

## ABSTRACTS

### Abstracts from the 45<sup>th</sup> Annual Meeting of Japanese Association for the Study of Taste and Smell (JASTS 2011), Kanazawa, Japan, October 5-7<sup>th</sup>, 2011 (The president of the meeting was Dr. Takaki Miwa, Kanazawa Medical University)

#### #1 Investigation of the conditions for large-scale purification of bitter taste receptor T2R16

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Bitter taste is received by T2Rs, a member of G-protein coupled receptor (GPCR) family, and the 25 variants are known as human bitter taste receptors with their possible ligands. However, both the accurate higher-order structures of T2Rs and their precise mechanism of taste reception are not adequately clarified yet. To obtain more information about these, it is necessary to analyze purified T2Rs. In this study, we designed *Saccharomyces cerevisiae*-aided, large-scale T2R16 purification protocols and examined each step of them by using the GFP tag-added C-terminal fragment for determination of the optimal conditions. As a result, the expression efficiency of T2R16 was better in BJ3501 than in BJ2168 of *Saccharomyces cerevisiae*. The optimal time of incubation for T2R16 expression was 72 hours. In the step of cell breaking, it was most appropriate to use glass beads with vortex machine CM-1000 for 20-40 minutes. We compared 6 detergents (Anzergent 3-14, DM, DDM, UDM, Triton-X 100, LDAO) in the T2R16 solubilization step and found the highest solubilizing ability in Anzergent 3-14. Although there was a problem of purified T2R16 stability because of its aggregation, the problem was solved by addition of 5 mM DTT as reductant. Moreover, we successfully removed the GFP tag from the purified protein by using TEV protease. It will thus be possible to purify an adequate amount of T2R16 for its structural and reception-mechanism analysis.

#### #2 Role of leucine repeats in interaction of T1R1 and T1R3

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Since umami taste receptors T1R1 and T1R3 have been identified, some reports revealed their structures and functions. Umami taste receptors are member of G protein-coupled receptors, belonging to family C with long N-terminal extracellular domain. T1R1 and T1R3 perceive umami taste as a heterodimer. We focused on leucine repeats of T1R1 and T1R3 in their transmembrane domains. We have reported the possibility of correlation between the food preference of animals and difference in the length of leucine repeats. Poly leucine (polyL) is called homo polymeric amino acid, so called HPAA. It is reported that 1~2% of proteins in prokaryotic and eukaryotic organisms contain HPAA. It is also suggested that polyL could interact with each other more than with the other HPAA. Some inherited diseases are caused by proteins in which HPAA such as polyQ, polyA are expanded to excessive length. Activity of the protein containing HPAA is thought to be increased by expansion of HPAA, but with expansion of more than the threshold, activity of the protein is suddenly decreased. HPAA are one of the structures to be seen frequently. It is suggested that HPAA are important structures for some proteins to function. On the other hand normal function of HPAA remains unclear. From the above point of view, polyL might affect interaction of umami taste receptor T1R1 and T1R3. We also hypothesize polyL play an important role in the perception of umami. Some odorant receptors also have the similar polyL structure, suggesting that under the hydrophobic condition some proteins are dimerized by interactions between leucine repeats. We named a leucine HPAA motif for protein-protein interaction as "Leucine Glue." We are discussing the effect of polyL on protein-protein interaction by using Split GFP assay.

#### #3 Comparative analysis of the umami receptor gene, *t1r1* from Cetaceans

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T1R1 and T1R3 are known to act as umami receptor. They are member of G protein-coupled receptors (GPCR) and have seven transmembrane domains. In 2010, it has been reported that the *t1r1* gene of giant panda has frameshift mutation, resulting in inactivation of umami receptor. Originally, they are used to be a carnivorous animal. It is suggested that the mutation of the *t1r1* gene might be associated with their current food preference. We have already analyzed genomic sequences of the *t1r1* genes from two different dolphins; *Lagenorhynchus obliquidens* and *Tursiops truncatus*.

The results strongly suggested that umami receptor genes, *t1r1* from both cetaceans are not functional, because frameshift mutations occurred in their coding sequences. Nonsense codon caused by these frameshift mutations appeared on their downstream region. In this study, we have also cloned two more *t1r1* genes from cetaceans, *Balaenoptera acutorostrata* and *Balaenoptera physalus*. Both the *t1r1* genes had frameshift mutation and various mutations in their coding sequences. In addition, we have also cloned two more *t1r1* genes from cetaceans, *B. acutorostrata* and *B. Physalus*. Both the *t1r1* genes had frameshift mutations and various mutations in their coding sequences as well as dolphins described above. Especially, in one allele from both *B. acutorostrata* and *B. Physalus*, we found point mutations at start codon ATG which is A to G substitution, resulting in GTG. By this mutation, *t1r1* could not be translated.

#### #4 Responses to amino acid solutions in T1R3 knockout mice

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Cell-based expression studies have shown that mammalian T1R1 and T1R3 G-protein coupled receptors combine to form a broadly tuned L-amino-acid receptor. However, contradictory data in T1R3 knockout (KO) mouse models have been reported on taste nerve responsiveness. One study showed that taste nerve responses to amino acid stimuli in T1R3-KO mice were totally abolished. The other reported reduced but not abolished responses to glutamate in T1R3-KO mice. In the present study, we have tried to recharacterize the role of T1R3 for the taste nerve responses to amino acids using a wide variety of taste stimuli. We have recorded from whole-nerve chorda tympani nerve responses from T1R3-KO mice to lingual application of various amino acid stimuli including sweet- and umami- tasting amino acids. Whole-nerve recordings of chorda tympani nerve in T1R3-KO mice showed robust responses to all the tested amino acids. Comparing to those from C57BL/6 wild-type mice, only small reductions of responses to glycine and L-alanine, but no reduction in responses to L-proline, L-serine, L-glutamine and L-glutamate salts have been observed. These results suggest that T1R3 mediated receptor system may play limited role for detection of amino acids.

#### #5 Sour taste and ATP activate subpopulations of taste cells separately

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Type III taste cells play to act to receive sour taste and also to act as "taste integration cell" to transmit taste information from type II taste cells through ATP. But, it is not known whether a type III taste cell has both roles and whether two kinds of type III taste cells share each role separately. Using calcium imaging and immunocytochemistry, we revealed that ATP activates type II and III taste cells and HCl activates type III taste cells, and also that HCl and ATP

activate separate subpopulations of taste cells. We also showed that sour-responding taste cells do not have P2X<sub>2</sub> and P2Y<sub>1</sub> as ATP receptors in taste buds. Moreover, we carried out double cell staining of mice circumvallate papillae using antibodies to ATP receptor (P2X<sub>2</sub>, P2X<sub>3</sub> and P2Y<sub>1</sub>) and sour taste receptor (PKD2L1) using an antibody to type II taste cell marker (PLCβ2) and type III taste cell marker (PGP9.5). It was shown that P2X<sub>2</sub> and P2X<sub>3</sub> are expressed in taste nerves, P2Y<sub>1</sub> is in type II taste cells, and PKD2L1 is in type III taste cells. No taste cell was stained by both ATP receptor and sour taste receptor. Although we could not cover all the ATP or sour taste receptors, these results suggest that there are the two separate subpopulations of type III taste cells: the one sensing sour taste and the other accepting the ATP released from type II taste cells.

#### #6 Development of gustatory papillae in the absence of *Six1* and *Six4*

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*Six* family genes encode homeobox transcription factors, and a deficiency in them leads to abnormal structures of the sensory organs. In a previous paper, *Six1* was reported to be expressed in the taste bud-bearing lingual papillae of mice, and loss of *Six1* affected the development of these gustatory papillae. We show here that embryos lacking both *Six1* and *Six4* revealed more severe abnormalities than those lacking *Six1* alone during morphogenesis of their gustatory papillae. By *in situ* hybridization, *Six4* was shown to be broadly distributed in the epithelium of the lateral lingual swellings at embryonic day (E) 11.5, and in the tongue epithelium, mesenchyme, and muscles at E12.5. From E14, *Six4* was similar in expression pattern to *Six1*, as previously reported; and in the fungiform papillae, *Six4* was expressed in the epithelium at E14-E16.5. In the circumvallate and foliate papillae, *Six4* expression observed in the trench wall of these papillae at E15.5-P0. Although *Six4*-deficient mice had no abnormalities, *Six1/Six4*-deficient mice showed distinct morphological changes: fusion of the lateral lingual swellings was delayed, and the tongue was poorly developed. The primordia of fungiform papillae appeared earlier than those in the wild-type or *Six1*-deficient mice, and the papillae rapidly increased in size; thus fusion of each papilla was evident. The circumvallate papillae showed severe defects; e.g., invagination of the trenches started asymmetrically, which resulted in longer and shorter trenches. The foliate papillae elevated initially, and showed stunted trenches. Therefore, *Six1* and *Six4* function synergistically to form gustatory papillae during development of the tongue.

#### #7 Analyzing cell turnover in taste buds with a new thymidine analog detected by fluorescence

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The life span of taste cells was previously estimated as 10-14 days by labeling newly proliferated taste cells with radioactive <sup>3</sup>H-thymidine or Bromo-d-uridine (BrdU) incorporated during DNA synthesis. To re-evaluate this observed lifetime of taste bud cells, we repeated the analysis of lingual tissues of mice after incorporation of ethynyl-d-uridine (EdU) instead of BrdU, followed by detection of EdU by a click reaction. We injected EdU into adult mice and sacrificed them 1, 3, 5, 6, 10, 15, 20, 25, 30, 35, or 40 days later. The vallate papillae were fixed, cryo-sectioned, immunostained for PLCβ2 (a marker for Type II cells) and examined with a confocal laser scanning microscope with optical sectioning at 2 μm. Taste buds were identified in DIC images captured in parallel. Numerous cells with brightly fluorescent nuclei (EdU+ cells) appeared at Day 1 near and within taste buds. The number of those cells declined rapidly toward Day 10. However, among Type II cells, very few appeared to be EdU+ at Day 1. The number of EdU+ Type II cells increased at Day 3, showed a broad peak around Day 10, and then declined. Occasional EdU+ Type II cells were still detected at Day 40. Because labeling of DNA with EdU is much faster, brighter and more consistent than with BrdU, this method will be useful to study proliferating cells in taste buds. By combining EdU labeling and immunostaining for appropriate markers, the longevity of taste cell types can be examined.

### #8 Expression of the adiponectin receptors in mouse circumvallate papillae

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Adiponectin is a white adipose protein that plays important roles in glucose homeostasis and lipid metabolism and is involved in cell proliferation and differentiation. The function of adiponectin is mediated by its receptors (AdipoR1 and AdipoR2), which have different expression patterns. In the gustatory system, adiponectin is contained in the saliva; however, the function of adiponectin signaling in gustatory tissues has not been elucidated yet. Therefore, we have examined the expression patterns of AdipoR1 and AdipoR2 in mouse gustatory tissues. Reverse transcription polymerase chain reaction assays have revealed that *AdipoR1* and *AdipoR2* mRNAs were expressed in the circumvallate papillae. Using immunohistochemistry, the anti-AdipoR1 antibody labeled at the taste hairs in the fungiform, foliate and circumvallate papillae. Double-labeling experiments have demonstrated an expression pattern of AdipoR1-positive cells in a subset of basal cells within the taste buds and extragemmal epithelium cells to marker proteins for taste cell types (including types I, II, III, and basal cells). For the marker proteins analyzed, only Shh was co-expressed with AdipoR1 in a subset of basal cells within the taste buds in the circumvallate papillae. These results show that AdipoR1 at taste hairs in gustatory tissues may detect saliva adiponectin and may play a role in taste sensing in the taste buds. In addition, AdipoR1 may play a role in cell proliferation and

differentiation for the Shh and AdipoR1 expressing basal cells within the taste buds.

### #9 Cell differentiation of taste bud and neuronal network formation

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*Mash1* is expressed in subsets of neuronal precursors in both the central nervous system and the peripheral nervous system. However, involvement of *Mash1* in taste bud cell differentiation remained to be demonstrated. In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation, we have investigated the role of *Mash1* in regulating taste bud cell differentiation using *Mash1* KO mice and forced expression of *Mash1* in lingual epithelial cells. In *Mash1* KO mice, AADC-IR cells are missing both in the *Mash1* mutant circumvallate papilla epithelium and in the taste buds of soft palate. In *Mash1* KO/GAD67-GFP mice, GFP-positive cells (GAD67 expressing type III cells) are also missing in the taste buds of soft palate. On the other hand, Gustducin, a type II cell marker of taste bud, is expressed in soft palate taste buds in *Mash1* mutant mice. These results suggest that *Mash1* plays an important role for expression of AADC and GAD67 in type III cells in taste buds.

Taste receptor cells are epithelial in sense that they have a limited life span and therefore must be replaced to maintain the structure of the epithelium. Therefore gustatory nerves need to make synapse with appropriate taste receptor cells. However, mechanism of recognition of taste receptor cells which express appropriate taste receptor is still unknown. The cadherin superfamily of cell-cell adhesion molecules controls a series of interactions that regulates synapse formation. In this study, in order to test whether the cadherins are required for formation of synapse between gustatory nerve fibers and taste receptor cells, we have investigated expression patterns of cadherin superfamily in the taste buds.

