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ABSTRACTS

List of Abstracts from the Thirty-ninth Annual Meeting of the Japanese Association for the Study of Taste and Smell

Growth factor and morphogen regulation of taste papilla and taste neuron development

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Taste papillae are ectodermal specializations in stereotypic locations on the tongue. Papilla localization determines the peripheral distribution of lingual taste buds and thereby is a key element in spatial aspects of taste coding. My laboratory studies are about development of fungiform papillae that form on the anterior two-thirds of the tongue and the ganglia that provide sensory innervation for the fungiform: the trigeminal and geniculate. To what extent do papillae and sensory neurons develop independently or via interactions? How is a developmental ''match'' established between the taste papilla organs and nerves that innervate these papillae? I used an organ culture of embryonic rat tongue to study papilla development in a system that excludes intact sensory innervation. I have demonstrated roles for sonic hedgehog protein (SHH), epidermal growth factor (EGF), and bone morphogenetic protein (BMP) family members and antagonists in papilla induction, development, and patterning. These data provide evidence for induction and patterning functions for SHH. I have shown that PI3K-AKT, ERK1/2, and p38 MAPK protein kinase cascades mediate fungiform papilla effects in response to EGF in culture and suggest roles for EGF in maintaining the interpapilla epithelium. I propose that BMPs act to inhibit new papilla development, thereby maintaining an interpapilla space, and that the inhibitory actions of BMP within forming papillae *per se* are opposed by noggin. In addition, initial data suggest that the same molecules that regulate papilla development are active in survival and differentiation of the ganglion neurons that innervate the papillae.

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Interaction of taste-aversive memory system and reward system during taste-guided learned behavior

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Conditioned taste aversion (CTA) establishes a robust long-term gustatory memory to the conditioned stimulus (CS) that has been previously associated with visceral malaise (unconditioned stimulus, US) even though a normally preferred taste stimulus such as sucrose, saccharin, or DL-alanine is used as a CS. In toxin-induced CTA, conditioned animals show aversive taste reactivity to reexposure of the CS, implying that the biological value of the CS is shifted from positive to negative after CTA acquisition. A benzodiazepine agonist, midazolam, impairs CTA expression to sucrose but not to 0.2 M NaCl or 0.01 M HCl, suggesting that CTA expression is differentially dependent on the original palatability of the CS. In our preliminary study, dopamine and opioid systems may be involved in midazolam-induced impairment of CTA expression because a D1 receptor antagonist, SCH23390, and a μ-opioid receptor antagonist, naloxone, impaired the effects of midazolam. The reward system, including dopamine and opioid systems, activated by midazolam could inhibit the neural substrate of CTA when taste of the CS is palatable.

Obsession with the luxury meal: an eating model for modern humans

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A luxury meal for modern humans has an abundance of palatable ingredients such as fat, sugar, and tasty bouillon. Obsession with luxury foods is a standout feature of humans in most industrialized countries. In this symposium, I deal with the addiction to such palatable ingredients as an eating model for modern humans. We have found that the rewarding effects of corn oil in the conditioned place preference (CPP) test are at least partly mediated via opioidergic and dopaminergic systems. Recently, we tested intact corn oil and sorbitol fatty acid esters, which have been developed as nondigestible fat substitutes with low energy. Palatability of the sorbitol fatty acid esters was similar to corn oil for over 30 min in the short-term two-bottle choice test in mice. However, mice did not continue to eat the fat substitute in a long-term two-bottle choice test. The low-energy fat substitute did not act as a reinforcer in the CPP test. Mice with 0.1 ml of corn oil placed into their stomachs just before conditioning showed reinforcing effects on taking sorbitol fatty acid ester in the CPP test. These results suggest that the postingestible effects of corn oil are involved in long-term preference and reinforcing effects. Japanese traditional ''dashi'' bouillon acts as a reinforcer as well as fat and sugar in the CPP test. In the case of the bouillon, however, the flavor was essential for inducing the rewarding effects, suggesting that flavor, taste, and energy are all involved in the effects.

Brain mechanisms of palatability

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Palatability consists of emotion and behavioral expressions elicited by ingestion of good-tasting edibles. Palatability is one of the

factors that regulates food and fluid intake, and it contributes to overconsumption. Although the neural mechanisms of palatability are yet to be clarified, previous studies in animals indicate the involvement of the cerebral cortex, limbic structures, and hypothalamus together with some neuroactive substances. Taste information is sent to the nucleus accumbens (NAcb) in the reward system and feeding center via the prefrontal cortex. The reward system contains the ventral tegmental area (VTA), NAcb, and ventral pallidum and finally sends information to the lateral hypothalamic area, the feeding center. The dopaminergic system originating from the VTA mediates the motivation to consume palatable food. The amygdala which receives taste inputs from the gustatory neuraxis also influences reward and feeding. The actual ingestive behavior is promoted by orexigenic neuropeptides from the hypothalamus. In terms of neuroactive substances, palatability is closely related to benzodiazepine derivatives and β-endorphin, both of which facilitate consumption of food and fluid by partly stimulating the reward system. The abovementioned brain regions are key components of the network underlying hedonic evaluation of taste.

Identification of novel taste bud–specific genes and their functions in gustatory sensation

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Information about taste-signaling molecules is still insufficient to understand the mechanisms of taste signal transductions, while novel taste receptors have been found recently. To identify novel taste-signaling molecules, we have searched for genes specifically expressed in taste buds by cDNA microarrays. We made a cDNA microarray containing about 3500 cDNA clones from mouse circumvallate papillae (CV) and synthesized Cy3/Cy5-labeled fluorescent probes from a single taste bud of CV or from the tongue epithelium without taste cells using the PCR-based cDNA amplification technique. The relative gene expression level was estimated from the intensity of signals of hybridization with Cy3/Cy5-labeled probes to the cDNA microarray and then candidates for taste bud–specific genes were selected. After *in situ* hybridization analyses of these candidate clones on the sections of mouse CV, 37 genes in the array were found to be specifically expressed in taste buds. Some genes expressed almost all cells of taste buds and others expressed a subset of taste cells. To predict the functions of the genes which expressed a subset of taste cells, we compared the expression pattern of these genes with known taste signal transduction–related genes. Four genes are coexpressed with taste signal transduction–related genes, raising the possibilities that the genes might have roles in taste signal transduction. To obtain more information about these genes, genetic information analyses were carried out. These studies showed that one of these gene products bind inositol triphosphate receptor subtype 3 (IP_3R3), and that it might play a role in sweet, bitter, or umami taste signal transduction.

Expression of ATP receptors in taste buds

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In taste buds, candidate neurotransmitters including acetylcholine, serotonin, noradrenaline, glutamate, dopamine, etc. were proposed. In addition to them, we hypothesize that taste bud cells utilize extracellular ATP as signaling molecules. ATP receptors can be divided into two major families based on their signal transduction mechanisms and their characteristic molecular structures: P2X ionotropic ligand–gated ion channel receptors and P2Y metabotropic G protein–coupled receptors. These receptors are expressed in a wide variety of tissues. Diverse responses to extracellular ATP have been reported in a wide range of biological systems, from single cells to whole tissues and include neurotransmission in the peripheral and central nervous system, smooth muscle contraction, exocrine and endocrine secretion, the immune response, inflammation, platelet aggregation, pain, and modulation of cardiac function. We are examining the expression patterns of ATP receptors using RT-PCR, *in situ* hybridization, and immunohistochemistry. Bo *et al.* (1999) reported that P2X2 and P2X3 receptors are expressed in nerve fibers innervating the taste buds. We have also demonstrated that P2X3 receptors were expressed in adult as well as developing rat taste buds. In addition, P2Y1 and P2Y4 receptors were detected in a subset of taste bud cells of fungiform, foliate, and circumvallate papillae. Furthermore, we performed double immunolabeling to compare the expression patterns of P2Y1 and IP3R3, SNAP-25. Many P2Y1-positive cells coexpressed with IP3R3 or SNAP-25. Although the significance of ATP receptors is not fully understood, P2Y receptors as well as P2X receptors appear to be important regulators in taste bud functions.

Formation of taste neural coding channels

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The sensory pathways of taste begin with taste receptor cells (TRCs). The interaction between chemical compounds and channels or receptors on the apical membranes produces a depolarization of the TRC either directly or indirectly. TRCs contact axons of the primary sensory neurons and transfer their message onto these neurons. Then, the message is transferred to neurons of solitary tract nucleus, from where projections spread to a number of nuclei and cortical areas of the brain. Because TRCs are replaced with an average life span of about 10 days in mammals, primary gustatory axons must continuously seek and synapse with newly differentiated TRCs. However, little is known about how a constant afferent massage for taste quality coding is maintained under such continual synaptic reconnection. We compared taste responsiveness of mouse fungiform taste cells with that of chorda tympani (CT) nerve fibers. We found no major difference between them, suggesting selective taste information processing between corresponding classes of TRCs and fibers. We examined amiloride sensitivities of regenerated and cross-regenerated CT and glossopharyngeal nerves and found that a particular type [amiloride sensitive (AS) or insensitive (AI)] of regenerating fibers selectively innervate AS or AI TRCs. These results suggest that taste information detected by TRCs

may be transferred to primary gustatory fibers without major modification by selective innervation of fibers with TRCs. A constant taste message may, thus, be conveyed to the brain.

Neural basis for aversive olfactory learning

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Preweanling young rats prior to eye opening depend on somatosensory and olfactory function for survival as they can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and somatosensory stimulation is crucial. Noradrenergic activation through the locus coeruleus (LC) by a somatosensory stimulus is implicated in olfactory learning. Within the olfactory bulb (OB), the noradrenergic innervation modulates the efficacy of dendrodendritic synapses between the mitral and granule cells. At the dendrodendritic reciprocal synapses, mitral cell activity is inhibited by GABA released from the granule cells. It is noteworthy that disinhibition of the mitral cells is a crucial step in the formation of an olfactory memory. We previously showed that intrabulbar infusion of the GABA receptor antagonist, bicuculline, facilitates olfactory learning. These results implicate the OB as a critical site for olfactory learning. Since the transcription factor, CREB (cyclic AMP response element–binding protein) is well known to be involved in plasticity, we examined whether CREB is involved in olfactory learning. Behavioral pharmacology shows that only long-term olfactory memory was prevented by CREB antisense infusion, but short-term memory was intact. Western blot analyses reveal that phosphorylated MAPK/ERK (mitogen-activated protein kinase/extracellular signal–regulated kinase) was increased for 1 h after odor exposure paired with shock, followed by increase of CREB phosphorylation for 6 h. This may be evidence suggesting that synaptic plasticity in the OB underlies aversive olfactory learning.

Functional anatomy of olfaction in the awake monkey revealed by positron emission tomography

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Noninvasive imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) can visualize the activated brain regions by olfactory stimuli in the human. They have demonstrated that olfactory stimulation activates the prepyriform area (PPA) and the orbitofrontal cortex (OFC). On the other hand, electrophysiological studies have demonstrated patterns of neural activities during olfactory stimulation in the PPA and OFC of the monkey. To complement the gaps between the studies of human imaging and electrophysiology in the monkey, we developed the PET system to map the brain functions in the awake monkey. Our studies have revealed the olfactory stimulation–induced increment of regional cerebral blood flow (rCBF) not only in the PPA and OFC but also in the amygdala and cerebellum in the monkey. Using this system, we effectively measured the changes in rCBF during olfactory stimulation of a mixture of hexenol and hexenal, which are purified from green leaves and have physiological functions of calming autonomic stress response in the rat and decreasing the amplitude of event-related potential (P300) in humans. The odors of hexenol and hexenal, but not acetic acid and isoamylacetate, increased rCBF in the anterior cingulate cortex, though all of them commonly increased rCBF in the prepyriform area. These findings suggest that the selective increases of rCBF in the anterior cingulate cortex by hexenol and hexenal may contribute to stress release.

Neuronal identity and projection of olfactory sensory neurons in the mouse

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We have analyzed olfactory sensory neurons (OSNs) for their odorant receptor (OR) gene expression and their axonal projection to the olfactory bulb (OB). *In situ* hybridization with various OR gene probes demonstrated that each OR gene is expressed in a restricted area in the olfactory epithelium (OE). It has been reported that OSNs expressing a given OR gene are confined to one of the four OE zones but are randomly distributed within the zone. However, for most class II OR genes, the expression area in the OE appears to be unique to each OR gene and does not always fit into one of the conventional zones. No clear boundaries or borders appear to be present between the neighboring zones. For ~80 class II OR genes examined thus far, their expression areas are distinct and distributed in an overlapping and continuous manner along the dorsomedial/ventrolateral (DM/VL) axis of the OE. DiI retrograde staining experiments demonstrated that the dorsal/ventral (D/V) arrangement of glomeruli in the OB is correlated with the expression areas of corresponding ORs along the DM/VL axis in the OE. In contrast, the anterior/posterior arrangement of glomeruli appears to be independent of the epithelial locations of OSNs and more dependent on the expressed ORs. The present study indicates that the spatial information within the OE plays an important role not only in the axonal projection of OSNs along the D/V axis of the OB but also in the choice of the OR genes in the OSNs. The OR gene choice appears to be more restricted by the OSN location and not totally stochastic within the conventional zones.

Taste characteristics of sugar alcohols in C57BL/6 mice

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Some sugar alcohols are widely used as anticarcinogenic sweeteners. The behavioral and receptor characteristics of these sweeteners,

however, are not well understood. Therefore, in the present study, we conducted behavioral and electrophysiological experiments in C57BL/6 mice. As sugar alcohols, mannitol, xylitol, sorbitol, and palatinit were used. In the behavioral experiment, a 48-h two-bottle preference test, one of the above sweeteners versus distilled water was carried out to investigate the preference for sugar alcohols. The mice preferred 0.3 M sugar alcohols, except mannitol, rather than distilled water: the preference percent for xylitol, palatinit, and sorbitol was more than 50%. On the other hand, preference for mannitol was about 30%. When the short-term (10 min) two-bottle preference test was carried out to avoid postingestive effect, mannitol was also rejected rather than distilled water. In the electrophysiological study, to elucidate the stimulating effectiveness of these sweeteners on the sweet taste receptor, chorda tympani nerve responses to 0.3 M sucrose and four sugar alcohols were compared before and after tongue treatment with 2% pronase E or 80 μM gurmarin. The response to sucrose was suppressed by these treatments ($P < 0.01$, *t*-test), whereas pronase E did not suppress the response to mannitol, and gurmarin did not suppress responses to mannitol, xylitol, and sorbitol ($P > 0.05$, *t*-test). These results suggest that the taste receptor mechanisms for sugar alcohols are different from one another.

Central neuropeptide Y induces gastric relaxation via Y1 receptors in the dorsal vagal complex of the rats

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The effects of neuropeptide Y (NPY) on motility of the proximal stomach was examined in anesthetized rats. Intragastric pressure (IGP) was measured using a balloon situated in the proximal part of the stomach. The administration of NPY into the fourth ventricle induced relaxation of the proximal stomach in a dose-dependent manner. Administration of a Y1 receptor (Y1R) agonist (Leu31, Pro34) NPY induced a larger relaxation than NPY. The administration of a Y2 receptor agonist (NPY 13-36) did not induce significant changes in motility. Microinjections of (Leu31, Pro34) NPY into the caudal part of the dorsal vagal complex (DVC) induced relaxation of the proximal stomach. Contrastingly, similar injections into the intermediate part of the DVC increased IGP of the proximal stomach. The administration of NPY into the fourth ventricle did not induce relaxation after bilateral injection of the Y1R antagonist (1229U91) into the caudal DVC. These results indicate that NPY induced the relaxation in the proximal stomach via Y1R situated in the DVC. Since both the bilateral sectioning of the vagi below the diaphragm abolished the relaxation induced by the administration of NPY into the fourth ventricle, the relaxation induced by NPY is mediated by vagal preganglionic neurons. The intravenous injection of atropine methyl nitrate reduced the magnitude of the relaxation induced by the administration of NPY into the fourth ventricle; therefore, the relaxation induced by NPY is mediated by peripheral cholinergic neurons.

