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ABSTRACTS

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Modulation of the Threshold of Action Potentials in Olfactory Receptor Cells

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Olfaction begins with the transduction of the information carried by odorants into electrical signals in olfactory receptor cells (ORCs). The initial step involves the binding of odorant molecules to specific receptor proteins on the ciliary surface of ORCs. Odorant receptors coupled to G-proteins activate adenylyl cyclase leading to the generation of cAMP, which directly gates a cyclic nucleotide-gated cationic channel in the ciliary membrane. This initial excitation causes a slow and graded depolarizing voltage change, which is encoded into a train of action potentials. Action potentials of ORCs are generated by voltage-gated Na^+ currents and T-type Ca^{2+} currents in the somatic membrane.

Isolated ORCs that have lost their cilia during the dissociation procedure are known to exhibit spike frequency accommodation by injecting the steady current. This raises the possibility that somatic ionic channels in ORCs may serve for odor adaptation at the level of spike encoding, although odor adaptation is mainly accomplished by the ciliary transduction machinery. In this symposium, I will discuss current knowledge concerning the mechanisms of spike generation in ORCs, and also how neurotransmitters and hormones modulate ionic currents and action potentials in ORCs.

Brain Responses after Ingestion of Nutrients

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Monosodium L-glutamate (MSG) is an umami substance and its addition in adequate amounts to most foods enhances palatability and acceptability. Recently, the receptors for L-glutamate (GLU) are found both in the oral cavity and gastrointestinal tract. Taste and vagus nerves respond to stimulation of MSG. Especially, the gastric vagal afferent fibers respond only to GLU among 20 amino acids and NaCl administered in the stomach. To investigate responsive areas in the rat brain, we recorded single unit activity during ingestion of amino acid solutions, and also measured spatio-temporal activation in response to intragastric administration of taste substances by using functional magnetic resonance imaging. Some neurons in the lateral hypothalamic area (LHA) responded specifically to MSG during ingestion. These neurons discriminated between MSG and NaCl. Intragastric administration of taste substances (glucose, MSG, and NaCl in 60 mM) activated several brain areas including the cerebral cortex, basal ganglia, limbic system, and hypothalamus. Notably, the medial preoptic area, dorsomedial nucleus of the hypothalamus, and habenular nucleus were activated by MSG only. The nucleus accumbens was activated by glucose only. After vagotomy, the brain response to MSG was substantially abolished while response to glucose was unaffected. These results suggest that the dietary GLU activates the brain via taste and vagus nerves. The LHA is one of the higher brain centers for recognition of umami taste. The brain activation after the ingestion of umami substances may possibly link to regulation of several physiological functions such as body temperature, energy metabolism, emotion, learning, memory, and motor activity.

Availability of a Self-Administered Smell Questionnaire for Patients with Chronic Sinusitis

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This study demonstrated statistic correlations between a novel self-administered smell questionnaire and other olfaction tests in patients with chronic sinusitis presenting with olfactory dysfunction, and discussed usefulness of the questionnaire. The questionnaire proposed by Japan Rhinology

Society was self-administration test consisting of 20 smell items. We applied the questionnaire to 100 patients performed endoscopic sinus surgery (ESS) between December 2004 and December 2007. There were 66 men and 34 women. Mean age was 51.6 years old (15-78 years old). Postoperative olfaction was follow-up in 58 patients. In the preoperative stage (n = 100), the scores of questionnaire showed statistically significant correlations with those of visual analogue scale ($p < 0.05$, $r = 0.852$) and T&T recognition threshold ($p < 0.05$, $r = -0.337$). In the postoperative stage (n = 58), the scores of questionnaire showed statistically significant correlations with those of visual analogue scale ($p < 0.05$, $r = 0.853$) and T&T recognition threshold ($p < 0.05$, $r = -0.387$). On average, the preoperative questionnaire scores (19.5%) significantly improved to 67.1% after ESS (n = 58). Visual analogue scale (%) also significantly improved from 15.9% to 56.5% after ESS (n = 58). Mean T&T recognition threshold showed significant improvement from 5.4 to 3.8 after ESS (n = 58). In conclusion, utility of the smell self-administered questionnaire as an easy method to estimate the olfaction was indicated, though the questionnaire is still required to be modified.

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Glucocorticoids May Enhance Regeneration of Olfactory Epithelium

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Conclusion: Glucocorticoid (GC) administration enhanced apoptotic changes in mature olfactory receptor neurons (ORNs). GC administration may enhance regeneration of olfactory epithelium (OE).

Objectives: The mechanism underlying olfactory epithelial cells turnover involves apoptosis replaced by new ORNs. On regeneration of OE, we evaluate the apoptotic change in OE. To corroborate the enhancement of apoptosis of ORNs induced by GCs that are generally administered locally or systemically to patients with olfactory dysfunction.

Methods: In vitro study: We established cultured murine ORNs. Triamcinolone acetonide was added to culture supernatants. ORNs were then cultured for another two weeks. In vivo study: Triamcinolone acetonide was administered to mice 5 or 10 times. The mice were dissected 3 days after the final injection, and the olfactory regions were removed and embedded. All samples were examined by immunohistochemical staining and TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method.

Results: glucocorticoid receptors (GRs) expression of cultured murine ORNs were observed among ORNs with mature stage. GRs expression of murine OE were localized on mature ORNs and supporting cells. The proportions of apoptotic cells in both of GC-administered cultured ORNs and GC-administered mice were significantly higher than those in the control groups.

Cell Types and Response Properties of Mouse Fungiform Taste Cells

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Taste bud cells have been classified into type I, II, III and basal cells. Among them, Type II and III cells may transduce taste stimuli. Type II cells express sweet, bitter and umami taste receptors and transduction components but they do not form conventional synapses. In contrast, Type III cells possess synapses but not these taste related molecules. Recently, taste cells expressing markers for Type III cells were shown to be co-expressed with putative sour taste receptors, suggesting Type III cells may be sour taste cells. However, little is known about physiological responses of Type II and Type III cells. In this study, we investigated response properties of specified taste cells by means of transgenic mice which express green fluorescent protein (GFP) in gustducin-positive (Type II) and GAD67-positive (Type III) cells. We found that gustducin-GFP cells responded to sweet, bitter and umami stimuli whereas GAD67-GFP cells responded to sour stimuli and electrolytes. Single cell RT-PCR analysis demonstrated that gustducin-GFP cells responding to sweet stimuli expressed sweet taste receptor (T1r2/T1r3) and GAD67-GFP cells responding to sour stimuli expressed sour taste receptor (PKD2L1) and synapse related gene (SNAP25). These results suggest that both type II and Type III cells may function as taste receptor cells. Sweet, umami and bitter substances may be detected by type II cells, whereas sour substances and electrolytes may be detected by type III cells.

Recovery of Responses to Umami after Crush of the Mouse Chorda Tympani Nerve

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Recent molecular studies proposed that various receptors, such as a truncated type 4 metabotropic glutamate receptor (taste mGluR4), heterodimers of T1R1/T1R3, taste mGluR1, and brain-type mGluR4, might underlie umami taste. However, to date the roles in umami taste of each of these receptors and their downstream signaling molecules have not been made clear. In the present study, we examined recovery of umami taste responses in the mouse chorda tympani (CT) nerve after crushing the nerve. At about 2 weeks after the nerve crush, no significant responses to taste stimuli were observed in the CT. At about 3 weeks after the crush, taste responses reappeared and response to 0.1 M monopotassium glutamate (MPG) was significantly suppressed by AIDA and CPPG, mGluR1 and mGluR4 antagonists respectively. At about 4 weeks after the crush, although responses to MSG + 0.5 mM inosine monophosphate (IMP), 0.1 M MPG + IMP and 0.1 M L-Ala + IMP recovered to their control levels, synergism between 10 mM quisqualic acid (mGluR1 agonist) and IMP and/or that between10 mM L-AP4 (mGluR4 agonist) and IMP were not observed. After more than a month, the CT showed recovered responses to all stimuli tested including 10 mM quisqualic acid + IMP and 10 mM L-AP4 + IMP to similar levels to those shown by intact animals. These results suggest that the differential restoration of T1R1/T1R3, mGluRs and transduction pathways, providing additional evidence for existence of multiple receptors and transduction pathways underlining umami taste in mice.

Interaction Between Triterpene Glycoside and Human Sweet Receptor hT1R2/hT1R3

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Glycyrrhizin (GL) and Gymnemic acid (GA) are triterpen glycosides isolated from Glycyrrhiza glabra and Gymnema sylvestre, respectively. It is known that GL tastes sweet to human and that GA selectively suppresses taste responses to various sweet

compounds without affecting responses to salty, sour and bitter substances in human and chimpanzees.

In order to examine whether GL and GA directly interact with the human sweet receptor, we used the human sweet receptor hT1R2+hT1R3 assay in transiently transfected HEK293 cells. Similar to psychophysical studies in human, GI showed [Ca2+]i responses and 0.2 mg/ml GA inhibited the [Ca2+]i responses to 1mM GI. It has also been shown that in human psychophysical study, sweetness of GI is inhibited by γ-cyclodextrin (CD) and that the sweet-suppressing effect of GA is diminished by rinsing the tongue with γ-CD. So we examined the interaction between these triterpen glycosides and (α, β and γ-) CDs in vitro. The responses to 1mM GI were also inhibited by 1mM γ-CD completely and the effect of GA rapidly disappears after rinsing the cells with 10mM γ-CD. Our present study confirmed the previous finding in human psychophysical study and demonstrated that GI and GA directly interact with hT1R2+hT1R3 on the taste cell membrane and this interaction is inhibited by forming inclusion complex between these triterpen glycosides and γ-CD.

Conditioned Flavor Preference Learning Induced by Intragastric Administration of Glutamate in Rats

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Postingestive consequences as well as oronasal cues are the important factors on the preference for foods and fluids. This study demonstrates that the postingestive effect of glutamate by using conditioned flavor preference paradigm. Adult male Sprague-Dawley rats with chronic intragastric (IG) cannula were given daily 30 min one-bottle training to drink a flavored solution (CS+) paired with IG infusion of nutrient solution and another flavored solution (CS-) paired with IG water infusion on alternate 8 days. Nutrient solution was given 60mM monosodium L-glutamate (MSG group) or 60mM NaCl (NaCl group). Before and after conditioning, rats received 30 min two-bottle choice tests for CS+ and CS- solution. Before conditioning, both MSG and NaCl groups had no higher preference for the CS+ solution. During conditioning, the intake of CS+ at the last half of the session was significantly higher compared to the first half of the session in MSG group, but not in NaCl group. After conditioned, MSG group showed significantly higher intake and preference for the CS+ solution ($p<0.05$), while the NaCl group did not show any significant intake and preference for the CS+ solution. These results indicate that glutamate has a postingestive positive effect after ingestion.

Effects of Inactivation of the Central Nucleus of the Amygdala on Palatability-Induced Ingestion

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The central nucleus of the amygdala (CeA) is involved in the regulation of feeding behavior. However, it remains unclear whether the CeA is concerned with voluntary intake of palatable food stimuli. In the present study, we investigated the effect of transient inactivation of the CeA on palatability-induced ingestion. After bilateral microinjections of muscimol (0, 5 or 20 ng / side), an agonist of $GABA_A$ receptor, into the CeA, the intake of a highly palatable liquid diet (50% Ensure Liquid diluted with water) was measured. Microinjections of muscimol dose-dependently reduced the intake of Ensure Liquid and increased the number of paw treading which is considered to be an aversive response to taste stimuli. Since the lesion of the CeA is shown to enhance the aversive property of taste stimuli, it is likely that the decreased palatability of Ensure Liquid by inactivation of the CeA suppressed the intake and increased the number of paw treading. In a separate experiment, microinjections of muscimol into the CeA increased the number of c-Fos positive cells in subregions of the parabrachial nucleus and the nucleus of the solitary tract which receive visceral afferents. Because previous research showed that a malaise-inducing intraperitoneal injection of LiCl stimulates the number of c-Fos positive cells in the same regions, inactivation of the CeA may lead to nausea which prevents the intake of highly palatable Ensure. These results suggest that an intact function of the CeA is required to voluntary intake of palatable food stimuli.

