophila melanogaster*. We compared the proboscis extension reflex by tarsus gustatory stimulation with various sugar solutions between $norpA^{P24}$ one of the strong *norpA* mutant alleles, and normal flies. It was found that the threshold concentrations to glucose and fructose in the *norpA* mutant were significantly higher than those of the wild-type flies. The genetic and cytological mapping located the low sensitivity on the *norpA* gene locus. Double mutants of *norpA* and the two alleles of *Tre*, a taste gene for the sensitivity to trehalose, were then made to determine whether there was any interaction between the two taste transduction genes. Both double mutants showed remarkably lower sensitivity to trehalose in the reflex response as compared with the corresponding single *Tre* and *Tre* + hemi/homozygotes, suggesting that the two taste genes are involved in different molecular events of the transduction cascades that do not interact. Overall, single and double *norpA* mutants, however, still showed considerable sensitivity to all three sugars. It was concluded that NORPA is involved, but not exclusively involved, in activating the transduction mechanism by tarsus sugar stimulation.

21. Effects of stimulant mixture in the labellar sugar receptor of the fleshfly

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The taste receptor current of the fleshfly, *Boettcherisca peregina*, for the mixture of sucrose and fructose was examined by analyzing impulse frequency and current fluctuation.

For the mixture of high concentration stimulants, the impulse frequency and the variance of the receptor current were reduced markedly to the same extent compared to their sum of independent stimulations (i.e. expectations from independent receptor–channel complex model). For the mixture of medium -concentration stimulants the impulse frequency was not reduced but the variance was reduced significantly. For the mixture of low-concentration stimulants, the impulse frequency was increased synergetically and the variance was not reduced. Synergistic responses at low concentrations show the existence of complex interactions between the P receptor site and the F receptor site, and these interactions cause the different effects of mixtures on impulse frequency and fluctuation variance. At high concentrations, however, the interaction seems to be a simple inhibitory effect and might be accounted for by the channel inactivation hypothesis.

22. Receptor current noise analysis of the salt receptor cell of the fly, *Boettcherisca peregrina*

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To elucidate the mechanism of the salt reception of insects, receptor current noise of salt receptor cell of the fly, *Boettcherisca peregrina*, was analyzed. We used NaCl as stimulant and choline chloride as a control. To inhibit impulses evoked from water and salt receptor cells, the chemosensory hair was treated with 0.1 mM TTX. Receptor current fluctuation was recorded, A/D converted, and fed into a personal computer. When chemosensory hair was stimulated with 1 M NaCl, current fluctuation was significantly increased compared to the control. The data were analyzed by calculating their autocorrelation functions. The autocorrelation function curves decayed exponentially with a time constant of ~2–5 ms, which indicated the possibility that the fluctuation of the receptor current might be caused by the open and shut mechanisms of the salt-activated channels. The noise analysis of the receptor current fluctuation may be a useful tool to elucidate the salt reception of the fly. The dependence of the time constant and the magnitude of the fluctuation on the concentration and species of cations remain to be elucidated.

23. Detection of substances liberated from the lingual epithelium by gustatory stimulation

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We developed a device for automatically sampling substances liberated from the isolated lingual epithelium containing taste buds during repetitive taste stimulation. The device consists of three parts: (i) a chamber for isolating the mucosal side from the basolateral side of the lingual epithelium; (ii) a programming and driving apparatus for repeated stimulation of the mucosal side with taste stimuli and separate perfusion of the basolateral side with normal Tyrode solution (the number and duration of stimulation are programmable); and (iii) a sampling apparatus for transporting the solution containing substances liberated from the epithelium into the sample cups, which are cooled down to –20°C. Using this device, we detected two peaks of peptide-like substances by HPLC in a sample liberated from rat lingual epithelium during stimulation with 0.5 M NaCl or sucrose. The two peaks detected in the sample in rat are roughly consistent with the peaks for Met-enkephalin and CCK8 respectively. Two peaks of peptide-like substances were also detected in the sample in gerbil, but these peaks were not consistent with those from rat or any of three known peptides. The results suggest there is a species difference in the transmitters released from taste cells.

24. Measurement of cytosolic Ca^{2+} concentration change in gerbil taste bud cells induced by gustatory stimulation of the restricted receptive membrane alone

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To elucidate the dynamics of cytosolic Ca^{2+} in taste buds in physiological response to taste stimuli, we used Fura-2 imaging of the lingual epithelial sheets containing some fungiform papillae. We used gerbils, which are more sensitive to sweeteners than other animals. By using the lingual epithelial sheets, which enabled us to separately stimulate the apical receptive membrane and the basolateral membrane, we observed tastant-induced changes in the cytosolic Ca^{2+} concentration in taste bud cells under the normal physiological conditions. Sucrose, saccharin and NaCl increased the cytosolic Ca^{2+} concentration in 51.9, 33.3 and 30.0% of the taste buds tested respectively. Ca^{2+} responses to sucrose, saccharin and NaCl differed in differential parts of single taste buds as well as in the taste buds of different fungiform papillae.

These results suggest that in gerbils taste transduction mechanisms of sucrose, saccharin and NaCl may be carried out in differential cells in a taste bud or the taste buds of different fungiform papillae.

25. Quench flow studies for understanding bitter taste signal transduction

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To understand taste sensation, it is necessary to study the molecular mechanisms of signal transduction. Bitter taste is elicited by a variety of compounds with diverse chemical structures. Several recent studies have shown the involvement of the inositol triphosphate pathway in signal transduction of bitter compounds such as sucrose octaacetate and denatonium. The involvement of a gustducin-activated cyclic nucleotide phosphodiesterase in bitter taste transduction has also been proposed. The current investigation was undertaken to understand the signaling mechanisms of other bitter compounds, alone or in mixtures.

We have investigated the kinetics of formation of second messengers, inositol-1,4,5-trisphosphate (IP_3). A quench flow system was utilized for the measurement of the rapid kinetics. Using taste tissue from C57BL/6j mice, generation of IP_3 was monitored over the first 500 ms. The bitter stimulants used were: quinine sulfate (1 mM), naringin (1 mM), caffeine (25 mM), or a combination of caffeine (25 mM) and denatonium (2 mM).

All bitter compounds alone or in combination elicited production of IP_3, while non-gustatory tissue did not respond to any of the bitter compounds. Quinine elicited an IP_3 peak between 100 and 150 ms when tested in C57BL strain, while naringin stimulated peak IP_3 production at 50 ms in the same strain. A different chemical pathway would be existed in these two bitter compounds. These data argue for specific mechanisms that, at least in part, utilize the IP_3 pathway.

26. Electrophysiological responses of mouse taste cells to three bitter stimuli

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Several hypotheses have been offered for the mechanism of bitter transduction, e.g. increase of IP_3, decrease of cNMP followed by activated phosphodiesterase via G-protein (gustdusin), blocking of K^+ channels. The responses of taste cells to the three different kinds of bitter stimuli—quinine, denatonium and naringin—were recorded using whole-cell patch-clamp techniques. Since many bitter substances block K^+ channels, we substituted Cs^+ for K^+ in the pipette solution to eliminate this effect. Stimuli were applied to the cells by pressure ejection from a pipette. Quinine (10 mM) produced a rapid depolarization and increase in membrane

conductance in the taste cells. Denatonium (500 μM, but not 100 μM) reversibly inhibited both voltage-dependent inward and outward currents. Naringin, a food additive, had no affect on the cell at a concentration of 1 mM. Although the transduction mechanism for bitter is still unclear, these results suggest that several transduction mechanisms may be involved, each mechanism depending on the type of bitter stimulant.

27. Patch-clamp recording of glutamate responses in mouse taste cells

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Transduction mechanisms for umami are believed to involve membrane-bound receptors on taste cells. However, the mechanisms of transduction for umami are still unknown. In mammals, umami taste is probably transduced by means of several receptor types that may be related to glutamate receptors of the vertebrate central nervous system. To elucidate the taste receptor mechanisms for umami, we examined the responses of isolated mouse taste cells to monosodium glutamate (MSG) or a potent agonist of metabotropic glutamate receptors 4 (mGluR4), L-AP4 (2-amino-4-phosphonobutyrate), by the patch-clamp technique. Data were obtained from taste cells isolated from female mice (8–9 weeks of age) of the C3H/HeJ and C57BL/6J strains, which are highly sensitive to MSG. In the whole-cell voltage-clamp configuration (holding potential –80 mV), bath application of MSG (10 mM) elicited both inward currents with an increase in membrane conductance and outward currents with a decrease in membrane conductance. L-AP4 (2 mM) stimulation elicited only outward currents with a decrease in membrane conductance at a holding potential –80 mV. These results suggest that MSG acts both at an L-AP4-sensitive receptor (mGluR4), that mediates a membrane conductance decrease, and at another receptor type that produces a membrane conductance increase. These results suggest that at least two mechanisms, including a metabotropic receptor, are involved in the transduction of umami taste.

28. Change in membrane currents of mouse taste bud cells induced by cyclic nucleotide

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Recently many studies have strongly suggested that cyclic adenosine 3′,5′-monophosphate (cAMP) plays a major role in taste transduction mechanisms for sweeteners such as sugar and saccharin. A raise in intracellular cAMP activates cAMP-dependent protein kinase, which results in closure of the K^+ channel by phosphorylation and depolarization of taste cells. More recently, cyclic-nucleotide-suppressible conductance was demonstrated in frog taste cells. In the present study, therefore, to understand the action of cyclic nucleotide in mammalian taste cells, the conductance change in mouse taste bud cells induced by cAMP was investigated using whole-cell patch-clamp recording.

The taste bud cells were isolated from vallate and foliate papillae by treatments with collagenase and EDTA. The taste cells were identified by their spindle-like shape and long cell process. The change in whole-cell currents induced by membrane-permeable cAMP analogues such as 8-bromo-cAMP (8-br-cAMP) and dibutyryl cAMP (db-cAMP) were measured using a perforated patch-clamp technique and a CsCl patch pipette. Bath application of 125–500 μM 8-br-cAMP and 250 μM db-cAMP significantly suppressed the voltage-dependent K^+ currents in some perforated clamped taste cells. When K^+ currents were blocked by CsCl pipette solution, a remarkable conductance increase at negative membrane potential was induced by 8-br-cAMP and db-cAMP in some taste cells.

The results indicate that an increase in intracellular cAMP induced an increase in taste cell conductance. More potent enhancement of inward currents suggests that cation conductance may be increased by cAMP, showing the diverse actions of cAMP in taste cells.

29. Changes in cAMP and IP_3 mass levels of the fungiform papilla in response to saccharin in mice

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Recent findings suggest that the transduction pathway involves cyclic AMP (cAMP) for stimulation with sugars and inositol-1,4,5-triphosphate (IP_3) for stimulation with non-sugar sweeteners as well as bitter substances. We measured mass levels of both cAMP and IP_3 in the fungiform papilla in response to 0.3, 3 or 20 mM saccharin in C57BL mice. Within ~10 s after the onset of taste stimulation, each fungiform papilla was removed with fine forceps, and frozen under liquid nitrogen. This procedure was repeated to pool ~120 fungiform papillae from four mice. Non-sensory epithelial tissue was prepared identically and used as a control. Mass levels of both cAMP and IP_3 in each tissue pool were measured by a radiobinding assay system using commercially available kits. Stimulation with saccharin produced significant increases in cAMP levels of the fungiform papilla at 3 and 20 mM, while it produced significant increases in IP_3 levels of the tissue only at 20 mM. No increase in both cAMP and IP_3 levels was observed in the non-sensory epithelial tissue stimulated with 0.3–20 mM saccharin. The results suggest that cAMP is involved in the taste transduction for saccharin from low to high concentrations (3–20 mM), whereas IP_3 is involved in it only at high concentrations (20 mM).

30. Selective inhibition of bitter taste by acidic phospholipids

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The development of a masking additive for bitter taste is widely required in the pharmaceutical and food industries. However,

several techniques for masking bitterness are not always applicable and are insufficient to mask the bitterness of many drugs and foods. In previous studies, we found that a lipoprotein composed of phosphatidic acid (PA) and protein selectively inhibits taste responses to various bitter stimuli without affecting those to salts, acids and sugars in frog and humans. It was suggested that PA is a key material for the inhibition of bitterness because PA-containing lipoproteins showed inhibitory action irrespective of species of proteins. In the present study, we examined the inhibitory action on bitterness of PA itself without protein and other phospholipids in humans. The results showed that PA and phosphatidylinositol (PI) inhibited the bitterness of various bitter substances without affecting the taste sensation to other taste stimuli. Allowing for manufacturing on an industrial scale, we prepared a fraction containing PA and PI in high content from soybean lecithin with organic solvents. We found that the fraction also inhibited bitterness of many bitter substances. Since the fraction can be easily manufactured and is much cheaper than PA or PI alone, it is expected to be applicable to many drugs and foods as a masking additive for bitter taste.