The microinfusions of $GABA_A$ receptors antagonist into the ventral pallidum disrupt the retrieval of conditioned taste aversion in rats

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To elucidate the role of the ventral pallidum (VP) on the expression of conditioned taste aversion, we examined the effects of microinjections of a $GABA_A$ receptor antagonist, bicuculline, on the intake of conditioned stimulus (CS) in the retrieval test. We measured the intake of CS using the one-bottle test (Experiment 1) and observed the ingestive and aversive behavior to CS using intraoral cannula (Experiment 2). Rats received 5 mM saccharin or 0.3 mM quinine hydrochloride as CS along with an i.p. injection of 0.15 M lithium chloride. After this conditioning, vehicle or bicuculline (12.5–200 ng) was bilaterally infused into the VP immediately before reexposure to the CS. In Experiment 1, microinjections of bicuculline significantly increased the intake of the saccharin CS but not the QHCl CS. In Experiment 2, while the control rats injected with vehicle showed a variety of aversive responses (e.g., gapes, chin rubs, head shakes, forelimb flails), the rats with infusion of bicuculline failed to show aversive responses. These results indicate that the blockade of $GABA_A$ receptors in the VP by microinjections of bicuculline disrupts the retrieval of CTA, and this may be due to elimination of aversive responses to the saccharin CS. Thus, it is suggested that the GABAergic system in the VP plays an important role in the retrieval of CTA when the CS is saccharin, indicating the existence of distinct neural circuits between learned aversion and innate aversion.

Circadian rhythms in sweet taste sensitivities and their relation to plasma leptin levels in human

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Leptin is a hormone that regulates food intake, energy expenditure, and body weight. It is primarily produced in adipose cells but also has been found in some other peripheral organs. Recently, we found that the taste cells are one of the peripheral targets for leptin, and in mice leptin suppresses behavioral response to sweet substance through its action on Ob-Rb in taste cells. In humans, our previous study showed that there is a circadian rhythm in the sucrose threshold that may be parallel with that in the plasma leptin level. The present study further investigated the relationship between sweet taste sensitivities and plasma leptin level under various meal conditions. Analyses were made based on the comparison between two groups of subjects classified with their plasma leptin levels (the high and low leptin groups). Under normal food intake with three meals, the threshold for glucose gradually increased from noon to midnight. This circadian rhythm was clearly observed in the low leptin group, but the rhythm was not evident in the high leptin group. Circadian rhythms for sucrose threshold were

observed both the groups. In contrast, under fasting condition with one or two meals, the circadian rhythm for plasma leptin levels did not become, and the rhythm for glucose and sucrose thresholds disappeared in both the groups. These results suggest the possibility that the circadian rhythm for sugar taste sensitivities synchronizes with that for plasma leptin levels. Difference in the glucose sensitivity rhythms between two leptin groups may imply higher dependence of glucose sensitivity upon the plasma leptin level.

Swallowing reflex elicited by chemical stimulation of the pharyngolaryngeal region and tongue in humans

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In humans, distilled water (DW) applied to the posterior tongue is effective for elicitation of swallowing reflex, suggesting that excitation of water receptors (W-Rs) elicits the swallowing reflex. However, the precise sites of W-Rs remain unclear. In the present study, the properties of the W-Rs responsible for the swallowing reflex in humans were investigated. Each subject was instructed to repeat swallowing as fast as possible. DW or 0.05–0.3 M NaCl solution was delivered to the posterior tongue through a fine tube at a slow rate of 0.2 ml/min. The tip of the tube was located in the pharyngolaryngeal region (PL-R), where 0.3 M NaCl did not give rise to salty taste. The intervals between two consecutive swallowings in a test were measured. The swallowing intervals were shortest when DW was used, and the interval increased with increase in NaCl concentration. The mean interval when 0.3 M NaCl was used was the same as that in the case of olive oil, which does not activate chemoreceptors, suggesting that 0.3 M NaCl inhibits excitation of W-Rs. The present results suggest that W-Rs for swallowing reflex reside in the PL-R, whereas salt taste receptors (S-Rs) do not reside in this region. The tip of the infusion tube was moved to the anterior tongue. In several subjects, it was shown that infusion of 0.15 M NaCl led to shorter intervals than did DW. This suggests that S-Rs for swallowing reflex reside in the tongue, whereas W-Rs do not reside in this region.

Chemosensory behavior of the deep-sea isopod *Bathynomus doederleini*

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To demonstrate that the deep-sea isopods *Bathynomus doederleini*, collected from the 600-m-deep sea of the Suruga Bay, use keen chemical senses to localize their foods without visual clues, their searching behaviors for their food in an experimental arena in the dark were studied using a backpack-type infrared LED flasher tag. Twenty-one trials with different individuals starved for 3–5 days were taped with a digital video camera for a period of 10 min before and after introduction of meat extracts of saury fish into the arena. The recorded tape data were transformed into the image sequence data and analyzed with a newly developed software, PixelProbe 1.0 for Windows, on a computer. After introduction of the chemical stimulus, the moving distance for initial and the last 5-min periods increased in 80.9% (17/21) and 61.9% (13/21) of trials, respectively. The angular velocity of body axis and the moving velocity increased or decreased significantly, depending on the location of tested individuals in the arena when the stimulus was introduced in 57.1% (12/21) and 42.9% (9/21) of trials (Kolmogorov–Smirnov test, $P < 0.0001$), respectively. The success rate for localization of the stimulus introduction site was 93.3% (14/15). The results indicate that the deep-sea isopods can search and localize their foods solely by clues of the chemical senses.

Localization of a putative pheromone receptor and Gai2 in goat olfactory epithelium

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Most mammals have two nasal chemosensory epithelia [olfactory epithelium (OE) and vomeronasal epithelium]. In rodents, the apical surface of olfactory receptor neurons (ORNs) and vomeronasal receptor neurons (VRNs) are covered with cilia and microvilli, respectively. The olfactory signal transduction pathway is involved in Golf, whereas pheromonal signal transduction pathway is involved in Gi2 or Go. And these three G proteins are mainly located on cilia or microvilli. In previous study, the goat pheromone receptor gene (gV1ra1) was expressed in not only VRNs but also ORNs. In this study, we investigate whether goat OE has the ability to detect pheromones. We examined the expression pattern of the molecules in the pheromone signal transduction pathway such as Gi2 and transient receptor potential channel 2 (TRP2). Gi2 protein was located on the apical surface of a small subset of cells. Double-labeled *in situ* hybridization revealed that Gi2 and V1ra1 were expressed in the same cell. The expression of TRP2 could not be recognized in goat OE. It was also examined whether V1ra1-Gi2 (+) cells expressed olfactory marker proteins (OMP) and/or protein gene product 9.5 (PGP9.5) which are expressed in ORNs and VRNs. V1ra1-Gi2 (+) cells coexpressed with PGP9.5 but not OMP. Transmission electron microscopic observations revealed that there were microvillous neurons in OE and suggested that two subsets of receptor neurons exist in goat OE that are ciliated receptor neurons and microvillous ones. Thus it is concluded that goat OE has VRN-like ORNs. We would like to suggest the possibility of pheromonal detection in goat OE.

The mere exposure effect of fragrance

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It is known as the mere exposure effect, and humans come to form a favorable attitude to stimuli which they come into contact with repeatedly. This examination was done to prove the mere exposure

effect of fragrance as olfactory sense stimuli by examining the change in preference of fragrance to which one comes into contact with repeatedly. As a result, the mere exposure effect of fragrance was proved with higher preference of fragrance by those who smelled before rating than by those who did not smell. No effect was observed when one rated fragrance preferences every single time of smelling. Thus, it is suggested that it was indispensable to come into contact with stimuli subconsciously before rating it as the same as all the preceding researches carried out by using stimulation other than fragrances. Also the suggestion such as there is no relationship between recognition memories and arousal of fragrance preference was obtained from this examination. And regarding each fragrance particularly, the characteristic of fragrance was conceivable as the factor that the preference of fragrance changed. Therefore, we will investigate and clarify the characteristic of fragrance that will be functional on raising preference. And, also, we will keep working in this study field to utilize the results on developing new fragrance products which will be favorites for long.

Kansei evaluation and its structure analysis on processed meat products by fruits flavor

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Eating quality comprises several different factors: chemical (taste and flavor) and physical (temperature, appearance, and sound) actualized properties of the food as well as latent influences such as past experience and the mental and physical state of the consumer. In the field of Kansei engineering, the relationship between several evaluation factors is called a Kansei interaction. The purpose of this study is to clarify the possibility by using fruits flavors on processed meat products. The control sample is a plain sausage with no added flavors. Three samples are flavored with different fruits (Flavors A, B, and C). The eating quality of the sausages is rated using nine evaluation terms. After eating a sausage set, the panel assigns positive points if they rate the flavored sausage more highly in each of the eating quality categories. The two analytical methods used in this study are 95% critical intervals of means and graphical modeling, a type of causal analysis. The data obtained by panels with no difference in 95% critical interval of means were analyzed in detail using the graphical modeling method. Consequently, the hierarchy was found in a particular flavoring. The results suggest that fruits flavored on processed meat products developed more deliciousness.

Development of taste sensing with electric and optical measurements

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The experimental study of taste sensing has been carried out with the measurements of electric potential difference, admittance, and optical absorbance. The substances with different taste qualities were solved in the 10 mM KCl aqueous solution. The electric potential difference has been measured between two detecting electrodes immersed in the 10 mM KCl (containing taste substances) and 1 M KCl solutions isolated by the lipid/polymer membrane. Resulting from the increase in the ion concentration of the taste substance–added 10 mM KCl solution, the output shows different patterns for the different taste substances. The electric potential measurement, therefore, has sensitivities to electrolytes such as sour and salty substances and insensitivities to nonelectrolytes such as sweet substances. For improving the sensitivity to sweet taste substances, we measured the admittance and optical transmittance of the taste substance–added 10 mM KCl solution. Sweet taste substances such as sucrose decrease the admittance and the optical absorbance of the ~950 nm band. It should be indicated that the sweet substances adsorb the ions in the solution and break up the hydrogen bonds of aqueous molecules. We could make an improvement of the taste-sensing system by a combination of these different measurements.

Study of bad-smell sensing network using gas detector tube and CCD image sensor

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Recently, the deterioration of the environment caused by bad smell and volatile organic compounds has become one of the problems in our daily life. The ministry of the environment of Japan reported that the number of complaints against bad smells was increasing recently. Thus, a rapid and low-cost sensing system is required. Although the GC/MS (gas chromatograph/mass spectrometry) method is often used to analyze gases, it is expensive and time consuming. Moreover, skill for handling the equipment is required. We focused on a gas detector tube to perform bad-smell sensing for monitoring the living environment since the gas detector tube technique is well known as a simple method for gas measurement. It is cheap and is easy to handle. In the gas detector tube, the fundamental function of the chemical reaction between the analyte and the reagent system is to form colored compounds that make the reaction visible. Although it is generally difficult to obtain selective and stable sensor response to ppb-order gas, our system using a gas detector tube and a one-dimensional CCD image sensor enabled the detection of methyl mercaptan, hydrogen sulfide, and propionaldehyde with the concentrations down to a few tens of ppb. Since the developed system has wireless LAN capability, we can construct bad-smell sensing networks easily. We measured gas distribution of propionaldehyde indoors. The distribution of bad smell within the area [20 × 2 (m)] was monitored using our developed portable sensors. The gas plume shape caused by natural wind was clearly observed using the proposed bad-smell sensor network.

Bitter inhibitory effect of fatty acids: analysis by psychophysical, molecular biological and neuroethological studies

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The effect of unsaturated fatty acids on taste sensitivity was explored by a human psychophysical study and a molecular biological study using *in vitro* G-protein activation assay and a mouse neuroethological study. In the human psychophysical study, lingual application with docosahexaenoic acid (DHA) significantly suppressed bitterness of QHCl without affecting the perceived taste intensity measurement of NaCl, sucrose, and MSG. Consistently, the results from the mouse behavioral experiment using a short-term lick test (10 s) showed a significant increment in licks for bitter stimuli including QHCl and denatonium, but not for other salty sweet and sour stimuli, after DHA. Inhibitory effects of fatty acids on responses to bitter substances including QHCl and denatonium were also observed in the recordings from the chorda tympani (CT) and the glossopharyngeal (GL) nerve in wild-type mice, although responses to bitter-tasting L amino acids, NaCl, HCl, sucrose, and MSG were not inhibited by fatty acids. The order of suppressive effect of fatty acids was DHA > linoleic acid > eicosapentaenoic acid = oleic acid. In contrast, gustducin KO mice showed no such suppression in bitter taste responses both in the CT and the GL. Results from the *in vitro* G-protein activation assay using the bovine taste membrane showed that the activation of both gustducin and transducin by denatonium was significantly inhibited by DHA and oleic acid. The inhibitory effect of DHA was stronger than that of oleic acid. These results suggest that fatty acids specifically inhibit responses to bitter stimuli by suppression of activation of T2R receptors which coupled with G gustducin and G transducin.

Amiloride sensitivity of the chorda tympani nerve response to NaCl in 129X1/SvJ mice

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It is known that there is a prominent mouse strain difference in the effect of amiloride on the chorda tympani (CT) nerve responses to NaCl. For example, in C57BL mice, amiloride suppresses NaCl responses to about 50% of control, whereas no clear amiloride inhibition was observed in 129 mice. The 129 inbred strain, however, has a number of substrains derived mainly from two major parent stocks, 129/J and 129/SvJ. Recently, 129X1/SvJ (formerly 129/SvJ) mice are reported to differ from the 129P3/J (formerly 129/J) strain by 25% of sequence length polymorphisms. In the current study, therefore, we examined possible substrain difference between 129P3/J and 129X1/SvJ in the amiloride sensitivity of the CT response. The results suggest that amiloride is effective in 129X1/SvJ mice. CT responses to 0.3 M NaCl were significantly suppressed by amiloride at the concentration of 10 μM or more, and the inhibition reached the maximum (about 50% of control to 0.03–0.3 M NaCl) at 100 μM. In contrast, no such amiloride inhibition was evident in 129P3/J mice. These results suggest that amiloride sensitivity of NaCl responses differ among 129 substrains.

Analysis of individual differences in human sweet taste sensitivity

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It is reported that the T1r2/T1r3 heterodimer is responsive to a wide range of sweeteners. Mice lacking T1r3 showed no preference for artificial sweeteners and had diminished but not abolished behavioral and nerve responses to sugars, suggesting that T1r3-independent sweetener-binding sites also exist in taste cells in mice. However, the numbers and functions of ligand-binding sites on T1r2/T1r3 (and/or other sweet receptor) remain largely unknown. In this study, in order to predict the number of sweetener-binding sites in humans, we measured sensitivity thresholds to various sweet taste substances (sucrose, glucose, fructose, saccharin, aspartame, acesulfame-K, glycine, D-phenylalanine, D-tryptophan, and L-proline) in 58 human subjects and examined the qualitative similarities among these sweeteners by using a hierarchical cluster analysis. We also used gymnemic acid and γ-cyclodextrin, which selectively inhibit sweet responses and reduce the inhibitory action of these responses in humans, respectively. The cluster analysis showed that individual sweet sensitivities were classified into 15 different groups. The 10 sweet compounds were classified into five groups [1) sucrose, glucose and fructose, 2) saccharin, aspartame, acesulfame-K and glycine, 3) D-phenylalanine, 4) D-tryptophan, (5) L-proline]. These results suggest that there may be at least five different sweetener-binding sites on T1r2/T1r3 (and/or other sweet receptor) in humans. The individual differences in human sweet sensitivities may be due to the differences in the binding function of these sites. So, we are investigating the relationships between these individual differences and single-nucleotide polymorphisms in T1r2/T1r3.