P-009

Maternal Zinc Deficiency during Lactation Period Affects Postweanling to Adulthood Sodium Preference in SD Rats

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It has been well known that maternal dietary NaCl intake influences weanling rats' salt preferences, and brief exposure to NaCl during early postnatal development enhances adult intake of sweet and salty compounds. However, few data have been published concerning about the maternal milk nutrients such as zinc level and later NaCl preference in their grown pups. We have already shown that short term zinc deficiency clearly causes the increase of NaCl preference, so we demonstrated in the present experiment whether or not the maternal zinc deficiency during lactation period cause changes in NaCl preference of the rats grown to adulthood using this zinc deficient system with SD/Slc rats. Low zinc (4.0 mg Zn/Kg) or zinc sufficient diet (33.7 mg Zn/Kg) was fed to the lactating mother during lactation period only (for 3 weeks after birth), and zinc sufficient diet was fed to the all group's rats after weanling. Taste preference study with water and 0.5M NaCl solution in the 2-bottle preference system was undertaken, and it was shown that maternal low-zinc diets during lactation period caused the increased 0.5M NaCl preference in their developing pups up to 11 week-old (after 8 weeks from weanling) as reported last year, even though after their recovery from zinc deficiency. After growing to adulthood (11 wk-old), significant increase of norepinephrine (NE) concentration in the hypothalamus was observed, so it was suggested that this phenomenon might affect the NaCl preference behavior, though further detailed clarification such as by microdialysis method must be required.

The Taste and Odor of Carp Sashimi

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A carp meat has been used as event foods e.c. ''a New Years holiday's cooking'', ''Buddist mass'' and ''meal attention of daily life'' on a formal occasion in Nagano Prefecture, especially, Saku City. In our previous study, younger generation claimed the carp meat had a muddy odor or fishy smell[1)]. Therefore, the carp meat's taste and smells are the weak-points as foodstuff. In this study, we considered the interregional differences (Saku City, Nagano Prefecture and Kooriyama City, Fukushima Prefecture) of the raw carp's taste and odor with the sensory evaluation. The carp meats were subjected to sensory evaluations of odor and taste by trained 41 panelists (ave. age was 22.9). As the results, the over half panelists suggested that both Saku and Kooriyama City's carp meats had the fishy and valueless smell. On the contrary, these carp meats did not smell the muddy odor by comparison. A Saku City's carp quality of odor was better than Kooriyama. On the other hand, umami taste of Kooriyama City's carp was stronger than Saku. In addition, there is a trend toward of the taste pleasant of Saku-Carp. As above, in interval area, there was a difference in taste and smell of the carp sashimi.

1) Hiroko Nakazawa, Kana Kogiso and Yumi Yoshioka; Freshwater Fish in Dietary Habits of Nagano Prefectural Collage Students, Journal of Nagano Prefectural Collage, *62*, 9-20(2007)

Evaluation of Canned Coffee by Taste and Smell Sensor Systems

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Validity of instrumental methods as the substitute for organoleptic method to evaluate the flavor of canned black coffee products with no added flavors was investigated. Five kinds of samples were produced in the actual line in the factory and the flavor of the samples was analyzed by using MS type smell sensor, taste sensor and GC/MS and then compared with the sensory evaluation by the trained panels. The obtained regression equations were tried to predict the average panel flavor score from the instrumental data. The ''umami'' score evaluated by the panels could be predicted from the analytical values by the smell sensor and the taste sensor significantly. These results suggest that the analysis by the smell sensor and the taste sensor is useful as the substitute for panel method in the actual line production of canned black coffee with no added flavors.

Transporting System of Glutamate and GABA on the Taste Bud that Expresses Glutamate Decarboxylase

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Glutamate decarboxylase (GAD) is an enzyme that produces γ-amino butyrate (GABA) from L-glutamate. We found that GAD67 is specifically expressed in the type III taste bud cell that is presynaptic cell by using GFP-GAD67 knock-in mice. We also found that GABA is synthesized inside the taste bud, and ionotropic $GABA_A$ receptor subunits and G-protein coupled $GABA_B$ receptor subunits are expressed in mouse circumvallate papillae.

In this study, we have investigated the path how the substrate, glutamate, is coming into the taste bud and how the product, GABA is utilized within the taste bud. We have employed RT-PCR and immunohistochemical analyses to demonstrate the presence of glutamate transporters and GABA transporters. In results, some of glutamate transporter subtypes (GLAST, GLT-1, EAAC1) and GABA transporter subtypes (GAT1, GAT3, GAT4) are expressed on taste bud cells. Our results suggest that glutamate is taken into the taste bud cells and GABA is synthesized by GAD. The produced GABA might act as a transmitter of the taste signal or might bind to Cl^- channel to affect the salt sensation. Our results offer a possibility that GAD may be involved in the taste signal transduction mechanism via releasing GABA molecule, where the inhibition of GAD activity may lead to an alternation.

Brain Mapping of Conditioned Taste Aversion using Manganese-Enhanced MRI in Rats

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We tried to detect the brain areas concerning the memory retrieving of conditioned taste aversion (CTA) using manganese-enhanced MRI (MEMRI). The Mn ions are taken into the cells on their activations and enhance the MRI intensity. Therefore, the MEMRI intensity reflects the activity of the brain tissues. Rats were conditioned by 5 mM saccharine as a stimulus (conditioned stimulus: CS). LiCl solutions (as a robust aversion chemical, unconditioned stimulus: US) of 0.15 M were injected i.p. 15 min after drinking the saccharine solution. After the two times conditionings, these rats showed a robust aversion to the saccharine solution (US). Rats of control group were injected a saline i.p. instead of LiCl solutions. The MRI signal intensities at GC (gustatory cortex), CeA (central amygdale), VP (ventral pallidum) and IPAC (interstitial nucleus of posterior limb of anterior commissure) of the conditioned group were higher than those of the control group ($p < 0.05$). There are no significant differences between the conditioned and the control groups in the intensities of the other regions, such as AcbC (acumbens nucleus core), AcbSh (accumbens nucleus shell), LH (lateral hypothalamus), BLA (basolateral amygdale anterior), MC (motor cortex) and ST (striatum area). These indicate that GC, CeA, VP and IPAC have important roles in the retrieval of CTA.

The Role of Mu-Opioid Receptors in the Ventral Pallidum in a Palatability Shift Induced by the Development of a Conditioned Taste Aversion

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A paring of a taste (conditioned stimulus, CS) with visceral malaise causes a palatability shift of the CS from ingestive to aversive (conditioned taste aversion, CTA). To elucidate a possible involvement of the opioidergic system in the ventral pallidum (VP) in the palatability shift in CTA, we examined the effects of the microinjections of mu-opioid receptors agonist D-Ala^2-N-Me-Phe^4-Glycol^5-enkephalin (DAMGO) into the VP on the palatability of the CS after the development of CTA using a taste reactivity test (TR test) and a single-bottle intake test. Rats received a paired presentation of 5 mM saccharin solution (CS) with 0.15 M lithium chloride. Two days after the conditioning, simultaneous bilateral microinjections of DAMGO (10 or 100 g/0.25 μl) or vehicle (Ringer solution) were given in the rats just before the re-exposure of the CS. We counted the ingestive (e.g. tongue protrusion) and aversive responses (e.g. chin rubbing) in the TR test and measured the consumption of the CS solution in the single-bottle test. In the TR test, the microinjections of 10 ng DAMGO significantly decreased the occurrence of aversive responses (gaping and head shaking) and tended to increase ingestive responses. In the single-bottle test, the DAMGO-injected group showed significantly higher intake of the CS than the vehicle-injected group. These results suggest that the administration of DAMGO into the VP reduced the aversion to the CS after the establishment of CTA, inducing the higher CS intake. The opioidergic system in the VP may be involved in the signal transmission of the aversiveness of the CS after the development of CTA.

Gustatory Responsiveness of the Insular Cortex in Awake Rats

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There have been few studies on taste responsiveness of the gustatory cortex in awake, behaving rats, although substantial lines of evidence for this issue is available from studies using anesthetized rats. In the present study, in order to acquire gustatory data under an awake condition, we isolated single neurons from gustatory area in the insular cortex of behaving rats. The head of the rat was held in place in the stereotaxic apparatus using a surgically pre-fixed cranioplastic cap. Fluid stimuli consisted of 50-ml samples of water, sucrose, NaCl, citric acid (CA) and quinine HCl (QHCl) were given to the rat via intraoral cannulae. From a total of 45 insular neurons, 21 responded significantly to at least one of the sapid stimuli (taste neurons). The mean spontaneous rate of the taste neurons was 3.84 ± 0.78 (mean ± SE) spikes/s; the mean response to water (above spontaneous rate), 0.25 ± 0.54 spikes/s. Based on their largest response, taste neurons were classified into NaCl best (N = 7), sucrose-best (N = 6), CA-best (N = 5), and QHCl best (N = 4). The mean breadth of tuning index (entropy) calculated from the data on all the taste neurons was 0.70. Some taste neurons displayed temporal fluctuation in the taste responses. These taste response properties of neurons in the insular cortex show clear contrast to the neurons in the medulla and pontine gustatory areas (showing smaller entropies and monotonic excitatory responses), suggesting the hierarchy of gustatory information processing form lower relays to the cortex.

Taste Sensitivity and Cortical Localization of Putative Pyramidal Neurons and Interneurons in the Rat Gustatory Cortex

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Previous studies have indicated that the extracellular spike waveforms and firing properties of cortical neurons differed between fast-spike (FS; inhibitory) interneurons and pyramidal (PY) neurons, the former tending to have narrower-spike widths and high-frequency activities without accommodation. PY neurons in somatosensory cortices were shown to have finer sensitivity to stimuli than FS neurons. In the present study, 85 taste-sensitive neurons in the rat gustatory cortex were classified primarily into 3 groups based on the spike width, i.e., narrow-, intermediate- and broad-spike neurons. Furthermore, narrow-spike neurons with high-frequency activity were categorized as FS neurons. FS neurons showed significantly higher spontaneous activity as well as higher taste response magnitude to NaCl compared with other cell classes. N-best FS neurons were characterized by persistently high discharges without accommodation during NaCl stimulations. These results suggest that FS neurons in the current study correspond to fast-spike inhibitory interneurons. The average ratio of responses to two concentrations of tastants was higher in PY neurons than in FS neurons, indicating that PY neurons had higher sensitivity to tastant concentration than FS neurons. The higher sensitivity in PY neurons may be partly due to inhibitory inputs from FS neurons that reduce the former neurons' background activities. Histological data indicated both taste-sensitive FS and PY neurons were observed more frequently in the superficial cortical layer (III, 0.5 mm from the pia mater) than in the deep layers (IV/V, 0.7-0.8 mm). Further studies are required to know whether such a layer-specific organization plays functional roles in the gustatory cortex.

Functional Connections between Olfactory and Gustatory Information Via the Endopiriform Nucleus in the Rat

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The endopiriform nucleus (EPN) is a large group of multipolar cells located in the depth of the pirifprm cortex (PC). Although many studies have suggested that EPN may play a role in temporal lobe epilepsy, the normal functional role of the EPN remains unknown. In a previous study, we have shown the convergence of olfactory

and gustatory excitation onto the EPN in frontal slice preparations under Mg^{2+} free condition (Fu et al., 2004). In this study, we used optical imaging with voltage-sensitive dye to study spatiotemporal excitation spread from PC or gustatory cortex (GC) to the EPN in Mg^{2+} containing condition. Paired-pulse stimulation (interpulse interval: 20-100 ms) of the PC induced signal propagation from the PC to the EPN through the excitation in the agranular division (AI) of the insular cortex, whereas paired-pulse stimulation of the GC did the same from the GC to EPN via the AI. Excitation extended further to the claustrum. Double-site paired-pulse stimulation of the PC and the GC also evoked the excitation in the AI, claustrum and EPN. In addition, using immunohistochemical detection of the c-Fos protein as a measure of neuronal activity, we examined the distribution of Fos-positive cells in the olfactory and gustatory cortical areas following feeding the food (an apple). Fos-positive cells were found in the PC, GC, EPN, AI and claustrum, consistent with the results of the optical imaging study. Thus these results together with our previous study, suggest that the EPN may be important as the integrative region of olfactory and gustatory information.

Expression of Sox2 in the Genesis and Differentiation of the Taste Buds and Circumvallate Papillae

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Sox2, which encodes an HMG box transcription factor, is known to function to regulate the differentiation of progenitor cells of the tongue into taste bud cells versus keratinocytes during development. To determine the neural dependence of Sox2 expression, glossopharyngeal nerves of mice were cut bilaterally. In unoperated mice, the expression of Sox2 mRNA and protein was restricted to a subset of taste bud cells, and the epithelium surrounding the taste buds of the circumvallate papillae. During the period of denervation, the taste buds largely disappeared; and the taste bud cells and the epithelial cells with Sox2-immunoreactive (IR) nuclei decreased in number and totally disappeared from the epithelium by 16 days after the denervation. When regenerated nerve fibers entered into the epithelium, Sox2 expression reappeared first in the epithelial cells and then in the regenerating taste bud cells. Also, in prenatal mice, Sox2 was expressed in the epithelium of the dorsal surface of circumvallate papillae, where numerous nerve fibers entered. The results suggest that Sox2 expression is dependent on gustatory innervation. Moreover, Sox2-IR cells in the taste buds were examined by double immunolabeling using BrdU and cell- type markers such as CK14, NCAM, IP_3R3, and blood group H antigen. Sox2-IR cells were found in the population of basal cells, as well as immature and some mature taste bud cells. A large number of Sox2-IR cells were identified as type-I cells, and a few were in type-II and type-III cells.