31. Characteristics of the gustatory neural responses in the senescence-accelerated mouse

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It is generally accepted that sensory function in mammals substantially declines with age. Such changes should be reflected in the electrophysiological responses from taste cells and the behavioral responses from animals. It is well established that the SAMF-1 /TA/Aud strain with accelerated senescence and age-related pathologies appears to be the sole animal model available for research on ageing. The mean lifespan of this strain of mouse is 1 year and senescence begins 20–30 weeks after birth. In the present study, this animal model using taste sensitivity was studied by analysis of integrated responses of the chorda tympani nerve and two-bottle preference behavioral tests to various taste stimuli [sucrose, NaCl, HCl and quinine–HCl (QHCl)]. We compared results obtained from comparable age groups (10, 25 and 50 weeks after birth). The results were as follows: (i) electrophysiological results indicated that the sensitivity of NaCl is decreased in 50 week animals, but that of the sucrose, HCl and QHCl are not changed; (ii) two-bottle preference test results indicated that the sensitivity of the NaCl , HCl and QHCl decreased remarkably at 50 weeks but that of the sucrose is not changed.

32. The gustatory effects of eight alcohols in rats as revealed by electrophysiological and behavioral studies

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To study the gustatory effects of eight alcohols—methanol, ethanol, ethylene glycol, 1-propanol, 2-propanol, propylene glycol, 1,3-propandiol and glycerin (all 1 M)—electrophysiological and behavioral studies were designed. In the electrophysiological study, neural responses to each alcohol were recorded from the chorda tympani and glossopharyngeal nerves of Wistar rats. The responses to alcohols with two or three hydroxyl groups were larger than those to the other alcohols in both nerves. The responses patterns of 93 single chorda timpani fibers were similar to those of sucrose and quinine–HCl. In the behavioral study, the rats which acquired conditioned taste aversions to alcohols with two or three hydroxyl groups also avoided 0.5 M sucrose and 10 mM quinine–HCl. However, the aversion was not generalized to NaCl and HCl. These results suggest that alcohols exert their gustatory effects by binding to a receptor with binding sites for more than two alcoholic hydroxyl groups, and that alcohols have a taste similar to the taste of both sucrose and quinine.

33. Laryngeal taste responses to beer and soda water in the rabbit

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Laryngeal afferent signals are important to produce the satiety of thirst. However, to our knowledge, no report is available about taste responses of the laryngeal nerve to beer and soda water, although it is well known that the laryngeal nerve responds to water. This work was therefore carried out to examine the responsiveness of the superior laryngeal nerve (SLN) to these liquids. Afferent neural activity was recorded from the SLN of urethane-anesthetized rabbits. The test solutions were applied to the laryngeal surface of the epiglottis. Fresh beer elicited a marked response greater than the response to water. Soda water produced marked transient discharges. The beer- and soda water-induced responses were abruptly terminated by rinsing the larynx with saline, but not with water. The water-induced response was eliminated in the same way with saline. It seems likely that the excitatory mechanism of the water response is similar to that for beer-induced response.

34. Effects of sour taste on chorda tympani nerve responses to sweet, salt and bitter tastes in rats

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To clarify the interaction between sour taste and the other basic tastes, we have examined the effects of HCl on the chorda tympani nerve responses to sucrose, NaCl and quinine–HCl in rats. In the presence of 2 mM HCl, a concentration that exerts a only modest effect on the nerve activity, the response to sucrose was synergistically enhanced. The enhancement was observed not only for high concentrations of sucrose (0.5–1 M) but for concentrations as low as 0.05 M, indicating that HCl decreases the threshold of sucrose reception. In contrast, co-existence of HCl during the stimulation with NaCl or quinine–HCl onto the tongue suppressed the responses of the chorda tympani nerve compared with the effects of these stimuli alone. The responses to glucose and fructose were also increased synergistically by HCl as to sucrose. Interestingly, HCl did not show the synergistic effect on the response of chorda tympani nerve to glycine, a non-sugar sweet tastant. These results indicate that sour taste enhances the sweet taste evoked by sugars, but not by amino acids, and suppresses salt and bitter tastes.

35. Different sensitivity to the sweet taste inhibitor gurmarin between the chorda tympani and the glossopharyngeal nerve in C57BL/KsJ mice

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The effects of the sweet taste inhibitor gurmarin on taste responses of the glossopharyngeal nerve were compared with those of the chorda tympani nerve in C57BL/KsJ mice. The lingual application of gurmarin suppressed the responses to 0.5 M sucrose and other six sweeteners examined in the chorda tympani. Significant inhibition was achieved at >3 μg/ml of gurmarin, maximum inhibition being ~50% of the control, which was comparable with our previous observation in C57BL/6 mice. On the contrary, gurmarin did not show any significant change on the sweet taste responses to various sweeteners in the glossopharyngeal nerve even at 100 μg/ml. On the other hand, a proteolytic enzyme, Pronase, suppressed the response to sucrose to <20% of control in both nerves. These results suggest that the gurmarin-sensitive receptor is expressed predominantly in the taste cells innervated by the chorda tympani nerve, and little in those innervated by the glossopharyngeal nerve.

36. Effects of isoproterenol pretreatment on intakes of capsaicin-containing diet in rats

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The previous study demonstrated that capsaicin (a pungent principle component of hot pepper) contained in diet induces salivary components in the rat and this induction disappears after glossopharyngeal nerve denervation. The induced components are similar to those of saliva of isoproterenol (β-agonist, IPR)-treated animals. We therefore exainined the effects of IPR pretreatment on intakes of capsaicin-containing diet in the rat. Male Wistar rats weighing 300–350 g had a daily injection of IPR (1, 5, 10 and 20 mg/body wt) for 5 days and were fed a diet containing 0.05% capsaicin. The submandibular glands of some animals were removed under pentobarbital anesthesia prior to experiments. Submandibular saliva samples were analyzed by porous silica gel column chromatography. The production of cystatin fraction was recognized depending on dosage in IPR-pretreated groups but not in the control group. There was not difference in intakes of CAP-diet among three IPR (1–10 mg)-pretreated groups and control group until the 7 days after feeding. However, the 20 mg IPR-treated group showed significant increases compared with the control group after 5 and 7 days. Body weight loss followed decreases in food intakes in all groups without the 20 mg IPR-treated group, the weight of which transiently decreased and returned to a level at the beginning of feeding 2–4 days later. Removal of the submandibular gland decreased intakes of ordinary chow to 20% of preoperation levels after 1 day. The intakes returned to preoperation levels by 4 days after the operation. The CAP diet were not at all took in by the sialo-adenectomized group for at least 7 days after operation. These results suggest that chemosensony information conveyed by the glossopharyngeal nerve indirectly stimulates β-adrenergic receptors of the submandibular gland to induce several salivar and components (cystatins and so on). The components of submandibular saliva may supress burning sensation and irritation to the oral mucosa at time of intake of a spiced diet.

37. Depressing activity to *Hydra* R4 response which increases after food intake in body fluids

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We have reported that depressing activity to *Hydra* R4 response increases after food intake in the cerebrospinal fluid of the rat. This activity is considered to be closely related to acidic fibroblast growth factor. We have found another R4 depressing activity in extract from the adrenal medulla, and by purifying this activity, we found this was amphoterin from its N-terminal amino acid sequence. We raised an antibody to amphoterin in a rabbit. The

antibody detected amphoterin in the extract from adrenal medulla as a single band in the protein blotting analysis, showing its specificity. A similar activity also increases in the human serum after food intake. In contrast to the activity in the cerebrospinal fluid, the activity in serum was not diminished by anti-aFGF-IgG, while this activity was diminished by anti-amphoterin IgG. With use of the antibody, the amount of amphoterin-like activity in human serum was estimated to be not detected before food intake, increased to 0.95 ng/ml 1 h, and decreased to 0.05 ng/ml 3 h after food intake (breakfast). Immunohistochemical localization was also examined with this antibody. Amphoterin immunoreactivity was found in neurons in the paraventricle and supraoptic nucleus of the rat brain, as well as in the cells of the adrenal medulla.

38. Isolation of olfactory receptor neurons from the rainbow trout and their chemical stimulation with the pressure ejection system

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Techniques of cell isolation to obtain morphologically different olfactory receptor neurons, ciliated and microvillous neurons, and their chemical stimulation, which were suitable for patch-clamp and single-cell polymerase chain reaction studies, were developed in the rainbow trout *Oncorhynchus mykiss*. Finely minced individual olfactory lamellae were treated with two different proteolytic enzymes in different conditions, and then olfactory receptor neurons were dissociated mechanically by pippetting with a thin-tipped (<0.3 mm opening) Pasteur pipette. After 68 trials, two different treatment conditions were found suitable for the purpose: (i) Papain, Worthington 3126, 56Uu/ml; DNase I, Sigma D-4263, 100 U/ml; L-cysteine, 1 mM; EDTA-4Na, 0.5 mM; 20 mg/ml bovine serum albumin, Sigma A8022: 37°C, 40 min; (ii) collagenase/dispase, Boehringer Mannheim 269638, 1 mg/ml; DNase I, Sigma D-4263, 100 U/ml; 20 mg/ml bovine serum albumin, Sigma A8022: 37°C, 20 min. Five-barrel micropipettes, which were fabricated from WPI P-5-50 (tip openings ~2 μm diam.), filled with five different chemical solutions, and connected to a home-built pressure control system,were successfully utilized to give a successive application of five different chemical stimuli to a patched olfactory neuron in the perfusing chamber.

39. Specific reception of odorants at high concentrations in the turtle olfactory system: concentration dependence of odor discrimination

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The ability of the turtle olfactory system to discriminate between various odorants of different concentrations at the olfactory bulb was examined by the cross-adaptation technique. The concentration dependence of the odor-discriminating ability varied among the odorant pairs examined. The degrees of discrimination between odorants having different molecular structure and odor quality were unchanged or decreased only slightly with an increase in odorant concentration, suggesting that odorants are received specifically even at high concentration in the turtle olfactory systems. Discrimination between odorants having similar molecular structure and odor quality was decreased with an increase in concentration of odorants. Odorants increased membrane fluidity monitored with a fluorescence dye (1,6-diphenyl-1,3,5-hexatriene) of liposomes made of lipids extracted from the epithelia in a dose-dependent manner. There was a good correlation between the degree of discrimination and the membrane fluidity change induced by odorants, suggesting that an increase in membrane fluidity of the lipid layers of olfactory receptor membranes affects the odor discrimination between similar odorants.

40. Effects of cGMP and NO on odor response in turtle olfactory receptor cells

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In isolated turtle olfactory receptor cells, we have examined the effects of cGMP and NO on odor response using whole-cell voltage-clamp recordings. Dialysis of cGMP into the cell induced inward currents in a dose-dependent manner. After the response to 1 mM cGMP had been desensitized, 3 mM cpt-cAMP, a membrane-permeable cAMP analogue, did not elicit a response, indicating that both cAMP and cGMP activated the same channels. Extracellular application of sodium nitroprusside (SNP), an NO donor, evoked inward currents in a dose-dependent manner. Application of SNP did not induce responses after desensitization of the cGMP-induced currents, suggesting that NO-induced responses are mediated via the cGMP- (cAMP-) dependent pathway. Application of the cAMP-increasing odorant mixture to the cell induced a large inward current after desensitization of the cGMP-induced or SNP-induced responses. These results suggest that a cAMP-, cGMP- and NO-independent pathway greatly contributes to the generation of odor responses.

41. Calcium-activated conductances in rat olfactory neurons

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Olfactory neurons have a few kinds of conductance activated by Ca^{2+}. In the present experiment, we studied the effect of the Ca^{2+}-ionophore ionomycin on the membrane properties of

olfactory neurons isolated from rat using conventional and perforated whole-cell patch-clamp techniques. In conventional whole-cell clamp methods with internal 137 mM Cl^-, ionomycin (3 μM) induced three kinds of response in rat olfactory neurons: inward current (4/11 cells), outward–inward current (3/11 cells) and outward current (4/11 cells). The early component of ionomycin-induced inward current was almost eliminated by decrease of intracellular Cl^- concentration from 137 to 10 mM, but outward current and outward–inward current were still observed. In gramicidin (100 μg/ml)-perforated whole-cell clamp under which intracellular Cl^- level could be maintained in natural conditions, ionomycin elicited inward current (3/8 cells). Intracellular dialysis of 1 mM Ca^{2+} with 10 mM Cl^- induced a sustained outward current which was composed of NPPB-sensitive and Ba^{2+}-sensitive components (7/11 cells). The data suggest that intracellular Ca^{2+} can activate Cl^-, K^+ and cationic conductances in rat olfactory neurons.

42. Subcellular localization of glycoconjugates with terminal α-D-galactose sugar residues in the vomeronasal sensory epithelium of rats

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To extend our previous observations (Takami *et al.*, *Cell Tissue Res.*, **277**, 211–230, 1994; **280**, 211–216, 1995), the subcellular localization of terminal α-D-galactose (α-Gal) containing glycoconjugates was examined cytochemically. Adult Sprague–Dawley rats were transcardially perfused with 2.5% glutaraldehyde in phosphate buffer and dissected vomeronasal organs were embedded in Lowicryl K4M. Ultrathin sections were cytochemically labeled using biotinylated *Griffonia Bandeirae simplicifolia* I-B_4 isolectin (BS-I-B_4) and streptavidin–10 nm colloidal gold. Colloidal gold particles were abundant in microvilli of vomeronasal receptor neurons, as well as within the cytoplasm of their dendritic terminals. In contrast, only a few gold particles were observed in microvilli and cytoplasm of sustentacular cells. Quantitative analyses demonstrated that the density of gold particles in the sensory microvilli (mean: 3.4/1 μm, $n = 200$) was 9 times greater than that in microvilli of sustentacular cells ($P < 0.0001$, Mann–Whitney *U*-test). Furthermore, the density of gold particles in the proximal portion of the sensory microvilli was significantly greater than that in their distal portion ($P < 0.001$). The present results suggest that α-Gal-containing glycoconjugates that were present in the sensory microvilli play an important role in vomeronasal sensory processes.