Gustatory responses from the soft palate in mice

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Gustatory responses from the soft palate in C57BL mice were studied electrophysiologically. Integrated taste responses from the greater superficial petrosal nerve (GSP) and chorda tympani nerve (CT) to NaCl, HCl, sucrose (Suc), and quinine HCl (QHCl) at various concentrations were recorded. The results showed that QHCl produced large phasic and tonic responses relative to the other three stimuli in the GSP, although NaCl was most stimulatory in the CT. Similar results were obtained in C3H mice. To determine the relative importance of the taste information mediated by the GSP for QHCl, the effects of bilateral transection of the GSP, the glossopharyngeal nerve (GL), the CT, or both the GSP and CT on licking behavior in mice for QHCl at 0.0001–0.003 M was studied. The effect of the CT transection was less as the number of licks to 0.003 M QHCl was ~20% of that to deionized water (DW); however, for GSP-transected mice, the number of licks to 0.003 M QHCl increased by ~50% and by ~75% in the GSP + GL-transected mice. The large responsiveness to QHCl in mice is completely different from that in the GSP of rat and hamster which respond greatly to sweet substances. Functional differences in soft-palate taste buds for bitter substances among species of rodents suggest that not only

are there gustatory receptive mechanisms but also fundamental differences exist in the development of the associated neural networks since the behavioral responses are instinctively different—that is, aversive for QHCl and preferable for sweet substances.

Participation of Giα family in umami transduction

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Umami is one of the basic tastes, which is proposed for the receptor-mediated mechanism. Monosodium glutamate (MSG) and inosine monophosphate (IMP) are umami substances, and this mixture produces a synergistic effect. Several G protein–coupled receptors for umami substances have been identified. The umami signal is thought to transduce via these receptors. On the other hand, although G protein coupling to umami receptors is suggested, it is not still identified. In order to estimate G protein involvement in umami transduction, using mouse taste receptor cells (TRCs) treated with inhibitory G proteinα-subunit (Giα) inhibitor pertussis toxin (PTX; 500 ng/ml at 37°C for 4 h), we measured the intracellular Ca^{2+} level change by umami stimuli (10 mM MSG and 10 mM MSG + 0.5 mM IMP). In TRCs untreated with PTX, 23% of TRCs (15/62) responded to 10 mM MSG and 27% of TRCs responded to 10 mM MSG + 0.5 mM IMP. In TRCs treated with PTX, 9% of TRCs (6/66) responded to 10 mM MSG and 9% of TRCs (6/66) responded to 10 mM MSG + 0.5 mM IMP. The proportion of TRCs that respond to umami stimuli decreased to approximately one-third by PTX treatment. These results suggest that umami signal transduces mainly via Giα containing α-gustducin.

Responses of taste disk cells elicited by arachidonic acids in frogs

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Lingual lipase is secreted into the oral cavity, digests fat into free fatty acids and 2-monoglycerides, and free fatty acids may in turn stimulate taste cells. In the present study, we investigated the responses elicited by arachidonic acid (typical unsaturated fatty acid) while recording the electrophysiological properties of isolated taste disk cells in bullfrogs. When wing cells were exposed to 10 μM arachidonic acid (AA), the cells displayed parabolic inward currents. The currents conducted poorly at the membrane potential of −80 mV despite the presence of AA, but their conductance increased at a resting potential of −50 mV. AA (10 μM) elicited inward currents of −19 ± 5 pA ($n = 4$) at −50 mV in wing cells. Higher concentrations (50 μM) of AA induced larger inward currents and subsequent outward currents in two wing cells but only parabolic inward currents in the other two wing cells. Rod cells (four cells) displayed transient decrease of outward currents and subsequent increase of the currents in response to 50 μM AA. A stable analog of arachidonic acid, eicosatetraynoic acid (ETYA, 10 μM), decreased the outward currents in wing cells but did not induce inward currents as did arachidonic acid. AA (10 μM) and ETYA (10 μM) decreased the voltage-gated Na^+ and K^+ currents to 40–60% of the controls. The results suggest that arachidonic acid or its metabolite can induce a novel conductance in frog taste disk cells.

Taste responses in frog taste disk cells and the glossopharyngeal nerve

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Taste disk cells in the taste organ of frog tongue have been classified into several types on the bases of morphological and electrophysiological features. However, the function of each type of cell has not yet been clarified. This lack of clarity is at least partly due to the fact that taste responses have been separately recorded either from taste disk cells or the glossopharyngeal nerves. The present study simultaneously recorded taste responses from taste disk cells and the glossopharyngeal nerve in the frog, *Rana catesbeiana*. A taste disk in the fungiform papilla together with a branch of the glossopharyngeal nerve was dissected from the isolated tongue. Using fine scissors under binocular vision, the taste disk was cut vertically into a slice containing the nerve. Neural activities were recorded extracellularly from the glossopharyngeal nerve using a glass capillary suction electrode. Responses in taste disk cells were obtained using the whole-cell patch-clamp technique. The cell bodies of those cells were located at the middle or lower layers of the taste disk. Injection of current pulses triggered action potentials in those cells under current-clamp conditions. When Ca^{2+} or quinine was applied to the taste disk, depolarization was evoked in those taste disk cells and action potentials were recorded in the glossopharyngeal nerve. Experiments using the present sample will provide a better understanding of the connection between the taste disk cells and the nerve.

Characteristics of A-currents of morphologically identified cells of the frog taste disk

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Frog taste disks contain morphologically and physiologically diverse types of cells. It has been reported that type Ib, type II, and type III cells were electrically excitable. Although the presence of transient outward currents (A-currents) were reported in type Ib cells, little is known about the correlation between properties of A-currents and cell types. We report here the properties of A-currents in three types of cells. Whole-cell patch-clamp recordings have been made from frog fungiform papilla slice preparations. The electrode contained Alexa Fluor 488 hydrazide to fill the recorded taste cells for subsequent identification. We recorded voltage-gated K^+ outward currents from all recorded type Ib, II, and III cells. By application of 600-ms depolarizing voltage-clamp steps from 200 ms holding potential of −130 mV, we could record A-currents. Of the six type Ib cells tested, four cells did not show A-currents; however, all type II and III cells exhibited A-currents. The inactivation process of A-currents was different among cell types. Time

constants of inactivation could be fit using a single exponential in type II cells. On the other hand, time constants of inactivation could be fit using a double exponential in type Ib and II cells. The present findings suggest that the kinetics of A-currents is different among the three types of cells.

Effects of osmotic pressure on bullfrog salt responses

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Taste stimulations simultaneously change chemical compositions and osmotic pressure on taste receptors. We investigated the effect of osmotic pressure on bullfrog taste nerve responses to inorganic salts where the osmotic pressure was increased by the addition of either urea or sucrose to stimulating salt solutions. Both urea and sucrose alone elicited negligible neural responses up to 1 M. The magnitude of tonic response to 0.5 M NaCl was increased by a factor of 2.7 with 1 M urea. The addition of 1 M urea to the concentration series of NaCl increased response magnitude without changing its threshold concentration. In contrast, the magnitudes of tonic responses to 1 mM $CaCl_2$ were suppressed with urea at concentrations higher than 0.6 M. The control concentration–response relation for $CaCl_2$ peaked at 1 mM. The addition of 1 M urea suppressed the peak magnitude and increased the response magnitude with increasing $CaCl_2$ concentrations. Sucrose similarly modified the response magnitude to these salts as did urea. The addition of 1 M urea to various salts of 0.5 M differently increased the response magnitude of the salts. The extent of increase depended on the differences between the mobility of each cation and anion forming the tested salt. It is suggested that the osmotic pressure increases the conductance of tight junctions and enhances diffusion potentials across these junctions, which depolarizes or hyperpolarizes the basolateral membrane of taste receptor cells and modifies the neural responses.

Inhibitory effects of *l-cis*-diltiazem on the responses of the taste cells in the blowfly, *Phormia regina*

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The taste organs in flies are hair-shaped sensory units individually housing four functionally differentiated taste cells, which preferentially respond to sugars, salts, water, and possibly repellents, respectively. Our recent researches have suggested that the sugar receptor cells in the blowfly, *Phormia regina*, employ the nitric oxide (NO)–regulated transduction mechanism. Soluble guanylyl cyclase (GC) was raised as a possible target of NO in the transduction based on pharmacological analysis of the electrophysiological records. Soluble GC is known to produce cGMP that has also been predicted as a second messenger in the sugar receptor cells. To explore a target of cGMP, we examined the effects of a cyclic nucleotide–gated (CNG) channel blocker, *l-cis*-diltiazem (kindly presented by Tanabe Seiyaku Co., Ltd.), on the responses of the sugar receptor cells in *P. regina* toward sucrose or fructose. The responses were recorded by the "tip-recording" method which enabled us to examine the impulse activity of fly taste cells. Application of 10–50 μM *l-cis*-diltiazem into a stimulating solution partially and reversibly suppressed the responses of the sugar receptor cells in a dose-dependent manner. However, the same concentration of *l-cis*-diltiazem did not affect the response of the salt or water receptor cells. The results support the hypothesis that CNG channels function in the transduction mechanism of the sugar receptor cells.

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Depression of the neural taste responses by chloroform and bromoform in carp

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To check the effectiveness of chloroform and bromoform on the taste-receptive membranes of carp (*Cyprinus carpio* L.), we electrophysiologically recorded the neural taste responses to L-amino acids during applications of several concentrations of the two chemicals in the artificial freshwater bathing the taste epithelia. The two compounds slightly elicited neural excitation by themselves and do have some effects on taste responses. Taste responsiveness for L-Ala and L-MSG in 1 mM were significantly depressed with the increase of concentration of chloroform and bromoform (0.001–0.1 mg/l). It is obvious that the latter strongly affected the taste responses than the former at the same concentrations. These results indicated that although chloroform and bromoform depressed the neural responses of the taste system somehow, it is not clear from the present experiments whether these chemicals might cause an anesthetic state on the taste membrane.

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Synapse-associated proteins in the developing taste buds of the rat

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Traditionally, taste bud cells were categorized into at least three types: spindle-shaped dark, light, and rounded basal cells. Spindle-shaped light cells are thought to be taste cells and are further subdivided into two types: type II and type III. Type II cells have connection but do not make synaptic contacts with taste nerves, and type III cells make synaptic contacts with taste nerves. In the central nervous system various synapse-associated proteins are present. These proteins have been also present in the peripheral gustatory system. However, the relationship between these two types of light cells is still unknown. In the present study, we employed double immunohistochemistry for synapse-associated proteins, such as SNAP-25, syntaxin, synaptobrevin and Gα-gustducin, and protein-gene product 9.5 (PGP 9.5), a marker for type II cells and type III cells to rat circumvallate papillae. All synapse-associated

proteins were localized in both intragemmal cells and perigemmal nerve elements, and not only PGP 9.5–immunoreactive cells but also Gα-gustducin–positive cells were immunopositive for these synapse-associated proteins. The presence of these synapse-associated proteins in both type II (Gα-gustducin positive) and type III cells (PGP 9.5) suggests a cell lineage between type II and type III cells.

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Jacalin bindings in the gustatory epithelium of the adult rat

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Lectins are often used as markers of several types of epithelium or mesenchymal cells to identify particular cell populations on the basis of different abilities for binding with high specificity to different glycohydrate epitopes. Several lectin histochemical studies have been performed in the taste buds of various mammals. However, the binding pattern of Jacalin (*Artocarpus integrifolia*), a specific lectin for galactosyl (β-1, 3) *N*-acetylgalactosamine, has not been studied. In the present study, we report the distribution of Jacalin bindings and their relationship with other biochemical markers in the adult rat. Adult Spargue–Dawley male rats, weighing 200–250 g, were perfused with 4% paraformaldehyde and lingual papillae, nasoincisor papillae, and soft palates were embedded in paraffin wax. The lectin histochemistry of Jacalin was applied. In some cases, combined lectin histochemistry for Jacalin and immunohistochemistry for α-gustducin or protein gene product 9,5 (PGP 9.5) were performed. In ordinary lingual epithelium, Jacalin labeled the cell membrane from basal to granular cell layer. In the gustatory epithelium, Jacalin labeled membranes of rounded cells at the basal portion of taste buds, and there was no apparent difference in the binding pattern in the taste buds of the lingual papillae and those of the palatal epithelium. Occasionally, few spindle-shaped cells were labeled with Jacalin. Double labeling revealed that Jacalin-labeled spindle-shaped cells were devoid of α-gustsducin and PGP 9.5. The present results indicate that Jacalin is a specific marker for type IV cells.

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Expression of the marker genes for the basal cells of taste buds during mouse embryogenesis

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Taste buds are maintained under continuous cell renewal. BrdU pulse-labeling studies showed that Sonic hedgehog (Shh)–expressing cells differentiated earlier than Mash1-, gustducin-, and T1r3-expressing cells in the taste buds of adult mice. Examination of the change of gene expression in taste buds after taste nerve transection revealed that, although gene expression in fusiform cells was almost autonomous, Shh expression in the basal cells was strongly dependent on the taste nerves. These results suggest that the differentiation of the basal cells in taste buds may have an important role in taste bud maintenance. However, relatively little is known about the basal cell differentiation in the taste buds, including the timing of differentiation. Shh-expressing cells in the basal region of taste buds coexpress the homeodomain transcription factor, Prox1, and sometimes Mash1. And we have previously reported that the coexpression of Shh and Prox1 was observed at 0.5 days after birth in circumvallate papillae. Then we examined the expression of the basal cell marker genes for taste buds such as Shh, Prox1, and Mash1 during embryogenesis and analyzed the coexpression patterns of these genes.

The expression of type III cell marker NCAM and possible cell lineage relationships in mouse taste buds

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Mammalian taste buds are composed of cells in various states of differentiation since taste receptor cells are continuously differentiated from their precursor cells in taste buds. The cells in taste buds are classified into four cell types, type I, II, III, and basal cells, based on morphological characteristics, and the molecular markers for each cell type have been reported. However, the lineage relationships among these four cell types remain uncertain. NCAM has been regarded as a type III cell marker; and about 98% of Mash1-expressing cells showed NCAM immunoreactivity, suggesting that Mash1-expressing cells should be categorized as type III cells. We also found, however, that NCAM was expressed in 12.9% of gustducin-expressing cells and 7.7% of T1r3-expressing cells of adult mice, although gustducin has been thought to be expressed in type II cells. Examinations of developing taste buds showed temporal changes in the ratio of NCAM-IP cells in gustducin- and T1r3-expressing cells; the ratio of NCAM-IP cells in these gene-expressing cells were about 90% at 0.5 days after birth and decreased markedly during development. In contrast, the majority of Mash1-expressing cells showed constant NCAM immunoreactivity throughout development. In addition, BrdU-labeling experiments showed that the differentiation of Mash1-expressing cells precedes those of gustducin- and T1r3-expressing cells in the taste buds of adult mice. These results suggest that type II cells expressing gustducin or T1r3 might be derived from type III cells expressing Mash1.

Detection of transcription factor and neurotrophic factors in the taste buds by laser-capture microdissection

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Taste buds were collected from mouse circumvallate papillae by laser-capture microdissection. We examined whether NeuroD, nerve

growth factor (NGF), glial cell–line derived neurotrophic factor (GDNF), neurturin (NTN), artemin (ARTN), persephin (PSPN), neurotrophin (NT)-3, NT-4/5, and their receptors, that is, TrkA, p75NTR, GFRα-1, GFRα-2, GFRα-3, GFRα-4, TrkC, and/or RET were expressed in the taste buds by using real-time PCR and RT-PCR as well as the immunohistochemical approach. NeuroD, a transcription factor, expressed immunohistochemically in Type II cells, and its mRNA was exclusively expressed in the taste buds. NGF and its receptor TrkA, which were expressed in all cell types in taste buds, also were detectable at the mRNA level in the taste buds. GDNF, which is expressed immunohistochemically in Type II cells, and its receptors GFRα-1 and -2, found in all cell types of taste buds, were detected in the taste buds at the mRNA level. The immunoreactivity for RET was observed in the taste buds, and its mRNA was also present in them. The immunoreactivity for NT-3 was reported to be expressed in the taste buds of hamster, and its mRNA was also detected in the mouse taste buds. TrkC, the receptor for NT-3 was detected at mRNA level. NT-4/5 was detected immunohistochemically as well as by PCR. The results suggest that these neurotrophic factors, their receptors, and the transcription factor, NeuroD are synthesized by the taste buds themselves. The mRNAs of p75NTR, NTN, ARTN, PSPN, GFRα-3, and GFRα-4 were not expressed in the taste buds.