### #10 Induction of Type III-like taste cells in three-dimensional co-culture and analysis of excitability of clonal cell lines derived from murine taste buds

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We have collected taste buds from a tongue of a *p53*-deficient mouse and established clonal cell lines (TBD cell lines). Each of TBD cell lines expresses gustducin and neural cell adhesion molecule (NCAM), suggesting that TBD cell lines have characteristics of both Type II and Type III taste cells. To reveal whether TBD cell lines have excitability or not, we analyzed the expression pattern of voltage-dependent sodium channels in one of TBD cell lines, TBD-a5 cells and performed patch clamp recording. *SCN2A*, *SCN3A*,

*SCN5A* and *SCN9A* genes coding voltage-dependent sodium channels were detected in TBD-a5 cells by RT-PCR. Patch clamp recording revealed that TBD-a5 cells had tonic outward currents, but lacked transient inward currents. These results suggest that functional voltage-dependent sodium channels are not expressed in TBD-a5 cells.

Next, we tried to establish a culture model mimicking the lingual taste cells of the mouse. TBD cell lines were co-cultured with a lingual epithelial cell line (20A cell line) and a lingual mesenchyme-derived cell line (TMD cell line). TBD, 20A and TMD cell lines were maintained in a triple co-culture, in which TBD cells were pre-seeded as aggregates or in suspension on the collagen gel containing TMD cells and 20A cells were laid over the TBD cells. TBD cells in the triple co-culture expressed NCAM, however gustducin was not immunohistochemically detected in TBD cells. This result suggests that TBD cells in the co-culture exhibited a characteristic of Type III taste cells. This triple co-culture model would be useful to study morphogenesis and functions of the gustatory organ.

### #11 Immunoreactivities of the gustatory cell in rat vallate taste buds

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Immunohistochemistry of taste-signaling molecules is useful for the distinction of taste-bud cell types. However, the optimal immunohistochemical detection depends on specimen-preparing conditions. Therefore, this study aimed to examine differences in immunoreactivities under various tissue-preparing conditions in rat vallate taste buds for some typical markers of gustatory cells as follows: gustducin, type III inositol triphosphate receptor (IP<sub>3</sub>R3), synaptobrevin-2 (VAMP2), protein gene product 9.5 (PGP9.5), and neural cell adhesion molecule (NCAM). Staining patterns and intensities of immunoreactivities for these molecules varied according to tissue-preparing conditions, especially the fixation.

Confirming our previous finding, gustducin immunoreactivities were localized to the upper part of the taste bud called taste hairs in case of short fixation, but to the cell body cytoplasm excluding the taste hairs in case of long fixation. The specific immunostaining for IP<sub>3</sub>R3 was strong in short-time fixation. On the other hand, those for VAMP2, PGP9.5, and NCAM were intense in long-time fixation. The present data suggest that different experimental protocols cause the discrepancies in immunoreactivities for these markers. Further investigations based on this study should provide important information on the classification of the gustatory cell type.

### #12 Immunohistochemical localization of NK1 and VPAC1 receptors in the frog fungiform papilla

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Although the presence of substance P (SP) immunoreactive (IR) nerve fibers and vasoactive intestinal peptide (VIP)-IR nerve fibers has been reported in the frog fungiform papilla, the functional roles of these fibers remain to be elucidated. To clarify whether these SP-

IR and VIP-IR fibers play any efferent roles in the taste disc in the fungiform papilla, we immunohistochemically examined the existence of neurokinin 1 receptor (NK1R), which is also a SP receptor, and VIP and pituitary adenylate cyclase activating hormone 1 receptor (VPAC1R), which is also a VIP receptor, in the fungiform papilla of the frog, *Rana catesbeiana*. Many NK1R-IR structures were observed in the taste disc. These were conical in shape in the middle layer of the taste disc and had a rod-like thin apical process toward the upper layer of the taste disc. Double-labeling immunohistochemistry showed that only a few NK1R-IR structures were located adjacent to the SP-IR nerve fibers in the taste disc. VPAC1R immunoreactivity was observed in the taste disc cells and the glossopharyngeal nerve bundle beneath the taste disc. VPAC1R-IR cells in the taste disc had either a thin or thick apical process. VIP-immunonegative nerves beneath the taste disc showed VPAC1R-IR. VIP-IR neuronal cell bodies colocalized VPAC1R in the glossopharyngeal nerve bundle within the tongue. The presence of the NK1R and VPAC1R in the taste disc suggests that SP-IR and VIP-IR nerve fibers play efferent roles in the taste disc in the frog fungiform papilla.

### #13 Cesium-permeable potassium channel in the rod cells of frog taste disc

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In animal cells, the resting membrane potential is maintained by random open-shut gating of a leaky potassium channel. Frog taste disc cells display the resting membrane potential of about -50 mV. The magnitude is not consistent with the equilibrium potential of potassium (-90 mV). When internal potassium was replaced with cesium in whole-cell configuration, the rod cells in frog taste disc increased a parabolic outward current gradually and displayed the resting membrane potential of  $-49 \pm 2$  mV (n=23). The wing cells did not possess the outward current and displayed the resting potential of  $5 \pm 1$  mV (n=41) in the condition of internal cesium solution. The replacement of external normal saline solution with cesium saline solution turned the parabolic outward current to the inward current. The inward current reversed at  $2 \pm 1$  mV (n=10) which is close to the equilibrium potential of cesium (4 mV). Several experiments on cationic replacement showed the permeability ratio of  $P_K:P_{Cs}:P_{Na}:P_{NMDG}=1.67:1.00:0.29:0.15$ . Carbenoxolone (CBX, a blocker of pannexin) inhibited the parabolic outward current moderately (IC<sub>50</sub>, 26 μM). The dialysis of 20 μM arachidonic acid in internal cesium solution changed the parabolic outward current to the exponential one and depolarized the membrane potential by 15 mV. The results suggest that the outward cesium current in the frog rod cells may flow through a novel channel different from mammalian cesium-permeable potassium channel.

### #14 Reception mechanism of pungent sensation of capsiate and capsaicin analogs

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Capsaicin, which is the main component of chili pepper and has many physiological functions such as increasing perspiration and energy metabolism, has negative effect of its strong pungency. Capsiate was identified from 'CH-19 Sweet' (*Capsicum annuum* L.), a non-pungent cultivar of red pepper. Like capsaicin, capsiate is thought to enhance energy metabolism by activating the sympathetic nervous system. Human sensory evaluation tests demonstrated that capsiate and that analogs (capsinoids) had very weak pungency compared with capsaicin and capsaicin analogs (capsaicinoids), and the difference of threshold level between capsinoids and capsaicinoids was about 1000-fold. However, the *in vivo* studies focused on the reception of them in oral cavity are few because the reception mechanism of hot taste is unclear, and the reason of weak pungency of capsinoids is not well understood. Therefore, this study focuses on the reception of capsinoids and capsaicinoids in the oral cavity. Firstly, we performed two-bottle preference tests using rats and confirmed that they began to avoid capsinoids at about 1000 times higher concentrations than that of capsaicinoids. In addition, we recorded the lingual trigeminal nerve responses to capsinoids and capsaicinoids and found that capsinoids induced little responses while capsaicinoids evoked apparent intensive responses. Such differences were also found in immunohistochemical detection of p-ERK in the trigeminal ganglion after stimulation of capsinoids and capsaicinoids.

### #15 Immunoreactions of brain reward system in mice obtained overconsumption of sweet solutions

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Recent studies using animal models of binge eating shows that the accumbal dopaminergic neurotransmission is elevated in bingeing. It suggests that the experience of bingeing modifies the activity of midbrain dopaminergic neurons in the ventral tegmental area (VTA). However, few evidences directly clarify whether the activity of the VTA is elevated anticipatorily, that is, before receiving the oral (taste) and/or post-oral (viscerosensory) cues of palatable food. To address the issue, we explored possible changes in the activity of the VTA neurons in mice bingeing on sugar by immunohistochemical detection of *c-fos* expression as a marker of neuronal activation. Under food restriction, mice were subjected to a binge-type eating training for 10 days (day 1-10), in which their intake of sucrose solution gradually increased day by day, resulting in its overconsumption. On day 11, mice were deeply anesthetized and transcardially perfused with 4% paraformaldehyde before receiving the sugar exposure. Brain sections including the ventral midbrain were immunohistochemically processed to detect the *c-fos* gene expression product, Fos protein. The number of Fos immunopositive neurons in the VTA of trained mice was larger than that of untrained naïve mice. The finding suggests that the activity of the VTA is elevated anticipatorily before sugar exposure and contributes to the stimulation of the mesolimbic dopamine system, resulting in sugar bingeing.

### #16 Activation of output neurons in the basolateral nucleus of the amygdala by a learned aversive taste: a manganese-enhanced MRI study

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The basolateral nucleus of the amygdala (BLA) is known to play an important role in conditioned taste aversion (CTA). Anatomical studies have shown that the BLA has reciprocal neural projections with many brain regions. It is still unclear, however, which connections are involved in the retrieval of CTA. To elucidate the neural circuits for the retrieval of CTA, we examined the enhancement of activity of the efferents from the BLA by the presentation of learned aversive taste, using a manganese-enhanced MRI technique. Rats received a pairing of 5 mM saccharin with 0.15 M lithium chloride or saline (CTA or sham group, respectively). On the test day, thirty min after an injection of 40 mM MnCl<sub>2</sub> into the BLA, rats were intraorally infused with saccharin. Sixty min after the MnCl<sub>2</sub> injection, we acquired triple T1-weighted MR images at hourly intervals. The signal intensities of MR images, which reflect the movements of manganese by the activated BLA neurons, were measured. The CTA group showed significant time-dependent increase of signal intensity in the CeA than did the sham group, indicating that the retrieval of CTA activates efferents from the BLA to the CeA. Furthermore, the analyses based on the injection site of MnCl<sub>2</sub> (rostral or caudal) demonstrated the larger movements of manganese toward the CeA and cortices (insular and piriform) in the rostrally-injected CTA group. Therefore, the outputs of efferent neurons in the rostral part of the BLA may be separated in two directions, medial (CeA) and lateral (cortices). This work was supported by KAKENHI (22700748).

### #17 Validation of <sup>13</sup>C-Acetic Acid Breath Test to Evaluate Gastric Emptying of Liquid Diet in Conscious Mice

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The <sup>13</sup>C isotope-labelled acetic acid breath test is a common clinical method to evaluate gastric emptying and is a non-invasive and repeatable method in rodents. However, the sensitivity of this test to detect changes in gastric emptying of liquid diet in mice has not been studied thoroughly. The present study aimed to validate this method by using Bethanechol chloride, atropine sulfate, clonidine hydrochloride, CCK-8S and phenylthiocarbamide.

Male C57BL-6J mice after overnight fasting were placed in the metabolic chamber. Commercial liquid diet containing <sup>13</sup>C-acetic acid was used as the test meal. After intragastric administration of the test meal, the expired air was collected at 5-min intervals for four hours by a fully automated system. The <sup>13</sup>CO<sub>2</sub> / <sup>12</sup>CO<sub>2</sub> levels were measured by the mass spectrometer. The <sup>13</sup>CO<sub>2</sub> excretion data were

analyzed for calculation of gastric half excretion time ( $T_{1/2}$ ) as the gastric emptying parameter. T-test was used for statistical analysis ( $p < 0.05$ ). Bethanechol chloride decreased  $T_{1/2}$  of liquid diet and atropine sulfate and clonidine hydrochloride increased  $T_{1/2}$ . CCK-8S increased  $T_{1/2}$  and CCK1 receptor antagonist, Devazepide, attenuated the delay of gastric emptying by CCK-8S. Bitter tastant, phenylthiocarbamide increased  $T_{1/2}$ .

Our study indicated that the  $^{13}\text{C}$ -breath test was sensitive to detect difference in gastric emptying of liquid diet induced by pharmacological agents, hormones and tastants. We concluded that this technique could be used to determine the effect of physiological, pharmacological and nutritional intervention on the motility of the gastrointestinal tract.

### #18 Suppression of the chorda tympani nerve response to sucrose by acrinol hydrate in C57BL/6 mice

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In oral surgery treatments, acrinol hydrate is often used as a disinfectant for oral membranes; however, there are few reports about the effect on taste nerve response to this disinfectant. In this study, we tested if tongue treatment with acrinol hydrate affects taste nerve responses in mice. The chorda tympani nerve responses of C57BL/6 mice to 5 basic taste stimuli before and after treatment with acrinol hydrate were recorded and compared. The results of the treatment with acrinol hydrate indicated that the chorda tympani nerve response to 0.5M sucrose was suppressed by 60%; however, there was no change in the responses to 0.1M NaCl, 0.01M HCl, 0.02M quinine hydrochloride and 0.1M monopotassium glutamate. The suppression of the sucrose response by this treatment recovered within 1 minute after washing. These results suggest that acrinol hydrate has an ability to suppress sweet taste.

### #19 Suppression of sweet, bitter and umami responses by spearmint in the C57BL/6 mice

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Some mints, such as peppermint, are used as flavoring substances for foods and toothpaste widely. However, it is not clear whether mints affect the taste receptor mechanisms or not. In the present study, therefore, the chorda tympani nerve responses to 5 basic tastes were compared before and after mixing with 3 types of mints, such as 0.18% peppermint, 0.18% spearmint and 0.10% menthol, in C57BL/6 mice. When spearmint was mixed with 0.5 M sucrose, 20 mM denatonium benzoate, 0.1 M monopotassium glutamate (MPG), 5 mM 5'-inositol monophosphate (IMP) and the mixture of 0.1 M MPG and 5 mM IMP (M+I), the neural responses to these stimuli were suppressed. But mixing of spearmint did not change the responses to 0.1 M NaCl, 20 mM quinine-hydrochloride and 10 mM HCl. Mixing of peppermint

or menthol did not change any responses to all tested stimuli in the present study. These results suggest that spearmint may work as a suppressant for some kind of receptors for sweet, umami and bitter.