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43. Voltage-dependent and putative second messenger-dependent currents in rat vomeronasal receptor neurons

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Using the whole-cell mode of the patch-clamp technique, we recorded putative second messenger-activated currents in receptor neurons in the vomeronasal sensory epithelium of female rats. The resting membrane potential and input resistance were –45.5 ± 2.5 mV (mean ± SE, $n = 39$) and 1.5 ± 0.2 GΩ (mean ± SE, $n = 37$). Current injection of 1–3 pA induced overshooting action potentials, indicating that rat vomeronasal receptor neurons sensitively generate action potentials in response to a small receptor potential. Under voltage clamp, voltage-dependent Na^+ inward current, inward Ca^{2+} current, sustained outward K^+ current and Ca^{2+}-activated K^+-current were identified. Dialysis of D-inositol-1,4,5-trisphosphate (IP_3) induced inward currents with an increase in membrane conductance in ~54% of the cells and inward current fluctuations in 15% of the cells. L-IP_3 also induced inward currents and current fluctuations. The IP_3-induced responses were blocked by elimination of Na^+ and Ca^{2+} in the external solution or application of 10 μM ruthenium red. The present study suggests that an IP_3-mediated transduction pathway exists in rat vomeronasal sensory neurons.

44. Existence of multiple receptors in single bullfrog olfactory neurons

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The responses of single bullfrog olfactory neurons to 10 μM hedione, citralva and lyral were measured with the whole-cell patch-clamp technique. Fifty-six per cent of neurons responded to all three odorants. Sixty-seven per cent of neurons responded both to cAMP-dependent odorants (hedione and citralva) and to a cAMP-independent odorant (lyral). A cross-adaptation experiment was undertaken to examine whether different receptors exist in single olfactory neurons. Application of hedione to a single neuron after desensitization of the current in response to lyral or citralva induced an inward current and vice versa. Pre-application of an odorant did not practically inhibit the generation of responses to other odorants applied subsequently. The ratios of the number of neurons which responded to hedione (58%) were not decreased by pre-application of lyral (57%) or citralva (52%). Magnitudes of inward currents to odorants were not significantly suppressed by other odorants applied previously. Mean magnitudes of inward currents induced by 10 μM hedione after Ringer's solution and 10 μM citralva were 26 ± 12 and 18 ± 10 pA respectively. It was suggested that most single olfactory neurons carry multiple receptors and at least dual transduction pathways.

45. Measurement of Cl^- concentration in the olfactory cell with Cl^--sensitive fluorescent dye

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We have tried to measure directly $[Cl^-]_i$ in vertebrate olfactory cells with the fluorescent Cl^- probe MQAE. At the last JASTS meeting, we reported that the resting $[Cl^-]_i$ appeared to be ~100 mM. However, we had a problem in that the fluorescence from the cell loaded with MQAE continuously decreased during measurement. This decrease interfered with the determination of $[Cl^-]_i$. Hence, we checked the mode of the decrease by intermittent monitoring of the fluorescence. We repeated the 5 s irradiation with the excitation light followed by 10 min dark incubation of the cell. Up to 50 or 60 min, the fluorescence intensity stayed almost constant, which suggests that the leakage of the dye is negligible in 1 h and that the decrease was mainly due to the dye being bleached by the excitation light.

We applied this intermittent monitoring to measuring the resting $[Cl^-]_i$. We repeated the 5 s irradiation followed by 3 min darkness. The normal Ringer at the initial step was changed during the dark period to $[Cl^-]$ standard solutions including Cl^- ionophores. This procedure greatly improved the reliability of the measurement and revealed that the averaged concentration across the cell from the body to the knob was ~20 mM. If Cl^- flows out, as suggested before, there may be the accumulation of Cl^- in the cilia.

46. Adenyl cyclase activity in turtle and rat vomeronasal epithelium

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Many vertebrates have two olfactory systems such as the main olfactory organ and the vomeronasal organ. In a previous study, we showed that dialysis of cAMP and cGMP into the turtle vomeronasal receptor neuron induced inward currents, suggesting that cAMP-mediated transduction pathways exist in the vomeronasal receptor neurons. In the present study, we measured adenylyl cyclase activity in vomeronasal and olfactory epithelia preparations of the turtle and rat. Forskolin and GTP induced cAMP accumulation in vomeronasal membrane preparations of the turtle and rat in a similar dose-dependent manner to that in olfactory membrane preparations of the turtle and rat. In the turtle vomeronasal organ, common odorants, which previously were reported to induce electrophysiological responses, did not induce cAMP accumulation in the vomeronasal preparations. The present results suggest that the cAMPmediated transduction pathway in the vomeronasal organ is not involved in transduction for common odorants and probably plays a role in perception of specific chemosignals.

47. Optical imaging study of GABAergic action on oscillatory signal propagation in the guinea-pig accessory olfactory bulb

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An optical imaging study, using a voltage-sensitive dye (RH 482), was undertaken to investigate the GABAergic action on signal propagation and oscillatory response evoked by electrical stimulation of the vomeronasal nerve layer (VNL) in the guinea-pig accessory olfactory bulb slice. An oscillatory response (several periodic increases and decreases in neural activity) to VNL shocks was evoked at the EPL/MCL and the granule cell layer (GCL). Application of a $GABA_A$ agonist, muscimol (20–50 μM), markedly reduced the oscillatory response in the EPL/MCL with a small decrease in the glomerular layer (GLL) response. A $GABA_A$ antagonist, bicuculline (40 μM), reversibly suppressed the periodic decreases in the EPL/MCL and the GCL, resulting in a large and long-lasting activity, while it also increased the GLL response to some extent. This suggests the involvement of $GABA_A$ action in the oscillatory response. A $GABA_B$ agonist, baclofen (2–50 μM), blocked the GLL response and further propagation disappeared. GABA (200–500 μM) induced a moderate decrease in neural activities in the GLL and EPL/MCL. These results suggest the presence of $GABA_A$ action mainly in the EPL/MCL and also in the GLL, and the presence of $GABA_B$ action in the GLL.

48. Ion dependence of carnosine-induced inward currents in organotypic cultured neurons in the rat olfactory bulb

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It was reported that carnosine exists in olfactory neurons and is released from synaptosomes of the olfactory bulb by depolarizing stimulation. In a previous study, we found that carnosine induced excitatory responses in organotypic cultured neurons in the rat olfactory bulb. These results suggested that carnosine released from olfactory neurons plays a role as an excitatory transmitter or a modulator in the olfactory bulb. In the present study, we studied the ion mechanism of carnosine-induced responses in organotypic slice cultured neurons in the rat olfactory bulb under the whole-cell voltage-clamp condition at −70 mV. Application of carnosine induced inward currents in a dose-dependent manner. The mean reversal potential of responses induced by 5 mM carnosine to the neuron was 14 ± 4 mV (± SE, n = 13). The response to 5 mM carnosine was completely inhibited by substitution of Na^+ in the stimulating solution with choline ion. Elimination of Ca^{2+} in the stimulating solution did not practically affect the response. These results suggest that Na^+ may be a main current carrier during the response induced by carnosine.

49. Relationship between thresholds for the proboscis extension reflex and blood sugar level in the blowfly, *Phormia*

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Some insects such as flies have contact chemosensilla on their legs (tarsi). When the sensilla come into contact with a sugar solution whose concentration is over a threshold, the proboscis extension reflex (PER) is brought about.

Dethier and his co-workers reported that the blowflies (*Phormia*) injected with sugars including trehalose, which is the blood sugar of the insects, did not show any difference in tarsal PER-threshold for sugar. However, we obtained different results from similar experiments on the blowfly. Injection of 500 μg trehalose with saline into the haemocoele of unfed flies was effective in elevating the tarsal PER-threshold. On the contrary, no effect was found when mannose was injected instead of trehalose.

The blood sugar levels in the fly haemocoele determined by the enzyme assay showed a coincidence with the PER-threshold value, suggesting a possibility that the blood sugar is a regulatory factor of PER in the fly.

We also obtained the PER-threshold values for both glucose and fructose on the single labellar chemosensilla. By reading the two crossing concentration–response curves for glucose and fructose respectively, we could determine the firing level of the neuron in the central nervous system. We may study, by this method, the effect of chemicals injected onto the firing level of the neuron in the brain.

50. Taste responses of parabrachial nucleus neurons in chorda tympani-sectioned rats

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Responses of single parabrachial nucleus (PBN) neurons (n = 38) to the four basic tastes and distilled water applied to the posterior oral cavity (PO) were extracellularly recorded in chorda tympani-sectioned rats (CTs). The responses were compared with those (n = 45) of intact rats (CTi). The response magnitudes evoked with the five stimuli used as well as spontaneous activity of PBN neurons in CTs were lower than those of CTi, suggesting that afferent signals from the CT nerve increase spontaneous activity and taste responsiveness of PBN neurons. The most effective stimuli, 0.5 M sodium chloride and 0.03 M hydrochloric acid, in evoking taste responses in CTs were the same as those of CTi. Responses of PBN neurons to these taste stimuli were highly correlated each other in both CTi and CTs. The response characteristics of PBN neurons observed in the present study were very similar to those of the glossopharyngeal (GP) nerve. Although the PO is innervated not only by the GP but by the superficial petrosal and superior laryngeal nerves, the present results suggest that afferent signals from the GP nerve are mainly responsible for responses of PBN neurons to taste stimuli applied to the PO.

51. Neural mechanisms of salivation induced by rejectable taste in rats: importance of the parabrachial nucleus

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Rats execute taste rejection behavior that consists of, for example, gaping, chin rubbing, face washing and forelimb flailing in response to an orally injected bitter substance (quinine–hydrochloride). We have recently found that vigorous salivation is evoked during the taste rejection behavior. This study sought to evaluate the role of the parabrachial nucleus (PBN) on such salivation. Through behavioral, histochemical and electrophysiological studies, the following results were obtained: (i) vigorous salivation was present during the rejection behavior in rats decerebrate at the precollicular level; (ii) neurons in the PBN and the reticular formation ventral to the PBN were labelled by Fluoro-Gold injection into the superior salivatory nucleus (SSN), but the neurons in the so-called taste area were sparsely labelled; (iii) ~20% of the taste-responsive neurons recorded from the decerebrate rats were activated antidromically by electrical stimulation of SSN; (iv) these neurons were scattered in the reticular formation ventral to the PBN; (v) the rest of 80% taste-responsive neurons were not activated antidromically by stimulation of SSN. These results suggest that the so-called taste neurons in PBN project to SSN via the reticular formation ventral to the PBN.

52. Responses of insular cortex neurons to gustatory stimulation of the pharyngolarynx in rats

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Extracellular unit responses to gustatory stimulation of the pharyngolaryngeal region were recorded from the insular cortex of anesthetized and paralyzed rats. The taste simuli were deionized water, 1 M NaCl, 30 mM HCl, 30 mM quinine–HCl and 1 M sucrose. Of the 29 neurons identified, 19 (65.5%) responded to at least one of the taste stimuli and 10 (34.5%) showed no response. Of the 19 taste-sensitive neurons, eight showed an excitatory response, four showed an inhibitory response, and the remaining seven showed both types of response. Four neurons responded specifically to one stimulus and 15 neurons were responsive to two or more stimuli. These taste-responsive neruorns were located in the insular cortex between 0.2 mm posterior and 1.7 mm anterior [mean = 1.0 mm anterior to the anterior commissure (AC), n = 19] to the anterior edge of the joining of the AC. These results indicate that gustatory afferents from the pharyngolaryngeal region project to the insular cortex, where general visceral afferents also project,

and suggest that gustatory information from the pharyngolarynx may contribute to autonomic integration in the insular cortex.

53. Effectiveness of searching stimulus for identification of cortical taste neurons

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In searching for taste neurons in the central nervous system, a solution containing four basic taste solutions (0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl and 0.02 M quinine–HCl) has been used. We have felt that this searching stimulus (SS) was quite effective in identification of taste neurons in the taste relay nuclei from the NTS to VPMpc, but not in the cortical taste area (OTA) in rats. Thus we studied, by means of regression analysis, with what responses the SS responses were correlated. The SS responses were well correlated, in every relay nucleus and OTA, with the response to the best stimulus among the four basic taste solutions as well as with the sum of the response to each of four taste solutions, though the regression coefficient was higher for the equation with the former variable. Failure ratio of identification of taste neurons on the basis of SS responses was lower than 15% in the relay nuclei, but higher than 40% in the OTA. Microiontophoretic application of bicuculline ($GABA_A$ receptor antagonists; 5–15 nA) to OTA neurons reduced it to ~25%. Thus, interaction between taste qualities may operate through GABAergic interneurons in the OTA, which probably increased the failure ratio of identificaton of taste neurons in the OTA on the basis of SS responses.