Taste preferences in male and female rats

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Sex-related variations in taste preferences have long been known to exist in humans and animals. However, the neurophysiological basis of these variations has not yet been fully analyzed. Therefore, behavioral and electrophysiological studies were performed in male and female Wister rats. In Experiment 1, the intake of each taste solution (0.03, 0.1, 0.3 M NaCl; 0.025, 0.1, 0.3, 0.5 M sucrose; 2.5, 1, 5, 10 mM Na-saccharin; 0.01, 0.1 mM quinine-HCl; 0.01, 0.03 M HCl; 0.01 M inosine monophosphate, IMP; 0.01 M monopotassium glutamate, MPG, IMP + MPG; and 2.5%, 5%, 10% corn oil) was measured for 48 h by the two-bottle test with distilled water as the counterpart liquid. The conventional preference ratio for each taste solution was calculated. The preference ratios for 0.025 M sucrose and 5% and 10% corn oil were higher ($P < 0.05$) in females than in males. The preference ratios for 1 and 5 mM saccharin and 0.03 M NaCl were also higher, but not statistically significant, in females than in males. In Experiment 2, taste responses of the chorda tympani to each taste stimulus were recorded. No significant difference in taste responses was detected between males and females. The present results showed that sexual differences existed in taste preferences especially for sucrose and corn oil in rats and suggest that the differences originate at the central level rather than the peripheral level.

Relationship between taste palatability and the brain monoamine nervous system in rats

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It is known that taste plays a role in appetite, food choices, and nutrient intake. To elucidate the role of taste in these respects, we investigated changes of the monoamine transport systems in the rat brain (the hypothalamus, amygdala, limbic forebrain, hippocampus, striatum, cerebral cortex, septum, midbrain, and pons) by taste stimuli using high-performance liquid chromatography (HPLC). In Experiment 1, we used preferred 5 mM saccharin and aversive 0.3 mM quinine hydrochloride as the taste stimuli. The levels of 5-HT in the hypothalamus, amygdala, and septum in the rats that received saccharin were higher than those that received quinine hydrochloride. In addition, the levels of dopamine and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in the hypothalamus, amygdala, septum, and limbic forebrain showed similar patterns to those of 5-HT. In Experiment 2, we measured the monoamine levels using the conditioned taste aversion as the experimental paradigm. Rats received 5 mM saccharine as conditioned stimulus, followed 15 min later by an i.p. injection of 0.15 M LiCl (unconditioned stimulus). After the conditioning, the subjects showed a robust aversion to saccharin. These conditioned animals showed similar aversive responses to saccharin infusion as did the subjects that received quinine infusion. The levels of monoamines in the conditioned group in the septum area including the nucleus accumbens were lower than those in the unconditioned group, but the difference was not statistically significant. These results suggest that changes in the level of several monoamines in the septum area are involved in taste palatability.

Effect of the noradrenergic signal from the brainstem on memory retrieval of conditioned taste aversion

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In our previous studies, we suggested that noradrenergic (NAergic) transmission in the amygdala facilitates the retrieval of lithium-induced conditioned taste aversion (CTA) to 0.5 M sucrose (conditioned stimulus, CS). NAergic inputs to the amygdala originate mainly from the locus coeruleus (LC) and nucleus of tractus solitarius (NTS). We examined which NAergic input to the amygdala contributes to the facilitation in C57BL/6J mice. First, the number of c-*fos*-like immunoreactivity (c-FLI)–positive cells was increased in the LC of conditioned animals following intraoral reexposure of the CS. In contrast, fewer catecholaminergic NTS cells expressed c-FLI following CTA retrieval. Bilateral infusions of clonidine, a α2-adrenergic receptor agonist, into the LC before the test inhibited retrieval-induced increase of c-FLI in the LC. The same infusions into the LC increased the latency to reject the intraorally infused CS in conditioned animals. The intracranial infusions did not modify both unconditioned aversion to quinine and innate preference to sucrose in naive mice, implying that intra-LC infusions of clonidine modify the memory-related mechanism but not general taste information processing. The present study suggests that the NAergic signal from the LC plays an important role in memory retrieval of CTA, implying that NAergic input from the LC to the amygdala contributes to the facilitation of CTA retrieval.

Gender differences in liking of sweetness among young and old people in Japan

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A questionnaire survey examining possible differences between men and women in the degree of liking for sweetness was carried out with regard to young and old people. The total of 1427 participants were students of the Tokyo University of Agriculture at Tokyo and Nihon University at Fujisawa, Kanagawa Prefecture, and attendants of culture course of adults' schools for senior citizens in Tokyo and in the Niigata Prefecture (mean age: male, 66.0; female, 64.6). The survey consisted of 38 statements such as ''I like sweets very much'' or ''I would like to have some sweets at least once a day.'' The statements were concerned not only with the liking for sweet food but also the liking for fat, meats, fish, vegetables, and alcohols. There were significant differences in sweet liking both in gender and age groups. Young women most liked sweets in most aspects, including eatable quantity, eating frequency, feeling of happiness, etc. Men liked meats and fatty foods, which contain more protein and high energy and take time to digest them more than women. This means that men need not to supply quick-acting sugars more frequently for preference than women. Liking for sweetness as well as for meats and fat declined in old age and the gender difference was also decreased, although old women liked sweetness slightly more than old men. Old men who liked alcohols showed a tendency for sweet liking similar to women. It was also shown that the degree of sweet liking was slightly high in the big city of Tokyo than in the other cities.

Changes in comfort and preference levels after continuation use of mouthwashes

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Aimed at studying the physical condition inside the mouth cavity of 36 healthy male and female subjects, long-term observations on changes in comfort and preference levels after use of strong-stimulus commercial mouthwash and average-level commercial mouthwash every day for 15 days were carried out. The observation results of the strong-stimulus commercial mouthwash showed that the comfort and preference levels of some of the subjects increased through daily use. Moreover, according to an objective evaluation of comfort (obtained from brainwave and heartbeat cardiographs) regarding the strong-stimulus mouthwash, greater discomfort was felt while the mouthwash was in the mouth compared to after it had been expelled. After the mouthwash has been expelled, however, the comfort level went up. From this rise in comfort level, it can be concluded that, with daily use, the comfort and preference levels regarding the strong-stimulus mouthwash improve.

Bitter taste recognition after transaction of taste nerves in C57BL/6J mice

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This study focused on bitterness in food which is acceptable and bitterness in medicine and toxins which is unacceptable. After transaction of two main taste nerves, the chorda tympani (CT) branch of the facial nerve and the glossopharyngeal (GL) nerve, the two-bottle preference tests for 10 min and 48 h were carried out in mice. In this preference test, at higher concentrations, isohumulones and caffeine which are included in food were significantly disliked in mice. At lower concentrations, however, isohumulones and caffeine were selected as much as distilled water. And the sensitivities for isohumulones and caffeine were reduced by the CT and GL nerves transaction. On the other hand, for quinine and denatonium which are in medicine and toxins, there were no significant differences between the CT and GL nerves of transaction mice and sham mice. According to the results, their perceptions may be deeply involved in the activation of factors other than CT and GL nerves, for example, the superior laryngeal nerve, the greater superficial petrosal nerve which is the other taste nerve, and the trigeminal nerve which is one of the sensory nerves. Therefore, to confirm the two possibilities that mice did not either dislike caffeine at lower concentrations or mice did not recognize the bitterness, we did the conditioned taste aversion (CTA) test. In the test, mice recognized the bitterness of caffeine at lower concentrations which are of similar concentrations to those actually contained in food. This result showed that the bitterness of caffeine in food is acceptable in mice.

Behavioral analysis of responses to quinine in Trpm5 KO mice

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It is reported that Trpm5, a member of the transient receptor potential channel, is expressed selectively in taste receptor cells. Mice with a partial deletion of the Trpm5 protein, which retained intact the amino terminal portion, have been shown to be unresponsive to bitter, sweet, and umami compounds. To avoid any confounding effects of this amino terminal fragment, we generated knockout (KO) mice null for Trpm5 protein. In previous studies, this Trpm5 KO mice showed reduced, but not abolished, responses to quinine hydrochloride in both nerve recording and two-bottle preference tests. In this study, in order to examine behavioral responses to quinine hydrochloride in Trpm5 KO mice in further detail, we used a short-term (10 s) lick test for measurement of consumption of its solutions. Trpm5 KO mice showed significantly reduced responses to 0.1–10 mM quinine hydrochloride, but not abolished at high concentrations (3.0 and 10 mM), although no such difference was evident in response to DW. These results may be almost consistent with previous nerve recording and two-bottle preference tests, suggesting that there may be Trpm5-dependent and independent pathways in the signal transduction mechanism for quinine hydrochloride.

Behavioral analysis of the psychological effects of green odor on mice

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Green odor (the equivalent mixture of *cis*-3-hexenol and *trans*-2-hexenal) is thought to have healing effects on mammalians including humans. Recent studies have reported that green odor inhalation influences autonomic and hormonal responses to stress in rodents. However, the psychological effects of green odor have not been accurately evaluated in experimental animals. In the present study, we investigated the neuropharmacological basis of the psychological effects of green odor by assessing the effects of acute inhalation of green odor on locomotor activity, pentobarbital-induced sleeping time, and the forced swimming test in mice. In addition, two different mouse strains, DBA/2J and C57BL/6N, were used for investigating the influence of genetic factors on behavioral patterns. Exposure to green odor significantly reduced locomotor activity in DBA/2J mice but did not show a similar effect in C57BL/6N mice. In contrast, pentobarbital-induced sleeping time was prolonged by green odor in both strains. In the forced swimming test, in which desipramine (5 mg/kg) treatment significantly decreased the immobility time only for C57BL/6N mice, the green odor–scented water condition significantly decreased the immobility time for C57BL/6N mice in comparison to the no-scented water condition. However, DBA/2J mice showed no difference between the two conditions. These results indicate that acute inhalation of green odor has sedative and/or antidepressant effects in DBA/2J and C57BL/6N mice and the effects are genotype dependent. As these strains have known genetic differences in brain GABA, monoamine, and hormonal systems, these differences may play a critical role in the psychological effects of green odor.

Exploring olfactory processing through odor discrimination behavior in mice

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In the olfactory system, sensory signals generated in the olfactory epithelium (OE) are relayed through the OB to the olfactory cortex (OC), which transmits information to higher cortical areas. Recent studies have shown that the first step in odor discrimination appears to transform molecular features of odors into a spatially organized pattern of input to olfactory bulb (OB) glomeruli. Although much has been learned about the encoding of odor identities in the OE and OB, relatively little is known about how olfactory information is processed to recognize and discriminate odors in the OC. To understand how the olfactory system enables us to discriminate different odors, we conducted a Y-maze behavioral assay. During training and testing, mice had been deprived of water for 23 h. A drop of water is given to mice only when the choice is concordant. At first, water-deprived mice were trained to choose a particular odor of the odor-source material. After successful training, each mouse was tested to see whether it could discriminate the learned odor from others. In this study, we showed that mice preferred stronger one in case of the discriminative task between the identical odors. Further, mice focused on a particular feature of the learned odor mixture for odor discrimination. If each mouse noted different features of the odor, different responses for the same task could occur. And it really happened. Through learning experience, mice might have olfactory attention for odor discrimination.

Olfactory responses to a sex pheromone identified in masu salmon

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Steroids and prostaglandins have been reported as sex pheromones in several species of fish. Recently, however, we identified a novel sex-attracting pheromone in ovulated female urine in the masu salmon, *Oncorhynchus masou*, by using chromatographic and spectral tactics. This pheromone induces sexual excitement and locomotive behavior in spermiating males. We investigated behavioral responses of immature males, spermiating males, sexually regressed males, and ovulated females when the synthetic pheromone was introduced into the experimental trough. Collaterally, electroolfactograms to the pheromone were also recorded in fish groups used for behavioral tests. Behavioral responses were observed in only spermiating males. Response thresholds for the pheromone were $10^{-14–15}$ M in precocious spermiating males, $10^{-10–14}$ M in immature males, $10^{-10–11}$ M in sexually regressed males, and $10^{-9–10}$ M in ovulated females. These results show that behavioral and olfactory responses to the pheromone are specific for males.

Physiological and behavioral studies of eel (*Anguilla japonica*) olfactory responses to amino acids

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The Japanese eel is very popular with the Japanese as food material, but its life history and reproductive physiology still remain mysteries. It is suggested that the Japanese eel migrates to the western sea area of Mariana Islands for spawning. In general, fish olfactory systems play important roles in survival and reproduction. Anguilliformes have well-developed olfactory organs. Eel olfactory systems may strongly influence their migration and spawning; however, studies on eel olfactory response properties have not been enough. In this study we examined the magnitudes and sensitivities of eel olfactory responses to amino acids by electroolfactogram

recording and observed the behavioral change when amino acids were applied to their nose. 1) Twenty L-amino acids and two related substances tested elicited olfactory responses of various magnitudes. 2) Betaine, cysteine and aspartic acid were the most potent odorants that elicited responses three times as large as others. Arginine, methionine, and isoleucine elicited only small responses. 3) The threshold concentrations determined varied for different amino acids. The thresholds were between 10^{-8} and 10^{-7} M for serine, histidine, cysteine, and betaine; between 10^{-6} and 10^{-5} M for aspartic acid; and between 10^{-5} and 10^{-4} M for methionine. 4) Eel behavioral responsiveness obviously increased when there was food (gold fish, goby) odor stimulation, while small change was observed in responses to betaine and cysteine. 5) These results show that single odorants, even potent amino acids, have small stimulative effects on eel behavior. Further investigations of olfactory and behavioral responses to various odorant mixtures are necessary.

Olfactory recognition of prey fish *Plecoglossu altivelis* by largemouth bass Micropterus salmoides

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This study was designed to investigate whether largemouth bass can detect their prey, ayu (*Plecoglossu altivelis*), by the olfactory system. Electroencephalograms (EEGs) of largemouth bass were recorded for two stimulus solutions: extracts from injured and noninjured skin of ayu. The response intensities to both stimulus solutions were expressed as the relative value to the response of 0.1 mM L-gln. The average relative response intensities to injured and noninjured skin extracts were 1.39 and 1.70 times larger than the response to L-gln, respectively. There are no significant differences, however, between the response intensities to injured and noninjured skin extracts. The results indicated that skin extract is highly stimulatory to the olfactory system as well as amino acid, L-gln, suggesting that largemouth bass can recognize ayu by the olfactory system in their turbid habitat. It is also suggested that there are no specific stimulants to predator fish in ayu epidermis.

Effects of shochu (Japanese rough distilled spirits) distillery by-product on attracting and feeding behavior in rudderfish, *Girella punctata*

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Tank experiments were conducted to examine the effect of the distillery by-product of Japanese spirit (shochu) (solution-A) on the attracting and feeding behavior of the rudderfish, *Girella punctata*. The attraction was examined in a Y-shape FRP testing tank. All the fish in a vestibule in the tank moved into a stimulation area when the solution-A was introduced into the area, indicating a strong attraction of solution-A. When solution-A and the extract of the krill (solution-B) were introduced, the fish invaded both stimulation areas. There were no statistical differences on the invasion frequency, number of fish invading, and time spent in the areas between two stimulants, showing the same attraction for solution-A with the krill extract. The feeding of the fish on the test agar diet contained in solution-A and solution-B of different concentrations was examined in an outdoor concrete tank. The fish showed the highest feeding rate on the agar diet contained with no stimulant-A, and a similar feeding rate was obtained for the agar diet with solution-A at 20% concentration. Lower feeding rates were observed proportionally with higher solution-A concentrations, indicating a reverse effect of solution-A on the feeding at high concentrations. A similar reverse effect was also found in a feeding experiment with the krill preserved in solution-A and untreated fresh krill. It is concluded that the distillery by-product of Japanese spirit, an industrial waste from distilleries, is a strong attractant for marine fish and can be partly replaced with fish meal in the commercial diet for fish farming.