Taste Bud Cell Differentiation in Mash1 Knockout Mice

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Mash1 is expressed in subsets of neuronal precursors in both the central nervous system and the peripheral nervous system. Recently, it has been shown that Mash1 is expressed in cells of the taste bud lineage, and that the expression of Mash1 in rat taste buds is dependent upon gustatory innervation. However, involvement of Mash1 in taste bud cell differentiation had yet to be demonstrated. In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation in lingual epithelia, we have investigated the role of Mash1 in regulating taste bud cell differentiation using *Mash1* KO mice and forced expression of Mash1 in lingual epithelia. At E18.5, taste buds are observed in soft palate in both Mash1 mutant and wild type mice. Gustducin, a type II cell marker of taste bud, is expressed in soft palate taste bud in Mash1 mutant mice. In contrast, AADC-IR cell is not detectable in soft palate taste bud of Mash1 mutant mice. Forced expression of Mash1 in tongue epithelial cells induced type III cell markers (AADC and GAD1) expression. These results suggest Mash1 play an important role for differentiation of type III cells in taste buds.

Expression Analysis of CD36 Homologs in Fasted *Caenorhabditis elegans*

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CD36 is a membrane-integrated protein that facilitates fatty acid uptake into cells. CD36 is found in adipocyte, skeletal and heart muscle, intestinal cells of mammal, and is presumed to play an important role in regulating lipid status by affecting fatty acid metabolism. To further gain information about the role of CD36 in lipid metabolism, function of CD36 homologs in nematode *Caenorhabditis elegans*, a useful model organism for genetic analysis of multicellular organism, was analyzed. There are six CD36 genes in *C.elegans*, and by knocking down the expression of the genes by RNAi, some homologs are shown to affect the fat accumulation in the nematode indicating that these CD36 homologs are involved in lipid metabolism as are in mammals. We then analyzed the expression pattern of the homologs by RT-PCR upon food deprivation. Upon fasting, mRNA level of one homolog among the six showed significant decrease within 12 hours, and the decrement of the expression completely returned to normal level within 2 hours after refeeding. The data strongly indicates that the expression of the homolog was closely linked to food availability. Further study is being conducted to identify the role of the CD36 homolog in responding food availability.

Cell Differentiation in the Taste Bud after the Cross-Regeneration between the Chorda Tympani and Greater Superficial Petrosal Nerves

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Three gustatory nerves, the chorda tympani (CT), glossopharyngeal (GL) and greater superficial petrosal (GSP) nerves, innervate taste buds in the fungiform papillae (FF), circumvallate papillae (CV) and soft palate (SP), respectively. Denervations of these gustatory nerves result in the disappearance of the taste buds, and reinnervations induce regeneration of the taste buds. Although the maintenance of the taste buds apparently depends on the gustatory nerve, the role of the nerves in taste cell differentiation still remains unclear. We

reported that the expression of gustducin reached 96.7% of IP3R3-expressing cells in the SP taste buds while only 42.4% in the FF taste buds. Based on these results, we analyzed taste buds regenerated after the cross-regeneration of the CT and GSP in rats using immunohistochemistry of IP3R3 and gustducin. The results showed no difference from the co-expression in the taste buds of control rats. These results suggest that the regional difference in the co-expression patterns of IP3R3 and gustducin in the taste buds does not depend on the taste nerves but depend on some kind of regional factors in the epithelium where taste buds regenerated.

Strain Differences of Preferences for Sugar-Alcohols in Mice

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Some sugar alcohols are widely used as anti-cariogenic sweeteners. The behavioral and receptor characteristics for these sweeteners, however, are not well understood. Our recent study showed that C57BL/6 mice felt mannitol, which is a sweetner for human, bitter. Some recent reports also demonstrated that there are strain differences for receptivity of sweetners in mice. Therefore, in the present study, to investigate whether there is a strain difference between C57BL/6 and BALB/c mice for receptivity of sugar alcohols, we conducted a behavioral study. As sugar alcohols, mannitol, xylitol, sorbitol and palatinit were used. In the 48 hr two-bottle preference test, one of the above sweeteners vs distilled water (dw), was carried out. C57BL/6 mice preferred 0.1-0.3M xylitol, sorbitol and paratinit rather than dw. On the other hand, BALB/c mice did not prefer these sugar alcohols in all tested concentrations rather than dw. Either strain did not prefer 0.03-0.5M mannitol rather than dw. These results suggest that there are strain differences for receptivity of anti-cariogenic sugar alcohols.

The Necessity of Masticatory Stimuli in the Induction of Rat Salivary Proteins by Dietary Quinine

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We previously demonstrated that dietary quinine induced cystatin S in submandibular saliva of rats. To explore the mechanism underlying induction of this salivary protein by a dietary constituent, we investigated the effects of quinine on the submandibular gland and its saliva in rats with molar teeth extraction on the bilateral upper jaws or with placement of cannula for perfusion of the oral cavity. Detection of cystatin S was performed by electrophoresis and affinity chromatography on a papain column. Dietary quinine (972 and 3240 ppm) increased the weight of the submandibular gland and induced cystatin S in normal intact rats and the teeth-extracted rats fed dry diet. In the teeth-extracted animals fed wet quinine diet, induction of cystatin S significantly decreased (possibly owing to decreased masticatory stimulation). Animals treated intermittently with 972 or 3240 ppm quinine solutions through an oral cannula produced no cystatin S as well as in the intact control animal fed plain diet. These results suggest thatneither gustatory stimuli nor masticatory stimuli individually increase full production of cystatin S. Namely, some integration of sensation to both stimuli is perhaps required for its full production.

The Effect of Umami Taste on Saliva Secretion

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Taste stimulation causes the reflex of saliva secretion, and sour taste is used clinically for the artifitial saliva to stimulate salivation for the care of dry mouth. We measured the temporal properties of salivary secretion by the stimulation of umami taste by comparing to other basic tastes. Twenty-four healthy adults participated in the study as subjects. They held 3 ml of each aqueous taste solution for 30 sec without swallowing, and spit whole saliva into cups at every 30 sec for 10 min. Taste stimuli were 100 mM monosodium glutamate (MSG) (umami), 3.8 mM citric acid (sour), 150 mM NaCl (salty), and 200 mM sucrose (sweet). The concentration of each solution was adjusted to have 'moderate' in intensity evaluated using a labeled magnitude scale. The weight of saliva per min was assumed to be saliva flow per min. In the cases of all tastes, salivary flow increased during first one min and dropped immediately during second min after the taste stimulation, then dropped gradually. MSG stimulation of oral cavity caused a sustained saliva secretion following to the transient increase. In contrast, when stimulated by citric acid, saliva flow increased transiently very much, and decreased to the resting level immediately. The total saliva secreted by MSG was larger than the case of citric acid. In conclusion, there was a possibility for the application of umami for the oral care by stimulating sustained salivary secretion of the people with low salivary flow such as the elderly.

Effect of the Oral Umami Taste Stimulation on the Rat Gastric Secretion

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Some reports indicate that taste stimulation could regulate the gastrointestinal functions such as gastric and pancreatic exocrines. In this paper, we investigated the gastric exocrines (gastric acid and pepsinogen) of the fifth taste, umami, in the conscious Sprague-Dawley rats. Rats were surgically mounted the Thomas gastric fistula under ketamine anesthesia. During experiment the stomach was continuously perfused with saline at rate of 2 ml/min through the fistula and measured the acid and pepsinogen contents in the perfusates. Sham feeding stimulation evoked gastric acid secretion but not pepsinogen secretory response. In contrast, oral stimulation of taste substances such as saltiness (NaCl), sweetness (sucrose) and

umami (monosodium glutamate: MSG) all induced pepsinogen secretory response witout gastgric acid secretion. However, bitter taste stimulation using quinine hydrpchloride induced both the gastric acid and pepsinogen secretions. The potency in the pepsinogen secretion were in order; sucrose > MSG = NaCl > quinine. Thus, it is very interesting that nutrient linked tastants (salty, sweet and umami) induced pepginogen secretion, in addition to acid secretion by sham feeding stimulation (vision and smell). Taste and smell seems to have a different role in the cephalic phase response for the gastric food digestion.

Changes in Membrane Currents and Intracellular Calcium Concentration of Frog Taste Cells Elicited by NaCl Taste Stimulation

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Salt taste transduction involves both amiloride-sensitive sodium channels and amiloride-insensitive nonselective cation channels. However, salt taste transduction mechanism has not been fully understood. To elucidate NaCl salt taste transduction mechanism, we recorded changes in membrane currents and intracellular Ca^{2+} concentration of morphologically identified taste cells in the bullfrog (*Rana catesbeiana*) taste disc elicited by taste stimulation of NaCl. We used patch electrodes containing calcium green-1 dextran for labeling cells and Ca^{2+} imaging. We used 110 mM NaCl solution containing 2 mM $NiCl_2$ (Na-Ni solution) as NaCl taste stimulating solution. The Na-Ni solution was chosen because addition of small amount of $NiCl_2$ to NaCl stimulating solution enhances the Na^+ responses of the frog glossopharyngeal nerve and because the Na-Ni solution used is isotonic. The Na-Ni solution was applied focally to the apical ends of taste cells. We recorded 3 type Ib, 13 type II and 9 type III cells. Of the 9 type III cells tested, six cells showed transient inward current at the holding potential -80 mV and intracellular Ca^{2+} concentration increasde in Ca^{2+}-free extra bath solution. None of the type Ib cells and type II cells showed detectable current and intracellular Ca^{2+} responses. These results suggested that increases in Ca^{2+} by NaCl in type III cells are due to release from the internal Ca^{2+} store and not due to influx through voltage-gated Ca^{2+} channel. NaCl taste transduction mechanisms in frogs may include receptor-related second messenger pathway.

Firing Frequency-Dependent ATP Release from Type II Taste Cells in Mice

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Taste cells transmit information to gustatory afferent fibers through synapses. Taste cells are classified into four types according to cell electron density and morphology: Type I, II, III and basal cells. Conventional synapses are observed only in Type III cells, some of which express putative sour receptors. Sweet, bitter and umami receptors are expressed in Type II cells and the mechanisms have not been elucidated. Recent reports have highlighted the role of ATP as a key neurotransmitter. In previous work, we presented that taste cells with action potentials release ATP in response to sweet compounds. Here we tried to measure ATP release from Type II and Type III cells of mouse fungiform papillae. The action potentials were recorded with the electrode basolaterally attached to a single taste cell. The electrode solution was collected and applied for luciferase assay to determine the ATP just after an increase in the action potentials was observed in response to a taste compound. To identify taste cells, we used transgenic mice expressing GFP in gustducin-positive (Type II), or glutamic acid decarboxylase 67-positive (Type III) cells. When Type II cells responded to saccharin, quinine or glutamate, ATP was detected in the electrode solution in a firing frequency-dependent manner. The ATP release was inhibited by a hemichannel blocker, carbenoxolone. When Type III cells responded to HCl, ATP was below the detection limit of our luciferase assay. The results suggest that the amount of ATP released from single taste cells differ with the response properties, or that acid-sensitive taste cells release another neurotransmitter.

The Taste Response of Medaka Fish Expressing PLCβ2 Promoter- Induced Tetanus Toxin

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Recent studies have revealed that phospholipase C β2 (PLCβ2) are expressed in receptor cells in taste buds and are required in sweet, bitter and umami signal transduction in mammal (and fish), tough the mechanisms of signal transmission between PLCβ2-expressing cells and afferent nerves remain unresolved. To determine whether the exocytosis is involved in neurotransmitter release in PLCβ2-expressing cells or not, we developed transgenic medaka fish carrying tetanus toxin (TeNT) gene downstream of PLCβ2 promoter. TeNT is known to block exocytosis. The extracellular recordings were made on the facial nerves of wild-type and transgenic medaka fish with a glass suction electrode. The magnitudes of taste response were measured as the peak heights (in millimeters) of the integrated responses. The response magnitudes were standardized to the response to 100 mM KCl, as a standard.

The response magnitudes for amino acids (1 mM L-Pro, 10 mM L-Ala) and a bitter compound (1 mM denatonium) found to be significantly lower in transgenic fish than in wild-type fish, though the difference of the response in these fish were not statistically significant when stimulated by 1 mM HCl or 100 mM NH_4Cl. Electrophysiological threshold for L-Proline was about ten times higher in transgenic fish (10^{-7} M) than in wild-type fish (10^{-8} M), but the thresholds for KCl were 2.5 mM in both fish. Our results suggest that PLCβ2-expressing receptor cells may secret transmitter(s), stimulated by amino acids and denatonium, via exocytosis at least in part.