54. Disruptive effects of glutamate receptor antagonist injected into the amygdala in conditioned taste aversion in the rat

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To study whether conditioned taste aversions (CTAs) are dependent on glutaminergic neural transmission in the amygdala, we injected two different types of glutamate receptor antagonists into both sides of the amygdala (Amy) in Wistar rats: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) as an α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor antagonist, and D-2-amino-5-phoshonovalerate (APV) as an *N*-methyl-D-aspartate (NMDA) receptor antagonist. Soon after rats ingested 0.005 M sodium saccharin (conditioned stimulus, CS), they received CNQX or APV by intra-amygdala infusion. Thirty minutes later, they received an i.p. injection of LiCl (unconditioned stimulus, US; 0.15 M, 2% of body wt). The injections of CNQX significantly disrupted the acquisition of CTAs in comparison with the injections of the vehicle in control rats. In contrast, CNQX infusion into the vicinity of Amy was ineffective. CTA acquisition was attenuated, but not significantly blocked by the infusion of APV. These results suggest that glutaminergic neuronal transmission in the Amy participates in the brain mechanism of CTA acquisition.

55. Effects of lesions of the midbrain ventral tegmental area on taste-guided behaviors in rats

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It is well documented that the mesocorticolimbic dopamine system is primarily involved in hedonically positive reinforcement. However, little is known about the possible function of this system in hedonic responses to taste stimuli. In the present study, we examined the effects of electrolytic lesions of the midbrain ventral tegmental area (VTA), which is the origin of the meso-corticolimbic dopamine system, on three taste-guided behaviors in rats. (i) Twenty-four hour two-bottle preference tests revealed that the consumption of palatable taste solutions (0.1 and 1.0 M sucrose, and 0.1 M NaCl) in lesioned animals was significantly less than that of control animals. The consumption of unpalatable taste solutions (HCl and quinine–hydrochloride) was not different between lesioned and control animals. (ii) When body sodium was depleted by injections of a natriuretic drug, furosemide, lesioned animals consumed significantly less NaCl than control animals did. (iii) Although control animals reliably acquired a conditioned taste aversion following pairing of 0.3 M alanine with i.p. injection of LiCl, lesioned animals did not. Taken together, it is suggested that the VTA functions in normal expression of taste-guided behaviors, including hedonically positive responses to palatable taste stimuli.

56. Chemosensory evoked potential of four qualities of taste

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The evoked potential of four taste qualities (sweet, salty, sour and bitter) are measured in solution at various concentrations. The moment at which the liquid substance of the taste quality touches the surface of the tongue is instantly detected by an apparatus containing a laser. In the measurement, the individual threshold levels of concentration for four tastes are obtained. The resultant maximum evoked potentials do not depend statistically on the kinds of quality taste. However, the latencies at maximum evoked potential show a minimum value between 120 and 150 ms for sweet, salty and bitter tastes within the range of presented relative concentrations of taste quality, the values of which are calculated from the ratio of the presented concentration to the individual threshold level of concentration. The latency for sour taste shows a decreasing tendency as the presented relative concentration increases.

57. The laterality of the gustatory area of human cerebral cortex with measurment of evoked magnetic fields

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Little information is available about the neural gustatory path in humans. By analogy with monkeys, it is assumed to be projected ipsilaterally. It is assumed, however, to be projected contralaterally, from the clinical observation that gust-sensory damage appeared contralaterally. We therefore used an apparatus which stimulates a narrow part (4 × 10 mm), on a right or a left part of a tongue (~2 cm from the center), and investigated the neural projection of the gust-sensory system.

Magnetic fields from gustatory stimulation with 0.5 M NaCl were recorded from the human brain by using a whole-cortex SQUID system. Stimulus duration was ~800 ms and the inter-stimulus interval ~15 s. Three neurologically healthy volunteers (male) were used as subjects (two were right-handed, and one was left-handed).

We did not obtain clear evidence of the laterality on the neural projection of gust-sensory system. In the cases that a left or a right part of a tongue were stimulated, both hemispheres were activated. There were no remarkable differences of amplitude or onset latency of magnetic fields between both hemispheres.

58. Determinations of the center of olfactory neuromagnetic fields evoked by odorant stimuli

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We have measured olfactory evoked potentials and event-related potentials. However, as the electrical potentials were distorted by the differing resistance in various regions of the human brain, we could not obtain the more localized signal source precisely. In the present study, we detected the olfactory evoked magnetic fields and somatosensory fields from six healthy subjects using a 122-channel whole-head type SQUID gradiometer.

Odorant pulses were given synchronized with the subject's respiration using a non-magnetized olfactory stimulator. Amyl acetate (banana-like odor) was used as odorant stimuli for a duration of 300 ms. The stimuli were delivered into the right or left nostril at one session of the experiments at random every 3–12 respirations. The generators of olfactory magnetic fields were estimated at two regions located near bilateral frontal areas. In this experiment evoked potentials and event-related fields were often dominant on the ipsilateral side than the contralateral side at the lateral orbital sulcus in the deep frontal area, which was clearly different from the contra-laterality of the trigeminal area obtained by fresh air stimulation.

59. Neuronal responses of olfactory bulb and pyriform cortex to odors of mushroom flavors in rats

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The present study was conducted to clarify the response properties of olfactory bulb (OB) and pyriform cortex (PC) to stimulation of various kinds of odorants applied to the rat olfactory epithelium.

The volatile compounds of shiitake (*Lentinus edodes*) and matsutake (*Tricholoma matsutake*) mushrooms were used as compound flavors, and 10^{-2}% 1-octen-3-ol, 10^{-3}% methyl cinnamate (MC) and 10^{-3}% dimethyl disulfide (DMDS) were used as simple substances. Volatiles of shiitake and matsutake were extracted at 30–35°C by reduced steam distillation, and each volatile was diluted to contain 10^{-2}% 1-octen-3-ol. Extracellular responses of single units in the OB (71 units) and PC (41 units) to odors were recorded electrophysiologically, and these responses were calculated as the ratio of the number of spikes obtained 0–30 and 31–60 s after stimulation to the number obtained 30 s before stimulation. The responses to each odorant in the OB and PC were classified into three types: excitatory, inhibitory or no response.

In the OB, the distribution of each response type differed between compound substances (volatiles of shiitake and matsutake) and simple substances (1-octen-3-ol, DMDS and MC). In the PC, the no response type to each odorant was observed in 33–54% neurons (in the OB 63–73%), and the excitation type to compund substances was 8–10% in the OB and 38–41% in the PC.

These results suggest that PC neurons respond to compound substances more remarkably than OB ones.

60. Effects of olfactory stimulation on the electroencephalograph in chicken

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To characterize the olfactory sense in the chicken, we have recorded electroencephalographs (EEG) after olfactory stimulation with n-pentyl acetate, limonene, methyl antranilate, geraniol or ionone, without anesthetizing the animal. Among the odorants used, metyl antranilate and n-pentyl acetate induced clear dissynchronization, which was composed of increased alpha and theta waves and decreased beta wave. In contrast, the EEG was not influenced dramatically, when limonene or geraniol was adminislrated into the nasal cavity. Electroencephalographic changes in response to olfactory stimulation were classified into three patterns: (i) response starting immediately after the odor

stimulation; (ii) response starting with a time lag; and (iii) response starting after the odor stimulation. These response patterns, however, were not always observed clearly when the same odorant was applied. It is indicated that this heterogeneity of responses was derived from the animal condition or the application method of the odorants. Therefore, the results obtained show that the chicken olfactory system preferentially receives some specific odors, and EEG recording serves as a valuable tool for analysis of the olfactory system in the chicken.

61. Taste receptor proteins directly extracted by liposome from intact epithelium of bullfrog tongue 2

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Direct extraction of taste-receptor proteins from frog tongue epithelium by liposomes containing an artificial boundary lipid (DDPC, 1,2-dimyristoylamido-1,2-deoxyphosphatidylcholine) has been studied by utilizing a quartz crystal microbalance (QCM). Binding of proteoliposomes (liposomes containing membrane proteins of bullfrog tongue epithelium) onto the surface of the QCM coated with L-alanine was measured in order to check the presence of L-alanine receptor proteins in the proteoliposomes. Frequency change of the modified QCM due to the adsorption of DDPC/1,2-dimyristoyl-phosphatidylcholine (DMPC)-proteoliposome was obviously larger than DMPC-proteoliposomes as well as proteins extracted with pure water. Furthermore, binding rate constants (k_1) of above three samples for QCM coated with L-alanine were calculated and the k_1 of DDPC/DMPC-proteoliposome was found to be the biggest of them. The results of adsorption experiments support the hypothesis that an L-alanine receptor protein can be directly extracted with DDPC/DMPC-liposomes from frog tongue epithelium onto their bilayer membranes.

62. Study on the relationship between ethanol episodes and odor sensor (SnO_2 type) responses

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The relationship between ethanol episodes and odor sensor (SnO_2 type) responses was studied. Standard gases were prepared by the evaporation method in 10 l Tedlar bags. The concentrations of ethanol in the bags were 6.1 p.p.m. (at which the odor is recognizable) and 132 p.p.m. (equivalent to the breath air from an alcohol drinker). The odor sensor responses are as follows : 434–447 counts for direct laboratory room air; 645–657 (~+200 counts) for Tedlar bag filled with laboratory room air; 773–786 (~+350 counts) for ethanol (6. 1 p.p.m.) and 1503–1509 (~+1050 counts) for ethanol (132 p.p.m.) respectively.

These data showed good repeatability and the times needed for maximum counts obtained were ~5–6 min; recovery times to background level counts were ~7–8 min.

63. Application study of metal oxide semiconductors for odor analysis

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By using an odor analyzer which consists of six kinds of metal oxide semiconductors of SnO_2 and ZnO, three experiments were performed: (i) the difference of odor character of optically active chemicals, D- and L-tetrahydro-ethyl-ionol, D- and L-tetrahydro-methyl-ionol; (ii) the masking effect of a particular fragrance against tobacco smoke smell; and (iii) the application to foodstuffs.

The sensor showed distinctly an odor difference between the optical isomers; in addition it showed a distinct difference in the volatility of the D and L isomers. Furthermore, odor quality and strength calculated from the radar chart are almost equal to human sense. The sensor also showed interesting results on analysis masking the effect of tobacco smell by fragrance and freshness of foodstuff.

These results indicate that the sensor is useful as an aid for odor quality analysis in human olfactory sensory tests.

64. Sensing film characterization for a QCM array in an odor sensing system

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Since odor sensing systems are required in many fields, we have developed a system using a quartz crystal microbalance (QCM) array and neural-network pattern recognition. The system has an automatic sampling stage for measuring many samples with good reliability and reproducibility. In the present study, the characterization of the sensing films deposited on QCMs was performed. Responses of four kinds of films exposed to 12 sorts of organic vapors were studied to classify sensing films and investigate sensor response models. As a result of the present research, it was found that the frequency shifts can be expressed by the multiple-regression equations with six material parameters of odor samples as explanatory variables.

65. Discrimination of aromas from alcohols using a polymer-film-coated quartz resonator gas sensor

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Transient response curves for aromas from several kinds of alcohol (Japanese *sake*, wine, fruit liquor and whisky) were measured using an acrylic resin film-coated quartz crystal resonator gas sensor. The sensor exhibits different shape of the transient response curve for each aroma. The pattern recognition analysis using principal component analysis or neural network analysis is carried using four parameters which characterize the transient response curve for each aroma. The recognition probability of the neural network for four kinds of alcohol is 100% for 50 trials. The sensing method using the acrylic resin film-coated quartz crystal resonator gas sensor in conjunction with pattern recognition is one of the most attractive candidates for aroma sensing and identification of alcohols.

66. Distribution of trigeminal fibers in the facial lobe of the channel catfish, *Ictalurus punctatus*

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In order to reveal the distribution of trigeminal fibers in the facial lobe, the carbocyanine dye DiI was applied to the stump of the trigeminofacial root in isolated, paraformaldehyde-fixed brains of channel catfish, *Ictalurus punctatus*. After a diffusion period of 10–600 days, the brains were serially sectioned on a vibratorne and examined with epifluorescence. The entire trigeminal root was clearly labeled and the whole central projections of the trigeminal root were easily traced. The afferent fibers of the trigeminal nerve turn caudally after its entrance to the brain to form the descending root. Through the descending root trigeminal fibers were found to project onto the facial lobe as well as the principle sensory nuclei of the medulla and the funicular regions of spinal cord. The trigeminal projections to the facial lobe were through fascicles which arise from the descending root at the level of the posterior third of the facial lobe. In preparations where trigeminal root was selectively stained, these trigeminal fibers were found to be distributed throughout the entire region of the facial lobe except the trunk-tail lobule. These results show that trigeminal fibers contribute to make a sharply defined somatosensory map in the facial lobe of the catfish as well as the mechanosensory fibers of the facial nerve.