### #20 A method for recording neuronal activity in the nucleus of the solitary tract of mice

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The majority of the experiments for investigating gustatory information processing in the nucleus of the solitary tract (NST) have been performed using rats as experimental animals. Although the importance of mice in this kind of study is widely recognized from the view point of recent, rapid advance in gene manipulation techniques, there are very few studies using mice in this academic field. Also, it is desirable for physiological studies on neural mechanisms of taste perception to use awake, or at least, weakly anesthetized animals. Therefore, in the present study we tried to establish an experimental model for *in vivo* recording of NST neurons in slightly anesthetized or awake mice. For this, we surgically fixed a head restraining holder to the mouse skull, which made it possible to place the mouse head precisely in a stereotaxic position repeatedly without pain. After the recovery from this surgery, the gustatory area in the NST was localized electrophysiologically under anesthesia. Then, single neuron activity was recorded in this area under slight anesthesia, and responsiveness to 4 standard taste solutions (NaCl, sucrose, citric acid and quinine HCl) was tested. As a result, 9 gustatory neurons were recorded; of these, 4, 4 and 1 neurons were classified into NaCl-best, sucrose-best and citric acid-best, respectively. Furthermore, we found that the mouse could be easily trained to lick taste stimuli with the head restrained in the stereotaxic device. These results indicate that the present method is applicable to the investigation of gustatory information processing in the NST of mice under a physiological condition.

### #21 Comparison of preference between sucrose and sugar alcohols in C57BL/6 mice

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Some sugar alcohols are widely used as anti-cariogenic sweeteners. It is known that C57BL/6 mice prefer xylitol, sorbitol and palatinit to distilled water. But it is not clear whether these sugar alcohols are preferred or not in comparison with common sugars, such as sucrose. Therefore, in the present study, we compared the preferences between one of these sugar alcohols and 0.3 or 0.1 M sucrose with the 2 bottle preference test. The results show that each of the sugar alcohols, xylitol, sorbitol and palatinit, was not preferred to 0.3 M sucrose in the mice. They also did not prefer these sugar alcohols compared with 0.1 M sucrose. When preferences were examined among the 3 sugar alcohols with the 2 bottle preference test, no particular difference was detected among the sugar alcohols,

although sorbitol tended to be less preferred compared to palatinit. The present study suggests that the sugar alcohols are less palatable than that to common sugars, such as sucrose, in mice.

### #22 Novel peptides, dRYamide-1, dRYamide-2: Comparison in regulatory effects on feeding behavior of the blowfly *Phormia regina*

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Because the *Drosophila* orphan receptor CG5811 is similar to the vertebrate neuropeptide Y receptor, we screened its putative ligands and purified two novel peptides: dRYamide-1 and -2. To investigate their function, we injected these peptides into the thorax of the blow fly, *Phormia regina*, and observed the subsequent changes in feeding behavior and taste sensitivity to sucrose. The blow fly was used for technical reasons, to facilitate injection.

Before and after peptide injection, we measured 1) the sucrose intake as an indicator of appetite, 2) the proboscis extension reflex (PER) as an indicator of feeding motivation, and 3) the electrophysiological response of the labellar contact chemosensilla to measure the magnitude of the gustatory input inducing PER.

There was no significant change in sucrose intake after injection. Injecting both peptides depressed PER and reduced the percentage of flies that performed PER. The electrophysiological response of the labellar taste sensillum to sucrose seemed to decrease after injecting dRYamide-1, but not dRYamide-2. Therefore, once feeding starts, these peptides might not limit the food intake of the fly. Nevertheless, the results of the PER test suggest that these peptides contribute to feeding initiation. Together with other peptides that restrict food intake, dRYamide-1 and -2 might contribute to the overall regulation of feeding behavior in *Phormia regina*.

### #23 Stand off effect between the noble bioactive peptide and appetitive odor on feeding behavior in blowfly

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We compared the behavioral effects of feeding suppression by the two kinds of new peptides (dRYamide-1, 2) in blowfly. Both of gustatory and olfactory systems are required to elicit appropriate motor pattern in feeding behavior. Feeding behavior was also controlled by internal condition based on many bioactive peptides. Proboscis extension response (PER) is indicator of feeding behavior that is generally controlled by the chemical cues from the maxillary palps. The behavioral experiment showed that they detect palatable chemical signals increasing their appetite

in blowfly, *Phormia regina*. *Phormia* usually have detected the appetite odor like mushroom (1-octen-3-ol) by maxillary palps. However, some kinds of newly found peptide have an effect on feeding behavior. Therefore, we compared the effect of two kinds of newly found peptides in *drosophila*. First, we test whether they affect feeding behavior by the amount of sucrose intake. After injection of one kind of peptide, food intake amount was obviously increased. In the next step, we investigated the odor of 1-octen-3-ol by maxillary palps did not work as appetitive odor when they are starved sufficiently in behavioral experiments. The results showed that these peptides work as the appetite-suppressant in blowfly feeding behavior, and it did work as counteractant to odor stimulation.

### #24 Effects of repeated sodium depletion on salt intakes in C57BL/6J mice

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Sodium-deficient animals ingest sodium chloride at concentrations normally rejected, and this motivated behavioral state is known as sodium appetite. For neural mechanisms of sodium appetite, there is a line of electrophysiological evidence in rats, whereas there is less evidence in mice. In order to develop a model for sodium appetite in mice, we examined the effects of repeated sodium depletion on salt intakes in C57BL/6J mice. Adult male C57BL/6J mice (n=5, 8 weeks, 21-26 g at the beginning of the experiment) were used; each animal was housed individually in a metabolism cage during the experiment. A combination of furosemide injection with sodium-deficient diet was used to induce acute sodium deficiency. In 3 of the 5 mice, furosemide was injected in the 1st week and saline was injected in the 2nd week. In the remaining 2 mice, the injection order was reversed. This pattern was repeated 4 times over 2 months. After 24 hours following the injection, intakes of 0.3 M NaCl and distilled water were measured. The furosemide injection shortened the latency of, and increased the amount of, 0.3 M NaCl intake. Furthermore, the increase in 0.3 M NaCl intake was tended to be enhanced at later furosemide injections. Both urine volume and 24-h urinary sodium excretion were greater after the furosemide injection than after the saline injection, but there were no significant differences of these measures across repeated depletions. These results show that present procedure reliably elicits sodium appetite in C57BL/6J mice, suggesting that this is a suitable experimental model of sodium appetite in mice.

### #25 Contribution of learning process on preference of dried-bonito *dashi*

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The sensations of taste and smell are the important determinants guiding preference for foods and fluids. The dried-bonito *dashi* is a traditional Japanese fish broth that enhances palatability of various dishes due to its specific flavor. However, the scientific evidence why dried-bonito *dashi* is important for Japanese dishes is little known. In general, the main stimulus is considered as inosinate (an umami substance) but the *dashi* is a complex mixture of many taste substances such as lactate (sour), histidine (bitter), sodium (salty) and other minerals (generally bitter) as well as macromolecules (proteins and peptides) and over 400 odorants. Here we investigated mechanisms of preference for dried-bonito *dashi* using 48-h two-bottle choice tests in adult male Sprague-Dawley rats. We found that preference for *dashi* was influenced by current nutrition, i.e., ingestion of high fat or high sucrose diet suppressed preference for *dashi*. In addition, past experience/learning of *dashi* ingestion was the most important determinant that could overcome the influences of current nutrition. For example, preference of the threshold concentrations (0.4%) of *dashi* increased gradually during repeated exposure to *dashi* solution. After the repeated ingestion of *dashi* solution, ingestion of high fat or high sucrose diet has no influence on *dashi* preference. In the descending concentration series, the concentration-preference function shifted 100-fold to the lower concentrations compared with the ascending one. These results suggested that postingestive consequences as well as oronasal sensations guide the preference for *dashi* solutions that are under the control of current nutrition and past experience/learning.

### #26 Involvement of histamine H<sub>1</sub> receptor of the central nucleus of amygdala in the retrieval of food aversion learning

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Food aversion learning is the long-term memory established after association of the properties of food, such as texture and taste (conditioned stimulus, CS), with visceral malaise (unconditioned stimulus, US). After the acquisition of the learning, the re-exposure to the CS induces the retrieval of the memory and the rejection of the CS occurs. Previously, we showed that histamine release in the central nucleus of amygdala (CeA) during the re-exposure to the CS was increased in soft pellets-conditioned rats, but not in sweet pellets-conditioned rats. In the present study, we studied the role of the histaminergic transmission in the CeA in the retrieval of food aversion learning induced by two patterns of the CS.

Fos expression, a marker of neuronal activation, following the retrieval of food aversion learning induced by each CS (soft pellets or sweet pellets) and US pairing was examined. Few Fos-positive cells in the CeA were observed by consuming both types of pellets in naïve rats. In the conditioned rats, Fos expression in the CeA was increased in response to the re-exposure to soft pellets while it was not elevated by the re-exposure to sweet pellets. In addition, the microinjection of H<sub>1</sub>-antagonist to the bilateral CeA just before the re-exposure to the CS impaired the aversive response to soft pellets but not to sweet pellets. These findings indicate that the

neuronal activation through the histaminergic system via H<sub>1</sub> receptor is involved in the retrieval of texture aversion learning but not in that of taste aversion learning.

### #27 Food entrained feeding behavior to highly sweet food in non-food deprived mice

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Light-regulated circadian clock in each animal species affects its original feeding rhythm. Nocturnal rodents mainly consume food during the dark phase under ad libitum food condition; however, their daily feeding rhythm is entrained to mealtime when they are restricted of food. Food-entrainment is suggested to be mediated by increased arousal and food-oriented motivation derived from calorie deficiency, but recent studies indicate that taste palatability seems to be important in food-entrainment. However, the role of taste palatability (hedonic aspect of taste) in food-entrainment remains not fully understood. To study the issue, we developed a new, simple mouse model. Ad libitum-fed C57BL/6J male mice received daily limited access to highly sweet chow (HSC) containing 65% sucrose for 2 hours in the light phase (9:00-11:00 a.m.). They received the regimen for 14 days as training. The intake of HSC gradually increased and was about 11-fold greater than their basal intake of normal chow at the corresponding period of time during pre-training phase. On the other hand, the intake of normal chow in another group during the corresponding timing did not increase. Intake of normal chow during the dark phase in mice group receiving HSC, but not normal chow, significantly decreased during the training. The entrained increase of HSC intake was also observed in genetically hyperphagic strain, *db/db* (leptin receptor gene deficiency) mice. These results suggest that taste palatability and/or hedonics of sweetened food play a role in shifting of daily feeding rhythm.

### #28 Sexual difference of gene expression of androgen receptor in the brain region related to extinction memory after conditioned taste aversion learning in immature mice

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Mice acquire the conditioned taste aversion memory (CTAM) by application of the conditioned stimulus (CS) such as a novel taste, sodium saccharin (Sac), followed by the unconditioned stimulus (US) such as an malaise induced by an i.p. injection of LiCl. After conditioned taste aversion (CTA) learning, the extinction of CTAM is induced by repeated presentations of CS without US. Recent studies have demonstrated that the extinction is a process of relearning, where mice acquire the extinction memory of CTA.



We have reported that the sexually mature male mice (C57BL/6) represented significantly higher retention of extinction memory (REM) than sexually immature male mice. In contrast, the significant difference was not observed in female mice. We have also reported that chronic administration of androgen into castrated mice during pre-maturation period results in an enhancement of REM in both sexes. Here we investigated whether the sex difference of the gene expression of androgen receptor (AR) is observed in the ventral medial prefrontal cortex (vmPFC) and amygdala between sexually immature and mature mice by real-time PCR. The sexually immature male mice presented significantly higher level of AR gene in vmPFC and amygdala than the sexually mature male mice. However, there was no such difference in vmPFC and amygdala of the female mice. These results suggest that the sex difference in maturation process of REM is induced by androgen acting on vmPFC and amygdala during sexual pre-maturation period in mice.

### #29 Conditioned flavor preference with different concentrations of sucrose in weanling rats

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Our previous study showed that weanling rats acquired conditioned flavor preference when the flavor was associated with low concentration of sucrose; while flavor aversion when associated with high concentration. The present study aims to confirm and extend this finding with various concentrations of sucrose. Wister male 3-week-old rats were used. Half of the rats received unsweetened grape-flavored water on odd-numbered days and sweetened (sucrose) cherry-flavored solution on even-numbered days. The remaining rats received sweetened grape-flavored solution on odd-numbered days and unsweetened cherry-flavored water on even-numbered days. During the acquisition session, the liquid was presented to each rat for 15 min daily across 6 consecutive days. In the following test session, each rat was presented with unsweetened cherry- and grape-flavored water simultaneously for 15 min daily across 4 consecutive days. The rats showed a significant preference for the flavor associated with 2% or 10% sucrose, but showed a significant aversion to the flavor associated with 30% sucrose, suggesting a hedonic shift from positive to negative with increasing the concentration. The association learning acquired at the age of 3 weeks was preserved when retested at the age of 20 weeks. Short-term (5 min) drinking test showed that the volume of intake was significantly smaller for 30% than for 2% and 20% sucrose, indicating that 30% sucrose is aversive because of its oropharyngeal sensation rather than its post-ingestive effects. The present study suggests that weanling experience of food is important in the formation of feeding behavior in adulthood.