Analysis of human salivary volatile components detected by HS-SPME

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It is well known that there are many kinds of proteins and amino acids in saliva, and their degradation has become one of the main causes of oral malodor. However, the volatile components in saliva have not been exactly clarified. In this study, we first established the detection method of human salivary volatiles with the headspace solid phase microextraction technique (HS-SPME). We detected over 100 volatile chemicals in healthy human whole saliva (three males and two females: mean age 33.6 years). In these chemical components, over 60 chemicals were identified with GC-MS. In these chemicals, the most prominently observed chemicals were C2–C10 volatile fatty acids. For clarification, the origin of these chemicals was traced from parotid and submaxillary–sublingual gland saliva directly and analyzed volatiles were detected from them and compared with volatiles in whole saliva. According to the results, only 10–20% of C2–C6 acids were observed in submaxillary–sublingual gland saliva, but in the case of C8–C10 acids, almost the same amount of chemicals was contained in the parotid and submaxillary–sublingual gland saliva compared to whole saliva. These results suggested that oral bacteria mainly supplied these volatile fatty acids and endogenous supply was also observed but it was restricted. To compare these volatile organic acids among 27 of subjects without rampant caries and injury of oral soft tissue, some volatile organic acids namely, acetic, propionic, butyric, methyl butyric, and pentanoic acids were significantly increased with age, suggesting that increase of these chemicals could be an indication of some age-related physiological and/or pathological change of oral circumstances in an indirect manner.

Influence of the reduction of nitrogen application in an organic tea field on quality of *matcha*

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Matcha—powdered green tea—requiring a better *umami* and sweet taste than the other types of green teas is produced from leaves that

require a large amount of nitrogen fertilizer for growth in order to increase the amino acids which are the *umami* and sweet components. However, water pollution caused by the excess nitrogen application in the tea fields is now a concern. The tea was cultivated to reduce the amount of nitrogen fertilizer from 635 kgN/ha/year (control) to 435 kgN/ha/year in an organic tea field from the viewpoint of environmental preservation. The chemical analyses of the taste component contents of *matcha* showed that the *matcha* after the nitrogen reduction contained more bitter taste components than the control. A sensory evaluation of the two *matchas* prepared for drinking used for the tea ceremony showed that the one after the nitrogen reduction received lower evaluations than the control due to its lack of flavor, bitterness, etc. The paired difference tests on the two kinds of *matcha* as the ingredients for sweets, however, showed that the two *matchas* received equivalent evaluations from the viewpoint of total preference. The chocolate prepared from white chocolate using the *matcha* after the nitrogen reduction was found to have its green–yellow color more visible like *matcha* than that of the control. On the other hand, the chiffon cake using the control was found to have its color preferable to the other. This indicated that the two *matchas* have to be selected to suit a specific use in food processing.

Flavor of caramel sauces prepared from granulated sugars with different thermostabilities

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It is expected that commercial granulated sugars made in Japan generally have the same thermostabilities because of the high purity of over 99.9%. However, we reported that the melting points and the shapes of the endothermic curves by differential scanning calorimetry (DSC) of the sugars from different sugar refineries are different from one another, and also in spite of the temperature being far lower than the melting point, some with lower melting points are colored light yellowish brown. Furthermore, from a previous study, we postulated that the difference in behavior of the granulated sugars or sucrose crystals upon heating is due to the structure of the crystal rather than impurities included in the crystal. Caramel sauces were prepared from the granulated sugars of two sugar refineries that significantly differ in the melting points in spite of the same origin, by heating at 190°C for 60 s followed by adding water. The caramel sauces were analyzed by HPLC for estimation of their sugar compositions and then had sensory evaluations. It was shown that the degree of degradation of the sucrose molecule brought about by heating was different between the two sugars. The caramel sauces had some significant differences in the sensory evaluations due to the differences in color, the quantity of the sugars, and the degree of molecular decompositions between the two caramel sauces. It was determined that the difference in behavior of the sugars upon heating might have an effect on the taste and flavor of cooked foods.

Does taste affect preference for sodium in zinc-deficient rats?

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Several reports have demonstrated that zinc-deficient (ZnX) rats enhanced their preference for sodium solution. In the present study, we conducted behavioral, electrophysiological, and hematological experiments to investigate whether or not taste affected the enhancement of preference for sodium. The results were as follows. In the long-term (48 h) two-bottle preference test, the preference percents for 0.1 and 0.3 M NaCl in the ZnX rats were higher than those in the control rats. But in the short-term (10 min) test, there was no significant difference in the preference percents between ZnX and control rats. When the ZnX rats transected, the taste nerves were used for the long-term two-bottle preference test, and there was no significant difference between the preference percents for 0.1 and 0.3 M NaCl in the ZnX and the sham rats. There was also no significant difference in the neural responses of chorda tympani nerves to some sodium solutions between ZnX and control rats. The assay of sodium and aldosterone in the serum of the ZnX rats revealed a sodium metabolism disorder in these rats. These results suggest that the enhancement of preference for sodium is caused by the disorder of sodium metabolism rather than taste.

Zinc deficiency alters lick rate responses to $CaCl_2$ in C57BL mice

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Zinc deficiency has been shown to induce growth retardation, decreases in plasma zinc level, and salt preference for NaCl in rats. However, only a few studies have been performed on mice. We measured body weight, plasma zinc level, salt preference for NaCl (48-h two-bottle test), and the number of licks (10 s) to 0.03–1 M NaCl, 0.03–1 M KCl, or 0.0001–0.1 M $CaCl_2$ after the mice were fed a zinc-deficient or control diet (d 0). The number of licks to water and 0.3 M sucrose was also measured. The mean body weight and plasma zinc level were significantly lower in the experimental group than those in the control group after 1–2 weeks. The two-bottle test revealed that a preference for 0.03 M NaCl was significantly greater in the experimental group than in the control group after 3–4 weeks. The number of licks to 0.01 and 0.03 M $CaCl_2$ was significantly lower in the experimental group than in the control group after 6 weeks. There were no significant differences in lick responses to increasing concentrations of NaCl and KCl between control and experimental groups. There were also no differences in lick responses to water and sucrose. Thus, the results indicate that zinc deficiency can be induced in mice by feeding them a zinc-deficient diet for 3–4 weeks. The results also suggest that long-term zinc deficiency alters taste intensity and/or taste quality of $CaCl_2$ in mice.

Relation of aldosterone and angiotensin II to strong salt preference in zinc-deficient rats

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Zinc-deficient rats have an increased preference for sodium chloride. Previous studies have suggested that in sodium-deficient animals aldosterone and angiotensin II are involved in increased sodium appetite. We investigated the relationship of endocrine status to sodium preference in zinc-deficient rats by analyzing serum concentrations of aldosterone, angiotensin II, and sodium. Animals were fed a zinc-deficient diet (group 1) or the normal control diet (groups 2 and 3). Groups 1 and 2 were fed *ad libitum*. Group 3 was pair-fed with group 1. Serum constituents and preference for NaCl solutions (48-h two-bottle choice test) were determined at 1 and 4 weeks after the onset of feeding. The intake of 0.3 M NaCl solution in preference to water occurs in group 1, but not in groups 2 and 3, from 1 week after the onset of feeding. There was no significant difference in serum concentrations of sodium among all groups throughout the experiments. However, estimates of group 1 tended to be lower than those of other the two groups 4 weeks after the onset of feeding. Angiotensin II concentrations decreased significantly in group 1 compared with the two other groups, whereas aldosterone concentrations in group 1 were approximately two times as high as those of the two other groups. These results suggest that in zinc-deficient animals with low serum concentrations of sodium, aldosterone is upregulated through synthetic pathways different from the rennin–angiotensin system. Increased central action of aldosterone may lead to increased sodium appetite of such animals.

Effect of zinc deficiency on umami taste preference in SD rats

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We have demonstrated that the short-term zinc-deficient signal after 4 days of the feeding increases NaCl preference in rats significantly, but very little data have been reported about the umami taste preference in this model. Therefore, the present study was performed to elucidate the impact of zinc deficiency on the regulation of monosodium glutamate (MSG) appetite and the taste nerve sensitivity in the zinc-deficient rat model. Four-week-old male SD rats were fed the zinc-deficient (1.2 mg Zn/kg, Zn-Def), low-zinc (4.1 mg Zn/kg, Low-Zn), and pair-fed control (33.7 mg Zn/kg, Pair-fed) diets for 6 weeks, and the two-bottles of water and MSG solutions (30 mM) preference test was undertaken in the first experiment. The Zn-Def rats showed increased preference for MSG solution only after 2 days of diet feeding, and Low-Zn rats showed intermediate preference for MSG solution throughout the experimental period. However, the increased sensitivity of the chorda tympani (CT) nerve was observed in the Zn-Def rats especially after 4 weeks and later in the Zn-Def group, though the Low-Zn group showed no difference in the taste nerve sensitivity compared with the Pair-fed group. The hypothalamic catecholamines profile after 2 weeks did not show any difference among the groups. Surprisingly, after the long-term intake of the MSG solution as a drinking solution in the second experiment (4 weeks or longer feeding of Zn-Def diet), it was shown that the decreased CT nerve sensitivity returned to the control level, and retarded growth was seen in the MSG solution–given rats compared with the water-given group, which might be included in the total phenomena in the present study.

Maternal zinc deficiency during lactation period modifies weanling and grown rats' NaCl preference

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It has been well known that maternal dietary NaCl intake influences weanling rats' salt preferences and that brief exposure to NaCl during early postnatal development enhances adult intake of sweet and salty compounds. However, few data have been published about maternal milk nutrients such as zinc level and later NaCl preference in weanling and adult grown rats. We have already shown that short-term zinc deficiency clearly causes the increase of NaCl preference, so we demonstrated whether maternal zinc deficiency during the lactation period was the cause for weanling and grown rats' NaCl preference using this zinc-deficient system with SD/Slc rats. Zinc-deficient (0.7 mg Zn/kg), Low-zinc (4.0 mg Zn/kg), and Pair-fed control (33.7 mg Zn/kg, and fed against Zinc-deficient rats) diets were fed to the lactating mother during the lactation period only (for 3 weeks after birth), and a zinc-sufficient diet was fed to all rats of all groups after weanling. For the water and 0.15 M NaCl solution, the two-bottle preference experiment showed that maternal zinc-deficient and low-zinc diets during the lactation period caused decreased NaCl preference in weanling and grown rats, even though this was after their recovery from zinc deficiency. After 3 weeks of feeding with a zinc-sufficient diet, significantly increased epinephrine concentration in amygdala and decreased plasma oxytocin concentration and decreased chorda tympani nerve response to NaCl were observed in the Zn-Def and Low-Zn groups. These results suggest that not only the central nervous system but also the peripheral mechanism of taste reception were strongly involved in the grown rats' taste preference by early zinc deficiency during the lactation period.

Detection of gustin in saliva by ELISA

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A zinc metalloprotein (M.W. 37,000) was isolated from human saliva and named "gustin" due to its possible function in taste. Thereafter, carbonic anhydrase (CA) VI was found to be identical with gustin. Thus, this investigation intended to establish clinical measurement of concentrations for CAVI in saliva and comparison of CAVI levels in whole and parotid saliva of healthy subjects and patients with taste dysfunction. Unstimulated whole saliva

and parotid saliva were obtained from 18 healthy subjects and 5 patients with taste dysfunction. The concentration of CAVI in saliva was quantified by ELISA using the polyclonal antibody against the synthetic peptide designed from 93–111 chains of human CAVI. The detection systems were biotinylated anti-rabbit IgG, avidin DH, biotinylated alkaline phosphatase, and *p*-nitrophenyl phosphate. And taste thresholds evaluation between healthy subjects and the patients were investigated. For the ELISA, CAVI concentration was calculated by reference to the typical standard curve of the synthetic peptide, and its detection range was 12.5–400 ng/ml in this study. CAVI in saliva was found among all subjects. The results showed that CAVI levels in parotid saliva were four times higher than that in whole saliva in healthy subjects and that in healthy subjects it was four times higher than that in patients with taste dysfunction. These results suggested that the ELISA using this antibody can be a probe for the quantitative measurement of CAVI, which may be useful to clinically diagnose taste dysfunction.

Recognition of energy amount of dietary fat is involved in preference and reinforcing effects in mice

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Previously, we showed that mice prefer to eat vegetable oils. Voluntary intake of corn oil in the light box produces place preference in the conditioned place preference (CPP) test on mice. However, mice did not continue to eat the low-energy fat substitute in the long-term two-bottle choice test, which included postprandial feedback effects. These results suggested that the energy of dietary fat is involved in long-term preference and reinforcing effects. In the present study, short-term preference for and reinforcement effects of various concentrations of fat solution were investigated by means of a two-bottle choice test and operant conditioning test in mice. In the 10-min two-bottle choice test, mice (BALB/c male) were offered two different concentrations (0% vs. 25%, 25% vs. 50%, 50% vs. 75%, 75% vs. 100%) of fat emulsified with 0.3% xanthan gum solution. In all the tests, mice largely preferred the higher concentration of fat solution between them. In subsequent test sessions, mice were offered various concentrations of fat solution (8.9%, 50%, and 100% in 0.3% xanthan gum solution) in the progressive ratio operant schedule. The break point correlated with the concentration of fat. These findings suggested that the energy of fat is involved in the reinforcing effects, although further study is required to show which is recognized by mice: the energy concentration or the total energy content in fat solution.

Involvement of D2, but not D1, receptors in dietary fat-seeking behavior

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We recently demonstrated that dopamine D1 receptors are involved in the conditioned place preference (CPP) test in mice. Here, we investigated whether D1 or D2 receptors also are involved in the lever responding under the progressive ratio schedule by testing the hypothesis that blockade of dopamine D1 or D2 receptors attenuates the rewarding effects of corn oil. Twenty-four food-restricted mice were trained to press a lever for sucrose solution under the schedules of reinforcement including fixed ratios (FR1-10) and for corn oil under the schedules of reinforcement progressive ratio (PR). After stable break point (BP: cessation to respond to the increasing criterion of instrumental effort) was established, the *ad lib* fed mice received injection of SCH 23390(0.03 mg/kg i.p.), a D1 receptor antagonist, or sulpiride (100 mg/kg i.p.), a D2 antagonist. Sulpiride ($n = 12$) led to a significant reduction of the BP compared to saline infusions ($P < 0.001$); SCH23390 ($n = 12$) did not. The preference test revealed no drug effects on the amount of consumed corn oil. These data suggest that D2 receptors but not D1 receptors are involved in corn oil–seeking behavior.

Involvement of β-endorphin in the formation of preference for dietary fat

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In the present study, we investigated the contribution of β-endorphin in the formation process of preference for dietary fat in rats. We trained rats to learn that they would be given 5% corn oil for 20 min starting at 1600 (dark phase: 1500–0300). We found that corn oil preference was acquired after the processing of this information. After the acquisition of the preference for corn oil, the ratios of mRNA expression of proopiomelanocortin (POMC) was increased just before corn oil intake, and the concentrations of β-endorphin in serum and cerebrospinal fluid (CSF) were increased 15 min after corn oil intake. POMC mRNA expression ratio increased 0 and 30 min after corn oil presentation when rats were given only anticipation of corn oil. Increase in corn oil intake was inhibited dosage dependently by i.p. injections of a nonselective antagonist of opioid receptors, naloxone (1 and 10 mg/kg BW), a selective antagonist of μ1 receptors, naloxonazine (1, 3, and 10 mg/kg BW), a selective antagonist of δ receptors, naltrindole (1 and 5 mg/kg BW), a selective antagonist of δ1 receptors, BNTX (0.5 mg/kg BW), or a selective antagonist of δ2 receptors, naltriben (0.5 mg/kg BW), 1 h before solution intake during sessions. These results suggested that the POMC was produced by anticipation for corn oil before corn oil intake and that β-endorphin was released after corn oil intake. Furthermore, we showed that binding of β-endorphin to μ1 receptors transiently at least in part was involved in the formation process of the preference for corn oil.