Contribution of cAMP to the Bitter Taste Transduction in Bullfrogs

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The transduction pathway for bitter taste has been proposed that bitter substances activate PLC beta2, rising intracellular levels of inositol 1,4,5-triphosphate (IP_3) to release Ca^{2+} from intracellular Ca^{2+} stores. On the other hand, it has been pointed out that bitter substances also activate phosphodiesterase (PDE) to decrease the concentration of cAMP in mice taste bud cells. In this study, we focused on adenylate cyclase (AC) and investigated whether cAMP contribute to the bitter taste transduction in bullfrog by measuring neural responses to bitter substances after treatments of the tongue with AC inhibitors and an activator. The neural activities to bitter substances were recorded as summated responses and the tongue surface was treated with the solution containing an inhibitor or an activator for 1 hour. The magnitude of taste nerve responses to bitter substances such as quinine, denatonium, strychnine and caffeine were increased by factors of 1.7-2.2 after treatments with 10 μM SQ22536 or 10 μM MDL-12,330A or 30 μM 2‘,5’-Dideoxyadenosine, inhibitors of AC. In contrast, Forskolin, AC activator, suppressed the magnitude of responses to bitter substances by a factor of 0.6. The magnitude of the taste response to denatonium was also suppressed by U-73122, PLC inhibitor, whereas it had no effects on NaCl and $CaCl_2$ responses. These results are suggesting that bitter substances not only activate PLC, increasing the level of IP_3, but also activate PDE or inactivate AC, decreasing the level of cAMP in bullfrog taste receptor cells.

Study of Membrane Potential Change in Surface-Modified Lipid/Polymer Membrane using Phenol Compound

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In this study, we investigated the sweet-sensitivity sensor with lipid/polymer mamebranes. In our previous study, we found that the lipid/polymer membranes composed of tetradodecylammonium bromide, dioctyl phenylphosphonate and polyvinyl chloride had the sensitivity to sweeteners. The surface of the membrane was modified with phenolic compounds in order to enhance the electric response to sugars. It also indicated that the structural of the phenolic compounds contributed to the membrane potential change to sugars. In this study, the membrane surface adsorption state of the phenol compound was examined using quartz crystal microbalance (QCM) method. Further, some elements such as pH value, which would affect the absorption, were also discussed.

Promoter Analysis of Human Sweet Receptor, T1R2 Gene

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The T1R2/T1R3 heterodimer has a role in the detection of sweet tastes in taste buds. T1R2 and T1R3 are also expressed in small intestine, colon and liver. However, the mechanisms of transcriptional regulation of the human T1R2 gene have not been elucidated. In this study, we examined the promoter region of T1R2 using the luciferase reporter assay in the T1R2 expressing cell line, HuCCT1.

The luciferase reporter assay shwoed that the promoter region of the T1R2 gene was located between -1256 and +200 bp relative to the start codon in HuCCT1. Deletion analysis of the T1R2 promoter showed that the T1R2 promoter had two major cis-regulatory elements. Site-directed mutagenesis indicated that the putative C/EBP family binding site in the cis-regulatory element might represent a binding site recognized by the specific positive regulatory element. These results show that the member of C/EBP family may play a role as the transcription factor regulating T1R2 promoter activity in HuCCT1.

Analysis of Temperature, Gurmarin and Pronase Effects on the Chorda Tympani Responses to Sweeteners in T1R3-, Ggust-, TRPM5-KO Mice

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Sweet taste responses are known to occur through the pathway involving T1R2/T1R3 receptors, Gα subunit, gustducin (Ggust) and temperature-sensitive TRPM5 channels. However, several studies demonstrated that mice lacking T1R3, Ggust or TRPM5 (KO mice) display largely diminished, but not abolished, the chorda tympani (CT) nerve responses to sweet compounds. In addition, sweet responses of the CT nerve could be classified into two components; one is inhibited by gurmarin (Gur) [Gur-sensitive (GS)] and the other is not [Gur-insensitive (GI)] in mice. To examine existence of additional pathways for sweet taste responses, we investigated Gur-inhibition of the CT nerve responses to sweeteners at 15, 25 and 35°C in T1R3-, Ggust- or TRPM5-KO mice. In T1R3-KO mice, residual responses to Sucrose (Suc) and Glucose (Glc) exhibited temperature dependent-increase (TDI) and GS. In Ggust-KO mice, Suc and Glc responses exhibited TDI but no GS. In TRPM5-KO mice, Glc responses exhibited both TDI and GS. In all KO mice, Saccharin (Sac) responses exhibited neither TDI nor GS. Moreover, the lingual application of another sweet inhibitor pronase, a proteolytic enzyme, almost fully abolished the residual responses to Suc and Glc but did not affect the residual responses to Sac in all KO mice. These results suggest: (1) existence of multiple pathways for sweet taste responses, including T1R3- or TRPM5-independent GS pathways, (2) an indispensable role of Ggust on GS sweet taste responses, and (3) existence of the sweet-independent reception pathway for responses to Sac.

Effects of Gastro-Intestinal Infusion of Taste Substances on the Afferent Activity of the Gastric and Celiac Branch of the Vagus Nerve

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This report deals with responses of vagal afferents to taste substances.

To observe the effects of infusion of five basic taste substances into the stomach or intestine, afferent nerve activities were recorded from peripheral cut end of gastric or celiac branch of the vagus nerve in anesthetized rats. As the five basic taste substances, glucose and sucrose (sweet), sodium chloride (NaCl) (salty), quinine hydrochloride (QHCl) (bitter), acetic acid (sour) and inosine monophosphate (IMP) (umami) solutions were used.

Results: Infusion of 5% glucose solution (sweet) activated vagal celiac afferents (VCA) as previously reported. Infusion of 0.9% and 1.8% NaCl solution (salty) showed no remarkable effects on vagal gastric afferents (VGA) and VCA. Infusion of 30 mM QHCl solution (bitter) strongly inhibited the activity of VGA and VCA. Infusion of 0.6% acetic acid solution (sour) and 30 mM IMP solutions (umami) activated VGA and VCA. The results of experiments suggest that taste substances in the gastro-intestinal canal send their information through VGA and VCA to brain with different mode of signaling system of the oral taste nerve and may play some role in reflex regulation of visceral function.

Taste Transmission Pathway for Bitter Taste in the Frog Taste Discs: Analysis of Quinine-Sensitive Fibers in the Glossopharyngeal Nerve

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Vertebrate taste buds contain diverse types of cells. It has been shown that some taste cells communicate with taste cells within taste buds via gap junctions or paracrine secretions. The cell-cell communications in taste buds would reflect neural responses. There are quinine-sensitive fibers (Q-fibers) and calcium-sensitive fibers (Ca^{2+}-fibers) in the frog glossopharyngeal nerve. Q-fibers show the phasic responses to quinine-HCl and Ca^{2+}-fibers show the sustained responses to $CaCl_2$. The different characteristics of response pattern suggest that Q-fibers and Ca^{2+}-fibers innervate the different types of taste cells. If quinine receptor cells communicate with Ca^{2+} receptor cells, quinine responses of the single taste fibers would be modulated by Ca^{2+} taste stimulation. In this study, we examined whether quinine responses are altered by Ca^{2+} stimulation. Anesthetized bullfrogs (*Rana catesbeiana*) were used. Antidromic unitary impulses of Q-fibers and Ca^{2+}-fibers were recorded from a single fungiform papilla drawn into a suction electrode. Quinine responses (the latency between onset of stimulation and appearance of the first impulse, and the frequency of impulses) of single Q-fibers were not altered by neural response of Ca^{2+}-fibers when a mixture of quinine-HCl and $CaCl_2$ was applied as taste stimuli. The present results suggest that there are no communications between quinine receptor cells and Ca^{2+} receptor cells in taste organs.

Functions of the Gustducin in the Soft Palate Taste Buds differs from those in the Tongue in Mammals

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Gustducin is a taste-specific G-protein mediating bitter, sweet and umami tastes. Based on the expression patterns of gustducin in the tongue taste buds of mice and rats, the functions of gustducin has been implicated primarily in bitter taste in the circumvallate (CV) papillae and in sweet taste in the fungiform (FF) papillae. We recently reported that gustducin was expressed in 96.7% IP3R3-expressing cells in the taste buds of the soft palate (SP) in rats, suggesting that gustducin is involved in signal transduction of all tastes of sweet, umami, and bitter in the SP, in contrast to its limited function in the tongue taste buds. To confirm a broad role of gustducin in SP taste buds, neural responses from the greater superficial petrosal nerve (GSP) and chorda tympani nerve (CT) of gustducin-KO mice were recorded and the response properties were compared between the two nerves. In consistent with the immunohistochemical results in rats, nerve responses to both sweet and bitter stimuli were markedly reduced in the GSP of gustducin-KO mice. The CT showed reduced responses to the sweet but not to the bitter stimuli. Immunohistochemistry of gustducin and IP3R3 in mice showed that 91.1% of IP3R3-expressing cells in the SP were gustducin positive. Also, in situ hybridization showed the gustducin-expressing cells co-expressed both T1r3 and/or T2r taste receptors. These results demonstrate the broad role of the gustducin on the SP taste buds, indicating the consistency between gustducin function in taste transduction and its expression pattern.

Polymorphism Analysis of the Umami Receptor Genes, T1R1 and T1R3 in Flesh-Eating Mammals

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Expansions of trinucleotide repeat cause several diseases, such as Huntington disease by polyglutamine expansion in the Htt gene, congenital central hypoventilation syndrome by polyalanine expansion in the phox2b gene. These mutations could be associated with protein misfolding and dysfunction, but the role of most homopolymeric amino acid (HPAA) tracts remain to be determined. Recently HPAA database has released and showed approximately 1% of total protein had HPAA in their sequences, suggesting that HPAA tracts had important roles in maintenance of the function of proteins. Leucine repeat number in the taste receptor, T1R1 gene is

seven in human and chimpanzee, six in mouse, five in dog and chicken and two or three in fishes. Because T1R1 has function for perception of umami such as glutamate, it was suggested that these size difference may be associated with preference of food and appetite. It is also known that T1R1 interacts with T1R3, which is a component of sweet receptor. T1R3 has leucine repeat as well. The leucine repeat region of the T1R1 and T1R3 gene from mammals are isolated and sequenced to determine the repeat number, resulting in the evolutional change in length. This change in evolution suggested that the difference of function or activity of taste receptors, which may be related to the preference for food. Flesh-eating animals definitely have five repeats, and omnivorous animals have more than five in the T1R1 gene, suggesting relationship with food preference of animals.

Influence of Color on Taste

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Purpose: This research was conducted in order to know the influence of the color on the taste.

Method: 1. The color that makes subjects imagine the taste was chosen from the 13color samples. The subjects spontaneously wrote the name of food which the taste made them imagine. 2. The color of drinking water for sale was changed intentionally by coloration, afterward taste evaluation was done. The subjects guessed the name of original drinking water, by which influence of color on the taste was tested. 3. The drinking water with sweetness, sourness, salty taste, bitterness was colored in red, green, yellow, and blue respectively. And how the coloration influenced on taste and deliciousness was considered by the score method.

Results: 1. The following is connection between tastes, colors and food. Sweet taste, red and pink, and chocolate are connected with each other. / sourness, yellow, and lemon / bitterness, brown and green, and coffee / salty taste, light blue, and salt / UMAMI, brown and orange colored, and stock /hot taste, red, and red pepper/ astringency, green, and tea / rich taste, brown, and curry. 2. Judgment of the taste quality for the sweet drink was possible by smell sense. As for the sour drink, it was unable to judge the taste quality.3. Sweet taste was considerably strengthened by red color. Bitterness increased a little by green color. Feeling of sour taste was weakened by the color, salty taste was not influenced by any color.

Conclusion: Connection of the taste and the color was confirmed.

Psychogenic Taste Disorders -the Relationship between Psychological Factors and the Effectiveness of Treatment

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We investigated the relationship between psychological properties and treatment outcome of taste disorders in 64 patients (14 males and 50 females) whose symptoms were assumed to be induced by psychological factors. Patient's age ranged from 25 to 83 years, and the average was 58.7 years.

The most frequent chief complaint was lack of taste (hypogeusia, n=51). Almost 1/2 of the patients reported pantogeusia, tongue pain or dry mouth. By the taste assessment using the paper disk method, 65.1% of the patients were classified into normal or slight disorder. Salivary secretions measured by gum chewing revealed that 17.2% of these patients secreted less than 10ml. Low serum zinc concentrations were observed in 23.4% of the patients by blood tests.

CMI, SDS and MAS were used to evaluate the psychological properties of the patients. As the results of CMI tests, 72.4% of the patients were in or levels, and according to the SDS and MAS, 62.1% and 80.5% of the patients were diagnosed as depressive neurosis state and high anxiety, respectively.