### #30 Age-related changes in taste preference in rats

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Tastes play important roles in detecting nutrients and irritant materials in food. Preference for food tastes is critical for ingestive behavior because greater palatability is to evoke greater food intake. Animals generally prefer sweetness but not bitterness. Such taste preferences are known to be affected by aging which causes changes in the dietary and energy requirements. However, the mechanisms of shift in taste preference by aging still remain unclear. Therefore, to elucidate differences in taste preference among the life stages, we measured the amount of intake of taste solutions by the two bottle test. We used juvenile (3-6 weeks), young-adult (8-11 weeks), adult (17-20 weeks), and middle-aged (34-37 weeks) Sprague-Dawley male rats. All rats were fed ad libitum during all tests. We used 0.1 M or 0.3 M NaCl, 0.01 M or 0.1 M HCl, 0.3 M or 0.5 M sucrose, 5 mM saccharin-Na,  $3 \times 10^{-4}$  M or  $3 \times 10^{-5}$  M quinine-HCl, and 0.1 M monosodium glutamate (MSG). The preference ratio for 0.5 M sucrose and 0.1 M MSG in middle-aged group was lower than that in juvenile and young-adult groups. On the other hand, the preference ratio for  $3 \times 10^{-5}$  M quinine-HCl in middle-aged group was higher than that in juvenile and young-adult groups. There were no significant differences in the preference for HCl and NaCl tastes among groups. These results suggest that aging affects taste preferences for sucrose, MSG and quinine-HCl which represent sweet, umami and bitter tastes, respectively, in humans.

### #31 Effect of Maillard reaction products on umami and sweet taste preference in mice

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We investigated the effects of furanones as the Maillard reaction products flavors on umami and sweet taste preferences in mice. 5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one; sotolon and 4-hydroxy-2,5-dimethyl-3(2H)-furanone; furaneol (Food-Grade Certified Products, SAFC Supply Solutions, USA) were chosen as the Maillard-borne odorants. In the short time two-bottle tests, 0.001, 0.003, 0.03, 0.1  $\mu$ g/kg sotolon flavored umami solutions containing 10 mM monosodium glutamate (MSG) and sweet solutions containing 1 mM saccharin were significantly consumed more than distilled water. Sotolon tended to enhance umami and sweet taste preference in a dose dependant manner. Whereas there were no differences of intakes between sotolon flavored water and distilled water. In addition, furaneol and citral flavor agents did not enhance umami solution preferences. These results suggested that sotolon, one of the Maillard reaction products, induced enhancement of umami and sweet taste preference in mice. Supported by Society for Research on Umami Taste and KAKENHI (22780127).

### #32 Effects of dietary zinc on food intake and insulin secretion in type 2 diabetic GK rats

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Zinc is one of the essential trace elements and its deficiency causes anorexia, growth retardation, and taste disorder. Insulin in pancreatic beta-cells is known to be stored as microcrystals of the zinc-

containing hexamer, and zinc is thought to be fundamentally important for assembly and release of insulin from beta-cells. However, the effect of dietary zinc on food intake or insulin secretion of model rats of diabetes has not been well elucidated. In this study, we followed the levels of food intake and insulin secretion in Type 2 diabetes model GK (Goto-Kakizaki) rats fed on diets containing different levels of dietary zinc for 27 weeks. All the experimental rats were divided into four experimental groups of zinc-deficient (Zn-Def, rats fed on 2.2 mg/kg diet), zinc-sufficient (Zn-Suf, rats fed on 33.7 mg/kg diet), high-zinc (High-Zn, rats fed on 67.4 mg/kg diet), and Pair-Fed (rats fed on zinc-sufficient diet with the same amount of diet consumed by Zn-Def one day later). Food intake in Zn-Def GK rats followed typical cyclic-pattern seen with normal rats such as SD rats. Plasma zinc concentrations and plasma insulin concentrations were found to have positive correlations in High-Zn rats.

### #33 Effect of central orexin on the reflex swallowing of the rat

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Orexins are neuropeptides synthesized mainly in the lateral hypothalamic area and are implicated in the regulation of food intake in addition to arousal. Since swallowing is early step of feeding behavior, orexins might modify swallowing. In the present study, we examined the effects of orexins on the reflex swallowing using anaesthetized rats. Orexin, which was dissolved in Ringer's solution, was administered into the fourth ventricle. Orexin-1 receptor antagonist (SB334867), which was dissolved in dimethyl sulfoxide, was administered into the fourth ventricle. Three  $\mu$ L of drugs were administered using a microsyringe. Swallowing was induced by the electrical stimulation (20 Hz, 20 sec) of the central cut end of the superior laryngeal nerve (SLN) and was monitored by recording the electromyogram of the mylohyoideus muscle. The frequency of swallowing during the electrical stimulation of the SLN decreased after the administration of orexin-A in a dose-dependent manner. Significant reduction in number of swallowing was observed after the administration of orexin-A at the dose of 1 or 3 nmol. The latency of the response tended to be longer in the presence of orexin-A. The administration of orexin-B did not affect the frequency of swallowing. Pre-administration of SB334867 attenuated the magnitude of inhibition of swallowing frequency induced by the administration of orexin-A. These results suggest that orexin-A but not orexin-B inhibits reflex swallowing via type 1 receptors situated in the dorsal medulla where swallowing center is housed. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No: 22592064 to MK).

### #34 Effect of stimulation of the laryngopharynx with unstimulated saliva on voluntary swallowing in humans: involvement of water receptors

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Stimulation of water receptors in the laryngopharynx (LP) with water shortened the swallowing interval in voluntary swallowing in humans. We previously found that excitation of water receptors was due to absence or reduced concentration of Cl<sup>-</sup> in fluids applied to the LP. Unstimulated saliva contains a low concentration of Cl<sup>-</sup>. Thus, unstimulated saliva may excite water receptors in humans. The aim of the present study was to evaluate the role of unstimulated saliva in initiation of swallowing in humans. Solutions were infused through a fine tube into the LP at a slow infusion rate (0.2 mL/min) that minimized the mechanical effect of infusion. Facilitation of voluntary swallowing by infusion of unstimulated saliva was almost the same as that by infusion of water. Topical anesthesia of the throat produced loss of facilitation of voluntary swallowing by both water infusion and unstimulated saliva infusion. The similar effects of water and unstimulated saliva on voluntary swallowing suggest that water receptors are responsible for facilitation of voluntary swallowing by unstimulated saliva. Since facilitation by water infusion appeared without mechanical effect of infusion, it is likely that excitation of water receptors in the LP by a small amount of unstimulated saliva precedes excitation of mechanoreceptors caused by a certain amount of unstimulated saliva accumulated in the throat. Excitation of water receptors by unstimulated saliva may initiate spontaneous swallowing and spontaneous swallowing probably plays an important role in clearance of the mouth of saliva. Therefore, it appears that excitation of water receptors by unstimulated saliva may contribute to avoidance of aspiration.

### #35 Peripheral chemical stimulation to trigger the swallowing reflex and its neuronal mechanisms in humans

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Initiation of swallowing is mediated by the swallowing central pattern generator (CPG) in the brain stem. Both peripheral and central inputs can activate the CPG. Previous studies demonstrated that the ability to initiate repetitive voluntary swallowing varies even among healthy humans and proposed that appropriate chemosensory inputs, e.g., activation of water receptors in the pharynx and larynx, may compensate for difficulty in initiation of swallowing. However, whether variation also exists in reflexively evoked swallowing is unclear, and the nature of interindividual variation has not been clarified. The aim of this study was to investigate interindividual variation of swallowing initiated by central and peripheral inputs and to clarify the neuronal mechanisms that cause this variation. Voluntary and reflexive swallowing activity was recorded during infusion of fluid (distilled water [DW] or 0.3 M NaCl solution) into

the pharynx and larynx at a very low rate in 20 healthy volunteers. DW and NaCl were used to activate and inhibit the water receptors, the activation of which facilitates initiation of swallowing. Swallowing intervals (SIs) were measured between the fourth and ninth swallows, and facilitatory effects (FEs) were calculated by subtraction of the SI of DW from that of NaCl. There was a significant positive correlation between SIs of voluntary and reflexive swallowing with NaCl. FEs of both voluntary and reflexive swallowing appeared strongly in subjects with longer SIs. The present results suggest that interindividual variation in the ability to initiate swallowing depends on the potency of the swallowing CPG.

### #36 The effects of various taste stimuli on prefrontal cortex blood flow in humans: a near-infrared spectroscopy study

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The effects of taste stimuli on the change in regional prefrontal cortex blood flow (rCBF) measured by multichannel near infrared spectroscopy (NIRS) were evaluated in 10 healthy women volunteers. The test solutions were sucrose, NaCl, citric acid and monosodium glutamate at three different concentrations each. Six pairs of source and detector NIRS probes were placed on the forehead with a 3 cm separation which allowed the measurement of 16 different channels every 0.65 sec. The changes in oxygenated hemoglobin (oxy-Hb), which is the most sensitive indicator of changes in rCBF, were analyzed. Subjects were positioned in a chair that supported their back and head while wearing a blindfold (eyemask) and headphones to minimize motion, visual and auditory disturbances. The entire oral area was exposed to 5 ml of solution for 15 sec applied using a syringe and rubber tube positioned above the head. Another solution was applied after rinsing with deionized water, subjects were tested consecutively for 12 different solutions in random order. Each subject was tested for the same solution three times, the data were averaged and the value for the water test data subtracted in order to determine subject's value for a given solution. The taste hedonic tone with a five-rating scale was also measured for the preference test. Oxy-Hb in all channels increased during all taste stimuli. The increases in oxy-Hb and preference scale values showed significant negative correlation. These findings indicate that the prefrontal cortex activation evaluated by NIRS is possibly related to pleasant or unpleasant emotions associated with taste stimuli.

### #37 The intake effect of cooling / warming chemicals for human central nervous system

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We receive various different stimulus modalities including 5 tastants and other gustatory and / or somatosensory sensations in

eating. Sensory information from these modalities would be integrated and recognized as a 'Flavor'. It is useful to understand their psychophysiological properties because it may help us to develop new functional foods. We investigated the effects of vanillyl butyl ether (VBE), l-menthol (LM) and carbonated water (CW) to the arousal level of 12 healthy subjects. These 3 stimuli were used as hot, cold, tingling stimulus respectively. The results of Contingent Negative Variation showed that VBE, LM and CW increased subject's arousal level just after ingested by aqueous solution whereas no significant change was observed after the water ingestion. The increase of the arousal level still remained for 20 min after ingesting VBE and LM. On the other hand, the arousal level of 20 min after ingesting CW decreased to the same level as before ingestion. Apart of the subjects were asked to rate the general stimulus intensity by 9 point likert scale. All samples including water showed maximum intensity just after ingestion and decreased with time course. However, the significant increase of the stimulus intensity still remained for 20 min after ingesting 3 stimulus solutions. These discrepancies between the arousal level and subjective stimulus intensity of CW suggest that only stimulus intensity itself cannot explain the change of the arousal level. In order to understand the property of each stimulus, we need to look each stimulus into detail in the derived modality of the sensation, intensity, timing of appearance.

### #38 The effect of umami taste on saliva secretion – The effect of repetitive taste stimulation

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We had reported that umami taste induced sustained saliva secretion. In this study, to investigate the effect of repetitive taste stimulation on saliva secretion, we compared the saliva weight secreted by single taste stimulation (Single Stimulation methods; SS) with repetitive taste stimulation (Repetitive Stimulation method; RS), using fourteen healthy adult subjects. In SS, the subjects sprayed the taste solution (approx. 0.25 ml) using a spray bottle into the mouth, and held for 30 seconds and spat the whole saliva into cups at every 30 second for 10 minutes. In RS, the subjects sprayed the taste solution into the mouth, and held for 30 seconds, then, spat the whole saliva into cup, and rested for 1 minute until the next stimulation. After this procedure was repeated ten times, the subject performed the same procedure as SS. The stimuli were 100 mM monosodium glutamate (MSG) (umami), 3.8 mM citric acid (sour) and 440 mM xylitol (sweet), which had 'moderate' taste intensity on a labeled magnitude scale. During the repetitive stimulation in the RS of MSG, the salivary flow was reduced gradually according to the decrease of umami taste intensity by adaptation. However, the MSG stimulation caused a sustained salivary secretion after repetitive stimulation, and there was no significant difference in the 10 minutes saliva between SS and RS. Thus, the repetitive umami stimulation did not reduce saliva secretion and could produce sustained salivary secretion. There was no adaptation in RS of citric acid and xylitol, and the saliva secretion pattern of RS was similar to that of SS, with less saliva secretion than MSG.

### #39 Effects of dried-bonito *dashi* (a traditional Japanese fish stock) on gastric myoelectrical activity and satiety in humans

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Dried-bonito (DB) *dashi* is a traditional Japanese broth that enhances palatability of various cuisines due to its specific flavor. DB *dashi* contains many components, such as histidine, lactate, inosinate, minerals, and proteins. Ingestion of DB *dashi* has been reported to have various physiological functions, e.g., effectiveness against physical and mental fatigues, enhancement of peripheral blood flow, and suppression of oxidative stress. In this study, we investigated the effects of DB *dashi* on gastric electrical activity and hunger-satiety states in healthy Japanese males. We performed a randomized crossover study for 19 healthy males (21.5 ± 0.5 yr). Subjects were fasted for at least 10 h, and all measurements were performed in the morning. Before and just after a single ingestion of 150 ml of DB *dashi* (“*Hondzukurī Ichiban-dashi Katsuo*”, Ajinomoto Co., Inc., Japan) or energy adjusted water as a control, gastric myoelectrical activity was measured by electrogastrography for 20 min. Subjective motivation to eat (hunger and satiety) was rated by visual analogue scales before, immediately after, and 35 min after the ingestion. Ingestion of DB *dashi* significantly increased both electrogastrographic normogastric power and percentage in postprandial state. In addition, DB *dashi* produced higher satiety sensations at 35 min after the ingestion, compared to the control diet. In conclusion, DB *dashi* intake enhances the gastric motility and satiety in humans.