67. Preferential introduction of an anionic fluorescent probe into taste cells

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Preferential dye introduction into the taste cells of taste buds was qualitatively examined using various water-soluble fluorescent probes such as fluorescein, 5 (and 6)-carboxyfluorescein (CF), calcein, rhodamine B, rhodamine 6G, sulforhodamine B, fura-2, calcium green-1, BCECF and pyranine. Taste buds dissected from bullfrog tongue were incubated in a medium that contained a fluorescent probe. Anionic probes, except fluorescein, were preferentially introduced in taste cells, while cationic probes were introduced in supporting cells. Confocal microscopic observation revealed that CF was introduced into cytosol of the cell. With fluorescein and CF, the introduction was dependent on the pH of the medium, while this was not the case with calcein. The dye introduction was not affected at 4°C, but was significantly prohibited by pretreatment of the bud with 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene, an inhibitor of anion transport. This suggests that introduction of fluorescent probe into the taste cell is mediated not by an endocytic route but by an anion transport one. In any event, the present results would be of benefit for the investigation at the taste cell level without destruction of the taste bud structure.

68. Taste disk in metamorphosed salamanders

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The end organ of taste in frogs is distinct from taste buds in other vertebrates, and is thus termed a taste disk. However, tadpoles have taste organs similar to the taste bud in aquatic salamanders, such as mudpuppies. We studied if transformation from taste buds to taste disks occurs during metamorphosis of Ezo salamanders (*Hynobius retardatus*) and axolotls (*Ambystoma mexicanum*). Neotenic axolotls were artificially metamorphosed with thyroxine (T4) treatment. The non-distensible tongue of salamanders changed its structure progressively during metamorphosis: a small area of the rostrum protruded and developed caudally with recession of the flat area of the tongue. The emerged protrusion had grooves on the surface and numerous foliate papillae as seen in the mammalian tongue. The apical end of the papillae had a cell mass similar to the taste disk in frogs. On the flat area the taste buds maintained a bud-like shape, but their taste pore became wider. The taste disks in metamorphosed salamanders proved to be innervated by the glossopharyngeal nerve: taste cells were transneuronally labeled with fluorescent carbocyanine dye (DiI) and chemosensitive afferent responses were recorded from the nerve. Therefore, during metamorphosis salamanders undergo rearrangement and transformation of taste organs on the tongue, possibly to adapt to the terrestrial environment.

69. Expression of ABO and related antigens in the taste buds and von Ebner's gland from some mammals

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The distribution of the ABO and related antigens, such as Lea, Leb, Lex and Ley in the taste buds and the lingual glands from human, Japanese monkey, dog, cat, rabbit and rat was examined using immuno- and lectin-histochemical methods. The ABO blood type of each individual was determined by the reactivity of A, B and O antibodies with secretory cells of lingual glands. The Japanese monkeys were grouped into blood group B or O, the dogs into A or O, the cats into A or A (O), the rabbits into A or AB, and the rats into AB respectively. The taste buds of the individuals belonging to the same species and the same blood group showed similar and good reactivity with A, B and/or O antibodies. The reactivity of antibodies against the related antigens with the taste buds and von Ebner's glands were recognized apart from the staining intensity. The finding of ABO and related antigen in the taste buds and von Ebner's glands may provide an important clue to the role of these antigens in chemoreceptor systems.

70. Immunolocalization of a metabotrophic glutamate receptor in rodent taste receptor cells

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To demonstrate and localize metabotrophic glutamate receptor type 4 (mGluR4), which is a candidate for umami receptor, vallate and foliate papillae of mice and rats were examined immunohistochemically. In the vallate and foliate papillae of both species, the immunoreactivity for mGluR4 was observed in the apical part of the bipolar- and pear-shaped taste bud cells. Laser scanning confocal microscopic observations on taste bud sections that were double-labeled with antisera to protein gene product 9.5 and mGluR4 demonstrated that taste receptor cells contained immunofluorescence for mGluR4 in their apical membranes. In contrast, immunoreactivity for mGluR4 was not observed in basal cells of those taste buds and non-taste epithelial cells between the vallate and foliate taste buds. These results indicate that mGluR4 is a taste receptor cell-specific protein and may play a role as a umami receptor.

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71. An immunohistochemical study of BDNF and TrkB in the rat taste papillae

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To obtain a cell biological basis for mechanisms that regulate differentiation and turnover of taste receptor cells, the localization of brain-derived neurotrophic factor (BDNF) and its high-affinity receptor, TrkB, was examined immunohistochemically in vallate taste papillae of adult Sprague–Dawley rats. A conventional single-labeling streptavidin-peroxidase technique demonstrated that immunoreactivity for both BDNF and TrkB was present in bipolar- and pear-shaped taste bud cells. Double-labeling immunofluorescence techniques in combination with laser scanning confocal microscopy demonstrated that taste receptor cells and perigemmal nerve fibers that exhibited immunofluorescence for protein gene product 9.5 contained TrkB immunofluorescence. These results suggest that both BDNF and TrkB are produced by taste receptor cells, and that BDNF produced by taste receptor cells plays a neurotrophic effect on intragemmal nerve fibers that express TrkB.

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72. Apoptosis in mouse taste buds after denervation and colchicine treatment

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Apoptotic cells in the taste buds of mouse circumvallate papillae after sectioning of bilateral glossopharyngeal nerves and treatment with colchicine (an inducer of microtubule depolymerization) were examined by the method of DNA nick-end labeling (TUNEL), together with standard electron microscopy. The taste buds decreased in number and size 3–11 days after denervation and disappeared at 11 days. The TUNEL method revealed only a few positively stained nuclei in normal taste buds but, in those of mice 1–5 days after denervation, the number of positive nuclei had increased to 3–5 times that of taste buds from normal mice. Electron-microscopic observation after denervation demonstrated taste bud cells containing condensed and fragmentary nuclei in a cytoplasm with increased density. The colchicine treatment after 15 h and 1 day showed many TUNEL-positive nuclei and the condensed and fragmentary nuclei in the taste bud cells. The results show that taste bud cells under normal conditions die by apoptosis at the end of their lifespan, and that gustatory nerve sectioning and colchicine treatment cause apoptosis of taste bud cells.

73. Morphological development of taste buds in the oral cavity of mammals

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Taste buds on the soft palate (SP) and the fungiform papillae (FP) of the tongue were examined histologically in rats (Sprague–Dawley) and mouse (C3H) at different postnatal ages. The number of taste buds with taste pores was expressed as a percentage of total number of taste buds. The percentage on the SP were significantly larger than those on the FP in both animals at birth, i.e. 57.8 and 14.1% in rats, and 46.7 and 24.1% in mice. Although the density of all taste buds in both SP in mice was 24.6/mm^2 at birth and rapidly decreased to 13.3/mm^2 with increasing age until 3 weeks of age, that of taste buds with pores increased from 11.8/mm^2 at birth to 16.9/mm^2 at 3 days, then decreased to 12.9/mm^2 at 3 weeks. The taste bud density in the FP at the tip of the tongue was 19.7/mm^2 at birth and decreased to 7.9/mm^2 at 3 weeks, while that of taste buds with pores was 1.0/mm^2 at birth and 1.3/mm^2 even at 3 weeks. Assuming that a taste bud is functionally matured by developing a taste pore, these results suggest that the taste buds on the SP might play an important role as a source of gustatory input at the quite early postnatal stage in mammals.

74. Rapid disappearance of actin filaments in the ring of taste pores after chorda tympani nerve resection

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Morphological changes of rat taste pores after unilateral chorda tympani nerve resection were studied to clarify the possible role of innervation in the maintenance of taste pores by the actin staining method under a confocal laser scanning microscope.

Epithelial cells in the top of the fungiform papillae were tightly connected with each other and were opened as the pore. Actin filaments were observed as a ring around the inside of taste pores, by staining with rhodamine–phalloidin, while the rest of the cells in the surface of these papillae were not stained. Actin filaments in epithelial cells forming the pore began to disappear 1 day after the chorda tympani nerve resection, and completely disappeared at day 4, whereas taste pores were clearly identified as an intact shape under a scanning electron microscope. Transection of the lingual nerve proper did not induce the rapid disappearance. In addition, marked changes were identified in peripheral cells around day 5.

It is concluded that actin filaments make the ring of the taste pore, and the chorda tympani nerve, but not the lingual nerve, play a predominant neurotrophic role in the maintenance of actin filaments in epithelial cells forming taste pores.

75. Electron microscopic study of the receptor cell replacement in the vomeronasal epithelium of the rat

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Receptor cells undergo continuous replacement in the dual olfactory system. Our previous study revealed that transection of the vomeronasal nerves resulted in the degeneration of the receptor cells and the number of the receptor cells reached the lowest level by recovery day 6 (M. Ichikawa *et al.*, in preparation). To analyze the structural changes of the degenerating cells, an electron microscopic study was performed on the vomeronasal sensory epithelium following a nerve transection. Male SD rats were examined for each postoperative recovery time of 4, 6, 10, 15 and 21 days. The microvilli of the receptor cells decreased in number at day 4 and could not be recognized at day 6 and day 10. Some dendritic endings of the receptor cells filled with many centrioles at day 10. The microvilli of the receptor cells were observed again at day 15 and increased in number by day 21. The number of these microvilli, however, did not reach that of control side by day 21. These results suggested that the receptor cells of the sensory epithelium degenerated by day 6 and gradually recovered the number of their microvilli after day 15, but these cells showed an immature state even at day 21. To determine the complete recovery time of the vomeronasal sensory epithelium, it will be necessary to examine a longer recovery time than used in the present study.

76. Vaginocervical stimulation induces Fos-like immunoreactivity of granule cells in the rat olfactory bulb around parturition

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Vaginocervical stimulation at parturition plays a critical role in the induction of maternal behavior in rats, because mothers experience a reduction in their aversion to pup odor immediately after parturition. We already have demonstrated that artificial VCS influenced the dendrodendritic interaction between mitral and granule cells in the OB. In order to observe the activity of each cell comprehensively, we carried out Fos-protein immunohistochemistry in the OB of rats. Prior to staining, their endocrinological environment around parturition was mimicked by the implantation of silicon capsules containing estradiol. Animals were stimulated with either or both of VCS and electrical stimulation of the lateral olfactory tract (LOT) which are axons of mitral cells. Fos-immunoreactive cells are observed in the whole OB. Only VCS could induce numerous signals in granule cells. Oxytocin, which is known to be secreted by VCS, was reported to enhance granule cell activity by electrophysiology. Noradrenergic excitation by the locus coeruleus activation induced by somatosensory stimulation might also participate in this phenomenon. Simultaneous LOT stimulation combined with VCS, however, does not affect the mitral cell activity. We failed to

observe the enhanced inhibition by granule cells on mitral cells immediately after VCS by Fos-protein immumohistochemistry.

77. An immunohistochernical study of BDNF and TrkB in the rat olfactory system

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Immunolocalization of brain-derived neurotrophic factor (BDNF) and its high-affinity receptor, TrkB, was investigated in the olfactory mucosa of adult Sprague–Dawley rats. Under deep anesthesia, animals were transcardially perfused with Zamboni's fixative and cryostat sections of nasal regions containing olfactory mucosae were collected for immunohistochemistry. A conventional single-labeling streptavidin-peroxidase technique demonstrated that most intense immunoreactivity for TrkB was localized in the mucociliary complex of the olfactory epithelium and moderate immunoreactivity in the olfactory receptor neuronal cell bodies, dendrites and olfactory nerve bundles. The most intense labeling for BDNF immunoreactivity was present in the perikarya of olfactory receptor neurons (ORNs), and olfactory nerve bundles and dendrites of ORNs were immunolabeled as well. The intensity of immunoreactivity observed in the olfactory mucosa was greater than that in neurons in the main olfactory bulb, which also exhibited BDNF immunoreactivity. Double-labeling immunofluorescence techniques in combination with laser scanning confocal microscopy demonstrated that olfactory knobs exhibiting immunofluorescence for olfactory marker protein contained TrkB immunofluorescence and were surrounded by the region that exclusively contained TrkB immunofluorescence. These results suggest that matured olfactory receptor neurons produce TrkB as well as BDNF.

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78. A new bitter diterpene isolated from *Isodon japonicus*

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A new bitter diterpene, rabdosianone, was isolated from the ethyl acetate extract of *Isodon japonicus* (Japanese name, emneiso). The chemical structure of rabdosianone ($C_{20}H_{22}O_5$) was studied on the basis of spectroscopic methods. Furthermore, the chorda tympani and glossopharyngeal nerve responses to rabdosianone were compared with those to quinine–HCl, a representative bitter tastant, in Wistar rats. Both responses to quinine–HCl were greater than those to rabdosianone. These results suggest that the receptor mechanisms for rabdosianone are different from those of quinine–HCl.

79. Flow injection analysis for the taste-related components of green tea

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The harmonization of astringency and umami taste is important for green tea. The substances which show the strongest astringency and umami are ester-type catechins and free amino acids respectively. As we have already reported a flow injection method to measure ester-type catechins in tea infusions, a flow injection analysis (FIA) to measure free amino acids in tea infusions is discussed here. In this system immobilized L-amino acid oxidase was packed in a reactor column and the decrease of oxygen concentration by the enzyme reaction was monitored by a downstream oxygen electrode. The linear measurement range was 0–1000 mg/l of theanine, which is the amino acid found in the highest amounts in tea, and the sample throughput was 12/h. Real infusions of various kinds of green teas were measured using this system and the previously developed FIA for ester-type catechins. The results obtained by these two FIA methods were consistent with the actual taste of these tea infusions.