The treatment mainly consisted of zinc prescription, dietary instructions and psychological care. The treatment outcome was examined by the paper disk method.

Clinical effects were excellent in 7.8%, and good in 42.2% of the patients. The tendency that the treatments were less effective in the patients who had psychological problems than the other patients was observed. These results suggest that the CMI, MAS and SDS tests are useful to diagnose taste dysfunction induced by psychological factors.

Formation Mechanism of Fishy Offflavor in Wine with Seafood

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White wine with fish and red wine with meat are known as appropriate pairings because red wine sometimes clashes with fish and instantly causes fishy off-flavor in the mouth. Although a large number of studies have been made on the gradual development of the fishy flavor in stored seafood, little is known about the instant fishy off-flavor. We examined the interaction between wine components and lipids in seafood on the formation of fishy off-flavor. The wine components tested were alcohol, acidity, pH, sulfur dioxide, total phenols, organic acids, anions, cations, and transition metals. The lipids were evaluated by the concentrations of n-3 polyunsaturated fatty acid (n-3 PUFA) and hydroperoxides, measured by gas chromatography (GC) and peroxide value, respectively. First, a strong positive correlation was found between the intensity of fishy off-flavor and the concentration of ferrous iron in wines. The addition of ferrous sulfate into a series of model wines generated the fishy off-flavor dose-dependently. Secondly, (E,Z)-2,4-heptadienal was identified as a potent odorant of fishy off-flavor by GC-olfactometry and GC-mass spectrometry. Thirdly, the formation of (E,Z)-2,4-heptadienal was statistically significant between raw and dried scallops. The drying treatment generated both n-3 PUFA and hydroperoxides in scallop. These results suggest that ferrous iron in wine can promote the instant formation of fishy off-flavor by the breakdown of pre-formed lipid hydroperoxides derived from

n-3 PUFA in seafood to generate (E,Z)-2,4-heptadienal via alkoxyl radicals.

A Mouse Model for Binge-Type Eating of a Sweet Solution

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Intermittent excess consumptions of sweet substances may induce eating disorder such as binge-type eating and result in obesity and metabolic syndrome. The study on a mouse model for bingeing may enable us to reveal genetic and neural mechanisms of overconsumption using many inbred stains and genetically modified mouse strains. Here, we aimed to develop a mouse model of binge-type eating using a limited access protocol to a sucrose solution. C57BL/6J male mice (experimental group) received a limited access to both 0.5 M sucrose (Suc) and food pellets only for 4 h under food deprivation for 8 days. The other mice (control group) allowed approaching 0.5 M Suc ad libitum but were restricted their access to food pellets for 4 h. The intakes of 0.5 M Suc for 4 h in the experimental group gradually increased day by day while those in the control group were unchanged. The intakes of 0.5 M Suc in the experimental group were significantly greater than those in the control group throughout the limited access protocol. Future research using this simple protocol in mice may provide new experimental approaches to study neural substrates underlying binge-type eating.

Behavioral Analysis of the Taste of Valine in C57BL/6J Mice and Distribution of Valine in Sea Urchin Gonads

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Valine (Val) has bitter and slightly sweet taste in human and contributes to taste of sea urchin (Komata, 1964). However, there are many unclear points in its taste quality. While, Val evoke only negligible activation of the mouse T1R1+T1R3 receptor, but activation of this receptor increases considerably when Val are mixed with IMP (Nelson et. al., 2002). This suggests that addition of IMP changes the taste quality of Val. We tested taste quality of Val and this hypothesis using a conditioned taste aversion (CTA) technique. Separate groups of C57BL/6J mice were exposed to 50 mM Val with or without 2.5 mM IMP, or to water (control) and injected with LiCl to form CTA. Conditioned mice were presented with five basic taste solutions, Met, Val, Lys and Arg with and without IMP, and their lick responses were recorded. An aversion to Val generalized to Val, Lys, Arg with and without IMP and quinine. An aversion to Val+IMP generalize to a mixture of 50 mM monosodium glutamate (MSG) and 30 μM amiloride (Ami; added to block sodium taste) with and without 2.5 mM IMP, but not quinine. This suggests that, as predicted by the in vitro study, addition of IMP changes the taste quality of Val in vivo. In addition the content of Val in mature and immature gonads (which are edible part) of sea urchin, *Hemicentrotus pulcherrimus* were examined. Val was 60-100mg/100g and its content was only 3-5% of total amino acids in the gonads. Thus there is no difference between mature and immature, also ovary and testis.

Analysis of Taste Characteristics of L-Theanine

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L-theanine, 5-*N*-methylglutamine, is a unique amino acid occurring in green tea. It is responsible for part of the taste of green tea. Recently, there has been an emerging the interest in psychological effects of L-theanine. However, little is known about the sensory characteristic of this amino acid. In order to optimally use L-theanine as a food ingredient, we need to characterize its taste. We investigated taste of L-theanine using human sensory evaluation method. Simultaneously, we observed synergistic effect with nucleotides. Furthermore, we performed gustatory nerve recording and 48h two bottle preference test in mice and examined synergistic effect. In human, L-theanine had mainly sweet and umami. When IMP was added to L-theanine, the taste intensity become strong, and ratios of umami among various taste quality increased. Taste intensity of L-theanine decreased significantly when human T1R3 inhibitor, lactisole, was added to L-theanine. These results suggest that L-theanine has the synergistic effect of umami and is accepted through T1Rs. In mouse, the synergistic effect to L-theanine + IMP was observed by chorda tympani nerve recording. However, when we performed 48h two bottle preference test, the synergistic effect was not observed. It is thought that an effect after absorbed L-theanine is related.

Study on the Formation of Macromolecular *Kokumi* Substances from Meat Extract-Function of Various Tropomyosin as a Precursor of *Kokumi* Substances

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Meat extract is used in food manufacturing industry to generate a meaty aroma and meaty flavor designated as *kokumi* flavor (continuity, mouthfulness, and thickness). This flavor is known to increase during the heating process of the extract. A previous study indicated that the precursors of macromolecular *kokumi* substances in commercial beef extract were collagen and tropomyosin, which is one of the components of muscular protein (Kuroda&Harada, J. Food Sci., 69, C542, 2004). In this study, the function of various tropomyosins as a precursor of *kokumi* substances was investigated. Tropomyosins were purified from the skeletal muscle of the cattle, rabbit, chicken, carp, skipjack tuna and scallop. Each tropomyosin was dissolved with porcine soluble collagen in a low-molecular-weight fraction of beef extract (DM 30%) and heated at 95 for 6hr. The macromolecular fractions were obtained by dialysis and then freeze dried. These fractions were then dissolved in a low-molecular-weight fraction of beef extract (DM 2.0%) at 0.2% concentration and the properties of taste and flavor were evaluated by a trained panel. The results of

sensory evaluation indicated that all the macromolecular fractions obtained from collagen and tropomyosins tested in this study had a similar *kokumi* flavor (continuity, mouthfulness, and thickness), suggesting that the tropomyosins tested in the present study can function as the precursors of macromolecular *kokumi* substances.

Practical Use of Riboflavin-Binding Protein in Food Bitterness Reduction

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We previously reported that riboflavin-binding protein (RBP) from chicken egg-white is a novel bitter inhibitor which has broadly tuned inhibition toward various bitter substances. In this study, we aimed to assess a prospect of RBP in bitterness reduction in foods or drinks. RBP was purified from chicken egg-white by ammonium sulfate fractionation, ion-exchange and gel chromatography. In order to evaluate the bitter inhibition of RBP on various tastants at same levels of bitterness, every bitter samples were prepared to elicit the bitterness as 0.125 mM quinine solution. RBP reduced bitterness of casein hydrolysate, soy protein hydrolysate, hop extract and bitter-gourd extract, in almost same manner to that toward simple bitter substances. RBP was heat-stable at neutral pH when treated at 100°C and 110°C for up to 120 min. Moreover, the bitterness reduction was also heat-stable even at 80°C for 6hrs. Sodium chloride (NaCl) and sucrose are major constituents in many foods and drinks, especially fermented products. NaCl has been known as a potential bitter inhibitor. Therefore, combinations of RBP and NaCl or sucrose were examined for their effects on bitterness. As the results, in a combination with 0.5% NaCl, the bitterness reduction of RBP increased synergically. And more than 7.5% of sucrose also enhanced the bitterness reduction of RBP effectively. These results suggested that RBP can be an useful bitter inhibitor for the foods having undesirable bitterness.

Nutritional Clue in the Terminal Care of the Cancer Patient – A Case Report of End-Stage Oral Cancer Patient

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Most patients of recurrent oral cancer with hypercalcemia will decease within 6 to 8 weeks. We experienced that a male 70s-year patient with the disease lived for 7 months without severe complication as constipation and with administering only one fifth of average of opioids. He could use gastric tube until the day before his death. We review the role of the content of enteral nutrition of the end-stage patients from the nutritional view. There could be four factors contributing a favorable course. First, fresh foods were used and made good smell. Second, ''*konbu*; seaweed'' ''*katsuobushi*; dried-smoked skipjack tuna'' which are specific to Japanese cuisine were used to make soup stock cooked dealing with "*umami* "carefully. Third, Aloes that Japanese use as phytotherapy was used. Fourth, comfortable surroundings to eat with his wife seemed effective. In rats, it is reported that taste stimulation has an effect on ß-endorphin levels. As in human it is considered that secretion of opioids within the brain is associated with gusto, we could contribute to the end of life from this aspect. Furthermore in human, not only the content of food and nutrition but also the occasions strongly affect the gusto or content, so it is considered that to make circumstance of eating better is effective. The specific point of this case is that aloes which was commonly used for disorder of digestive tract in alternative healthcare was eaten. In medicine, feeding service in which personal favour was esteemed contributes to fertilize QOL of the patient in the end of life.

Estimation of Salty Enhancing Effects Based on Licking Behavior

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Sensory evaluation tests by human being have been used for palatable salty enhancers or substitutes. However, there are no suitable evaluation methods by animal behavioral studies. We thought that behavioral studies should be conducted under restrictive conditions such as preferable concentration or sufficient salt appetite to evaluate palatable tastants. A water-deprivation or a sodium-deprivation elicits salt appetite. In this study, we investigated the preference for hypotonic NaCl solutions with sodium-deprived C57BL/6 mice, which were fed on a NaCl-deficient diet containing mineral-corticoid receptor antagonist spironolactone. In brief access tests, the sodium deficient mice showed the positive correlation between lick number and saltiness among 0 – 0.06 M NaCl solutions. The licking rate to NaCl solutions increased by 0.015 M or 0.03 M LiCl addition, but not by KCl addition. These results suggest that LiCl could enhance saltiness, but KCl could not. The NaHCO3 addition showed no effect to the licking rate to NaCl solution although the NaHCO3 solution supplied Na ion to the mice. This result suggests that the gustatory cue for finding Na by Na-depleted mice is not Na ion directly but sharp saltiness in solutions. We thought that this methods would be available to evaluate enhancing effects in sharp saltiness, and useful to find novel salty enhancers.

Relationship Between Body Weight Gains and Taste Change in Pregnant Women

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Objectives: We studied the changes level of electrogustometry during pregnancy and investigated the relationship between a level of electrogustometry and body weight gain in pregnant women.

Methods: 44 pregnant female and 30 non pregnant female volunteers were recruited to this study. Electrogustometry was performed with electrogustometer. Body Mass Index (BMI) was calculated by weight (kg)/ (height (cm))^2. Statistical analysis was performed by ANOVA and nonparametrics tests including Spermnan Rank correlation coefficient and Mann-Whitney U test .

Results: The level of electrogustometry in pregnant women was decreased according to progress of pregnancy and the level of

electorogaustometry in puerperal women was lower than that in non pregnant or pregnant women. The level of electrogustometry was inverse correlated with The Body Mass Index (BMI) and weight gain in pregnant women.

Conclusions: It is suggested that the change of electrogustometry in pregnant women was related to the physiological weight gain such as increased need of fat and water according to progress of normal pregnancy and taste may be an objective indicator of weight gain for pregnant women.

Alleviating Effects of Inchin-Goreisan on Spontaneous Bitter Taste

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Pantogeusia, or spontaneous taste with no taste substance in the mouth, comes across at about 15 % of patients with taste disorders. Among the taste qualities they complain, bitterness is most frequent, followed by saltiness and sourness. The spontaneous bitterness is easily alleviated with supplement of zinc when the suffering period is short, say 2 months, but it is difficult to deal with zinc alone when the period is longer. In the present study, 6 patients with spontaneous bitter taste were treated with a Kampo formula, Inchin-goreisan together with zinc as polaprezinc, after several week treatment with zinc alone. They did not show any specific clinical findings common to them, except for decreased serum zinc. Five of them were successfully alleviated, but the rest was alleviated only for a short period after administration of Inchin-goreisan. Allthough Kampo formula containing Saiko (Bupleuri Radix) is reportedly effective to spontaneous bitterness, this was not effective to the present patients except for changing spontaneous taste quality from bitterness to saltiness or sourness. Spontaneous bitter taste may originate from bitter saliva, or disorders in suppression of error neuronal signals in the brain associated with taste sensation. However, Inchin-goreisan is not known to act at any level of taste system. This medicine is recommended to alleviate spontaneous bitter taste when any other useful means are available.