### #40 Influences of physical surroundings on feelings of deliciousness during having sweet foods: an EEG frequency analysis study

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We usually take sweet foods with drinking tea or coffee not only to increase nutrition but also to produce feelings of pleasantness and restfulness that belong category of emotion. When we take sweet foods, comfortable audio-visual environments, e.g. consonant interior decoration, good music and fine view may increase feelings of deliciousness. Thus, it is hypothesized that environmental factors modify feelings of deliciousness. Comprehensive delicious feelings during having sweet foods might be effectively produced in the comfortable environments by integration of various palatable sensory perceptions. Electroencephalograms (EEGs) were recorded from frontal region of the scalp of healthy participants under virtual scenes of tearoom and construction work, respectively. The participants were asked to rate deliciousness after the recordings. Frequency analyses were performed from the EEGs. During having the foods, occupancy rates of beta frequency band between tearoom scenes and construction work scenes were markedly different, but

not in other frequency bands. During having no food, in contrast, there was no difference of occupancy rates in respective frequency bands between the two different scenes. With regard to deliciousness during having sweet foods, all participants rated high scores under the scenes of tearoom than those under the scenes of construction work. Interestingly, there is a positive correlation between occupancy rates of beta frequency band and scores of deliciousness. These findings suggest that comfortable audio-visual environments play an important role for increasing delicious feelings during having sweet foods, in which beta frequency rhythms may be concerned with producing comprehensive feelings of deliciousness.

### #41 Relationship between dietary habit and taste sensitivity: Comparison of thresholds for 5 basic tastes and pungent taste between Thai and Japanese

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Though it has been reported that capsaicin possesses effects of stomach protection and appetite improvement, the influence of dietary habits of consuming pungent substance such as capsaicin on taste sensitivity has not been investigated. In order to elucidate the influence of dietary intake of capsaicin on taste and pungent sensitivities, we measured the thresholds for 5 basic tastes and capsaicin in Thai and Japanese, because Thai have a dietary habit of much more use of pungent substances for meal than Japanese. In addition, we conducted questionnaire study about dietary habits of both groups.

Regarding the recognition thresholds for 5 basic tastes, the threshold for umami tended to be higher in Thai compared to Japanese with no differences in other basic tastes. This result indicates that daily high intake of pungent substances hardly affect taste sensitivities. However, the threshold for capsaicin in Thai was significantly higher than Japanese, which was considered to result from much more use of hot pepper for meal in Thai. When the thresholds for 5 basic tastes were measured immediately after capsaicin stimulation, the thresholds for acid and bitter tastes were elevated in Thai whereas they did not change in Japanese. This difference may be due to the concentration of pretreatment capsaicin because suprathreshold concentration was used in the experiment, showing that high concentration of capsaicin may cause transient impairment in some taste sensitivities.

In conclusion, it was suggested that daily high consumption of pungent substances hardly affects taste sensitivities but high concentration of capsaicin causes transient hyposensitivity of some tastes.

### #42 Flavors which show interaction with umami taste

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This study aimed to investigate what flavors show interaction with Umami taste. Twenty-one female university students participated in

this study. Solutions of 0.25 % MSG were used as taste stimuli, and the 19 kinds of flavors were used as flavor stimuli. Participants were asked to taste a pair of flavor and MSG solution, and to evaluate the intensity of umami taste and the congruency of the flavor with the umami. When the participants taste MSG solution with flavor of dried bonito, and of garlic, they evaluated the umami taste stronger than when they taste it without flavors (potentiation effect). When the participants taste MSG solution with flavor of tomato, of garlic, and of onion, they evaluated those flavors more congruent with umami taste than when they taste it without flavors. They evaluated congruency of dried bonito and of matsutake less congruent with umami taste. There is a significant, but weak, interaction between the congruency with umami taste and the potentiation effect of umami taste ( $r=0.36$ ,  $p<0.05$ ). In general, the flavors that are experienced in our daily lives are evaluated more congruent with umami taste, and have potentiation effect on umami taste perception. Preceding studies also suggest that the interaction between olfaction and taste, and perception of umami taste are based on learning. This study also supports the view of "learned synesthesia" of olfaction and taste.

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#### #43 Seeing a person eating a food facilitate the appetite for the food

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Eating with someone makes the foods more palatable, and the family ties more strong. On the other hands, eating alone (KO-SHO-KU, in Japanese) has many problems, such as malnutrition, unbalanced meals, not good at communication etc. KO-SHO-KU is very severe problems in modern Japanese society. Thus, we aimed to study the effect of KO-SHO-KU on palatability of the foods.

In the study 1, ratings for taste and palatability of confectioneries were compared between when they were eaten with friends and/or family members and when eaten alone. Thirty six female university students participated in this study, and were asked to eat two kinds of chocolate and two kinds of cookies in a laboratory (alone) and in their home (with someone). Palatability ratings were higher when they were eaten with someone in their home.

In the study 2, ratings for taste and palatability of jellies were compared between when they were eaten with watching a child eating same jellies in a video and without watching it. Fifteen female university students participated in this study, and were asked to eat three kinds of jellies in a laboratory and to rate a questionnaire about their ability of sympathy. Students with high sympathy tend to rate the jellies more palatable.

These results suggested that human perceptions of taste and food palatability were affected not only by foods but also by contextual and social variables.

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#### #44 Taste and smell of the bread made using pre-germinated brown rice sake lees

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Pre-germinated brown rice is prepared by soaking brown rice in water at 37°C for 24-48 hours to initiate germination of sprouts not exceeding 0.5-1.0mm in length. In the traditional method of brewing sake, rice bran and embryo bud are separated from brewer's brown rice during sake brewing in order to remove the rough taste. This study tried to use sake lees in fermentation with pre-germinated brown rice, since the sake made from pre-germinated brown rice has hardly appeared on the market. Also, the use and benefit of pre-germinated brown rice-made sake and sake lees have not been reported. In this study, we made sensory evaluation to measure the difference in taste and smell between bread made using normal sake lees and bread made using pre-germinated brown rice sake lees.

The result showed that the bread made with pre-germinated brown rice sake lees had significantly better smell ( $p = 0.0365$ ) and stronger sake lees smell ( $p = 0.0026$ ) as well as a stronger sake less taste ( $p < 0.0001$ ) than the bread made with normal sake lees. The complex flavor ( $p = 0.0587$ ) and saltiness ( $p = 0.0511$ ) of the bread made with pre-germinated brown rice sake lees tended to be stronger than that of the made with normal sake lees.

#### #45 Quality evaluation of steamed and cured slices of sweet potato by female college students

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Female college students' preference to steamed and cured slices of sweet potato was evaluated to find their sensory characteristics. Evaluation was carried out with the 10 sweet potato cultivars and lines, Tamayutaka, Izumi13, Hoshikirari, Kanto131, Tamaotome, Quick Sweet, Hitachi Red, Hamakomachi, Purple Sweet Lord and Sakukei20 in terms of appearance, fragrance, texture and taste by a rank-scoring method. The most preferable cultivars were Hoshikirari, Tamaotome, and Kanto131; sweetness greatly contributed to the taste of the steamed and cured slices of these samples, while appearance and texture somehow influenced their taste scores.

#### #46 Method of extracting dashi for taste improvement and cooking energy saving

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Fish flakes are one of the traditional Japanese ingredients for dashi. Various methods are used to extract dashi depending on the thickness of flake. Especially, the thick type flake that is often used for Soba noodle sauce requires around 30 minutes of cooking time and extracting conditions vary as well. The energy saving has become recent tendency even in culinary field, and we focused on a cooking method using remaining heat. We analyzed the free amino acid and 5'-IMP. Sensory evaluation was carried out at the same time. As a result, we can suggest that there is a method for extracting the most effective use of remaining heat for 25 minutes after heating for 5 minutes. We were able to explain the taste improvement from the amino acid composition determined by AccQ-Tag™ Ultra method. This condition can be considered effective for both energy saving and flavor improvement of dashi.

#### #47 Oolong tea components contributing to reducing the residual oily sensation in the oral cavity

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Oolong tea is usually served with oil-rich Chinese food. Our group has been studying on the mechanism of oil rinsing in the mouth and, in particular, on the interactions between oolong tea components and oil. Our previous studies showed that oolong tea had a higher affinity for oil than deionized water, mineral water and green tea did. It may thus be more effective in washing out remaining oil from the mouth. The objective of this study is to determine the effective oolong tea components contributing to removing the residual oily sensation. For the human sensory evaluation, sweetened whipped cream was used as an aliment, and mineral water or oolong tea was used as a washer, with the result that oolong tea more effectively reduced the sense of residual oiliness. An aqueous solution of Epigallocatechin gallate (EGCG) and epigallocatechin (EGC) as tea constituents was used to measure its interfacial tension activity in the presence of soybean oil by a face surface tensiometer. Also, we emulsified soybean oil with EGCG or EGC and examined the resulting average particle size with a Laser Diffraction Particle Size Analyzer. It was found that EGCG and EGC did not influence the interfacial tension and average particle size. Further studies are required to find some contributory factor.

#### #48 Influence of physical changes in sucrose crystal on the taste of prepared caramel sauce

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Commercial granulated sugars are of 99.9% purity which is almost equivalent to the purity of a guaranteed reagent-grade sucrose. We observed that commercial sugars were different in melting point from one another and the tastes of caramel sauces prepared from these sugars were different.

A white coarse bright sugar with larger grain size than that of regular granulated sugar used as an experimental sucrose crystal was milled into powder. In a differential scanning calorimetry of the crystals, a higher peak in the curve of endothermic heat flow shifted to higher temperature side after milling. The powder crystal became more stabilized on heating than the coarse crystal. Using the coarse and the powder crystals, caramel sauces ("sauce C" and "sauce P", respectively) were prepared. Sensory tests showed that sauce C was more brownish in color and was stronger in sourness, bitterness and acidity. On the other hand, sauce P was better in aroma, stronger in sweetness and more preferable in taste and aftertaste. Sauce C was lower in pH, giving stronger sourness. The use of a color difference meter showed a large difference between the two sauces ( $\Delta E=6.4$ ). Coarse crystal was less stable on heating and sauce C was more brownish in color, having stronger bitterness.

#### #49 Effect of flavor presentation method on gustatory and flavor evaluation in human

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In the sensory studies for flavor development, commercial products or trial products are often used as samples, because sensory evaluation is conducted considering usual behavior of eating habit. But in the case of odor-taste interaction studies, it is desired that odorants and tastants are completely separated and presented to subjects simultaneously. In this study, we established a simple and effective way of presenting odor and taste stimuli separately under two model systems: beverage and soup.

Experiment.1: Evaluation of sweet/sour intensity in fruit flavor beverage

Firstly, we prepared simple equipment comprising clear plastic cup with scented papers attached on the upper inside edge. The use of non-flavored solution and flavor on the scented papers allow subjects to evaluate beverage. Sugar-acid water solution was used as taste stimulus and, Lemon and Strawberry flavors were used as odor stimuli. Untrained panel (n = 45) evaluated intensity of sweet and sour characters. The result showed that Lemon flavor enhanced sour character and decreased sweet character when compared with non-flavored condition.

Experiment.2: Evaluation for savory - citrus flavor interaction

We prepared ordinary soup solution as taste stimulus, with two savory flavor (Chicken and Dried bonito) and two citrus flavor (Lemon and Yuzu) as odor stimuli. Untrained panel (n=66) evaluated 6 characters (saltiness, umami, sourness, mild, fatty, animalic). The results showed flavor influenced taste intensity as is the case in experiment 1. In conclusion, we successfully showed the important effect of odor on taste perception by using this study.

#### #50 Added flavorings enhance salivary hemodynamic responses to tastes detected by near-infrared spectroscopy -Flavor creation using optical imaging-

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Dried-bonito (Katsuobushi) broth, an important seasoning in Japanese cuisine, has long been used to reinforce the flavor of appetitive foods. Not only the taste but also the aroma components of the broth contribute well to the enhancement. To elucidate the effects of aroma from dried-bonito on broth tastes caused by the central integration of flavor, we performed optical imaging of salivary hemodynamic responses using near-infrared spectroscopy (NIRS). When hemodynamic responses to a reconstituted dried-bonito flavored broth were compared to those of odorless broth taste solutions, the flavored broth produced a significantly larger response than the odorless broth. This was the case for five of the ten participants, who felt that the combination of the aroma with the tastes was congruent. In the remaining five who felt the combination incongruent, the flavored broth did not cause the enhancement of responses. Moreover, when (Z,Z)-4,7-Tridecadienal, one of the most important key aroma components of dried-bonito extracts, was added to the flavoring, the latter five as well as the former five participants came to feel congruency of the reconstructed aroma with broth tastes. The reconstructed flavored broth produced significantly larger hemodynamic responses than the odorless broth in both participants' groups. Accordingly, there is a positive correlation between the degree of congruency and increase of the salivary responses. These results indicate that NIRS offers a sensitive method to detect the effect of flavoring on the taste-related salivary hemodynamic responses, dependent on the perceptual experience of the combination of aromas and tastes.