Study on the relationship between sugar intake and fatty acid transporter/CD36 on the rat tongue

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In recent years, there has been an interest in the prevention and cure for obesity and diabetes because of their increase. It is well known that lipid accumulation induces insulin resistance and often results in diabetes. And it was reported that an obese person prefers sweet food including high fat to only sweets. This suggested that

recognition of lipids and sugars may be related. Therefore, we analyzed that there was a relationship between lipid uptake and sugar uptake through the recognition system. In this study, we targeted fatty acid transporter (FAT)/CD36 on the rat tongue as a putative transporter for fatty acid in order to recognize fat existence. This transporter might be regulated to change the recognition for fat in the oral cavity, and the uptake amount of lipid might vary subsequently. First, we observed the change of FAT/CD36 mRNA amount after sucrose intake on the rat tongue. This result showed that circumvallate FAT/CD36 tended to decrease, while foliate FAT/CD36 tended to increase. Similar changes were also observed after stimulation of insulin *in vitro*. These results suggested that sugar uptake might be affected by the expression of FAT/CD36 in the tongue epithelium. In conclusion, sugar uptake could change the lipid recognition through FAT/CD36 and uptake of lipid.

Effect of orally administered oxidized fatty acids on antioxidative enzymes in *Caenorhabditis elegans*

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Oxidized fatty acids, when orally administered, cause toxic effects in organisms. Although the adverse effect of oxidative stress upon cell and cellular components such as plasma membrane, protein, and DNA has been intensively studied, the exact molecular mechanism regarding the toxic effect of oral administration of oxidized fatty acids is not fully understood. To evaluate the effect of oxidized fatty acids from the standpoint of nutrition and biosafety, we fed *Caenorhabditis elegans* with oxidized fatty acids and observed the effect of the oxidized fatty acids upon life span and the expression of enzymes such as catalase and superoxide dismutase that are implicated in antioxidative stress. We also analyzed the oxidized fatty acids–fed worms with electron spin resonance (ESR) to measure the amount of endogenous free radical molecules produced by the treatment. Oxidized fatty acids reduced the survival rate of worms around 7–10 days after hatching, indicating that the susceptibility of worms towards oxidized fatty acids increased temporarily and specifically at this stage. ESR measurement can be used to monitor the redox status of worms by determining the amount of endogenous free radical molecules. Using ESR, it was suggested that the oxidized fatty acids exert their toxic effect by increasing endogenous free radicals. On short-term feeding of oxidized fatty acids, the mRNA expression of catalase and superoxide dismutase were inhibited. The mechanism regulating the expression of the antioxidative enzymes by oxidized fatty acids is under study.

Bitter-masking effect of riboflavin-binding protein

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Riboflavin-binding protein (RBP) from chicken egg-white masks sweetness of sweet proteins such as monellin, thaumatin, and lysozyme. Since it was found that RBP also masks bitterness, we studied the bitter-masking effect of RBP on the bitter tastants, quinine-HCl, naringin, theobromine, and denatonium benzoate. RBP was purified as a riboflavin-free form from chicken egg white by ammonium sulfate fractionation, ion exchange, and gel chromatography. More than 0.05 mM of RBP had a masking effect on the bitterness of 0.125 mM quinine, but other proteins such as albumin (0.125 mM), ovomucoid (0.25 mM), and myoglobin (0.5 mM) had no effect. Hence, the bitter-masking effect of RBP was considered to be specific. In order to evaluate the bitter-masking effect of RBP on various tastants at same levels of bitterness, the point of subjective equality (PSE) of each bitter tastant for quinine was determined. The bitterness intensity of 0.125 mM quinine was equivalent to that of 1 mM naringin, 5 mM theobromine, and 0.1 μM denatonium; those were masked completely by addition of RBP at 0.05, 0.125, 0.5, and 1.5 mM, respectively. Whereas the molar ratio of RBP required for masking bitterness to bitter tastants was wide ranging from 1:10 to 1500:1, and the amount of RBP required for masking bitterness was relatively narrow ranging from 0.05 to 1.5 mM at the same level of bitterness intensity. These results reveal that it was more likely that the bitter-masking effect of RBP was due to interaction with taste receptor(s) rather than with bitter tastants.

Expression of human lysozyme in *Pichia pastoris* and its sweetness

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Chicken egg-white lysozyme (CLz) as well as monellin and thaumatin are sweet-tasting proteins. It has been reported that lysozymes from other avian and reptile eggs are also sweet. We prepared human lysozyme (HLz) using the *Pichia pastoris* protein expression system and investigated its sweetness. The HLz gene amplified from human placenta cDNA was cloned into expression vector pPIC9 and integrated into *P. pastoris* G115. The expression of HLz was carried out in fermenter cultures with methanol induction. The HLz secreted into the culture supernatant was purified by cation exchange chromatography and obtained as three peaks. These three HLzs differed in their lytic activities and additional N-terminal amino acid sequences: Glu-Ala-Glu-Ala-, Glu-Ala-, and Ala-. Regardless of those differences, all of them elicited the same sweetness having faint astringency like CLz. As the result of SD analysis for the taste profiles, the sweetness of HLz was clearer, less in astringency and aftertaste, and therefore more pleasant than CLz. The threshold values of both HLz and CLz were determined as 10 μM. Hence, the sweetness intensities of those were estimated as being 2000 times greater than that of sucrose on a molar basis. Although lytic activity of HLz was 2.5–5 times greater than CLz, the sweet intensities of both were at the same level. This result suggested that the sweet taste of lysozyme was independent of lytic activity. Further, since it is known that lysozyme is contained in human saliva, it is interesting to know whether or not lysozyme could have an effect on food taste.

Effects of the hardness of materials containing sweet substances on sweet taste sensitivity

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Using mixtures of representative sweet substances and potato starch as specimens, we examined the change in sweet taste

sensitivity with an increase in the starch concentration by means of a sensory evaluation method. Each sweet substance was sampled in a sequence of decreasing concentrations and was tasted using the subjects' entire mucosal surface of the tongue and oral cavity. Values of the sweet taste threshold for D-glucose, D-fructose, sucrose, and D-sorbitol were 0.025, 0.05, 0.0125, and 0.0125 M, respectively, in the absence of starch. The significance test showed that these sweet substances tasted sweeter when they were mixed with 0.15625%, 0.3125%, 0.625%, 1.25%, and 2.5% starch and were administered as a solution. However, these substances tasted less sweet when they were mixed with 5.0%, 10.0%, and 20.0% starch and were administered as a gel.

Sweet taste stimuli and endocrine responses

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It is known that the food-related sensory stimuli induce cephalic-phase hormonal release. Thus, tasting sweet food elicits insulin release prior to increasing plasma glucose levels; this is called cephalic-phase insulin release (CPIR). Typically, increases in plasma insulin are observed within 3 min after oral sensory stimulation, peak at 4 min, and return to baseline in the 8–10 min post-stimulus time period. CPIR has been well documented in animals, with research being conducted mainly in rats. The functional role of CPIR is not known clearly. In this experiment, we examined any sweet taste stimulated in the tongue induced by CPIR or not. We used female Wistar rats. Rats reliably exhibit CPIR to sweet liquids such as glucose, sucrose, and the nonnutritive sweetener saccharine. However, fructose and nonsweetener nutritive starch do not elicit CPIR. Therefore, the rats showed a strong preference for starch. From these phenomena, insulin release during the preabsorptive time period improves glucose tolerance in nutrient metabolism, that is, CPIR plays a role in glucose homeostasis. Therefore, fructose which is a typical sweetener does not elicit CPIR. This needs more detailed study.

Smelling lavender and rosemary increases free radical scavenging activity and decreases cortisol level in saliva

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Free radical/reactive oxygen species are related to many biological phenomena such as inflammation, aging, and carcinogenesis. The body possesses various antioxidative systems [free radical scavenging activity (FRSA)] for preventing oxidative stress, and saliva contains such activity. In the present study, we measured the total salivary FRSA induced after the smelling of lavender and rosemary essential oils that are widely used in aromatherapy. Various physiologically active substances in saliva such as cortisol and secretory IgA activity were found to be correlated with aroma-induced FRSA. The subjects (22 healthy volunteers) sniffed aroma for 5 min and each subject's saliva was collected immediately. FRSA was measured using 1,1-diphenyl-2-picrylhydrazyl. The FRSA values were increased by stimulation with low concentrations (1000 times dilution) of lavender or by high concentrations (10 times dilution) of rosemary. In contrast, both lavender and rosemary stimulations decreased cortisol levels. A significant inverse correlation (−0.486) was observed between the FRSA values and the cortisol levels with rosemary stimulation. No significant changes were noted in sIgA. These findings clarify that lavender and rosemary enhance FRSA and decrease the stress hormone, cortisol, which protects the body from oxidative stress.

TRPV2, a nonselective cation channel, is localized to elongating olfactory axons

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The transient receptor potential (TRP) superfamily comprises a group of nonselective cation channels that sense and respond to changes in their local environments. In order to determine what member of TRP is expressed in the olfactory system in mouse, we perform RT-PCR analysis using specific primers for each 22 plasma membrane–associated TRP family, TRPC1-7, TRPM1-8, TRPV1-6, and TRPA1. In olfactory mucosa and vomeronasal organ extracted from the mouse nasal region, amplification of fragments for TRPC1, TRPC6, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPV2, TRPV6, and TRPA1 was clearly detected. Here, we found that the TRPV2 protein localizes at the axon bundles of the immature and mature olfactory sensory neurons in olfactory mucosa shown by *in situ* hybridization and double-immunofluorescence analysis. TRPV2-immunoreactivity was also detected in the olfactory nerve layer in the olfactory bulb. We also demonstrate that cell bodies of olfactory sensory neurons located in cell layer–expressing TRPV2 mRNA are IGF-I receptor immunopositive. Furthermore, IGF-I induces the elevation of intracellular calcium level in olfactory neurons isolated from adult olfactory mucosa. In embryonic stages, the TRPV2 protein was detected on axon bundles of differentiating and mature olfactory sensory neurons in the nasal region. These results suggest that TRPV2 is concentrated on elongating axons in the regenerative olfactory system and contributes to the elevation of the intracellular calcium level in olfactory neurons in response to IGF-I.

A deficiency of the α_{1B} or β3 subunits of voltage-dependent Ca^{2+}-channels decreased pheromonal responses at the accessory olfactory bulb of mice

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Information regarding pheromones, which affect gonadal functions and sexual behaviors, is received by the vomeronasal organ (VNO) and transmitted to the accessory olfactory bulb (AOB). We investigated the physiological role of the α_{1B} and β3 subunits of the N (neuronal)-type voltage-dependent Ca^{2+} channel in neurotransduction in the vomeronasal system using α_{1B}-deficient mice and β3-deficient mice. RT-PCR studies showed the existence of β1, β2, β3, β4, α_{1A}, α_{1B}, and α_{1C} subunits of voltage-dependent Ca^{2+} channels in the mouse VNO. Immunohistochemical studies showed that the α_{1A}, α_{1B}, and α_{1C} subunits of voltage-dependent Ca^{2+} channels exist in the sensory neurons and supporting cells of the mouse VNO. Exposure of the VNO to urine samples excreted from male mice induced lower Fos-immunoreactivity in the periglomerular (PG) cells of the AOBs in α_{1B}-deficient female mice than in those of wild mice. The density of Fos-immunoreactive (Fos-ir) cells after exposure to female urine samples at the periglomerular cell layer of α_{1B}-deficient male mice was lower than that of wild mice. Exposure of the VNO of β3-deficient female mice to male urine samples also induced low Fos-ir cells in the periglomerular cell layer of the AOB. The present results suggest the importance of the α_{1B} and β3 subunits of the N-type voltage-dependent Ca^{2+} channel for the pheromone signal transduction system.

Urinary responses revealed by optical imaging of intrinsic signals in the rat accessory olfactory bulb

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To investigate how pheromonal information is processed in the rat accessory olfactory bulb (AOB), we obtained high-resolution mapping of pheromone-induced activation by optical imaging of intrinsic signals. Urine samples collected from male or female rats were used as test substances. Application of volatile components in male urine (2–5%) with a syringe mainly induced activation in the anterior AOB (aAOB), whereas female urine–induced activation was observed in both the aAOB and caudal part of the AOB in the male rats. In the female rats, urine-induced activation occurred mostly in the aAOB. Application of nonvolatile components of urine was performed by putting the nostrils in contact with filter paper moistened with urine (3–5%) or by ejecting urine (3–5%, 30 μl) to the incisive foramen. Either male or female urine induced mainly activation in the posterior AOB (pAOB) and to a lesser extent in the aAOB. Urinary responses were also observed in a few glomeruli of the main olfactory bulb. The threshold for urine-induced activation in the glomeruli, however, was approximately 50%, which was higher than those obtained in the AOB (approximately 1%). These results provide the evidence that the aAOB may be activated by probably volatile components in male or female urine, whereas the pAOB may be activated by nonvolatile components in urine.

Cloning of the putative CSP and OBP cDNA from the antennae of the adult silkmoth (*Bombyx mori*)

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In the first step of olfaction in moths, volatile, hydrophobic odorant molecules are transported from the air to olfactory receptor neurons through the sensillum lymph in the antennal sensilla. Antennal-binding protein X (ABPX), chemosensory proteins (CSP1 and CSP2), general odorant–binding proteins (GAP and GOBP2), and pheromone-binding proteins (PBP) have been reported as the odorant-binding proteins (OBPs) that are presumed to transport odorant molecules in the silkmoth *Bombyx mori*. The expression sites of CSPs in the adult antennae and the larval head were examined by immunocytochemistry. CSP1 was detected in almost every sensillum in both male and female antennae. CSP2 was detected beneath the cuticle of the antennae. In the larval head, CSP1 and CSP2 were not expressed in the chemosensory sensilla but were expressed in the mechano sensilla. We analyzed the EST database of the silkworm and subtracted the cDNA library of the adult male antennae. As a result, we obtained cDNA clones encoding nine new CSPs and two new OBPs from the adult antennae of the silkmoth in this experiment. RT-PCR experiments showed that the mRNA of the new CSPs and OBPs was present in various sensory and nonsensory tissues of the adult and larval insects. Among the proteins of CSP and OBP families, there were several proteins that were expressed outside the olfactory organ. We suggested that members of the CSP and the OBP families were carrier proteins for various volatile and hydrophobic molecules.

Localization of a receptor for BDNF, TrkB, in the rat olfactory axons by immunoelectron microscopy

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To obtain an ultrastructural basis for understanding cellular mechanisms of the brain-derived neurotrophic factor (BDNF) in the olfactory mucosa, it is necessary to localize one of the receptors for BDNF, TrkB, at the electron microscopic level. The present study was aimed at comparing two different postembedding immunoelectron microscopic techniques using the olfactory mucosae of Sprague–Dawley rats at postnatal day 1. After transcardial perfusion of a mixture of buffered 4% formaldehyde and 0.5% glutaraldehyde, small pieces of the olfactory mucosae ($n = 6$) were processed using the Lowicryl K4M technique or the reduced osmium–LR-White technique. Ultrathin sections of the olfactory mucosae were immunolabeled with an antibody that binds only to the full-length TrkB and the 10-nm colloidal gold-conjugated secondary antibody. Then, these sections were double stained with uranyl acetate and Reynolds' lead citrate and observed under a transmission electron microscope. Using the Lowicryl K4M technique, the perikaryal and dendritic regions of olfactory receptor cells were preserved fairly well, and gold particles, which reflect the presence of the TrkB molecule, were observed in their cytoplasm. However, the ultrastructure of olfactory axons in the lamina propria were not well preserved; destruction of membrane and cytoplasm obscured subcellular components. In contrast, the reduced osmium–LR-White technique demonstrated well-preserved ultrastructure of both olfactory axons and the perikaryal and dendritic regions of olfactory receptor cells. It became clear in axons that gold particles were localized in their plasma membranes and within their cytoplasm. Thus, it can be concluded that the reduced osmium–LR-White

technique is superior to the Lowicryl K4M technique in which osmium cannot be used.