Development of the Taste Disorder Screening Method having Applied the Human Salivary Proteins Concerning Bitter Taste

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In clinical dentistry, we have often met with patient of taste disorders to bitter taste, but there is no definite standard in the diagnosis and cure for the disorders, so far. We have reported that Histatin 5 and PRP-PE quinine in saliva showed electrophoretically the same movement with quinine.

The aim of this study was analyses of human salivary proteins concerning bitter taste, in which a basic guideline to establish a certain parameter for screening patients with taste disorder

Stimulated saliva was temporarily obtained from the parotid glands of 10 healthy adult subjects in often loading the stress of the Kraepelin psycho-diagnostic test with a loud noise (5,000Hz, 100dB) for 30 minutes.

The LF/HF ratio on ECG was adopted as indices of sympathetic nervous activity to check a liability for the load of the stress.

The concentrations of Histatin 5 and PRP-PE in saliva were quantified by ELISA test.

As a result, Histatin 5 concentration in parotid saliva was significantly reduced right after the stress. On the other hand, PRP-PE concentration in the saliva was no significantly change for 2 hours after the stress.

These findings suggest that Histatin 5 in saliva might be a parameter for screening of patient of taste disorder to bitter taste. It is necessary to collect more samples with patients of a taste disorder to raise the reliability of the parameter.

Functional Expression of Miraculin, a Taste-Modifying Protein in *Arabidopsis thaliana* and *Escherichia coli*

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Miraculin isolated from red berries of *Richadella dulcifica*, a native shrub of West Africa, has the unusual property of modifying a sour taste into a sweet one. This homodimer protein consists of two glycosylated polypeptides that are cross-linked by a disulfide bond. Recently, functional expression of miraculin was reported in host cells with the ability to glycosylate proteins, such as lettuce, tomato, and the microbe *Aspergillus oryzae*. Thus, a question remains as to whether glycosylation of miraculin is essential for its taste-modifying properties. Here we show that recombinant miraculin expressed in *Arabidopsis thaliana,* one of the model organisms for studying plant sciences, including genetics and plant development, forms a homodimer and has taste-modifying properties, which are similar to the previous reports of other recombinant miraculins. Moreover, the recombinant miraculin expressed in *E. coli* identified as mixture of monomer and dimer in soluble fractions, and only the homodimer has taste-modifying properties (manuscript in revision). These results indicate that glycosylation is *not* essential for the taste-modifying property and the recombinant miraculin expressed in *Arabidopsis thaliana* and *E. coli* will aid in studying the molecular mechanisms underlying its taste-modifying activity.

Clinical Usefulness of the Card Type Olfactory Identification Test for Japanese Patients with Olfactory Disturbance

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The card type olfactory identification test kit (Open Essence) is a new test of olfactory function recently developed for Japanese. It

is consisted of twelve odors and alternatives same as OSIT-J. We evaluated this test kit in relation to Japanese standard olfactory test (T&T olfactometer) and OSIT-J for the Japanese patients with olfactory disturbance. Significant correlations were found between the score of Open Essence, the average recognition threshold of T&T olfactometer and the OSIT-J score, respectively. The examination time of Open Essence is shortest in these three tests. We conclude that Open Essence is useful for evaluating olfactory disturbance in Japanese people.

Immunohistochemical Analysis for the Pheromone-Coding Region in the Rodent Brain

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The accessory olfactory bulb (AOB) is the primary brain center for the vomeronasal system in which, coding and the memory formation of pheromone information would take place. The vomoeronasal receptor cell (VRC) axon derived from the vomoeronasal organ (VNO) makes the synapses onto a glomerular arbor (GA) of a mitral / tufted cell (MTC) in the AOB. It became clear that GAs of MTCs show various morphology by the rapid-Golgi method (Takami and Graziadei, J. Comp. Neurol., 1991). However, functional significance of this polymorphic GAs is still unclear. To obtain morphologic basis to understand the functional significance of polymorphic GAs of MTCs, the tissue slices of adult rat AOBs were stained with several molecular probes, such as monoclonal and polyclonal anti-MAP2 antibodies, anti-GAP43, anti-neurofilament 160kD, anti-neurofilament200kD, and anti-neurotubulin antibodies. As a result, the monoclonal anti-MAP2 antibody was the best one to visualize the morphology of GAs of MTCs. In images obtained by a confocal laser-scanning fluorescence microscope, at least three types of apical dendrites of MTCs were demonstrated; large and small GAs, and dendrite without GAs. Some dendrites formed GAs and then did another one, suggesting that these GAs are classified as *en passant* GAs (Takami&Graziadei, 1991). The present results indicate that monoclonal anti-MAP2 antibody is a strong molecular probe to study polymorphic GAs of MTCs in the AOB.

Analysis for Molecular Markers in Rodent Vomeronasal Receptor Cells

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In rodents, a pair of vomeronasal organs (VNOs), which are chemosensory apparatuses, are situated at the base of nasal septum in the anterior nasal cavity. The vomeronasal sensory epithelium (VNSE) that contains vomeronasal receptor cells (VRCs) faces to a non-sensory epithelium via a lumen. Neural cell adhesion molecule (NCAM) and NCAM that contains large amount of polysialic acids (PSA-NCAM) are reported to be localized in membranes of brain neurons. However, little is known about useful cell membrane markers for VRC that is a bipolar neuron. For our on-going research for studying subtypes of VRCs, it was necessary to find excellent molecular markers for VRC membranes. Thus, the present study was carried out to examine if the following molecular markers are a appropriate a membrane markers for VRCs; antibodies to NCAM, PSA-NCAM, and a lectin GS-I-B_4 that binds to terminal α-galactose-containg glycoconjugates (α-Gal-GC). As a result, it was found that immunofluorescense method using anti-PSA-NCAM/anti-NCAM antibody and an antibody to olfactory marker protein that is an unequivocal marker for matured VRCs, PSA-NCAM / NCAM immunoreactive cells were revealed to be immature VRCs. On the other hand, GS-I-B_4 bound to both immature and matured VRCs. The labeling pattern was membraneous, indicating that α-Gal-GC is present in cell membraneous of VRCs. Therefore, the present study suggests that GS-I-B_4 is an excellent marker to visualize VRC membrane.

The Influence of Aroma Essential Oil on Salivary Secretion

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Number of people who have the problem of oral dryness is increasing with development of stressful circumstances as well as aged society in recent years. Olfactory stimulation with aroma essential oil is known as one of effective means to increase saliva secretion. In the present study, therefore, we examined effects of three types of aroma essential oil such as cinnamon leaf, lavender and lemon, on salivary secretion. In addition, we investigated the influences of these aromas on autonomic activities by analyzing electrocardiogram.

Nineteen healthy students voluntarily took part in the study of measurement of saliva, and each subject's saliva was collected before and immediately after 5 min sniffing of aroma. The electrocardiogram was recorded from 8 female subjects, and changes in autonomic activities during aroma stimulation were analyzed. Salivary secretion was significantly increased by smelling lavender and lemon aromas in whole subjects. In female subjects lavender was effective for salivary secretion increment, whereas lemon was effective for it in male subjects. The analyses of electrocardiogram demonstrated that both parasympathetic and sympathetic activities increased during lemon sniffing and parasympathetic activity was remarkably enhanced with simultaneous decrease in sympathetic activity during lavender sniffing, indicating that lavender has a relaxing effect. Since the relationship between saliva increment and changes in autonomic activities was observed for lavender, it was suggested that effects on autonomic activities by lavender are involved in increase of saliva secretion. These results suggest that taking into account the differences in aroma effects between male and female when we use aroma oil to increase saliva secretion.

Effect of Essential Oil Aroma on the Brain Function

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Based on the great advances in odour neurosciences, it was found that an odour in imspised air will activate lateral and medial orbitofrontal cortex mainly by fMRI for human brain. For recognition of odour molecule, it is highly requested to use new system which can measure dynamical changes in brain function, such as EEG and MEG.

Opto-topography may be the strongest candidate for this purpose. Opto-topography utilize absorption difference between oxy-Hb (O_2Hb) and reduced Hb (RHb) in near infra-red ray, which indivectly express neuronal activity. So far it was already established in psychological physiology that some essential oils excite brain function, while others suppress it. We used Jasmine as a excitatory and Bergamot as sedative odour. Jasmine was found to increase O_2Hb concentration in the capillary, while Bergamot decreased O_2Hb concentration in it. The concentration of RHb was not changed, unexpectedly. Other types of excitatory odours, such as peppermint and black pepper showed the same pattern as that of Jasmine.

Geranium, which was known to have bidirectional function showed that O_2Hb level was increased, while RHb level was unexpectedly decresed.

Estimation and Measurement of Brain Activities of the Cognitive Response Regions Relevant to Smell Stimulus

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In this study, in order to investigate estimation of activated regions and brain activities relevant to the cognitive responses to a smell stimulus, the event related experiment was conducted using a few fruit odors.

As a cognitive response task, two kinds of fruit odors were presented by odd ball task, and these responses were measured by functional magnetic resonance imaging.

Odor gas was presented by the equipment using the electromagnetic valve with computer control which we manufactured. Gamma undecalactone (peach) and citral (lemon) were used as fruit odors. As one session, in scanning time for 5 minutes, gamma undecalactone (control) was presented at a total of 46 times, and citral (target) was presented at a total of 6 times. As an experimental task, ''control'' was disregarded and total number of ''target'' was only counted.

As the results of these analyses, inferior frontal gyrus and insular were activated in the olfactory cortex.

Moreover, superior tempolal gyrus which is generally activated as the cognitive response region to target, middle temporal gyrus, and parahippocampal gyrus were recognized as active areas. On the other hand, superior frontal gyrus, middle frontal gyrus, cuneus, lingual gyrus, and fusiform gyrus were also recognized as active regions.

From these results on these activated regions and fitted responses, it was shown that the significance of the brain activity for functional localization and the relevance among each regions were found.

Effect of Odor on Neocortical Responses to Sweet Taste in Fronto-Temporal Regions-Flavorings Creation using Optical Imaging

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To evaluate the effectiveness of flavorings on taste caused by central integration of flavor, we recorded neocortical activity during sensory evaluation using multi-channel near-infrared spectroscopy (NIRS). For this purpose, we tried to record cortical responses to sucrose solutions as sweet taste, and then assess how odorants modify the responses. First, we observed concentration-dependent increases of the amplitudes of the responses to sucrose solutions in the ranges of 1 to 8 % in specific bilateral regions of the frontal and temporal cortices. The observed responses were thought to be cortical responses to sweet taste. The subjects also showed cortical responses to ethylmaltol aqueous solutions. The responses to the odor solutions were observed in quite similar regions to the taste solutions. Moreover, when ethylmaltol in the ranges of 1 to 20 ppm were added to 2% sucrose solutions (odor/taste mixtures), statistically significant increase of the amplitudes of the responses to these solutions as compared to odorless 2% sucrose (taste) solution were observed. In other words, the odorants increased the amplitude of the cortical response to sweet taste. These results indicated that the odorants modified the cortical responses to taste, caused by central integration. We could thus record the modification as the differences of the amplitudes of the cortical responses using optical imaging. The observed cortical responses may help evaluating the effect of added flavorings on taste.

EEG Change According to Age Groups by Odors Stimulation

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Abstract: The purpose of this study is to analyze the changes of EEG classified by age groups when the odors stimulation is applied. The subjects in the study were fifty persons whose sense of smell is normal; 16 of young age group, 15 of middle age group and 19 of elderly age group. The odors stimuli used for the experiment are six kinds of perfumes provided with 100% volume of standard value such as Basil oil, Jasmin oil, Lavender oil, Lemon oil, Ylangylang oil, and Sketol. EEGs were measured for one minute at 4 channels(Fz, Cz, F3, F4) according to the international 10-20 system method. As a result of analyzing Fz, in young age group, the significant($p<0.05$) difference was seen between Basil oil and Ylangylang, and between Lemon oil and Ylangylang, respectively. In young and elderly age group, the significant($p<0.01$) difference

was seen between Lavender oil and Ylnagylang oil. Also we did the subjective evaluation classified by age groups about six odors and compared the two.