80. Tasting characteristics of low sodium chloride-containing soy sauce by using saltiness-enhancing substances

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In 1991, Muramatsu *et al.* succeeded in improving the soy sauce manufacturing process. Employing this process, low sodium chloride-containing soy sauce could be produced. Although the amino acid content was almost the same, the concentration of NaCl was reduced to one-third of that of commercial type sauce. Preparation of artificial soy sauce was attempted by mixing the reduced NaCl product and salty saltiness-enhancing substances such as Gly-OEt.HCl, Lys.HCl and so forth. The soy sauce obtained by adding Orn-Tau.HCl was very similar to commercial soy sauce in that it exhibited unadulterated saltiness and a good relative balance between salty and umami tastes. The soy sauce obtained by adding Gly-OEt.HCl exhibited a slightly stronger salty taste, while the one obtained by adding Lys.HCl gave a slightly weaker salty taste in higher concentrations than commercial soy sauce. The soy sauce obtained with added KCl produced stronger umami taste in all concentrations than commercial sauce. Replacement of NaCl by a large proportion of a salty substituting compound might affect the umami taste in soy sauce.

81. Expression of cDNA encoding a sweet protein, curculin, in *Escherichia coli* and structure and activity of recombinant curculin

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Curculin, which exists in the pulp of *Curculigo latifolia*, has a sweet taste and a taste-modifying activity. After curculin, water elicits a sweet taste and sour substances induce a stronger sense of sweetness.

The DNA encoding matured curculin was cloned into the expression vector, pMAL-c2. The curculin expression plasmid, pMCU-m, was transformed into *Escherichia coli* strain BL21. Expression of protein was induced by adding isopropyl-β-D-thiogalactoside (IPTG). The presence of expressed maltose binding protein–curculin fusion protein in the supernatant of the cell extract was confirmed by SDS–PAGE and immunoblotting using anti-curculin serum.

The activity of recombinant curculin in *E. coli* BL21/pMCU-m extract was assayed by human sensory test. The maltose binding protein–curculin fusion protein which was digested by factor Xa protease showed no sweet and sweetness-inducing activities.

The DNA encoding matured curculin was cloned into the expression vector, pET-32a. The curculin expression plasmid, pTCU-m, was transformed into *E. coli* strain AD494. The presence of expressed thioredoxin–curculin fusion protein in the supernatant of the cell extract was confinned by SDS–PAGE and immunoblotting using anti-curculin serum. Assay of the activity of expressed curculin is in progress.

82. Discrimination between mixed tastes and their components

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Since generalization of conditioned taste aversions provides a good measure of the behavioral similarities among gustatory stimuli (Smith and Theodore, *Physiol. Behav.*, 32, 983–989, 1984), this measure was employed to investigate whether or not mixed tastes are similar to the tastes of their component stimuli. Rats were trained to take their daily water intake within two 20 min sessions per day, during which the intake of solutions was recorded. During these sessions, a mixed taste solution (CS+: 0.5 M sucrose + 0.2 M NaCl) was presented, followed by an i.p. injection of 0.15 M LiCl to produce a conditioned taste aversion. Two component taste stimuli (CS–: 0.5 M sucrose or 0.2 M NaCl) were presented without the LiCl pairing. After presentation of two CS+ + LiCl and four CS-s (twice each CS-s), rats were tested for aversion to the CS+ and generalization to CS-s. As a result, rats could avoid only CS+ but not CS-s. Because this result depended on the presentation procedure, it is suggested that rats can differentiate the mixed taste from the component tastes as a result of discriminative learning.

83. The effect of age on preference for sweet substances in diabetic *db/db* mice

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The *db/db* mouse is known as a genetic model of non-insulin-dependent diabetes, in which a single gene defect (*db* gene) leads to the expression of diabetes with preceding hyperinsulinemia, hyperglycemia and extreme obesity, but very low insulin secretion in elderly animals. Our previous studies demonstrated that *db/db* mice aged from 7 days to 24 weeks show greater taste preference for sugars than lean control mice do. However, there are no available data for elderly animals. The present study, therefore, compared the preference for sweeteners between two age groups (8–24 weeks and 40–80 weeks) of each *db/db* and control mouse, by using a conventional two-bottle preference test. At 8–24 weeks of age, preference scores not only for sucrose but also for the non-sugar sweetener, sodium saccharin, were significantly larger in the *db/db* than in the control mice, whereas that for D-phenylalanine was not different between the two groups of mice. This selectivity for sweeteners in taste preference is comparable with that previously observed in enhanced chorda tympani responses of *db/db* mice. At 40–80 weeks of age, the preference score for sucrose in *db/db* mice decreased and showed no significant difference from that in the age-matched control mice, whereas their preference score for sodium saccharin did not change and was even higher than that in the control group. Preference scores for D-phenylalanine did not differ between *db/db* and control mice, similar to the results at 8–24 weeks of age. These results suggest that reduction of sucrose preference in the aged *db/db* mice is probably due to their impaired excretion of sugars.

84. Behavioral and physiological study of the abnormality of salt taste sensation in zincdeficient rats

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It is well known that zinc deficiency causes abnormalities in general taste sensation. We have reported that rats fed a zinc-deficient diet have a significantly higher preference for NaCl and carbonated water than those fed a zinc-sufficient diet. We wondered if there might be higher preferences for other minerals in zinc-deficient rats, and we therefore investigated the effect of zinc deficiency on the preference for NaCl, KCl and $CaCl_2$ solutions in SD rats. To clarify the mechanisms underlying any taste insensitivity in zinc-deficient rats, we measured the chorda tympani nerve responses to these mineral compounds.

Twenty male SD rats, 4 weeks old, were divided into four groups (Zn-Def, Low-Zn, Zn-Suf, and Pair-fed). Each group was housed in a cage with two bottles: the taste solution (154 mM of NaCl, KCl or $CaCl_2$) and distilled water, to each of which they had free access. After taking part in the preference tests for 6 weeks or more, the rats were used for the recording of chorda tympani nerve responses. The whole-nerve integrated response was assessed by calculating the ratio peak height/background noise height.

Interestingly, in zinc-deficient rats, an increase in preference was observed for NaCl on day 2 of the test, but for KCl and $CaCl_2$ not until days 8 or 21 respectively. Thus, in zinc-deficient rats, NaCl preference develops more rapidly than preference for the other minerals. The chorda tympani nerve responses to NaCl in the Zn-Def and Low-Zn groups, and to KCl in the Zn-Def group were significantly lower than the responses in all the other groups.

85. Effects of cold and carbon dioxide on the preference for bitter taste solutions in SD rats

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Bitterness and carbon dioxide (CO_2) are key components of beer, cola and some other soft drinks, all of which are often drunk cold. The mechanisms involved in the chemoreception of isohumulones and other bitter compounds in man and animals have not been well defined, especially in terms of the modulating effects of cold and CO_2. To clarify the interrelation between of the acceptability of bitter compounds and their solution's coldness and CO_2 content, we carried out preference tests in rats.

Five male SD rats per experimental group were reared together in wire-bottomed cages, and allowed free access to a commercial pellet diet and drinking solution. The latter comprised four kinds of test solution: deionized water (= 0×), and solutions containing 0.5×, 1.0× and 2.0× the concentration in the standard solution of a given bitter compound. These standard solutions contained the following: 0.01 mM of quinine sulfate, 30 mM of caffeine, 34 mg/l of isohumulones, 17 mg/l of tetrahydroisohumulones, 34 mg/l of hexahydroisohumulones or 68 mg/l of reduced isohumulones. The various analogues of isohumulones were a gift from Kalsec Inc., MI, USA. The test solutions were kept cold (5°C) or at a room temperature (23°C), with or without 800 p.p.m. of CO_2 throughout the experimental periods.

All the bitter taste solutions were avoided by rats at room temperature. However, coldness enhanced the preference for solutions containing caffeine, reduced isohumulones, tetrahydro-isohumulones or hexahydroisohumulones, and the combination of cold and CO_2 further enhanced the preference for caffeine solution. These results suggest that the coldness and CO_2 content of drinks can modulate the reception mechanisms for some bitter compounds.

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86. Behavioral and biochemical study of the effect on acid-preference of forced-swimming fatigue

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We have reported that forced-swimming changes the preference for sour and bitter tasting substances: fatigued rats had a greater preference than control rats for citric acid, ascorbic acid and caffeine solutions (but not for HCl and urea solutions). In this study, the preferences for four organic acids (citric acid, 2-oxoglutaric acid, malic acid and tartaric acid) were examined in rats, with or without forced-swimming, in 22 h two-bottle preference tests.

Six-week-old male SD rats were used for these tests, finally being killed for the quantitative analysis of the activity of liver citrate synthase (regarded as a major rate-limiting enzyme in the TCA cycle). After acclimatization for 5 days, the rats were divided into control and fatigue groups (20 rats in each). Each day for 20 days, the fatigue rats swam in a big pail filled with water (30 ± 2°C) for 2 h, for which period the control rats were kept in a cage without drinking water.

A two-bottle (test solution and water) preference test occupied the remaining 22 h of each day. A given rat was tested with only one acid, four concentrations of which (0.5, 1, 2 and 3 mM) were presented in ascending order on consecutive days (= one 'cycle'). The experiment involved an uninterrupted sequence of five cycles (4 days × 5 cycles = 20 days).

The fatigued rats preferred citric acid and 2-oxoglutaric acid, but not malic acid or tartaric acid. The activity of liver mitochondrial citrate synthase was significantly suppressed after the program of forced swimming. These results are consistent with the idea that the suppression of citrate synthase caused by forced swimming may alter the preference for these sour-tasting substances.

87. The effect of bitter and astringent taste on food selection in primates

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The sense of taste is one of the important senses for efficient choice of foods in primates. However, recent studies have suggested that, in general, herbivores are likely not to avoid the bitter/astringent taste. That is, they do not avoid selectively the toxic plant secondary compounds, which are highly related to the bitter/astringent taste. Therefore, this study investigated how 90 species of primates respond to bitter (phenylthiocarbamide and quinine chloride) and astringent (tannic acid) tastes. Apple slices soaked in the taste solutions of three different concentrations were presented to the animals. The rejection thresholds were estimated from the reaction as the fruit was eaten. Most species showed high tolerance to the bitter and the astringent taste. One of the most tolerant phylogenetic groups to bitter taste was Colobinae, which is a leaf-eater and has a ruminant-like ungulate to digest the

secondary compounds. The other tolerant species was Hominidae, which does not have such a physiological system but eats various foods in the course of a day. These results suggested that primates have two different strategies to acquire tolerance to bitter taste: (i) physiological adaptation (e.g. acquisition of ruminant); (ii) behavioral adaptation (reduction of the absolute intake of single toxic secondary compound by increasing daily food variation).

88. Effect of physical exercise on preference for sour and bitter taste solutions

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The effect of physical exercise on the preference of sour and bitter taste solutions was examined. After 30 min of exercise using a bicycle ergometer, at an intensity of 50% VO_{2max} (maximal oxygen uptake), a taste preference test and absolute threshold test were performed in 31 healthy university students. Test solutions were citric acid and caffeine.

The absolute threshold for both taste solutions did not differ much before or after exercise. Preference scale values of citric acid increased after exercise in one-third of the subjects, while the values of caffeine were not changed in most of the subjects. Those subjects whose preferece for citric acid increased, preferred higher concentrations of the solution after exercise than before exercise.

These results suggest that preference for sour taste may be affected by physical exercise.

89. Influence of taste stimuli on heart rate and blood pressure

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Relations between taste stimuli and heart rate or blood pressure were evaluated in 29 healthy university students. Test solutions were sucrose, NaCl, citric acid, quinine–HCl and monosodium glutamate (MSG).

Heart rate increased by 7.1–13.6% after taste stimuli compared with pre-stimuli level for all taste solutions except water. Heart rate showed maximum increase at ~25 s after the taste stimuli and recovered to pre-stimuli level by 80–100 s. The heart rate showed maximum increase for citric acid. Recovery of heart rate increase was delayed more for quinine–HCl and MSG than the other taste stimuli. Increased heart rate and hedonic scale values of taste solutions showed significant negative correlation except for sucrose.

Systolic blood pressure increased weakly by 3.5–6.1% compared with pre-stimuli levels for almost all tested taste solutions. However, diastolic blood pressure showed no change after all taste stimuli.

These results suggest that taste stimuli increased heart rate, and that the increased heart rates were different among the concentration and taste solutions.

90. Effect of stimulus volume on taste perception for umami

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Various stimulus volumes (10, 1, 0.1,0.01, 0.001 ml) were compared in their effects on taste perception. The 10 ml stimuli were sipped from a small plastic cup and expectorated. The others were licked by the tip of the tongue from a small plastic spoon. A series of test solutions of sucrose, NaCl, DL-tartaric acid, quinine sulfate, MSG and IMP were presented to 30 subjects in ascending order of concentration to evaluate the taste quality and the degree of the certainty on a four-point scale, from absolutely to no taste. The recognition thresholds were raised by decreases in stimulus volume, and these effects of volume varied according to the taste substances. Volume affected the thresholds for DL-tartaric acid most due to the change in pH. Thresholds for sucrose and NaCl were less effected than those for quinine sulfate, MSG and IMP. This suggests that sweet and salty taste receptors densely exist in the anterior part of the tongue. It was also notable that the effects of stimulus volume on the thresholds for MSG and IMP were not different from those for quinine sulfate, nevertheless the area sensitive to umami is especially localized in the posterior part of the tongue as we have already shown. This suggested that the umami substances spread over the tongue more effliciently than quinine sulfate.