### #51 Difference in brain responses between aroma of mints and its flavor

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Minty flavors are utilized in foods, beverages, toothpastes and tobacco. Minty flavors are considered to have a relaxing effect, which is necessary for us, living in modern and urban societies. The authors have reported that the participants evaluated themselves more relaxing after smelling minty odor, when they were tied out for solving hard puzzles. However, psychological and physiological effects of minty flavors are still unclear. This study was aimed to reveal brain responses to minty flavors and minty odors with NIRS (near infrared spectroscopy).

Sixty female university students participated in this study. They received detailed explanation about the experiment and wrote consent for participating in this study. The three minty stimuli, menthol, spearmint and peppermint and the other three odor stimuli, rose, skatol and orange, were administered to the participants via the olfactometer. The participants were asked to smell the stimuli by an orthonasal or retronasal route or to taste them. Brain responses were measured with NIRS (BOM-1, Omegawave, CO LTD) and recorded with AD converter (Powerlab) connected to the iMac. The participants were also asked to evaluate the intensity

of the odor continuously with the apparatus which outputs 0~5 V being based on the participants' evaluations.

The results showed that the stimuli were recognized faster and stronger when the stimuli were administered via orthonasal and retronasal routes than via oral cavity. The rose odor and orange odor were recognized faster and stronger than the menthol odor. The right forebrain showed greater responses to the stimuli via an orthonasal route than via the other routes, and greater responses to the rose odor than the menthol and the peppermint odors.

### #52 Development of odorant sensor elements using insect cells expressing insect odorant receptors

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Currently existing odorant sensors are mainly fabricated based on metal-oxide semiconductor devices, conducting polymers, quartz crystal microbalances, or surface acoustic wave detectors. These sensors have been studied for improving on various parameters, such as sensitivity, selectivity, response speed, and portability. However, it has been difficult to develop odorant sensor elements that incorporate a combination of desirable properties. In contrast, living organisms, especially insects, use numerous olfactory sensory cells, which express odorant receptors, to sensitively detect environmental odorants in real time. So far, we have developed a compact odorant sensor that consists of a multichannel fluidic device and *Xenopus laevis* oocytes expressing insect odorant receptors. Although this odorant sensor possessed fast responsiveness and good portability, it had some technical disadvantages in terms of the stability of response measurements in the oocytes. Here, we report the development of new odorant sensor elements that enable us to acquire stable odorant response measurements. We introduced insect odorant receptors and the olfactory receptor co-receptor (Orco) as well as a calcium indicator protein, GCaMP3, into *Spodoptera frugiperda* Sf21 cells to construct sensor cell lines. When these cells were stimulated with a set of odorants in solution, intracellular calcium as monitored by fluorescence imaging showed sensitive responses in accordance to the ligand specificity of the expressed odorant receptors. These results show that our sensor cells can be applied as odorant sensor elements that detect various kinds of odorants with high sensitivity, selectivity, and stability.

### #53 Analysis and estimation of sensory score using taste and odor sensors

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With promotion of globalization, our diet are changing in several directions (diversification, mass commercialization, or high quality). Because of the change, objective evaluation techniques for taste or odor of foods and beverages are needed for effective R&D and strict quality control. In recent years, biomimetic sensors, such as taste sensor, have been more developed as practical objective

evaluation tools for taste or odor. With development of these sensors, development of correlativity evaluation system between objective values (sensor outputs) and subjective values (sensory scores) is needed to realize objective quality evaluation of foods and beverages. Mainly developing artificial-lipids-based taste sensors with global selectivity, our research group have studied for realization of 'taste-odor fusion biosensor system,' which estimates quality (deliciousness and safety) of foods or beverages using several sensor outputs through analysis and evaluation of subjective-objective relation. In the studies, we have estimated relations between each taste sensory and each sensor output, but did not investigate about correlativity among quality of foods or beverages and sensor outputs. Hence, in this study, we investigated correlativity among sensory of deliciousness and several sensors' outputs on a particular sample as the next step of realizing 'taste-odor fusion biosensor system.' As a result, we found that sensory scores of deliciousness were able to be displayed on a superspace as a hyperplane and to be estimated by the hyperplane and sensor outputs. This means that a sensory score on the particular sample can be calculated using sensor outputs and an estimation equation indicating the hyperplane.

#### #54 Taste disorder in patients with systemic diseases

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It has been reported that taste disorder caused by systemic diseases is associated with zinc deficiency. This study included 101 patients with dysgeusia due to systemic diseases: 30 patients with diabetes mellitus (DM), 21 with renal failures, 19 with digestive diseases, 21 with digestive diseases post operation, and 8 with liver failure. Taste function was tested by electrogustometry (EGM) and filter paper disk method (FPD method) in the area controlled by the chorda tympani nerve. The severe grades of taste threshold before treatment were more than 60% for renal and liver failure patients. All patients were treated with zinc sulfate. The rates of serum zinc deficiency (< 80 µg/dl) were more than 80% for all systemic diseases. In patients with renal failure, the symptom improvement rate was the highest, and the curative period was shortest. However, in DM patients, the improvement rate was the lowest, and the curative period was longest. This study demonstrated that zinc deficiency is induced by systemic diseases, and might cause taste disorder. It is also necessary to consider the possibility of taste disorder caused by medication.

#### #55 Clinical investigation of burning mouth syndrome

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Recent studies have reported on taste function and the amount of saliva in patients with burning mouth syndrome

(BMS). However, the results of these studies have not reached a consensus.

This study analyzed 124 patients with BMS. The threshold of the taste function was measured by electrogustometry (EGM) and the filter-paper disk (FPD) method. Both basal and stimulated salivary secretions were measured. In addition, the self-rating depression scale (SDS) was assessed. Serum zinc and copper levels were also measured. The EGM threshold was more than 10 dB in 80 (67.2%) of 119 patients, and the taste threshold measured by the FPD method was more than 4 points in 61 (51.3%) of 119 patients. Basal and stimulated salivary secretions were less than 3 ml in 90 (78.3%) of 115 patients and less than 10 ml in 52 (45.2%) of 115 patients, respectively. Based on the SDS, 45 (56.2%) and 14 (17.5%) of 80 patients were diagnosed with neurosis and depression, respectively. Blood tests showed that the serum levels of zinc were less than 70 or that of copper were more than 130 µg/dL, respectively, in 61 (54.5%) of 112 patients. The clinical outcome was cure in 38.1%, improvement in 13.6%, recurrence in 11.9%, unchanged in 11.0%, and dropped out in 16.9%.

In this study, basal salivary secretions lower than the normal range compared with stimulated salivary secretions. This suggests that, even if the salivary secretory function is maintained to some extent, the sympathetic nerve activity is predominant due to tension. It is necessary to accumulate more clinical data, such as trigeminal sensitivity of the tongue in comparison with the electric taste threshold.

#### #56 Analysis of taste function and intraoral environment in postoperative oral tumor patients

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Postoperative oral tumor patients show a variety of conditions and often display taste dysfunction resulting from anti-cancer drugs, irradiation and defects of oral tissue. Prosthetic rehabilitation should be required for the recovery of oral functional disorders. However, no reports have investigated taste disorder for patients wearing maxillofacial prostheses.

The aim of this study was to clarify taste sensation in postoperative oral tumor patients using the filter-paper disc method, salivary secretions measured by gum chewing, serum zinc concentrations by blood tests.

We investigated patients who had undergone prosthodontic treatment in the Department of Prosthodontics and Oral Rehabilitation at Iwate Medical University Dental Center. Results were as follows: 1) taste disorder was seen in 90% of cases; 2) within 2 years after radiation exposure, the degree of taste disorder was severe; 3) disease severity did not necessarily accord with awareness of taste disorder, and some patients with serious taste decline were unaware of their taste disorder; and 4) patients who were aware of their taste decline also showed declines in chewing score. Objective inspection and liaison with other departments to include an otolaryngologist, doctor of psychosomatic medicine, and a dietitian is important.



## #57 Fundamental Evaluation of Immunochromatography as a New Rapid Diagnostic Method for Detection of Zinc-binding Protein (CA VI) in Parotid Saliva

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It is known that more than 70% of patients with taste dysfunction have zinc deficiency. Carbonic anhydrase (CA) VI, also known as gustin, is a 37,000-Da zinc metallo-protein found in human saliva and is involved in taste dysfunction. It can be detected by enzyme-linked immunosorbent assay, but the test takes time. Therefore, in this study, we developed a new quick and simple method of diagnosis using an immunochromatography assay to be used as an on-site test. First, reactivity with a serum antibody (prepared using a synthetic peptide designed from a 93-111 amino acid chain of human CA VI as a hapten conjugated to keyhole limpet hemocyanin and injected into two New Zealand white rabbits) was evaluated and compared to a control (swine anti-rabbit IgG) using a Hi-flow plus120 membrane and detected with colloidal gold. A difference in color development was observed between samples of PBS (-) and the positive control (synthetic peptide). Furthermore, it was density dependent, and in serially diluted parotid gland saliva, the color development was seen to be 640 times dilution density from 8 times dilution density. The results suggest that an immunochromatography assay using this novel antibody can be used to quantitatively measure CA VI, which may be useful in the diagnosis of taste dysfunction caused by zinc deficiency.

## #58 The epithelium of recessus olfactorius is a part of the accessory olfactory system in the Japanese toad (*Bufo japonicus*)

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The recessus olfactorius (RO) is a small pouch observed in the antero-lateral olfactory epithelium of anurans. RO is mostly covered with the epithelial cells with cilia, which is reminiscent of sensory function. However, if RO is a part of the main olfactory system has not been studied. We studied the epithelium of RO (RO epithelium) in Japanese toads regarding central projection, ultrastructure and expression of proteins involved in olfactory transduction. Fluorescent carbocyanine dye (diI) was placed on the RO epithelium to show a neuronal projection from RO. Fluorescent signal was not detected in the main olfactory bulb, but in the accessory olfactory bulb. In contrast to the olfactory epithelium which does not contain microvillous receptor cells, the RO epithelium contained both ciliated cells and micro-

villous cells. The expression of  $\alpha$ -subunit of G-proteins ( $G_{\alpha_{olf}}$  and  $G_{\alpha_o}$ ) in the RO epithelium was examined by immunohistochemistry and *in situ* hybridization. Regionally different expression of  $G_{\alpha_{olf}}$  and  $G_{\alpha_o}$  was detected in the RO epithelium, namely expression of  $G_{\alpha_{olf}}$  in the apical cell layer and  $G_{\alpha_o}$  in the basal cell layer. Such a pattern of G-protein expression in the RO epithelium was similar to that in the vomeronasal epithelium. These results suggest that the RO epithelium belongs to the accessory olfactory system. However, the RO epithelium contained secretory cells with granules that were not contained in the vomeronasal epithelium. Physiological experiments will further clarify if the RO epithelium is functionally similar to the vomeronasal epithelium.

## #59 Effects of a protein synthesis inhibitor on long-term potentiation at synapses in the mouse accessory olfactory bulb

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In mice, the female memorizes some urinary pheromones of the mating male, thereby preventing the pheromones from inducing pregnancy block. The memory depends on the neural changes in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system. Microcircuits in the AOB include the prominent reciprocal dendrodendritic synapse between mitral cell projection neurons and granule cell interneurons. A previous study has shown that *in vivo* infusion of a protein synthesis inhibitor, anisomycin, in the AOB is able to prevent the pheromonal memory formation. Other reports have indicated that antidromical stimulation of mitral cell axons induces long-term potentiation (LTP) at the mitral-to-granule cell synapse in slice preparations of the AOB. Using AOB slices, we measured field EPSPs derived from granule cells to examine the effects of protein synthesis inhibition on LTP at the mitral-to-granule cell synapse. High frequency stimulation, consisting of a 100 Hz, 100 pulse train applied four times at 3 min intervals, induced LTP lasting for 180 min. Under bath application of anisomycin (20  $\mu$ M), high frequency stimulation induced short-term potentiation and early-phase LTP, but failed to induce late-phase LTP. The results suggest that protein synthesis underlies late-phase LTP at the mitral-to-granule cell synapse in the AOB.

## #60 A mechanism of action of group II metabotropic glutamate receptors on dendrodendritic inhibition in the mouse accessory olfactory bulb

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The goal of our research is to understand the mechanism of synaptic transmission in the AOB that has been demonstrated to be critical to memory formation for male mouse pheromones. By measuring the reciprocal synaptic currents from mitral cells in the AOB, we have demonstrated that an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, suppressed dendrodendritic inhibition (DDI) in a reversible manner while the mGluR2/mGluR3

antagonist LY341495 enhanced it. The effects of these drugs were markedly impaired by genetic ablation of mGluR2, indicating that DCG-IV-mediated suppression of DDI is mediated by mGluR2.

In these studies, glutamate release was triggered by an application of a voltage step from  $-70$  to  $0$  mV. In the present study, to see whether mGluR2 has similar effects on the DDI elicited by more physiological stimuli, IPSPs triggered by spike trains in mitral cells in slice preparations were recorded using the patch-clamp technique in whole-cell configuration. AOB slices were prepared from 23- to 36-day-old Balb/c mice. LY341495 enhanced it. Together with previous results, the present result suggests that mGluR2/mGluR3 can be activated by endogenous glutamate release from mitral cells, which results in the suppression of the synaptic transmission from mitral to granule cells in the AOB.