Immunolocalization of testosterone in the rat vomeronasal organ

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Most vomeronasal receptor cells (VRCs) contain large quantities of smooth endoplasmic reticulum (sER) that is common in steroid-producing cells in the sex glands and the adrenal cortex. To examine if sER in VRCs is involved in steroid biosynthesis, the presence of testosterone was identified and localized immunohistochemically in the vomeronasal organs (VNOs) of rats. Young and adult Sprague–Dawley rats (n = 10) were deeply anesthetized and perfused transcardially with Zamboni's fixative. The nasal cavities that included VNOs were dissected out and were processed for cryostat frozen sectioning. As primary antibodies, a commercially available polyclonal antibody to testosterone and antiserum to protein gene product 9.5 (PGP) that binds to VRCs and nerve fibers were used in this study. Using epifluorescence microscopy, intense immunofluorescence for testosterone was detected in the mucomicrovillar complex of the vomeronasal sensory epithelium in both male and female rats. Further, a combined technique of triple-labeling immunofluorescence and triple-scanning confocal laser microscopy elucidated that testosterone immunofluorescence in the mucomicrovillar complex was mostly localized in PGP-immunorective dendritic endings of VRNs. In the perikaryal region of acinar cells in associated glands of VNOs, intense immunofluorescence for testosterone was present in a granular fashion. The above results suggest that testosterone plays an unknown role in dendritic endings and probably sensory microvilli of VRNs in which vomeronasal transduction takes place. In addition, testosterone may be included in secretory granules in the associated glands of VNO.

Role of homeobox transcription factor Six1 in mouse basal development

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Six1, a member of the Six gene family homologous to *Drosophila so*, encodes a homeodomain protein, and its gene product functions as a transcription factor with coactivator Eya proteins. Six1-deficient mice show defects in the ear and nose, which are derived from olfactory and otic placodes, respectively. The olfactory placode is unique in the sense that it gives rise to neuronal and nonneuronal components such as supporting cells, gland cells, and ensheathing glial cells. The molecular mechanism for generating divergent cell types from the olfactory placode has been little understood. Here, we addressed this issue by examining the expression pattern of Six1 during development of olfactory epithelia and by analyzing the phenotype of Six1-deficient embryos. Six1 was expressed in almost all olfactory placode cells at E10.5, and its expression was gradually repressed in the regions where olfactory sensory neurons (OSN) were born. At E16.5, Six1 was expressed solely in supporting cell and basal cell layers. The cell fate determination for the OSN in the olfactory epithelium was severely disturbed in the absence of Six1 as judged by histological analyses and the expression patterns of Mash1, Neurogenin1, and NeuroD. We conclude that Six1 is essential to generate OSN from the placode.

Differences in taste representation between two primary gustatory areas alert monkeys

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In nonhuman primates, two primary gustatory areas are identified: area G of Sanides (1968) in the lateral sulcus and the precentral extension of area 3. So far, most studies have been devoted to area G, and little has been known about the gustatory coding in area 3. The present study explored taste neurons in response to eight tastants (NaCl, sucrose, HCl, quinine, MSG, water, orange juice, and artificial saliva) in both areas G and 3 to find differences in taste coding between the two areas. Taste neurons were recorded from four hemispheres of three Japanese monkeys (45 neurons in area G and 46 in area 3). There was no difference in spontaneous discharge rates, average response magnitude to tastants, tuning to four basic tastants of taste neurons between the two areas, but the onset latency of taste responses was significantly shorter in area 3 than in area G. A larger number of taste neurons in area 3 were HCl-best, whereas a larger number of sucrose-best neurons were found in area G. Multidimensional scaling disclosed a two-dimensional representation of tastants using all data from the two areas and revealed that area G tended to represent one of the dimension-related pleasant and unpleasant aspects of tastants and area 3 tended to represent the other dimension orthogonal to that of area G. It is suggested that two primary gustatory areas complement each other by representing different aspects of taste stimuli.

Detection of primary gustatory cortices in humans by functional MRI

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Previous studies with MEG located the primary gustatory cortices (PGCs) at the ventral end of the central sulcus (cs) and the

transition between the parietal operculum and insula (area G) because of the shortest latency. However, studies using other noninvasive brain imaging methods such as PET or fMRI have been unable to confirm this. In the present study, while repetitively stimulating the tongue of subjects with a short pulse of NaCl, we examined cortical activation by means of fMRI. Eleven neurologically healthy volunteers (21–33 years old, right-handed) participated in the study. BOLD signals were detected by a 1.5T Siemens MRI scanner (Magnetom Vision, Siemens) and analyzed by SPM99. The single-subject analysis, disclosed activations at both area G and the frontal operculum in 8 of 11 subjects and at the rolandic operculum in 7 subjects (threshold at $P < 0.05$ FDR corrected across the entire volume). Activations were also found at the ventral end of the central sulcus ($n = 3$). Group analysis with random effect models revealed activation at area G on both sides and at the frontal operculum, rolandic operculum, and ventral end of the central sulcus on the left side ($P < 0.02$ FWE corrected, ROI analysis). Taking MEG findings into consideration, the present findings strongly indicate that the PGCs are present at both the transition between the parietal operculum and insula and the rolandic operculum including the gray matter within the ventral end of the central sulcus.

Analysis of pathways involved in discrimination of umami taste in mice

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Monosodium L-glutamate (MSG) and monopotassium L-glutamate (MPG), both of which are employed as umami substances, have considerably different taste qualities in humans, but the taste quality of MPG with inosine 5′-monophosphate (IMP) is close to that of MSG. We have demonstrated using a conditioned taste aversion (CTA) paradigm that mice, whose umami sensitivity is comparable with that of humans, discriminated MSG from MPG, but not from MPG with IMP (MPG + IMP). We examined whether mice can discriminate difference of taste qualities among MSG, MPG with various concentrations of IMP (0.01–10 mM), and IMP alone using a CTA paradigm to verify that the change of taste quality results from synergism of umami substances. Mice discriminated between MSG and IMP alone but not between MSG and MPG + IMP, with the exception of MPG + 0.01 mM IMP. We also examined whether they could discriminate between KCl and KCl with IMP (KCl + IMP). Mice did not discriminate between KCl and KCl + IMP. The distribution pattern of MPG-stimulated Fos-like immunoreactivity (FLI) in the parabrachial nucleus (PBN) was altered by the addition of 0.1 mM IMP to a similar pattern of MSG-stimulated FLI but that of KCl-stimulated FLI in the PBN was unchanged. These results suggest that the change of taste quality of MPG + IMP did not result from an effect of cations but from synergism of umami substances and that taste signals of MSG and MPG + IMP are transmitted through the different pathways from that of MPG.

Correlation between subjective assessment and EEG for essential oils

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In the present study, the correlations between subjective assessment and EEG for essential odors were investigated quantitatively. EEG signals were measured from 19 electrodes according to the International 10-20 system (Fp1, Fp2, F3/4, F7/8, Fz, C3/4, Cz, P3/4, Pz, T3/4, T5/6, O1/2) from eight healthy males subjects for four odor [Rose oil bulgarian, Lemon oil misitano, Jasmin abs, Laverder oil france (KIMEX Co. Ltd)] conditions. The result of the subjective assessment shows the most pleasant odor for each subject, and the power spectrum of $\alpha/(\alpha + \beta)$ of EEG signals from the most pleasant odor was compared with those from the control condition, which has no odor at all. The power spectrum of $\alpha/(\alpha + \beta)$ of EEG from the most pleasant odor was increased significantly at T4 and T6 compared to the control condition. This result implies that the parameter, power spectrum of $\alpha/(\alpha + \beta)$, could be an important index for signifying the levels of pleasantness for odors.

Multineuronal recordings of odor-evoked responses from the cockroach brain

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It has been thought that olfactory processing function in the insect brain is mediated by dynamic modulation of synchronized firing in groups of neurons. However, a lot is still unknown about dynamics of neuronal firing correlation and the functional meaning. We characterized firing correlations by simultaneous recordings of several single-unit activities from the antennal lobe (AL) and mushroom body (MB) to odor stimuli (periplanone-B, 2-hydroxy-3-methyl-2-cyclopenten-1-one, 3-hydroxy-2-methyl-4-pyrone, and C6 compounds) in the male cockroach (*Periplaneta americana*). We found odor-dependent synchronous activity correlations in the AL, in the MB, and between the AL and the MB. Cross-correlograms of the AL neuron pair consist of common excitatory synaptic input, shared reciprocal synaptic input, and monosynaptic inhibitory input in the AL circuit. Cross-correlograms of the neuron pair obtained between the AL and the MB reveal direct monosynaptic excitation from the AL neuron (presumptive projection neuron: PN) to the MB neuron (presumptive intrinsic Kenyon cell: KC). Each PN forms odor-specifically diverging excitatory functional inputs to different KCs. A different combination of PNs functionally connected to each KC. Cross-correlograms of the KC pair suggest that the cells receive common excitatory synaptic input from the PNs and reciprocal input from presumptive inhibitory extrinsic neuron. These results indicate that some of the functional synaptic connections in a group of neighboring AL neurons function depending on odor quality. A group of PNs may encode information of specific odor by sending their synchronized spikes from AL

to MB. The results support the view that odor representation can be accomplished by ensemble networks.

The expression of candidate taste receptors in human tongue of normal and taste disordered persons

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The human tongue's organization was acquired from the foliate papilla by scraping and surgery. The expression of THTR family (THTRs) and T2R family (T2Rs) that are candidate taste receptors was measured by RT-PCR and microcapillary electrophoresis. There were no differences in expression of THTRs and T2Rs between the scraping method and the surgical procedure. The scraping method can measure an expression of taste receptors in the tongue tissue simply and is noninvasive. The expression of taste receptors in the foliate papilla was measured using this scraping RT-PCR method. More than 80% of normal persons over 35 years of age expressed THTR2, 3, 5, 6, 11, 12, 14 and T2R1, 3, 7, 8, 9, 10, 16. Especially, THTR2, 3, 5, 6, and T2R3, 5, 7, 8, 9, 10, 16 were expressed in more than 90% of the people. In contrast, for people under 30 years of age some THTRs and T2Rs were expressed and there were no typical expressions. Taste disordered persons expressed few kinds of THTRs and T2Rs, and there are no or almost no expressed THTR2, 3, 5, 6 and T2R3, 5, 7, 8, 9, 10, 16 that were expressed in over 90% of normal persons. We suggested that measuring the expression of THTRs and T2Rs in foliate papilla by the scraping RT-PCR method can be used as a biomarker of human taste.

Taste preference and pain relief; case reports of terminal with oral cancer patients

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Individual taste preference is one of the aspects of human personality; it induces positive emotions and can be used to markedly improve terminal patient care. We present three patients with oral cancer in the terminal stage in whom a relation between nutrition based on individual dietary preferences and pain relief was observed.

Case 1. A 78-year-old female with schizophrenia and progressive buccal carcinoma was referred to our department for pain control and terminal care. Her severe pain was relieved by oral care and oral nutrition taking into account her personal food preference and the previous dose of morphine, 60 mg/day, was decreased to 20 mg/day after she became an inpatient of our department.

Case 2. An 80-year-old female with advanced multiple cancers in the oral region was followed up as an outpatient. Her pain severely increased and was controlled by morphine (5 mg). As an inpatient for oral care receiving oral nutrition according to her daily preferences, her response to the pleasant-tasting stimuli could be observed, and pain complaints disappeared (morphine became unnecessary up to death).

Case 3. A 70-year-old male with recurrently advanced lower gingival carcinoma complicated by humoral hypercalcemia due to malignancy (HHM) (plasma calcium level: 11.3 mg and TPH-rP value: 1.8 pmol/l) was the third case. He received stomach tube nutrition due to swallowing disorder, and feeding meals were based on his preference for seasonable fish, fluid, and vegetables. Despite the progression of more than 5 months since the onset of HHM, his pain was well controlled by a limited dose of morphine 16 mg/day. Pain relief was assumed to be due to the reflex effect of gastrointestinal sensors on vagal nerve activities, which induced positive emotion.

Oral nutrition and histological findings of tongue papillae in an oral cancer terminal patient—autopsy study

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Case. A 78-year-old female with progressive buccal carcinoma had been receiving palliative medicine, and she was able to enjoy fluid meals with family support until nearly the terminal point. Oral cancer patients in the terminal stage (prognosis: less than a few more months), ordinarily receive stomach tube feeding nutrition due to severe oral problems and swallowing disorders, but this patient refused insertion of the feeding tube and continued oral nutrition until 18 days before death, without complication of oral infection and mucositis, xerostomia, exofoliation, or taste disorders which are common oral problems in terminal patients.

Autopsy findings. Primary tumor lesion was severely destructive of the left maxilla and mandible and lower face including lip and mouth floor, and submandibular lymph nodes metastases (bilateral) had developed. Marked lung metastases were observed along with pulmonary infarct due to obvious metastatic tumor cell clusters. The cause of death was diagnosed as respiratory failure due to pulmonary tumor infarction. Tongue mucosa and papillae showed normal structures as did the taste buds in the epithelial layer. Minor salivary glands and lymphoid follicles under the epithelium were not complicated by inflammation. Palate, pharyngeal and suprahyoid/infrahyoid muscles were almost intact. The suggested, oral chemical senses were nearly preserved until the terminal endpoint, the same as the auditory senses. To preserve oral chemical senses in a terminal patient, good oral hygiene by oral care is necessary along with oral nutrition based on the patient's individual food preferences.

The treatment outcome in patients with taste disturbance

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The importance of taste has been recently evaluated from the standpoint of quality of life. However, there have been few reports on clinical status of taste disturbance. In this study, we classified taste disturbance by its cause and investigated the effect of treatment and

the duration of recovery. The subjects were 321 patients with taste disturbance, who consisted of 131 men and 190 women with a mean age of 59.9 years. We evaluated all patients about the degree of symptoms with the VAS (visual analogue scale). The patients were treated with zinc sulfate, ferrotherapy, herbal medicine, and minor tranquilizers. Idiopathic taste disturbance was the commonest cause of taste disturbance (125 cases, 38.9%), the second was drug induction (62 cases, 19.3%), and the third commonest cause was disturbance after common cold (38 cases, 11.8%). Recently, drug-induced and psychogenic taste disturbances have increased. The recovery rate was 79/103 cases (76.7%) in idiopathic taste disturbance, 24/33 cases (72.7%) in that after common cold, and 14/17 cases (82.4%) in that with iron deficiency and the recovery time were 22.2 weeks. On the other hand, it was 32/50 cases (62.4%) in the drug-induced one and the recovery time was 48 weeks. The cases which took more than 6 months from the onset of symptoms to medical examination showed significantly lower rates of improvement and longer recovery time than those cases that were less then 6 months ($P = 0.04$).

Optical recording of the intrinsic signal from the human olfactory mucosa

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Endoscopy of the human olfactory mucosa is important as clinical examination for olfactory disorders. However, ENT specialists could know only morphological changing of the olfactory mucosa and could not know its function. The intrinsic signal recording from the human olfactory mucosa via an endoscope was examined. A LED (617 nm) light source and a cooled CCD camera were prepared for endoscopy of the olfactory cleft. Exposure of Sniffin' Sticks (phenethyl alcohol) for 10 s in front of the nostril was used for olfactory stimulation. Nothing that was exposed in front of the nostril was used for control. When the subjects with normal olfaction sniffed the Sniffin' Sticks, absorption of 617-nm light on the olfactory mucosa was increased. Increasing of the absorption was not elicited in the control. Slowly decreasing change of the absorption was observed both in the odorant stimulation and the control. Because it is well known that activation of the brain induces increase of oxyhemoglobin level in the tissue, the increase of oxyhemoglobin level in the olfactory neurosensory epithelium due to of odorant stimulation was suspected. Increase of tissue oxyhemoglobin–induced absorption of the 617-nm wavelength light and the olfactory-activated mucosa were visualized. Because optical recording of the intrinsic signal from the human olfactory mucosa is also applicable for olfactory disorder patients, this method will be an useful method to research clinical problems.

The clinical usefulness of Chinese herbal medicine: toki-shakuyaku-san

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It is known that toki-shakuyaku-san (TSS) which is a kind of Chinese medicine is effective for menopausal disorders. In recent years it has been known that it is effective for a neurodegenerative disorder such as Alzheimer's disease. Although we had used corticosteroids intranasally in the treatment of sensorineural olfactory disorders, it was changed for TSS therapy for 7 years. The purpose of this report is to compare the clinical effectiveness of TSS therapy with steroid therapy for patients with olfactory disturbance which are caused by upper respiratory infection and head injury. Of the total of 28 patients who were treated by TSS, 19 (67.9%) improved more than 1.5 point their recognition threshold measured by the T&T olfactometer. There was a significant difference compared with the effective ratio of steroid therapy (43.5%). There was no difference in the patients group by head injury. We need a long period for the treatment of sensorineural olfactory disturbance, but it is reported that there is suppression of cortisol, with high probability, in the patients treated with corticosteroids. In conclusion, TSS therapy is more useful therapy for the patients with sensorineural olfactory disturbance.