91. The extraction of sensory information from finger plethysmograms by taste stimuli

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Finger plethysmograms were measured on 25 subjects (male students) who perceived five tastants (sweet, bitter, sour, salt and water). The finger plethysmograms were normalized such that the maximum was unity and the minimum was zero; the normalized finger plethysmograms were transformed into the frequency distribution of baseline and wave height. Discriminant analysis was applied to the frequency distribution of plethysmogram baseline and wave height. The five tastants were separated by discriminant analysis. From a group scatterplot of the discriminant analysis, it was found that the relatively strong taste group of bitter and sour were separated from the water group.

92. Effect of the odor of tea on bitter taste and astringency

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It is known that taste and smell interact with each other in food

and we evaluate this effect as food flavor. Although some research has reported that sweet odorants such as strawberry enhanced the sweetness of sucrose, the effect of the odor of food on bitter taste is not yet well known. In this paper, the relationship between the odor of tea and bitter taste and astringency was investigated.

Catechin mixture (Polyphenon 100, Mitsui Norin Co., Ltd) extracted from green tea for astringency and bitter taste and caffeine for bitter taste were used as the tastants. The odorant of tea was prepared from green tea or black tea by the Porapak Q resin adsorption method. Thirteen women subjects, 21–26 years old, were selected by gustatory and olfactory test. The intensity of taste and taste threshold were determined by scoring test and triangle test respectively. The sipand-spit technique with a rinse before each sample was employed and the subject sniffed the odorant during tasting.

The taste of catechin mixture was enhanced by the odor of both green tea and black tea, and the taste of caffeine was enhanced only by the odor of green tea. There was also a tendency for subjects to find it difficult to discriminate the odor of both teas at near-threshold levels.

93. Olfactory responses of masu salmon (*Oncorhynchus masou*) to amino acids in stream waters: electrophysiological evaluation of amino acids as olfactory cue for home stream detection in Lake Toya

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Salmonid fishes in spawning migration have the remarkable ability to return to their home stream. Many behavioral studies have shown that their olfactory systems play an important role in searching for the home stream in the final stage of their homing migration. However, the chemical basis of the olfactory cue in each river has been poorly established. To characterize the home stream odor, we determined the main cations and various amino acids in the stream, which are potent olfactory stimulants for fish, and recorded olfactory neural responses of masu salmon to natural stream waters and artificial stream waters constituted of only amino acids and salts. Natural stream waters elicited large responses. The cross-adaptation experiments demonstrated that the odor of each stream water was well discriminated by the olfactory system. Amino acid analysis of stream waters showed that each natural water had a different amino acid composition. The fish olfactory system responded to the artificial stream waters that contained amino acids and salts in the same manner as to the natural steam waters. The fish olfactory system discriminated between the artificial stream waters, but did not discriminate between artificial stream waters constituted only of salts. The results obtained suggest that amino acids are a major component of the olfactory cue in the home stream water.

94. Olfactory response of salmonid fishes on their downstream migration and homing migration

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It is generally considered that olfaction plays a dominant role in mature salmon when they select the home stream in which they spawn. Many behavioural observations and electrophysiological studies strongly support the olfactory hypothesis for salmon homing. In this study, we measured salmon olfactory response to various natural waters, and examined the ability of olfactory discrimination in river odor differences.

Lacustirne sockeye salmon, *Oncorhynchus nerka*, caught in Lake Shikotsu at the time of their homing migration, lacustrine masu salmon, *O. masou*, and lacustrine sockeye salmon cultured at Toya Lake Station were used in the present study. The olfactory responses were recorded from the olfactory nerve. At first, fish were immobilized using gallamine triethiodide (3 mg/kg body wt), and then olfactory nerve responses were recorded from twin tungsten electrodes inserted at the olfactory nerve and integrated by an custom-built electric integrator. Natural water used for stimulation were lake water from Lake Toya and two stream waters drawn from streams (River Poromoi, Toya Lake Station culture pond).

Each natural water generated a different magnitude of response in both sockeye and masu salmon, and cross-adaptation experiments demonstrated that their olfactory systems were able to discriminate odors. In sockeye salmon, responses in cultured fish were smaller than for wilds. We were unable to measure the characteristic reponse to maternal water.

These results suggest that salmon olfactory nerve response is reflected in river water odors, and that cultured salmon have a lower olfactory sensitivity than wild fish.

95. Changes of mouse urinary marking behavior with sexual experience

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Urinary marking patterns of mouse vary according to sex, social rank, reproductive state and developmental stage. Male urinary pattterns have been shown to be regulated by androgenic hormones. Sexual experience changes urinary marking patterns. In order to ascertain whether the changes in urinary marking accompanying sexual experience were the consequences of a shift in sex hormone balance, I investigated the effects of the gonad removal and sex hormone injection on the counter marking pattern and activity of the mouse. After sexual experience, urinary marking was facilitated in males, but depressed in females. Generally, the activity was reduced in both sexually experienced males and females. Gonadectomized males decreased the urinary marking, while gonadectomized female restored the urinary

marking. Overall, sex hormone injection could not overcome the effects of gonad removal on urinary marking behavior. Contrary to my expectation, a sham-operation caused similar effects to gonadectomy. It is concluded that the contraverse effects of gonadectomy on male and female urine marking behavior in this experiment are due to the failure of neural memory of sexual experience following from anesthasia and operation but not to the horomonal regulating mechanism.

96. Formation of hedonics of odor: preference and discrimination in infants

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We reported the preference between phenylethyl alcohol (P) and skatol (S) in 2-year-old infants at JASTS 1995. We measured the preference and discrimination again between P and S in the same infants a year and a half later. Twenty infants from 2 years and 11 months to 4 years 2 months participated in a preference experiment using a sniffing bottle method. Fifieen infants from the same group participated in a discrimination experiment using gloves containing an odor. Nine of the infants could significantly select a favored odor. Five of them preferred significantly P, and two of them preferred S significantly and two of them preferred S as a tendency. Sixty-seven per cent of the infants could significantly discriminate between P and S. The correlation between preference and discrimination was significantly high (0.706).

97. The effect of odors on emotion and frontal brain activity

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We examined whether there was anterior frequency fluctuation asymmetry in the alpha frequency band correlated with self-report measures (awareness and mood) against the presentation of four kinds of odor (sandalwood, perilla, terpen, peppermint). An electroencephalogram (EEG) was recorded from 21 scalp electrodes in eight normal subjects. Alpha wave cycles in the frontal locations (Fpl, Fp2, Fz, F7, F8, F3, F4) were extracted using the filtering and zero-crossing method. The fluctuation of cycle changes was evaluated by a frequency analysis (FFT) and a linear regression method. The result indicated that there was anterior frequency fluctuation asymmetry in the alpha frequency band which correlated with both a level of awareness and/or positive/negative mood. In most cases subjects felt a good mood, the rhythm of fluctuation became a $1/f$-like and anterior frequency fluctuation asymmetry was small. On the other hand, in some cases subjects felt a bad mood, the rhythm of fluctuation became $1/f^0$-like and anterior frequency fluctuation asymmetry was relatively larger. The left frontal side was more $1/f^0$-like than the right side. But we also observed another case. Although terpen aroused a high awareness and a bad mood, the rhythm of fluctuations was $1/f$-like. In an another case sandalwood aroused a low awareness and a good mood. In such cases the rhythm of fluctuations was also $1/f$-like, suggesting that a pattern of the rhythm of fluctuations might be related non-linearly to awareness and mood states.

98. Preference of perfume and CNV: psychophysiology of perfume

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Among the many perfumes favored by young heavy perfume users, two perfumes were selected for this experiment according to their preference—one is more favored and the other less favored. The effects of them on contingent negative variation (CNV) were studied by comparing the increase ratios of CNV basis wave on the right and the left hemisphere. Reported feelings induced by scented conditions did not show any significant difference between the two samples showing a pleasant type of feeling profile.

Electroencephalograms were recorded from 19 electrode sites on the scalp and CNV was recorded by conventional S1–S2+R paradigm with additional S1–S2 paradigm. Multivariate analysis of single trial CNVs extracted a couple of basis waves during the S1–S2 interval and after S2. The largest basis wave, which developed gradually during S1–S2 and peaked at 400–500 ms prior to S2 on every electrode site, increased its magnitude when the paradigm changed from S1–S2 to S1–S2+R.

The hemispheric mean of the basis waves decreased its magnitude (μV.ms), in both paradigms and on both hemispheres, when scented by the more favored one, and increased when scented by the less favored. R/L ratios of the increase ratio tended to be smaller in the former than in the latter.

99. EEG transition under odors of coffee and whisky

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It is well known that various odors affect physiological condition and brain activity. This study examined α1 and α2 of electroencephalograms (EEGs) in order to clarify the effect of the odors of coffee and whisky.

Subjects were 21 healthy adult females whose ages ranged from 18 to 59 (mean 30.7 ± 2.8). Their EEGs were recorded at resting state and under odors of coffee and whisky, and then the power and coherence of α1 and α2 were analyzed using fast Fourier transform. The power and coherence of each group which was separated by subjective evaluation of odor and daily liking and disliking were examined using Wilcoxson's signed rank test.

The major results were as follows: (i) α2 power of every location

decreased in the group which disliked the odor of presented coffee and in the group which disliked coffee itself, but α1 power increased in the group which disliked the odor but liked coffee. (ii) α power increased more under the odor of whisky than coffee. In particular, in the group which liked the odor of presented whisky, α power greatly increased. (iii) α1 coherence (right–left) increased under both odors in the presented odor liked group and the daily liking group.

These results suggest that the brain activity is affected by the odors of coffee and whisky, and not only subjective evaluation of odor but also daily taste take part in this effect.

100. Effect of green odor on mental function

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The 'green odor' of green leaves arises from eight volatile compounds, C_6 aldehydes and C_6 alcohols, and these are closely related to the life of insects. However, no relationships with human beings have yet been disclosed. The present experiments were performed to determine whether those odors affect humans psychophysiologically. Of these odors, two odors, *cis*-2-hexenal and *trans*-3-hexenol, and the mixture of these odors were selected as test samples. Those odors were diluted and tested to choose which concentration produces a good sensation for the subjects, and the concentration of 0.03% was adopted and used. The psychophysiological effect of the odors, one each of a sample and a mixture of it, were investigated using the event-related potential P300. Those odors were inhaled for 5 min, and the change in P300 before and after inhalation were compared. Both of the odors inhibited the amplitude of P300, suggesting the sedation of mental function. The present results suggest that the green odor affects humans mentally, and is effective as aromatherapy.

101. Olfactory evoked magnetic fields by a 64-channel whole-head SQUID system

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We tried to confirm the localization of the human cortical areas activated by odor stimulation. We recorded olfactory evoked potentials (OEP) from Cz and magnetic fields (OEMf) after phenyl ethyl alcohol stimulation of 200 ms duration using a Kobal olfactometer (Kobal, 1981). The 30 stimuli were presented once every 40 s asynchronously with the respiration cycle, thus it took ~20 min for each experimental session. For recording of OEMf, we employed a 64-channel whole-head SQUID system (CTF Systems Inc., Canada) in a magnetically shielded room. Four male subjects participated in this experiment. We have been able to localize equivalent current dipoles (ECDs) in the response which peaked at nearly 566–854 ms after stimulus onset bilaterally in the area around the superior temporal sulcus after separate stimulation of the two nostrils. These ECDs stayed at the superior temporal sulcus for 50–130 ms. Moreover these ECDs corresponded in latency to the P2 component (~700 ms after stimulus onset) of the OEP responses, which confirmed results obtained by Kettenmann *et al.* (1996). We will also investigate the activated area in the neocortex related to the process of odor recognition, such as pleasantness and familiarity.

References

Kettenmann, B *et al.* (1996) Odorants activate the human superior temporal sulcus. *Neurosci. Lett.*, **203**, 143–145.

Kobal, G. (1981) *Elektrophysiologische Untersuchung des menschlichen Geruchssinns*. Thieme Verlag, Stuttgart, p. 171.

102. The extraction of sensory information from finger plethysmogram by odor stimuli

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Finger plethysmograms were measured on 24 subjects who sniffed five odorants (rose, burnt, sweaty, peach and moldy) in a T&T olfactometer and a scentless control, and recorded their reaction to the five ordorants using a sematic-differential (SD) experiment. The finger plethysmograms were standardized such that the mean was zero and the variance was unity. The standardized finger plethysmograms were transformed into frequency distribution. Discriminant analysis was applied to the frequency distribution of the finger plethysmogram. Five odorants and the scentless control were separated by discriminant analysis. From the group scatterplot of the discriminant analysis, it was found that the pleasant odor group (e.g. peach) was close to the scentless control group, but the unpleasant odor group (e.g. sweat) were separated from the scentless group. These results of discriminant analysis corresponded to the results of emotion analysis using an SD experiment.

103. Estimation of optically active linalools for effective fragrances by sensory evaluations and a physical measurement

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We had reported that linalool (commercial) was effective for favorable impressions after hearing a natural environment sound,

in contrast to the case of mental work or exercise (stepping up and down), on the basis of both sensory evaluation and conventional electroencephalograph (IBVA EEG). In order to investigate the effects of linalool in detail, optically active linalools, (*R*)-(–)-, (*S*)-(+)- and (*RS*)-(±)-forms, were used for statistical estimation of sensory test and physical measurement (IBVA EEG) of these fragrances before and after hearing the sound.