### #61 Effects of olfactory stimulation with phenols on $\beta$ -endorphin levels in rat hypothalamus

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Phenols are predominant in the total flavor compounds of dried bonito (Katsuobushi). The influence of odors of 1,3,5-trimethoxybenzene (TM; M=168), 2,6-dimethoxyphenol (DT; M=154), and guaiacol (GU; M=124), which were the chemicals responsible for the Katsuobushi aroma, on the production of  $\beta$ -endorphin (BE) in the hypothalamus were examined in F344 male rats. Rats (5 weeks of age) were exposed to 0.1% of odors dissolved in either ethyl alcohol (EtOH; M=46) or triethyl citrate (TEC; M=276) for 10h/day for one or two weeks. BE levels in water soluble extracts of hypothalamus were examined by ELISA kits. Among three phenols, TM is the most potent odor to increase BE levels in rat hypothalamus, followed by DT and GU: the increasing rate as compared with the control was 170%, 160% and 140%, respectively. These phenols may activate glomeruli clusterd in the lateral domain of the dorsal surface of the mammalian olfactory bulb and probably suppress the activities of sympathetic nervous system. These results suggest that the long-term exposure to bonito bouillon-flavored diet enhanced rat's preference for bonito bouillon.

### #62 Reduction of neurogenesis in subventricular zone and olfactory functions by impaired mastication

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Neurogenesis occurs in the forebrain subventricular zone (SVZ) throughout life. These neurons migrate to the main olfactory bulb (MOB) via the rostral migratory stream (RMS), and upon reaching the MOB they differentiate into granule cells and periglomerular

cells. The information broadcast by general odorants is received by the olfactory sensory neurons and transmitted to the MOB. Newly generated neurons at the SVZ play important roles in odor discrimination and odor memory. Recent studies have shown that reduction of mastication impairs neurogenesis at the hippocampus and brain functions. In the present study, bromodeoxyuridine-immunoreactive (BrdU-ir) structures in the sagittal section of the SVZ, RMS, MOB and accessory olfactory bulb (AOB) of female adult mice fed a soft diet were studied to explore the effects of reduction of mastication on newly generated neurons at the SVZ and MOB. The numbers of BrdU-ir cells in the SVZ of adult mice fed a soft diet for 1, 3, or 6 months were lower than those of mice fed a hard diet. The odor preferences of individual female mice to butyric acid were tested in a Plexiglas Y-maze preference apparatus. Feeding the soft-diet affected the adverse responses of mice to butyric acid. The results suggest that feeding with a soft-diet reduces neurogenesis at the SVZ, which in turn reduces olfactory function at the MOB.

### #63 Facilitatory Effect of Histone Deacetylase Inhibition on Olfactory Aversive Learning in Young Rats

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Epigenetic mechanisms play an important role in memory formation and synaptic plasticity. Specifically, histone-associated heterochromatin undergoes changes in structure during the early stages of long-term memory formation. Before eye opening young rats depend on somatosensory and olfactory function for survival, as they can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and somatosensory stimulation is crucial. We have shown that synaptic plasticity in the OB underlies aversive olfactory learning. Our behavioral pharmacological experiments have shown that long-term olfactory memory requires activation of CREB. Western blot analyses have revealed that expression of P-MAPK/ERK is increased for 1 hour after odor-shock training, followed by increase of P-CREB lasting for 6 hours. Therefore, we examined whether intrabulbar infusion of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, facilitates olfactory learning in young rats. TSA infusion during odor-shock training enhanced a conditioned odor aversion in a dose-dependent manner. We further tested whether odor-shock training leads to histone acetylation in the OB and defined the time course of the activation. The acetylation of histone H3 was significantly increased for 1 hour after odor-shock pairing compared with odor only or no stimulation. TSA infusion significantly increased histone acetylation levels as well. These results show that HDAC inhibition is associated with aversive olfactory learning in young rats.

### #64 Multimodal-chemosensory convergence to the endopiriform nucleus, claustrum and agranular division of the insular cortex of the rat

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The endopiriform nucleus (EPN) is a large group of multipolar cells located in the depth of the piriform cortex (PC). Although many studies have suggested that the EPN plays a role in temporal lobe epilepsy, the normal function of the EPN remains to be elucidated. By using optical imaging with voltage-sensitive dye in slice preparations, we previously found that paired-pulse stimulation to PC and/or gustatory cortex (GC) evoked the excitation propagation to the EPN, agranular division (AI) of the insular cortex and further to the claustrum (paired-pulse facilitation). In the present study, we investigated the electrophysiological properties of neurons in the EPN, claustrum and AI in anaesthetized rats. In response to electrical stimulation of the olfactory bulb, the evoked potentials with a latency of about 50 ms were elicited in these regions. Paired-pulse facilitation of the evoked potential was also observed. Of the 52 neurons in these regions tested with odor and gustatory stimulation, 8 (15%) showed excitatory responses to both odor and taste stimulation, 14 (27%) had excitatory responses to odor but not to taste stimulation, 2 (4%) had excitatory responses to taste but not to odor stimulation, and the remaining 28 (54%) had no response. Further, we previously found significant increases in the numbers of c-Fos-positive cells in the PC, GC, EPN, AI and claustrum following natural stimuli, such as feeding an apple. These results, together with previous results, suggest that the EPN, claustrum and AI are the multimodal-chemosensory regions, where olfactory and gustatory information is centrally integrated.

### #65 Magnetoreception by abdominal dark hairs in honeybees

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We found that a simple and typical association learning of a flower odor with proboscis extension reflex to nectar in honeybees was inhibited by exposure to extremely low frequency (ELF) alternating magnetic fields. We postulated that the ELF magnetic fields could be detected by magneto-receptors. Honeybees have two types of putative magneto-receptors which either contain magnetite crystals, abdominal dark short hairs or abdominal trophocysts. We previously reported that a cutting of the abdomen longitudinal connective nerve between 3rd ganglion (first ganglion in abdomen) and 4th ganglion had no effect on the inhibitory action of alternating magnetic fields. And we concluded that the trophocysts had no role in magnetoreception. In this work, we confirmed that the dark hairs had a role in magnetoreception and inhibitory action of olfactory learning by magnetic fields. An exposure to 100Hz alternating magnetic fields of approximate 450 $\mu$ T inhibited the performance of conditioning learning in intact bees. When all dark hairs were pull out, an exposure to alternating magnetic fields had no inhibitory effects on the conditioning. So, it was shown that the dark hairs had a role in magnetoreception which impaired olfactory learning.

### #66 Prediction of glomerular activity patterns using the graph structure of odorant molecules

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Quantitative evaluation of odor qualities is an important process especially in the fragrance, food and beverage industries. However, most of the conventional odor sensing systems are unable to assess odor qualities perceived by humans. This study therefore aims to develop an artificial sensory evaluation system that can be used to evaluate odors and support sensory testing.

As the first step in the development of such a system, we proposed a neural network model for prediction of olfactory glomerular activity based on the results of previous studies, which reported that odors evoking similar glomerular activity patterns have similar odorant qualities. The proposed model consists of marginalized graph kernels and a multilayer neural network. The former is used to evaluate distances between odorant structures, and the latter converts these distances into glomerular activity patterns. However, a number of parameters included in the model required further discussion.

We therefore explored the two parameters that most significantly influenced the prediction accuracy of the model. These were the number of the Gaussian units and the number of neuron units in the neural network. The results indicated that optimal prediction ability was achieved when the number of the Gaussian units was approximately sixty and the number of neuron units in the hidden layer was approximately double than in the input layer. Based on these outcomes, future plans for further improvement of prediction ability were discussed.

### #67 A comparison between human sense of smell and neural activity of the olfactory bulb in rats

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In food and beverage industries, quantitative assessment of odors is an important process in developing new products. However, conventional odor sensing systems focus on identifying or detecting specific odors, and are unable to assess odor qualities perceived by humans. Against this background, our study aims to develop an odor assessment system that can be used to evaluate odors and support sensory testing.

Toward the development of a model for the evaluation of odor qualities, this study focuses on neural activity evoked by odorant stimuli in the olfactory system. This is because the results of previous studies suggested that odorants prompting common neural activity patterns in the olfactory system had similar odorant qualities.

As a first step, we conducted a series of human sensory tests to investigate perceptual similarities between odorants, and then compared the results with activity patterns evoked on the glomerular layer of the olfactory bulb in rats. These animals were chosen because the structure of their olfactory system is similar to that of humans, and measurement data relating to their glomerular activity is available online. We defined three indices (correlation, overlap rate of activation, and sum of squares of difference between histograms) to describe similarities and differences between activity

patterns, and also conducted sensory testing to determine similarities to human perception.

Comparison of the results revealed that each index showed a weak correlation to perceptual similarities. We therefore examined whether similarities between odorants could be predicted by combining the three indices using SVM (Support Vector Machine), and the results indicated a certain level of prediction ability.

### #68 Odor properties of novel compounds containing the tetrahydropyran ring

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Novel esters, alcohols, and ethers containing a tetrahydropyran ring were derived from (2,6,6-trimethyltetrahydropyran-2-yl)acetic acid (1) or 2-methyl-(2,6,6-trimethyltetrahydropyran-2-yl)propionic acid (2), and their odor properties were investigated. The relationships between the structures of the derived compounds and their odor properties were also examined. The methyl ester of carboxylic acid (1) had a refreshing, floral, and herbaceous odor, and the methyl ester of carboxylic acid (2) had a refreshing, woody odor. The propyl ester of carboxylic acid (1) had an adzuki-bean odor, and the propyl ester of carboxylic acid (2) had a vegetable and spicy odor. Thus, the odor of methyl ester of carboxylic acid (1) was thus similar to that of methyl ester of carboxylic acid (2), but considerably different from that of the propyl ester. Moreover, the odor of propyl ester of carboxylic acid (1) was considerably different from that of propyl ester of carboxylic acid (2). To determine the most stable structures of these esters, the molecular orbital calculation was carried out for each ester using MOPAC PM3. The bulkiness of the side chain of a tetrahydropyran ring of methyl ester of carboxylic acid (1) was comparable to that of methyl ester of carboxylic acid (2). However, the side chain of propyl ester of carboxylic acid (1) was bulkier than that of its methyl ester, and this increased bulkiness prevents the free rotation of the side chain of propyl ester. Similarly, the bulkiness of the side chain of propyl ester of carboxylic acid (2) makes its free rotation more difficult than that of propyl ester of carboxylic acid (1). Thus, the bulkiness and the degree of free rotation of the side chain of a tetrahydropyran ring influence the odor of compounds containing a tetrahydropyran ring.

### #69 Methodological Factors in Odor Detection Threshold

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Measured odor thresholds depend on both subject sensitivity and method. Yet, threshold-methods have received relatively little systematic attention in olfaction. We measured psychometric, i.e., proportion

correct detection vs. concentration, functions for acetic acid. A 2-out-of-5, forced-choice procedure was used. Stimuli were precisely controlled using an air-dilution olfactometer. The design had four factors, all randomized or counter-balanced: 1) practiced subjects vs. unpracticed subjects (between subjects); 2) 15-second inter-trial interval (ITI) vs. 30-second ITI (within subjects); 3) re-sampling allowed (i.e., subjects could smell each of the 5 stimuli presented during a trial as many times as they wished) vs. not allowed (within subjects); 4) concentrations presented in ascending order (lowest concentration first, moving up to the highest concentration, then starting again at the lowest concentration after a break) vs. random order (within subjects). A four-way ANOVA (the above four factors) revealed a significant main effect of re-sample condition. These results highlight the importance of methods for measured thresholds, and have implications for laboratory practice.

### #70 Effects of positive and negative information to the same odor

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Many researches have reported the influence of information to identify an odor. We tried to measure the prefrontal cortex activity during the smelling an odor using functional near-infrared spectroscopy (fNIRS) to find out the effects of positive and negative information with a same odor. We used three kinds of odors which have been evaluated as moderately hedonic values by a preliminary experiment. Eighteen subjects who had normal sense of odor were presented these odors with visual stimulation on a display. The visual stimulation showed the explanations of each odor with positive or negative information. During the experiment, subjects were measured for their prefrontal cortex activity with 42 channels (3x9 distributions) by fNIRS (FOIRE-3000, Shimadzu corp). These odors were presented by odor-stimulation system through tubes and a mask. The experiment was conducted by the following protocol: pre-resting period (20s), task period (15s), evaluation period (25s) and post-resting period (25s). We had 24 trials (3 odors x positive/negative information x 4 trials) and 4 control tasks with odorless. During the evaluation period, subjects evaluated the intensity, pleasantness/unpleasantness and familiarity of each odor. The stimuli were presented by random order. As a result, we showed the different evaluation value on the same odor but different information condition. The positive information condition showed significantly higher pleasant value than negative information condition ( $p < .05$ ). fNIRS data also showed the significantly different prefrontal cortex activation between positive and negative information conditions. We showed the effects of positive and negative information on a same odor.

### #71 First impressions of a woman depend on her wearing perfume - You are what perfume you wear

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First impression of a stranger is created by several perceptions, such as face, tone of voice, speed of words etc. There are many researches on

development of first impressions, but there are few researches that study a role of olfaction on development of first impressions. We wear perfume not only because we like it, but also because we want someone to have good impressions. This study aimed to investigate whether the perfume that we wear affects others' first impression for us. Sixty female university students participated in this study, and all of them were not acquaintance of the experimenters. CK One, citrus-floral perfume, and COCO Chanel, fresh oriental note perfume, were used as olfactory stimuli. The method to investigate for participants' impression for the female model (experimenter) was TEG II (Tokyo University Egogram ver. II, published by Kaneko Shobo). When the model wore CK One, she was evaluated as having stronger sense of responsibility and the makings of leader. On the other hand, when the model wore COCO, she was evaluated as having unique personality and strong self-assertion. These results show the perfume that we wear changes others' first impressions for us. The authors are continuing to study about this phenomenon, and confirm it with another models and with another perfumes.