Clinical characteristics and treatment of olfactory dysfunction after upper respiratory infection

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It had been reported that the rate of improvement in olfactory dysfunction after upper respiratory infection (URI) was about 50%. But the cure rate was not cleare. Sugiura *et al.* reported the monthly incidence of olfactory disturbance after URI and that the olfactory disorder occurred most frequently from spring to summer. This study investigated the cure rate and clinical characteristics of post-URI olfactory dysfunction. A prospective study was performed on 250 consecutive patients with olfactory dysfunction from July 1995 to December 2004. The patients consisted of 48 men and 202 women with a mean age of 55.7 years. The olfactory acuities of the patients were examined by T&T olfactometry and the intravenous olfactory test. The steroid nasal drip and suspended-steroid local injection methods were used for patients as the principal treatment. The overall cure rate of post-URI olfactory dysfunction was 19.2%. The cure rate of patients who were started on the treatment within 3 months from the onset was higher than the cure rate for those started on the treatment after 3 months. The improvement rate gradually improved in a year. The patients who presented with severe hyposmia and anosmia found it hard to recover. Olfactory dysfunction after URI frequently appears in June (17.3%) and July (17.3%).

Study of odor memory—correlation with association words

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Odor memory refers to several aspects of memory, including odor recognition memory, recall memory, and memory for specific details associated with a target odorant (e.g., serial position, or which odor was presented before or after the target odor). Existing studies suggest that odor memory has a short-term working

memory, a long-term permanent memory component, and also has a semantic/language component. There have been several studies reporting the relationship between recognition memory and various perceptive/cognitive perspectives. However there are no studies that have investigated the relationship between quantity or quality aspects of associations for odor and it's memory performance. In this study, 51 Japanese male and female subjects (ratio 3:2, mean age 31 ± 6) were presented with 10 odorants. They were asked to smell one odorant at a time and write down the things that come into their minds (free associations). They were given 45 s for smelling and writing for each odorant and a 15-s break in between all trials. Number of associations and word class were checked, and the correlation with memory performance was examined for each odor. From the quantitative point of view, loose correlation with memory performance was observed. There are several anomaly odors, and this indicates the existence of other important factors, which play a great role in memory. From the qualitative point of view, clear correlation with memory was observed as the more concrete the associations, higher the memory performance.

The influence of odor classification on naming odors

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This study investigated factors that facilitate odor-naming performance. Previously, we indicated that mere experience of odors would have little influence on associative learning between the odors and their names. In the present study, participants experienced and classified odors repeatedly. The classification was presented to give them some semantic information of odors; therefore, it was expected that associating odors with their correct names would be easier. Fourteen spices were used as odor stimuli. Through a screening test, 12 unfamiliar odors were selected for each participant. From the preliminary screening test to the next session, during a week, the participants were asked to memorize 14 spice names. In the classification session, they smelled six odors of the 14 spices repeatedly and classified them into three categories. Half of six odors were presented five times, and other half odors were presented once. Five minutes later, the participants named six experienced odors and six unexperienced odors by using memorized odor names. Correct feedback was given to every naming. The results indicated that odor experience and odor classification had little influence on the performance of naming odors. In terms of the performance score of classification, we divided the participants into two groups. One group classified odors in consistent manner, and another group classified them inconsistently. The consistent group indicated high odor-naming performance when they smelled odors that were classified five times. The inconsistent group indicated difficulty in naming odors that were classified five times. Correctly naming odors would need perceiving odors consistently in addition to repeating odor experience and classifying.

A study on image structure of odors using adjectives

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The present study compared the two experimental methods on inquiring into the image structure of odors: presenting a stimulus is one, and not presenting a stimulus is the other. For experiment 1, five odors were presented, and the subjects were instructed to evaluate the odors on a seven-point scale for each of the 25 adjectives. For experiment 2, the odor was not presented, and the subjects were instructed to perform pairwise comparisons for each pair of two adjectives on their similarities on a seven-point scale. The data from the two experiments were analyzed and compared using MDS (multidimensional scaling), correlation, and cluster analysis. The results showed that there were no structural differences between the two experimental methods in terms of the image structure of odors. But, minor disparity was found between two methods in terms of density of distribution of the adjectives. It was construed that the difference came from the difference of the memory that was used for each of the experiments; that is, short-term memory for experiment 1 and long-term memory for experiment 2.

The effect of a scent on the ability to acquire a language

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In this study, we examined the effect of a scent in promoting the acquisition of the relation of the thing, the text, and the speech at different situations of the language study by the healthy subjects and the speech and language therapy for the slight language-disordered children. Consequently, in the language study of the healthy subjects, it was suggested that the subjects who learned language with smelling a scent have higher acquisition of language (the number of correct answers) than the subjects who learned without smelling a scent and that the language study was promoted with a scent. And, it was suggested that there is an increasing tendency to make autonomous efforts to speak by using a crayon, and it was expected to promote the spontaneous utterance with the scent in the speech and language therapy for the slight language-disordered children. As mentioned above, the usefulness of a scent was suggested in the language study of a healthy subject and/or speech therapy for the slight language-disordered children. However, it means that the scent did not influence the speech center directly, but a scent stimulates a human affective and makes it change, having been continuously connected to action.

Discussion about the standard odors

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We recognize smell as the same as sight and hearing in our brain, and certainly accept it as objective intelligence. It has standard

colors and sound and can be given by language. We also think standard smell is possible. The Japanese make ''Kodo'', which is a game or art said to be a fusion of perfume and language. We have started choosing our common odor term. We estimated smell by the semantic differential (SD) method using 31 concrete odor-descriptive terms for the five standard odorants for three subjects (Sub1, Sub2, and Sub3). They were playing ''Kodo'', where a mother and her two daughters had similar food, life, and surroundings. The data for principal components analysis was extracted from three principal components. The result of Sub1 became ''sweet and flower'', ''rotten and camphor'', and ''fatty and resin''. That of Sub2 and Sub3 were ''sweet and vanilla'', ''urine and camphor'', and ''mint'' and ''flower'', ''sweet and aromatic'', and ''rotten and camphor'', respectively. We discussed two ''rotten'' expressions (Chinese and Japanese characters) for three subjects. Sub1 gave the constant evaluation for ''rotten'' in spite of a different expression. Sub2 did not have an influence on ''rotten'' because she was evaluated by ''urine'' instead of ''rotten''. The expression of ''flower'' and ''mint'' was also in disagreement with others. Sub3 had normal evaluation for ''rotten'' and ''flower'' in comparison with the mother, but the first principal elements consisted of the mixture of ''flower'' and ''rotten''. The difference in the mother and her daughters might be reflected biologically, that is, Amoore should suggest that ''flower'', ''mint'', ''rotten'' etc. were the standard odors.

The influence of taste imagined from odor on taste-intensity ratings: coexposure study using passion fruit flavor, sucrose, and citric acid

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When we eat or drink something, we imagine the taste from its odor and confuse it with the ''real taste'', and the confusion between the taste and the imagined taste from odor sometimes influences taste-intensity rating. The imagined taste from odor depends on whether or not we have previously been exposed associatively to both the taste and odor. We examined the latter phenomenon using citric acid (Cit) and/or sucrose (Suc) as sour and/or sweet taste stimuli and passion fruit odor (Pf) as a novel odor to our Japanese subjects. During a 1-week coexposure period, subjects in the sour group (SoG) ($n = 24$) tasted 10 ml of Cit + Pf solution once a day. Subjects in the sweet group (SwG) ($n = 24$) tasted Suc + Pf solution at the same rate. The subjects sniffed the Pf solution and then evaluated the ratios of imagined sourness (OSo) and sweetness (OSw) to the whole imagined taste before and after the coexposure period. After this period, the subjects tasted Pf-added Cit, Suc, and Cit + Suc solutions and rated the taste intensities of sourness (TSo) and sweetness (TSw) with and without nose clips. Ratings with nose clips were regarded as ratings without the Pf odor. In the case of SoG, OSo increased and OSw decreased by coexposure. TSo of the Suc solution was larger with Pf than without it. In the case of SwG, though coexposure did not alter OSo or OSw, TSw of the Cit and Cit + Suc solutions was higher with Pf than without it. Subjects in both groups rated the intensity of tastes that were coexposed with Pf significantly higher when they had barely sensed the real taste and thus the sourness of the Suc solution and the sweetness of the Cit solution.

Relationship between mechanical properties and perceived texture described by onomatopoeic words affecting food acceptability

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Food texture described using Japanese onomatopoeic words was correlated with the physical properties and also with acceptability. Eleven onomatopoeic words were selected to describe food texture applying dual scaling and cluster analysis on the cross-table of 45 words and related 450 foods collected in preliminary studies. Sensory evaluation using the 11 words was carried out on the 11 kinds of foods representing different textures: cookie, jerry, bread, marshmallow, raw radish, pickled radish, raw abalone, fried chicken, steamed sweet potato, sesame tofu, and stewed pork. A laboratory panel of 11 members described the food texture as well as the difficulty of chewing and swallowing and the acceptability of the texture on visual analog scales. A mechanical compression test was conducted using a universal testing machine (Instron 5564). The true stress was calculated as the detected load divided by the contact area between the sample ($25 \times 25 \times 10$ mm) and the plate of Instron (φ 150 mm), which was measured using an I-SCAN system (Nitta Co., Osaka, Japan). The evaluated intensities of food texture were correlated to the true stresses at different compression strains and also to the difficulty of chewing and swallowing and the acceptability. The results of ANOVA on the evaluated intensities ascertained the availability of onomatopoeic words to the sensory evaluation of food texture. The stress at 70% strain correlated positively with the intensity of ''kori-kori'' which was concerned significantly with the difficulty of chewing. The stresses at 10% and 30% strains correlated positively to the intensity of ''saku-saku'', ''shari-shari'' and ''pari-pari'' affecting the acceptability of the texture significantly.

The taste expression of monatin and the analogues

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Monatin [(2*S*,4*S*)-4-hydroxy-4-(indol-3-ylmethyl)-glutamic acid] is an unusual amino acid with a high-intense sweetness from natural sources. We have synthesized 18 kinds of monatin analogues. At the beginning, the taste of each monatin analogue was predicted from the result of computer modeling based on Goodman's theory, that is, the sweetness production of dipeptides and related compounds requires the proton donor AH, the proton acceptor B, and the hydrophobic group X, and the tastes decide the direction of their X. In the case of monatin and analogues, which were peptide mimetics, 2-NH group, 1-carboxyl group, and indole or phenyl groups were estimated as the AH, B, and X, respectively. Then, the sensed taste was decided by a sensory test to solutions of each synthesized compound. As a result of comparing both tastes, it was found that predicted and sensed tastes were fairly similar to each other. Most compounds generally expressed sweet taste, and some of them expressed bitter taste; however, the intensity differed. We concluded

that the qualities and intensities of sweet taste were decided by the direction of X when the conformation was L shaped. Thus, the taste expressions of monatin analogues follow Goodman's theory. In addition, the important factor for the sweetness expression on monatin analogues was the location of the sweet expression groups (AH, B, and X) and not those properties.

The effect of experience of mismatching between tastes and visual images following the gustation of water

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Experiments that illustrate plasticity of processing of taste in humans were conducted. Subjects were given samples, each of which consisted of an unusual combination of taste and visual image. It was investigated how such strange experiences affect the following processing of taste. In a matching experiment, 42 subjects were given 24 samples, each of which consisted of a usual combination of taste and visual stimuli (e.g., a cup of apple juice covered with a lid painted in an apple). They were required to drink the taste stimulus while looking at the visual one. After 30 s, they were given a cup of water covered with a lid painted in an impossible figure. They were required to drink the water while looking at the figure and were asked whether they could sense the taste of the water. In the mismatching experiment, the same subjects were given 20 samples, each of which consisted of an unusual combination of taste and visual stimuli (e.g., a cup of soup covered with a lid painted in an apple). They were required to drink the taste stimulus while looking at the visual one. After 30 s, they were given a cup of water covered with a lid painted in an impossible figure and required to drink the water while looking at the figure. They were asked whether they could sense the taste of the water. As a result, eight subjects sensed the taste of water ($P < 0.05$) after the mismatching experiment, whereas four subjects did so ($P > 0.05$) after the matching one.

Electric stimulation of the tongue evokes sour taste after presentation of miracle fruit

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Electric stimulation of the human tongue evokes taste sensation known as ''electric taste''. Anodal stimulation produces sensation described as ''metallic'' and ''sour'' taste. The mechanism of electric taste is not clearly clarified. Two notions of the mechanism of electric taste that had received support from several researches have been proposed. One notion is the ion accumulation mechanism: ions in the fluid medium bathing the tongue are iontophoresed to the receptors by the electric current. The other notion is electric current from the microvillus membrane of a taste cell to the synaptic area. The purpose of this study was to verify the mechanism of an electric taste. In this experiment, after a miracle fruit (after miraculin in the tongue, the presence of an ion such as the hydrogen ion induces sweet taste activity) had been presented, electric stimulation was presented to the participants who have sour taste induced by anodal stimulation. If the ion accumulation mechanism exists, sweetness is induced by the hydrogen ion. As a result, the electric taste quality (sourness) that the participants reported did not change after the miracle fruit presentation, though citric acid induced sweet taste after the presentation. This result suggests that electrical current from the microvillus membrane of a taste cell to the synaptic area produces electric taste. However, the possibility that miraculin is transformed by electric stimulation cannot be excluded. In future studies, therefore, operating miraculin quantitatively will be needed.

Comparative study of gustatory examinations by taste strips and filter-paper disks method

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We have used the filter-paper disks (FPD) method for differential diagnosis of taste dysfunction, but this method is not so popular and only 34% of all institutions used it clinically. The reason for this unpopularity is based on the complicated and time-consuming procedures. Furthermore, setting of tastant concentration is not quantitative enough. Mueller *et al.* developed a simple and easy gustatory examination using impregnated taste strips in 2003, and the major advantages of this examination are long shelf life and short period of time needed for testing. Salsave, a paper impregnated with salt solutions of various concentrations, has been used in Japan and this long shelf life kit takes only a few minutes for measurement. However this kit lacks evaluation of sweet, sour, and bitter tastes and has limitations in qualitative evaluation, so the ability of this kit seemed to be incomplete for clinical purposes. In the present study, we developed taste strips soaked with different taste solutions (four concentrations each for sweet, salty, sour, and bitter) and dried in accordance with the method reported by Mueller. The data obtained by using taste strips were compared with those measured by the FPD method. A total of 138 healthy volunteers (12–79 years old) measured their gustatory ability by both the taste strip and FPD methods. The mean scores for sweet, salty, sour, and bitter taste are 3.2, 3.4, 3.3 and 3.1, respectively. These scores are similar with those reported by Mueller in German subjects. The results from taste strips significantly correlated with the results measured by the FPD method ($r = -0.59$). These data suggest that taste strips method is a useful for gustatory examination in clinical conditions.

Influence of sweetness and energy of food on taste in human subjects

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The influence of sweetness and energy of food on taste in healthy university students was studied. Food materials were made from agar with sucrose (sweet and high energy), erythritol (sweet and nonenergy), maltoteraose (low sweet and high energy), and water (nonsweet and nonenergy). Taste test material was 10gust sucrose

solution. Taste pleasantness, its strength, stomach fullness, and blood glucose were measured before eating food material, at 10, 30, and 60 min after eating. Blood glucose in agars with sucrose and maltoteraose was higher than in agars with erythritol and water. Taste pleasantness for taste stimuli after eating agar with sucrose was decreased significantly than other food materials, while taste strength after eating agar with sucrose was lower than the other foods. Stomach fullness increased after eating all foods. These results showed that both sweetness and energy of food might be important for taste sensation after eating food.