(*R*)-(–)-Linalool ([α]D –15.1°; 97.0% on GLC with CP-cyclodextrine-β-236M-19) was isolated by repeated flash column chromatography from lavender oil. (*S*)-(+)-Linalool ([α]D +17.4°; 88.3% on GLC) and (*RS*)-(±)-linalool ([α]D 0°; (*R*)-form 50.9% and (*S*)-form 49.1% on GLC) were obtained from coriander oil and commercial linalool respectively by use of the same method. Each optically active linalool was administered to examinees before and after hearing the sound, and both the sensory test and the measurement of the forehead surface potential wave (EEG) were performed simultaneously. After fast Fourier transform analysis of the measurement, the effects of fragrances were estimated.

The favorable impressions of (*RS*)-(±)-linalool were suggested for the fragrances of (*R*)-(–)-linalool, but not for (*S*)-(+)-linalool, by sensory test. This suggestion was also supported by the physical measurement with IBVA EEG.

104. Screening of effective fragrances for work using a sensory test

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We reported that the subjective impressions of fragrances before and after work was changed by the nature of the work. In order to evaluate the change of impression, we have employed a sensory test based on the semantic differential (SD) method.

The fragrance oils used in this study were lavender, rosemary, linalool, peppermint, ylang ylang, lime, basil and cardamom. The Kraepelin mental performance test (for mental work), physical exercise (stepping up and down) and hearing an environmental sound were the tasks studied. As for the sensory test for impressions of a fragrance, 13 pairs of adjectives were used. The impression scores were marked after smelling the fragrance before and after the work and the statistical significance of the change of the score for 13 impressions was examined by *t*-test. The effectiveness of the fragrance for the work was further evaluated in terms of the number of items judged significant by *t*-test for 13 impressions using the sign-test.

Based on the *t*-test and sign-test, the fragrances judged effective for the work were basil, linalool, peppermint, lime and cardamom. Among these, it was revealed that there was a group of fragrance that enhanced impressions after the task, e.g. basil for mental work and linalool, peppermint and lime for hearing the environmental sound, and a group that decreased the impressions after the task, e.g. linalool, peppermint and lime for mental work and cardamom for hearing the environmental sound.

105. An evaluation of the odor of city gas

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This study examined the difference between the odor of city gas and five kinds of odors that can be mistaken for city gas and which are found in the living environment: the smell of garlic, onions, kerosene, kitchen garbage and cigarettes.

The subjects were 112 healthy Japanese people aged 18–61 ,and 24 Japanese people aged 65–89. After sniffing each odor from a squeeze bottle without any information on its contents, the subjects rated the perceived pleasantness, first impact, longevity, over impression and similarities between each odor and the odor of city gas from their memories.

The results were as follows: city gas in Tokyo had the most unpleasant and unbearable odor of all the samples. Factor analysis showed that the odor of city gas belonged to the cluster of extraordinary odors and the memory of the odor of city gas was more dangerous, unsettling and distinct than it actually is in Tokyo.

As far as the aged Japanese were concerned, the recognition of the odor of city gas was greatly influenced by individual differences.

106. Odor of new cars

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The odor of a new car is caused by a complex mixture of various compounds evolved from interior parts and materials such as seats, instrument panel, ceiling panel, floor board and adhesives. Since the odor is one of the most important factors for customer satisfaction, the interaction between the compounds of the odor and the sense of smell was investigated. The compounds of the odor were analyzed with GC and GC–MS using an absorbent canister. Sensory evaluation was also carried out in a car's cabin. From the instrumental analysis, >200 compounds of paraffinic and aromatic hydrocarbon oxygenated hydrocarbon, nitrogen compounds and sulfur compounds were characterized as compounds of the odor. From the sensory evaluation, qualities of the odor were classified into stimulus, fishy, solvent-like, rubber-like, etc. By analyzing these results, the compounds causing the unpleasant odor and the compounds masking the unpleasant odor were identified. The odor change during car storage was also investigated.

107. Evaluation of odor quality for ten odorants in the T&T olfactometer using 28 concrete adjectives

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Using the semantic differential method, odor quality was evaluated for five odorants in the T&T olfactometer (A–E) in the previous paper, in which 28 concrete adjectives were used. Applying principal component analysis to the ratings, component A–C were extracted as 'rotten smell', 'burnt smell' and 'floral odor' respectively.

In the present paper, the odor quality of ten odorants in the T&T olfactometer (A–J) was evaluated using 28 concrete adjectives. As a result of principal component analysis, components A–G were obtained as 'sweet odor', 'rotten smell', 'green smell', 'burnt smell', 'sour smell', 'musty smell' and 'mint odor' respectively. In order to observe shifts of odor quality due to concentration of odors, root mean squares of principal component scores (RMS of PCS) were calculated for each odor. Discrimination of odor quality could be checked by calculating RMS of PCS between odors.

In future, some adjectives should be added for accurate odor discrimination, and the number of subjects will be increased for generalization of the results.

108. Effects of fragrance on insomniac tendency in healthy humans: application of the fragrance Kyphi

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The effects of fragrances on the insomniac tendency of healthy subjects were studied. Fragrances applied in this study were a sedative type of fragrance (A), that was named Kyphi, composed of sandalwood, rose, juniper berry and others, and a stimulant type of fragrance (B) composed of spearmint, lemon and others. Both fragrances had the same preference level. One hundred and nine men and women were recruited and 16 subjects with mild psychophysiological insomnia were selected by screening procedure to exclude the other types and degrees of insomnia. Every morning subjects answered a sleep questionnaire regarding the quantity and quality of their last night's sleep. The items on the questionnaire were total sleep time, sleep onset latency, number of awakenings, satisfaction of sleep and others. In the experiment subjects slept without using any fragrance for the initial 2 weeks and for the following 4 weeks slept using fragrance A or B in the fixed schedules. The use of fragrance A significantly reduced sleepless time compared to sleeping without using fragrance. In the comparative study, fragrance A tended to shorten sleepless time more than fragrance B. These results suggest that the application of appropriate fragrance with a sedative effect is useful to help counter insomnia.

109. Taste distortion in patients with gustatory disorder

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On gustatory testing (filter-disk paper method and whole mouth gustatory test), patients with gustatory disorders often exhibit taste distortion. In the last 4.5 years, we have performed gustatory testing of 301 patients at the Gustation Clinic of the Osaka City University Medical School, and found 178 (59.1%) with taste distortion.

Seventy-six of these patients had taste distortion with two or more taste stimulants, and 92.7% of patients with taste distortion misrecogniozed NaCl and/or tartaric acid. These two stimulants thus appear to have similar patterns of stimulation of gustatory neurons. The most common expressions used for misrecognized tastes were bitterness and acidity.

There was no relationship between patient complaint and type of taste distortion. Thus, dysgeusia, spontaneous abnormal taste, specific ageusia and phantogeusia do not appear to cause taste distortion in gustatory testing. The etiology and severity of gustatory disorder were unrelated to frequency and type of taste distortion. Disorders of the taste buds due to zinc deficiency may cause the taste distortion. The most common complications in patients with taste distortion were psychiatric disease, metabolic disease, urological disease and circulatory disease.

110. Combined disorders of taste and olfaction

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We have experienced 72 patients with combined smell and taste disorder in the past 3 years. This number corresponds to 13 and 33% of the total numbers of patients in our olfaction and taste clinics respectively. Common etiologies of olfactory disorder in those patients were viral infection (post-common cold) and head injury, while those for gustatory disorder were disease of unknown origin and viral infection. There were 44 (61%) patients with so-called flavor disorder. Eighteen of the 44 patients had normal function on gustation testing. Others had decreased gustatory function, but their subjective gustatory disorder appeared to be increased by their olfactory disorder.

The percentages of patients with subjective improvement after treatment were 64 and 47% for taste and smell disorders respectively. For patients with improvement of both taste and olfaction, taste disorder improved earlier than smell disorder. The

turnover time of olfactory receptor cells is ~4 weeks, while that of gustatory receptor cells is ~2 weeks. The finding of more rapid improvement of taste than of smell disorder appears to be due to the difference in turnover time between the corresponding receptor cells.

111. Electrical olfactory evoked potentials in clinical cases

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We have tried to detect the olfactory evoked potentials produced by electrical stimulation of the normal human olfactory mucosa and reported that the evoked potentials seemed to be originated in the olfactory bulb. This evoked potential was named 'electrical olfactory evoked potential' (EOEP) by our group. The aim of this study is to examine the possibility of the EOEP as an objective olfactometer.

Informed consent patients who complained of olfactory disturbance were tested. Condition was 2 mA, 0.5 ms, plus stimulation and 300 trials were averaged. Results were compared to T&T olfactometry. In the smell normal and the light hyposmic group, EOEP was well evoked. The EOEPs were also detected from a parosmic patient, but no EOEPs were detected in the anosmic group.

The EOEP seems to be applicable as an objective olfactometer. Because this method is easy to perform in the general hospital without special expensive equipment, it seemed to be useful for clinical use.

112. Topography of the olfactory evoked responses in patients with smell disorder

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We have developed a device for odorous stimuli control to record olfactory evoked responses (OER) from the human scalp. Methyl-cyclopentenolone was used as the odorant element. The stimuli were delivered for 0.5 s once every three inspirations for a total of 50 stimulations. We obtained the normal OER using this apparatus, whose positive peak latencies were ~350 and ~700 ms (P1 and P2 respectively).

In this study, OER recording was carried out in 33 patients with smell disorder. A positive response at ~300–400 ms was recorded in seven patients (females, 15–59 years old), since the other 26 patients had no responses. The high potential area of this positive peak was located in the centro-occipital region of the scalp. The latency and the high potential area of this peak were same as P1 recorded from normal subjects. We regarded the source of this peak and P1 as identical. It may be a response to the trigeminal nerve during odor administration. P2 was not recorded from the patients with smell disorder, so that we thought P2 might be a response to the olfactory nerve.

113. Laterality of olfactory acuity in nasal and paranasal sinus disorders

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Although unilateral olfactory disturbance is frequently encountered in ENT departments, these patients are usually not aware of their impairments and the traditional method for the examination of olfactory acuity, which determines both sides simultaneously, is not useful for detailed evaluation. In order to clarify the relationship between the common nasal and paranasal sinus pathology (NPP) and the impairment of olfactory acuity, we evaluated each unilateral olfactory threshold in NPPs.

Patients who visited the ENT department of the Mayo Clinic (Rochester, MN, USA), Izumi-Sano City Hospital (Izumi-Sano City, Japan) and Ohtemae Hospital (Osaka, Japan) were subjected to the testing. Those included 49 nasal septum deviation (NSD), 16 acute and chronic rhinitis and 39 sinusitis patients. Forty-two healthy volunteers served the control values. Using a T&T olfactometer, the examinations were conducted on one side, while the other side of the orifice was completely obstructed by the patch.

The better value of mean threshold of control, NSD, rhinitis and sinusitis group were 0.58 ± 0.83, 1.19 ± 1.02, 1.39 ± 1.10 and 2.47 ± 1.68 respectively. The control group recorded lower threshold than those of NPPs, while sinusitis group showed higher values than those of NSD and rhinitis group. Eighteen cases (54.5%) of sinusitis revealed a difference of threshold of >1.0 between the both sides. In the rhinitis group, eight cases (50.0%) demonstrated the biased affection and 10 cases (25.6%) were unequally damaged in the NSD group, while only one volunteer (2.4%) showed uneven acuity.

Among NPPs, the inflammatory pathology such as sinusitis seemed to more severely affect the olfactory acuity than NSD, which mainly obstructs the air-way tract. Examination of each unilateral olfactory threshold may be useful in clinical practice for more detailed information and be beneficial for choice of treatment.

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114. Electron microscopical observation on the human olfactory neuroepithelium

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The olfactory neuroepithelia of patients presenting with severe hyposmia or anosmia were studied by electron microscopy. After informed consent was obtained from patients, specimens were

taken from the septum as close to the roof as possible in order to avoid serious complications. Before the biopsies were taken, patients underwent evaluation of their olfactory function. The tissues were immersed in 2.5% glutaraldehyde and 2% paraformaldehyde solution buffered with 0.07 M phosphate for 2–3 h. They were post-fixed with 1% osmium tetroxide solution for 1 h at 4°C. After *en-bloc* stained in 3% uranyl acetate, they were dehydrated with a graded ethanol series, and were then were embedded in Epon 812. Ultra-thin sections obtained from the Epon blocks were doubly stained with 3% uranyl acetate and Reynolds' lead and were observed in a transmission electron microscope. Some specimens were freeze-dried with the t-butyl freeze-dry method and examined in a scanning electron microscope. The olfactory neuroepithelia obtained from patients with sinusitis and allergic rhinitis showed almost normal appearance. On the other hand, the tissues obtained from patients with idiopathic olfactory disturbance and those with upper respiratory infection/cold showed a decrease or the absence of the olfactory cells. It was noteworthy that the cells with clear cytoplasm and many free ribosomes were sometimes seen in the olfactory epithelium. We considered that they might be the immature olfactory cells.