Histological Properties of the Nasal Cavity and the Olfactory Bulb in Jungle Crow (*Corvus macrorhynchos*)

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We analyzed anatomical and histological properties of the nasal cavity and the olfactory bulb (OB) in jungle crow. We also examined the lectin binding patterns in the crow OB by using 21 types of fluorescence-labeled lectin. The presence of the anterior and medial concha was found in the nasal cavity of the crow, but the posterior concha was not found. As same as pioneer reports, the whole size of OB was extremely small and bilateral lobes are fused at midline. Nevertheless, they received projection of the bilateral olfactory nerve bundle separately in front of lobes. Microscopically, the olfactory nerve layer (ON), glomerular layer (GL), mitral cell layer, and granule cell layer can be clearly distinguished. Presence of the external- and the internal-plexiform layer were unclear. At most rostral portion of OB, all layers keep a bilateral independent structure, but every layers joined in the middle line and formed ''one loop'' at the main body of OB. Moreover, this ''one loop'' structure redivides into two lobes at caudal portion of OB. Only four out of 21 lectins stained ON and GL of the crow OB, although ten and fifteen lectins binds to the quail and mouse respectively. We could not discuss about whether or not crows have a sense of smell. But, our results show that the crow have the basic olfactory system in the same as other vertebrate. Furthermore, in parallel with olfactory importance in an animal species, the number of the binding lectin increases is suggested.

Identification of Novel Unique Flavor Compounds Derived from Nelson Sauvin Hop and Synergy of these Compounds

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Nelson Sauvin (NS) is a unique hop variety that was bred and grown in New Zealand. This hop gives a specific flavor (exotic fruit-like, white wine-like) to finished beers. However, the key compounds of this flavor have not yet been identified. We have attempted to identify the specific flavor compounds derived from NS. We first compared the GC-MS peaks between the products made from NS (NS product) and those from typical aroma hop, and discovered three specific peaks from the NS products. These peaks were identified as isobutyl isobutyrate (IBIB), isoamyl isobutyrate (IAIB) and 2-methylbutyl isobutyrate (2MIB). These compounds had a floral flavor like green apple and/or apricot. However, the flavor characters of these compounds were different from the total flavor impression of the NS products. We next focused on certain volatile thiols that are well known to contribute to wine flavors, especially Sauvignon Blanc. The NS product lost its specific flavor by contact with copper. Copper is well known as an absorber of thiols in the field of wine flavor investigations. Therefore, it might point to the existence of thiols. We analyzed the NS product by GC-FPD, GC-Olfactometry and GC-MS, and identified two new volatile thiols, 3-mercapto-4-methylpentan-1-ol (3M4MP) and 3-mercapto-4-methylpentyl acetate (3M4MPA). These compounds have a grapefruit-like and/or rhubarb-like odor, similar to that of Sauvignon Blanc. We quantified these compounds in the NS products and determined their thresholds. As a result, 3M4MP was contained twofold of its threshold in beers and 3M4MPA and 2MIB were contained below their thresholds. However, it was confirmed that 3M4MP enhanced the flavors of 3M4MPA and 2MIB by synergy. Therefore, we concluded that all of these compounds would contribute to the specific odor of beers produced with NS.

Odor Responses of Descending Interneurons and Thoracic Motor Neurons in the Male Cockroach

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In insect central nervous systems, descending interneurons (DINs) carry the final information from the brain to thoracic motor systems to initiate specific patterns of behavior. Using extracellular recording method, we have studied neural activities of the DINs and the motor neurons (MNs) of the mesothoracic ganglion in the male cockroach (*P. americana*) to olfactory stimuli (pheromone) to the antenna. In this study, recordings were made from the DINs of both side connectives close to the prothoracic ganglion and the MNs from right-side nerve 5r1b and left-side 6Br4 in the mesothoracic ganglion. The MNs in the 5rlb and the 6Br4 mainly innervate extensor and flexor muscles in the pair of middle leg, respectively. Excitatory activities recorded from the DINs in the connective typically showed significant greater firing rates to pheromone stimulations of the antenna ipsilateral to the recording site. The increase of spike number of the DINs was dose dependent. Excitatory activities from the 5rlb (presumed slow excitatory extensor: Ds) followed by phasic inhibition, synchronized with the DINs activities, were recorded to stimulations of the right antenna.

Simultaneously-observed excitatory activities from the 6Br4 (presumed slow excitatory depressor: Ls) were recorded. To stimulations of the left antenna, phasic excitatory activities followed by tonic inhibition from the 5rlb and phasic excitatory activities from the 6Br4 were observed. These MN activity patterns may correspond to turning movement in the male cockroach to pheromone source. The results show that the male can make spatial comparisons between their two antennae, the male could be able maintain position themselves by the activities of the DINs and MNs to the odor source.

The Effect of Sniff Frequency on Odor Stimulation

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It is believed that the sniffing is one of the important behaviour in the animal. Many animals, such as dogs, rats, mice and horses commonly exhibit the sniffing behaviour when they are searching the foods or the odors. Standard respiration rate in rats is 1-2 Hz but sniffing varies between 4-12 Hz. Olfactory receptor cells are elicited the larger responses by the more absorption of the chemical substances. In our last report, we used H_2S, CH_3SH and $CH_{32}S$ mixtures as a basic odor. In this report, we used n-amyl acetate and lemonene mixtures as an odor stimulant. We applied with continuously or intermittent odor stimulation methods, and have investigated that changes in sniff frequency could be changed the level of odor behaviour or not, and how sniffing alters odor responses during the different sniffing behaviour.

The Detailed Expression of the Quality of Smell by Mixing the Additive of the Smell on SHIMADZU E-Nose System FF-2A

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We suggest a method to express quality of smell at electronic nose systems. When the smell that is common to two samples changes when we compare two samples in e-nose conventionally, it is expressed with another smell even if the quality of the difference is the same. However, it is thought that the nose of the person ignores the smell that is common to two samples unconsciously and may feel a difference. Therefore we mixed the additive of the smell to one sample and tried the method that this additive considered to be the ingredient of the one of the differences by comparing it with other sample. This method is application of a method taken by ASmell™ software of SHIMADZU e-nose system FF -2A.

ASmell™ software is absolute value representation software. This method involves the measurement of multiple category gases and evaluating unidentified samples based on these category gases.

We introduce the example which compared the ice cream with this method and FF -2A.

This expression may be possible for collaborating evidence data of the analysis type sensuality evaluation in a flavor wheel and QDA method. This method can extract a signal corresponding to the analysis type sensuality value to be inherent in a sensor and, only in a device, may demand analysis type sensuality value. In addition, we may revise drift in time of the sensor by using the additive smell gas which is stability.

The Evaluation of Citrus Fruits by means of an Electronic Nose

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A total of 39 aroma compounds were detected in the essential oil of *Citrus nagato-yuzukichi* Tanaka (nagato-yuzukichi) by GC-MS. The composition pattern of essential oil in *C. nagato-yuzukichi* was fairly similar to that of *Citrus sudachi* Hort. ex Shirai (Sudachi). Principal component analysis (PCA) of data obtained with an electronic nose indicated a variation of each oil along PC1. The oils of *Citrus junos* Tanaka (Yuzu) and *Citrus sphaerocarpa* Tanaka (Kabosu) showed a clear upward displacement as compared with those of *C. nagato-yuzukichi* and *C. sudachi*. However, in PC2, the oils of *C. nagato-yuzukichi* and *C. sudachi* showed a displacement in a negative direction and a positive one respectively. On the other hand, a total of 20 volatile organic compounds from the peel of citrus fruit Ohshima no. 1 were identified by GC-MS. Comparing the results, it was found that volatile components from both parent cultivars, Miyauchi iyokan and Yoshiura ponkan, were present in the peel of Ohshima no.1. PCA of data obtained with the electronic nose indicated that the odor of Ohshima no. 1 showed a clear upward displacement as compared with those of parent cultivars in PC1. The oils of Miyauchi iyokan and Yoshiura ponkan showed displacement in a negative direction, and a positive one in PC2. By PCA analysis, it was found that the odor quality of Ohshima no. 1 was very different from those of the parent cultivars.

The Effect of Music on Perception of Soap Odor

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We are using all five senses and integrating them to recognize external things in our daily lives. Although there are many studies considering the interaction and integration of olfaction with gustation, vision and somatosenses, an interaction between olfaction and audition remains unclear. To understand the interaction between audition and olfaction, the authors investigated whether the olfactory perception is affected by focusing attention to auditory stimuli or not. Twenty participants, who were female university students, were asked to continuously evaluate an intensity of soap odor (KAO White) presented by an olfactometer during listening to J-POP music (Music condition) or white noise (Control condition). After finishing evaluation, participants were asked to evaluate the odor intensity and the odor hedonics on visual analogue scale (VAS) and to rate auditory hedonics on 7-point scale. There were no significant differences and interactions in continuous evaluation for odor intensity among the conditions. However, there were significant effects of conditions in both evaluations on VAS. Participants evaluated the odor stronger and less pleasant during listening white noise as an auditory stimulus than those during listening to J-POP music. Thus, it is suggested that an attention for the auditory stimuli affects the olfactory information processes. This study also showed weak, but significant, correlation coefficient was found between the hedonic evaluation for odor and one for auditory stimulus. The latter finding seems to be an example of mood congruency effect.

Successive Contract of Fragrance Influence to Perception

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Objective nature of one stimuli is emphasized when two stimuli were given continuously, compared to when only one stimuli was given. This is known as successive contract. There have been many interests on successive contract poured in to adaptation as the change of sensory strength but any to the study of olfactory stimuli.

In this study, we focused on the change of fragrance characteristic, strength, and preference when continuous giving olfactory stimuli such as paired same kinds, paired different kinds, paired different dosages and paired mixed complex, regarding.

The results under each successive contract condition were as followed. The result of examining successive contract of olfactory stimuli of a pair of same kinds showed adaptation such as strength perception declined. The result of giving paired different dosages olfactory stimuli showed no effect to prevent adaptation by increasing dosage of successive stimuli even at 10times more. The result of giving a pair of different kinds olfactory stimuli showed fragrance characteristics of successive stimuli emphasized. Finally, giving a pair of mixed complex olfactory stimuli; tried relation clarification of adaptation and successive contract occurring simultaneously and clarified that adaptation occurred preferentially.

Effect of Association between Visual Images and Odors on Overall Evaluation of Personal Care Products

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When the odor is associated with some comfortable visual images, the odor it self would acquire emotionally positive properties. If the association is rised directly between those two different sensory modalities, it is presumable that the odor would first evoke images of the visual stimuli and thereby positive emotions would be accmpanied with it. In this case, it also has possibilities that the odor acquire not only emotional states but also image aspects of visual images as well.

In this study, 65 Japanese female (age 25~35) were divided into 3 groups and presented with the odor and the visual images (photos). They were asked to experience the odor and the photos simultaneously once in a day for one month (associative learning). The group 1 associated odor with pleasant nature photos, the group 2 associated with pleasant animal photos, and the last group with neutral building photos. Before and after the associative learning, they were asked to evaluate preference, familiarity and images of the odor and functions of the body cleanser with the tested odor. Familiarity of the odor was increased in all groups as the result of frequent contact with the odor. However only group 1 and 2 showed the increase in odor preference suggesting the association of emotional state of visual images with the odor. Furthermore, the changes in odor perception suggest that the odor perception have been affected by the associated visual images . These effects were partly seen in product evaluation, where in certain performances and groups, changes in evaluation were obserbed.

255 words

Odor-Active Components in Ground Roasted Sesame Seeds

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A purpose of this study is to elucidate the compounds contributing to a characteristic aromaof the ground roasted se same which is peculiar to Japanese foods. The aroma compounds are highly volatile and instable. To elucidate precisely which compounds contribute to the characteristic aroma, the compounds of these seeds were concentrated using Headspace-Solid Phase Microextraction (HS-SPME) method, and analyzed by Gas Chromatography - Olfactometry (GC-O). In the compounds contributing to the aroma, two or more peaks described as the roast and sulfur odors were found in the area of low boiling points. Among the sulfur odors, the peak of the catty odor was identified as 2-mercapto-3-pentanone (2M3P), an odor compound of coffee and cooked meat. This is the first study to detect 2M3P in sesame seeds. The sulfur odors of the low boiling points including 2M3P and the roast odors increased with the increase of the aroma of the ground roasted sesame from the short-roasted to the middle-roasted. On the other hand, the phenol and oily odors were dominant in the long-roasted. The 30 compounds contributing to the aroma of ground roasted sesame seeds were found at this time. It was thought that the balance, where the roast and sulfur odors of the low boiling point were dominate, was peculiar to the ground sesame aroma.