## #72 A study of the image congruency between bottle appearance and fragrance of perfume

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Appearance of bottles might influence on the evaluation of perfumes as visual information before sniffing fragrance. We investigated such congruencies/ incongruencies between visual and odor information in the market products of perfume through the two experiments. In the experiment1, we investigated broad constructs of image aroused from appearance of bottles and fragrance of perfumes, and reviewed congruencies between bottles and fragrances. We selected 10 products as samples, and used "Napping" for experiment1. In Napping, subject sort the samples on the space reflecting sensory similarities. 11 our subjects carried out Napping under the two conditions. Subjects were asked to sort samples according to similarities of scents. These scents were sniffed actually in the sniffing condition, but in the viewing condition, scents were imagined from appearance of bottles. Distances matrixes between samples were measured from results of Napping, and were used for Multi-Dimensional Scaling (MDS). The result of MDS showed that dimension1 could represent major image of perfumes (for men or female) in the both conditions, and that dimension2 in the viewing condition could represent lightness of scents related to transparencies of bottles. However, we could not find systematic feature change of scents on dimension2 in sniffing condition. It may suggest complexity of scents sniffed actually. We then examined the qualitative aspects in congruencies/ incongruencies between bottles and fragrances in the experiment2. We selected 5 among 10 perfumes used in experiment1, and used 10 descriptive terms to evaluate each of the samples. In the results of experiment 2, we could not find any relations between terms with perfumes.

## #73 The relaxation effect of the bark oil of *Lindera umbellata*

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Changes of the physiological parameters (saliva amylase, heart rate, and brain waves) of 10 subjects during the smelling of the bark oil of *Lindera umbellata* were measured. The value of saliva amylase did not change during the smelling of the bark oil. The proportion of the parasympathetic nervous system increased significantly during the smelling of the oil, while that of the sympathetic nervous system decreased. On the other hand, the proportion of  $\beta$ -wave decreased significantly during the smelling of the oil. Thus, the bark oil exhibited the relaxation effects for the subjects. The bark oils included monoterpene alcohols (73.19%) and ketones (6.99%), monoterpene oxides (5.59%), monoterpene hydrocarbons (5.18%), and others. Among of these, linalool (63.70%) was the most abundant component.

## #74 Prediction of odor pleasantness for single- and multi-component odors

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Here we investigated relationship between the number of constituent odors and odor pleasantness. We hypothesized that the stability of the olfactory perception depends on the number of odor components and their molecular variability. We especially focused on odor pleasantness, which best explains molecular variability of odors in the natural world. Three different categories of odors, aliphatics, aromatics and terpenoids, were used in this study. We used 5 or 10 odors for preparing multi component odors. Odor pleasantness for the multi component odors tended to be more stable against concentration changes than that of the single component odors. Notably, multi component odors containing odorants of all categories showed higher stability than odors containing single odor category. Future study for large number of components with different molecular variability will reveal rules for the prediction of the odor perception for odor mixtures.

## #75 fMRI of brain responses to complex odor and visual stimuli

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One of the most important features of odor perception is the hedonic or emotional component. The purpose of this study was to measure the brain activity associated with human olfaction using functional magnetic resonance imaging (fMRI) and to evaluate the influence of the pleasantness of odors by complex odor and visual stimuli.

Seventeen healthy right-handed subjects (9 men; 8 women) with normal olfaction participated in the study. An event-related MRI

method was used to analyze olfaction-induced responses on fMRI. A puff of odorant was delivered to the nose of each subject through a nose mask with electrically controlled pneumatic valves. For the event-related task in the fMRI experiment, two odorants (pleasant odor: amyl-acetate, unpleasant odor: iso-valeric acid) and two types of visual stimuli (pleasant image, unpleasant image) were randomly presented. Two cross-modal sensual interactions were tested using SPM-software for both matching/mismatching conditions and pleasant/unpleasant conditions, respectively. Results revealed specific brain areas that were activated by cross-modal interactions with odor and visual stimuli.

In advance of the fMRI experiment, psychological odor experiments (cross-modal, complex stimuli with a visual and odor evaluation test) were conducted using a subjective judgment method or pleasantness/unpleasantness, respectively. Based on the fMRI and psychological experiments, we estimated the most suitable conditions for evaluating the pleasantness of odors.

From analysis of the fMRI experiments, two responses (1: emotional response to pleasantness/unpleasantness, and 2: response to matching/mismatching) were obtained with the cross-modal, complex stimuli using odors and images.

From the analysis of our experiments, activity associated with the response to pleasantness/unpleasantness was seen mainly in the right orbital frontal cerebrum and the supramarginal gyrus, middle frontal gyrus and amygdala. On the other hand, activity associated with the response to matching/mismatching was seen in areas of the brain corresponding to memory and cognition.

Thus, cross-modal, complex effects were suggested to be an emotional response to pleasantness/unpleasantness and cognitive and memory responses to matching/mismatching.

### #76 Quality of life (QOL) in patients with olfactory dysfunction -the study using Health-Related QOL Measure (SF-36) and VAS

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As otorhinolaryngologists, it is important to grasp the grade of subjective symptoms and quality of life (QOL) when we examine outpatients with olfactory dysfunction. However, the influence that the sense of smell exerts on the QOL related to health is not clear. Therefore, we investigated outpatients' QOL using the "Health-Related QOL Measure (SF-36)," which is widely used around the world. Concurrently, we applied a 1) visual analog scale (VAS) to grade subjective symptoms (the smell of food and drink, perfumes, excreta and gases) and the deliciousness of meals (QOL), 2) smell questionnaire for daily life, 3) T&T olfactometry, 4) intravenous olfaction test (Alinamin test) and 5) card-type smell identification test (Open Essence). We then analyzed those tests and SF-36 for correlations. As the results, "Vitality (VT)," which is a subscale of SF-36, correlated weakly with the subjective symptom VAS for "smell of perfume" ( $r_s=0.262$ ) and the T&T recognition threshold ( $r_s=0.203$ ). "Role functioning emotional (RE)," which is another subscale of SF36, correlated weakly with the subjective symptom VAS for each of "smell of food and drink" ( $r_s=0.234$ ), "smell of perfume" ( $r_s=0.272$ ) and "smell of gas" ( $r_s=0.222$ ). No other correlation

was found for any of the olfactory tests. These results showed that, although olfactory dysfunction affected the VT and RE of the patients' health-related QOL, it was very slight. Even if olfactory dysfunction develops, the effect on the overall QOL of the patient is slight compared with other diseases. This likely explains why these patients are slow to consult a doctor.

### #77 Olfactory Dysfunction in Patients with Eosinophilic Chronic Rhinosinusitis (ECRS)

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**[Introduction]** Olfactory dysfunction is most commonly caused by chronic rhinosinusitis (CRS) in Japan. Within CRS, eosinophilic chronic rhinosinusitis (ECRS) is said to cause severe olfactory dysfunction. However, there have been few reports that have compared ECRS with non eosinophilic chronic rhinosinusitis (NECRS).

**[Patients and Methods]** A prospective study was carried out at three institutions. A total of 621 patients underwent endoscopic sinus surgery between April 2007 and March 2008 and were pathologically diagnosed with CRS. Of those, 464 patients (318 males, 146 females; mean age: 48.2 years) were included in this study because they had undergone olfactory evaluation preoperatively. Olfactory dysfunction was assessed using the standard olfactory threshold test (T&T) and subjective symptom score (0~6). Data were analyzed using SPSS software ver. 11.

**[Results]** The 464 patients consisted of 228 NECRS patients, 190 ECRS patients, 21 patients with allergic fungal rhinosinusitis (AFRS), 13 patients with odontogenic maxillary sinusitis and 12 patients with fungal rhinosinusitis. T&T found that 54.9% of the total patients had olfactory dysfunction with a mean recognition threshold of  $\geq 4.1$  (107 of 228 NECRS patients; 46.9%, 119 of 190 ECRS patients; 62.6%), while 57.2% of the total patients had olfactory dysfunction with a subjective symptom score of  $\geq 3$  (100 of NECRS 228 patients; 43.9%, 135 of 190 ECRS patients; 71.1%). Both the T&T result (OR: 1.895) and the subjective symptom score (OR: 3.309) were significantly worse in the ECRS patients than in the NECRS patients.

### #78 Duration of Olfactory Dysfunction due to Chronic Rhinosinusitis, and its Prognosis

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**[Introduction]** We investigated the hypothesis that the prognosis of olfactory dysfunction (OD) due to chronic rhinosinusitis (CRS) becomes worse as the duration of OD becomes longer.

**[Patients and Methods]** 113 patients (70 males, 43 females; age range: 21-74 years; mean age: 50.9 $\pm$ 12.7 years) with OD due to CRS underwent appropriate surgery and postoperative treatment, and were followed up for at least 3 months. A visual analog scale (VAS) was used to evaluate their pre- and post-treatment symptoms of OD.

**[Results and Discussion]** Patients with an OD duration of  $\geq 5$  years showed significantly less improvement compared with an OD duration of  $< 5$  years. The OD duration and the post treatment VAS showed a significant negative correlation ( $r_s = -.399$ ,  $n = 113$ ). It was particularly strong in male patients aged  $\geq 60$  years ( $r_s = -.632$ ,  $n = 27$ ), and in the aspirin-intolerant asthma ( $r_s = -.624$ ,  $n = 11$ ), peripheral eosinophil count of  $\geq 800/\text{microL}$  ( $r_s = -.528$ ,  $n = 17$ ) and current smoking ( $r_s = -.731$ ,  $n = 11$ ) subgroups. Multiple logistic regression analysis of 19 patients with severe OD even after appropriate treatment identified the following as poor prognostic factors: 1) male gender (OR=18.996), 2) OD duration  $\geq 5$  years (10.023), 3) age  $\geq 60$  years (9.349) and 4) peripheral eosinophil count of  $\geq 800/\text{microL}$  (8.234) (predictive rate: 89.4%). These results indicate that the prognosis of OD due to CRS becomes poorer as the duration of the disease becomes longer. In particular, patients who have these risk factors should be started on treatment as soon as possible and should be advised to quit smoking.

### #79 Causative viruses of postviral olfactory dysfunction

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Causative viruses of postviral olfactory dysfunction have not yet been identified. We investigated causative viruses in patients with postviral olfactory dysfunction. We examined the presence of viruses in nasal discharge and examined the time course, with regard to changes in olfactory dysfunction and nasal obstruction in 24 patients with postviral olfactory dysfunction, using questionnaires, acoustic rhinometry, and olfactory tests. Nasal discharge was collected from patients with postviral olfactory dysfunction. Rhinoviruses were detected in 10 patients. Viral serotypes were identified by nucleotide sequences to be human rhinovirus-40, human rhinovirus-75, human rhinovirus-78, and human rhinovirus-80. One of the four patients complained of dysosmia, whereas another complained of anosmia. Results of acoustic rhinometry significantly improved in the four patients, although olfactory testing did not show significant improvement. Two of the four patients complained of olfactory dysfunction even 6 months after the first visit. Coronavirus and parainfluenza virus were detected in one patient each, and Epstein-Barr viruses were detected in three patients. The present study detected rhinovirus, coronavirus, parainfluenza virus, and Epstein-Barr virus in nasal discharge of patients with postviral olfactory dysfunction. Furthermore, this study suggests that rhinoviruses cause olfactory dysfunction through mechanisms other than nasal obstruction.

### #80 The clinical evaluation of olfactory disorders derived from central pathway

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In this study, we evaluated 10 patients with olfactory dysfunction induced by central nervous damage. Six of 10 patients developed hyposmia after head trauma, 2 patients by brain tumor, one patient after subarachnoid bleeding and one patient by encephalitis. Five of 10 patients did not sense any smell by venous injection of prosultiamine. Psychophysical evaluation of olfactory function was performed by T&T olfactometry, and the average threshold score of recognition was 5.2 (severe hyposmia). Eight patients got MRI brain scans and each of them had one or more intracranial lesions. Frontal lobe abnormalities were seen in 7 of 8 patients. Intracranial lesions were also observed in 3 temporal lobes and one parietal lobe and one occipital lobe. These MRI findings suggested most of the olfactory disorders were come from damage of orbitofrontal cortex in frontal lobe, which is thought to be the olfactory center. On the other hand, 8 out of 10 patients developed taste disorder, despite no pathogenic lesion was found in medullary, thalamus and insula, which are thought to be the central pathway of taste and taste cortex. Currently, we have yet to establish effective treatment of olfactory loss caused by central pathway damage. In clinical practice, some of these patients show improvements in olfactory function with several years, thus long-term follow-up of patients is needed to develop new clinical studies.

### #81 The clinical study of patients with drug-induced olfactory disturbances

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There are many causes of olfactory disturbance such as chronic sinusitis, common cold (virus), allergic rhinitis, head-trauma, congenital, drug-induced, unknown and others. Out of 2326 patients who visited an olfactory clinic at Showa University Hospital between 2000 and 2009, 32 cases (1.4 %) of drug-induced olfactory disturbance were investigated. Anti-cancer drugs occupied 81 % in the frequency of causative drugs. Tegafur was most common in the causes of olfactory disturbance by anti-cancer drugs. We showed the characteristics of drug-induced olfactory disturbance by Tegafur. A total of 21 patients were identified. The averaged recognition threshold was 5.29 and the averaged detection threshold was 4.67 in T&T olfactometry. After treatment, re-examinations were performed in 15 patients. The averaged recognition threshold was 5.12 and the averaged detection threshold was 4.01. Two patients (13.3%) showed improvement of olfactory functions, but 13 patients showed no improvement by T&T olfactometry.