Mixture Interactions at Threshold Level among Coffee Odorants

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A previous study examined detection of peri-threshold mixtures of aliphatic carboxylic acids (C_2, C_4, C_6 and C_8) and selected binary mixtures(C_2-C_4, C_2-C_6 and C_2-C_8). These results suggest that structural similarity may play a role in mixture-interactions. However, it is possible that the short carbon chain acids(C_2 and C_4) interact more strongly with other compounds(C_6 and C_8). The current study included three coffee flavor compounds very different in structure from the carboxylic acids: furfuryl mercaptan (FM), maple lactone (ML), and 3-methyl-3-sulfanylbutyl acetate (MSA). Subjects attempted to detect (2-out-of-5, forced-choice method) each flavor compound mixed with each of the four carboxylic acids (six peri-threshold concentrations of each binary mixture). Predictions for response addition, i.e., statistical independence, were calculated based on detection of the unmixed compounds. For FM and MSA, ANOVA revealed significant deviations from additivity for mixtures with C_2 and C_4, but not with mixtures of C_6 and C_8. There were no clear

deviations from additivity for any mixtures of ML and fatty acids. These results suggest that, while molecular structure is important for mixture-interactions, carbon chain length is not the only factor involved.

Evaluation on the Quality of Tea Odor using Sensual Test and Odorantsensor System

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Recently it has been very important that we are making to evaluate the artificial odorant-sensor system using odor sensual test. The purpose of this study is to obtain the possibility of evaluation on the quality of tea odor using sensual test and an odorant-sensor system (FF-2A: which was made in Shimadzu Co., Japan).

Twenty six olfactory normal students (20-24 olds: male 24, female 2) were participated as the subject, and their agreements for the experiment were demanded after the informed consent.

Odorant samples in five tea bottles which were mixed with different rates of Japanese green tea (Ito-en Co.) and oolong tea (Suntory Co.) were evaluated using sensual test. All subjects estimated the value of strength (ratio percentages for maximum 100%) of olfactory quality in each mixed tea bottles for the quality (words) selected by himself with magnitude estimation method.

From these experiments, ''Sweetness'' quality (word) was selected out most much by 21 persons, and the characteristic tendency under the ratio of tea mixture was found but except for large deviation. Statistic t-test and cluster analysis were also applied to these data and the confidence for a few olfactory qualities was calculated in this sensual test. On the other hand the measurement values using odorant-sensor system were good correlated with the evaluating data at all five ratio levels of mixed tea samples for the quality of ''Sweetness'' in sensual test.

From these results it was suggested that the olfactory-sensor system could show the capability of the expression for a kind of selected olfactory quality using the correlation with the sensual test.

Effects of Long- or Short-Term using of Shampoo on Memory Consolidation for their Fragrances

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We investigated formation and consolidation of memory for fragrances of several kinds of shampoo purchased in a market. In an acquisition period, subjects were presented with fragrances of shampoo for tens of seconds. After 15 minutes or three days interval, the subjects were tested for their memories of fragrances. Subjects in the 15 minutes retention group showed better recognition for more characteristic fragrances. On the other hand, subjects in the 3 day retention group showed low recognition for the fragrances, and there was no tendency for the correlation between the character of the fragrances and the accuracy of the recognition memory. Thus, the procedure used in this experiment is suggested to be appropriate for the evaluation for the consolidation of memory for the fragrances of the shampoo.

In the second experiment, the subjects used a kind of shampoo provided by experimenters for four weeks in their home, and were tested for memory of fragrance of the shampoo 1 week after ending the shampoo use. The results obtained in this study were content with those in the studies described above. It was suggested that the 15 minutes retention test is suitable for the model for investigating formation and consolidation of the memory for fragrances, and that recognition for fragrances is related to character and pleasantness of the fragrances.

Olfaction Presentation Based on Odor-Emitting Device using Capsules

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The information processed by the sense of smell is directly transmitted to the cerebral limbic system, which governs emotions and memory unlike any other sense. If olfactory information is effectively transferred in addition to vision and audio information, the quality and quantity of information improves spectacularly. For the purpose of the application of odor to information technology, an odor-emitting apparatus needs to meet the following conditions. (1) Any odor ingredient can be used. (2) The apparatus is small as much as possible. (3) Little vibration and noise during odor emission. (4) Amount and strength of odors can be controlled along with on-screen images. (5) Long-term and stable odor emission. We have developed an odor-emitting apparatus to overcome these issues. This apparatus consists of a chemical capsule cartridge including chemical capsules of odor ingredients, valves to control odor emission, and a temperature control unit. There are nine kinds of chemical capsules containing natural fragrances. They were encapsulated by alginic acid polymer, which was a dietary fibers in polysaccharide. The valve is made of artificial metal muscles, which contracts and returns to original length by turning the electricity on and off. It occurs little noise and vibration. We have succeeded in generating different odors and changing strength of odor using this apparatus. We have developed an integrated system of vision, audio and olfactory information in which odor strength can be controlled coinciding with on-screen images.

Study of Mixed Odor by Analyses of the Molecules and by the Olfactory Evaluations

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In order to clarify the change of odor perception by mixing odorants, four rigid molecules were selected and a simple method of olfactory evaluation was performed. The selected molecules were acetophenone (F3), 1,8-cineole (F47), *p*-methylacetophenone (F58),5-methylquinoxaline (F80). Moreover, the size of the molecules, the electronic feature, and molecular vibration (the IR and Raman spectrum), etc. were investigated by the computer calculation using Gaussian R 03W program, and it was examined whether the calculated molecular features would explain the results obtained by the olfactory evaluations. As a result, the smell of F80 was particularly disliked, and when this was mixed with other compounds, there was the tendency which odor offensiveness increases. This odor peculiarity was not able to be explained from the size of the molecules, the electronic features, and log P values. The odors of F3 and F58 showed some similarity, and it was found that the correlation in both their IR spectrum and the Raman spectrum.

Effects of Gonadotropin-Releasing Hormone Antagonist on Urine Marking Behavior of Mice

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House mice use urine marking for variety of social communication. Urine marking varies with sex or dominance status. The number of urine marks alters in presence of other mice. Removal of the vomeronasal organ results in a significant reduction in urine marking, so odor cues affect on marking behaviors. The nervus terminalis which containing gonadotropin releasing hormone (GnRH) neurons innervated to the vomeronasal organ and the GnRH facilitates vomeronasal receptor neuron responses to odors. Then, to investigate the role of GnRH in urine marking behavior in house mice, mice were tested for marking before and after GnRH antagonist, cetrorelix acetate, injection in response to stimulus male, stimulus female or non stimulus animal on opposite side of mesh partition. Before GnRH antagonist injection, male mice produced large number of urine spots dispersed widely, and the number was increased when a stimulus female was around but decreased to a stimulus dominant male. Females produced relatively fewer spots with none stimulus animal, and they deposited many spots to a stimulus male. GnRH antagonist injected males diminished marking activity like females, and GnRH antagonist injected mice showed no difference in marking response to different stimulus animals. GnRH antagonist injection diminished the sensitivity of vomeronasal receptors to odors, so this hyposensitivity should be responsible these decreases of urine marking activities.

Aversive Conditioning to a Mixture of Monopotassium Glutamate and Glutamate Receptor Antagonists is Generalized to Sucrose but not to Glutamate Receptor agonists in C57BL Mice

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Monosodium glutamate (MSG) elicits the taste called umami. Recent studies have identified at least five candidate receptors for umami: taste-mGluR4, T1R1+T1R3, and taste-mGluR1 responding to the millimolar concentration range of MSG and brain-expressed mGluR4 and mGluR1 with higher sensitivities to MSG. Our previous behavioral and electrophysiological studies in mice showed (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG, mGluR4 antagonist) and 1-aminoindan-1,5-dicarboxylic acid (AIDA, mGluR1 antagonist) suppressed the responses to MSG or monopotassium glutamate (MPG) at concentrations of 0.0001-100 mM. In this study, to confirm the possibility of brain-expressed mGluR4 and mGluR1 as receptors for umami, we compared aversive responses to various taste stimuli in the mice conditioned to avoid either MPG alone or MPG mixed with CPPG and AIDA (M+C+A). The aversive conditioning to MPG showed aversive responses to 0.01-100 mM MPG alone, and mixtures with inosine 5'-monophosphate (IMP), whereas the conditioning to M+C+A showed aversive responses to 1-100 mM MPG mixed with IMP, but not to 0.01-100 mM MPG alone. The aversive conditioning to MPG was strongly generalized to sucrose, MSG, L-AP4, quisqualic acid, M+C+A, and MPG, MSG, L-AP4 or quisqualic acid mixed with IMP. The aversive conditioning to M+C+A was strongly generalized to sucrose and MPG, MSG, L-AP4, or quisqualic acid mixed with IMP, but not to MSG, MPG and L-AP4 alone. The results suggest the possibility that brain-expressed mGluR4 and mGluR1 may mediate the umami component of MPG, and that T1R1+T1R3 receptor may play major roles in mediating the sweet component of MPG alone and mixture with IMP.

Development of a Software for the Evaluation of Odor Impact Spectrum (OIS) and an Application of the Program for Determination of the Ripening Period from *Citrus* Aroma

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An add-in software into Microsoft Excel designed for the evaluation of GC-Olfactometry was developed and named it as "odor impact spectrum (OIS)". This software composes of 1) data sheet for filling in chemical name, retention time and concentration of each chemical, 2) calculation unit of Uo and OIS by using the odor detection thresholds installed in the database and then 3) visualization of OIS. OIS was formulated by following equation (2) on the basis of Stevens' law and recorded it visually into a figure.

Uo = (concentration of aroma chemicals)/(detection threshold)·····(1)

OIS = sqr(Uo)/sqr(Uomax) ·····(2)

where sqr means square root.

This evaluation system was applied to the determination of the ripening period in aroma of *Citrus sudachi,* one of Japanese citruses. In August, OIS of *Citrus sudachi* increased a lot in chemical number and chemical concentration. Along with the increasing of OIS values, sensory scores of those aroma oils harvested at June, July, August and September were evaluated by the 9-point scale method

with 21 panel members and finally showed higher similarity between *C. sudachi* aroma of August and September. As the result of cluster analysis of OIS and sensory scores of the citrus, we could prove the adequacy of OIS system by the consistency with the consequence of sensory test.

Clinical Analysis in Patients with Recurrent Olfactory Dysfunction

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Background: It is known that some patients who recovered from their olfactory dysfunction by medical treatment lose their olfactory function again. OBJECTIVES: The present study was designed to clarify clinical features and prognostic factors of recurrent olfactory dysfunction. MATERIALS&METHODS: We retrospectively analyzed 347 patients presenting to the Mie University Hospital Smell and Taste Clinic over 7.5 years (2000-2008). They underwent clinical examination by interview, endonasal fiberscopy and olfactory function tests (T&T olfactometry) and intravenous olfactory test (the AlinaminR test). A total of 150 patients underwent the smell tests more than twice and were followed up their course of olfactory dysfunction. We defined the recurrent olfactory dysfunction as that olfactory function is reduced again after recovery by medical treatment.

Results: Of 150 patients, 89 (59%) restored their olfactory function after medical treatment. Percent recovery was 63% (40 of 64 cases) in olfactory dysfunction induced by sinusitis and 78% (32 of 41) by upper respiratory infection (URI). The incidence of recurrent olfactory dysfunction was 6.0% (9 of 150), with a range of 32 to 72 years old, three males and six women. The etiologies of the recurrence for the nine patients were sinusitis (7 patients), URI (1) and nasal allergy (1). Causal diseases for the recurrence were the same as caused for the primary olfactory dysfunction in individual patients. Five of the nine recurrent cases were complicated bronchial asthma. The incidence of recurrent olfactory dysfunction was significantly higher in patients with bronchial asthma than others.

Conclusions: Asthma and sinusitis are critical for recurrent olfactory dysfunction. We must carefully treat and follow up the patients with these diseases when we treat their olfactory dysfunction.

Investigation of Odor Identification Abilities of Disabled People in the Non-Residential Workshop

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While it is well recognized that ability of smell declines with aging and at early stage of certain dementia, the smell sense of disabled people has not been studied yet. To clarify how well disabled persons can identify the smell, we investigated abilities of odor identification by means of four-choice test using 12 types of odors which are familiar to Japanese. The subjects comprised of 31 physically-disabled volunteers and 27 intellectually-disabled volunteers who utilize non-residential workshop and 65 healthy students. The mean identification rate with 12 test odors were 73.0% for physically-disabled subjects and 57.1% for intellectually-disabled subjects, while it was 89.2% for healthy subjects. Although the intellectually-disabled subjects collectively showed significantly lower ability of odor identification compared with other groups, 21.4% of them had quite high ability showing identification rate above 90%. Comparing ratio of subjects who could identify each smell, no significant differences were found among physically-disabled, intellectually-disabled and healthy subjects for curry, orange and rose smells which are familiar to all groups. On the other hand, the smells which were poorly experienced by disabled subjects like condensed milk, cypress and city gas were difficult to identify for disabled subjects, especially for intellectually-disabled subjects. The present results suggest that the ability of odor identification of disabled people can be improved by increasing the opportunities for smelling a variety of odors in their daily lives.