

## ABSTRACTS

### Abstracts from the 43rd Annual Meeting of the Japanese Association for the Study of Taste and Smell, Asahikawa, Japan, September 2–4, 2009

#### 1. Effects of dietary zinc on taste sensitivity and salt preference in rats

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Zinc is one of the essential trace elements. Anorexia, growth retardation, epilation, skin parakeratosis, failure of immunological competence, failure of sexual maturation in adolescent males, and hypogesia (decreased taste acuity) are prominent symptoms of zinc deficiency in animals. We previously reported that feeding of the zinc-deficient (Zn-Def) diet causes a sudden increase of salt preference in rats in a short-term period of 4 days, however, short-term zinc deficiency did not alter the taste sensitivity for NaCl. Therefore, the change in salt preference at the early stage of zinc deficiency occurs before any change in salt taste sensitivity. We also demonstrated that in rats, long-term zinc deficiency reduced taste sensitivity at the level of the chorda tympani nerve, total carbonic anhydrase (CA, zinc enzyme) activity in the tongue epithelium and submandibular gland, and salivary secretion. Moreover, expression of CA II protein and mRNA in the rat submandibular gland was significantly reduced with long-term zinc deficiency, while expression of CA VI protein and mRNA was not affected. These results suggest that decreased CA II expression underlies the decreased total CA activity observed in the submandibular gland in long-term Zn-Def rats, and may cause reduction of regular salivary secretion and taste sensitivity.

#### 2. 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. On the other hand, Gustducin, a type II cell marker of taste bud, is expressed in soft palate taste bud in *Mash1* mutant mice. Forced expression of *Mash1* in tongue epithelial cells induced type III cell marker (AADC) ex-

pression. These results suggest *Mash1* play an important role for expression of AADC in type III cell 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 regulate the dissociation, synapse formation. In this study, in order to test whether the cadherins are required for formation of synapse between gustatory nerve and taste receptor cell, we have investigated expression patterns of cadherin superfamily in the taste buds.

#### 3. Voltage-gated sodium channels expressed on fungiform taste bud cells in mice

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Taste bud cells (TBCs) elicit receptor potentials with action potentials in response to taste stimuli. In type III cells, action potentials effectively open voltage-gated  $\text{Ca}^{2+}$  channels, and taste information is conducted to taste nerves by conventional chemical synapses. In contrast, action potentials in type II cells open voltage-gated hemichannels, which release ATP to activate neighboring TBCs or taste nerves by paracrine systems. In both cell types, action potentials play an important role to taste transduction. Since voltage-gated  $\text{Na}^+$  channels form the rising phase of action potentials on TBCs, I investigated their electrophysiological properties and related them to cell types by immunohistochemical staining. Both IP<sub>3</sub>R3- immunoreactive (-IR) cells (type II cells) and SNAP-25-IR cells (type III cells) elicit voltage-gated  $\text{Na}^+$  currents. There were significant differences between IP<sub>3</sub>R3-IR and SNAP-25-IR in the midpoint of activation and the recovery time constants of  $\text{Na}^+$  current inactivation. However, the difference between these TBCs and neuronal cells in the recovery time constant was much larger than that among TBCs. These results suggest that the firing frequency on TBCs was much smaller than that on neuronal cells.

#### 4. The odorant-pattern for individual recognition is acquired during the early developmental stages

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In mice, territorial males show vigorous aggression toward intruders, based on the chemical investigation to access the sex and familiarity of the intruder. We found that unfamiliar males even castrated were objective of the attack of the resident male mouse, indicating that familiarity of the subject is important for the aggression in mice. Aggressive behavior toward unfamiliar castrated B6 mice (UFCs) was lowered when UFCs were swabbed with urine collected from B6 familiar cage mate or urine from a cage mate's littermate, suggesting that familiarity information in urine has an inhibitory property for mice aggression.

Because resident mice did not show aggressive behavior to cage-mate's littermates, we cross-fostered B6 litters and examined whether the pre- or postnatal period was important for formation of individual urine odor. As a result, the resident male displayed attack bites toward UFCs that were his cage mate's littermates but were fostered by another B6 dam. In addition, castrated males that were reared with a cage mate (but not his littermate) were also attacked by the resident male, suggesting that littermates that share the same pre- and postnatal environments provide with similar olfactory information, which inhibits aggression. In adulthood, even after dietary changes, the resident male showed less aggression toward UFCs when they were swabbed with the cage mate's urine collected before the dietary change, indicating that individual information was not affected by dietary condition in the adulthood.

Taken together, these results suggest that olfactory cues containing individual information are shared among littermates, and that both the pre- and postnatal environments are important for the formation of individual information that can inhibit aggressive behavior among family and/or group members.

## 5. Mechanisms of imprinting and homing in salmon: animal behavioral, reproductive endocrinological and sensory physiological research approaches

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Salmon have an amazing ability to migrate thousands of kilometers from the open ocean to their natal stream for reproduction after several years of oceanic feeding migration. Three different research approaches from behavioral to molecular biological studies have been applied using four anadromous Pacific salmon (*Oncorhynchus* species; pink, chum, sockeye, and masu salmon) migrating from the North Pacific Ocean to Hokkaido as well as lacustrine sockeye and masu salmon in Lake Toya, Hokkaido Japan. The first was behavioral approach by means of biotelemetry techniques. We investigated swimming profiles of maturing Japanese chum salmon migrating from the Bering Sea to the eastern Hokkaido using a micro-data logger with a propeller that can measure swimming speed, swimming depth, and ambient temperature of fish. The second approach was endocrinological research on hormone profiles in the brain-pituitary-gonadal axis of Japanese chum salmon migrating from the Bering Sea to the spawning ground in Hokkaido. Levels of two types of gonadotropin-releasing hormone (sGnRH and cIIGnRH), two types of gonadotropin (LH and FSH) in the pituitary, and gonadal steroid hormones were measured by specific time-resolved fluoroimmunoassay. The third approach was molecular and physiological research on olfactory functions that play a crucial role in discrimination of the natal stream odorant during

upstream migration. The results demonstrate the navigation ability in open water, the hormone profiles in the brain-pituitary-gonadal axis, and the olfactory functions related with imprinting and discriminating ability of natal stream odorants in salmon.

## 6. Promoter analysis of mouse sweet and umami receptor, T1R3 gene

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The human T1R3 promoter has C/EBP binding site and TATA box. C/EBP  $\beta$  binds the C/EBP binding site and activates the transcriptional activity of human T1R3 gene. However, the mechanisms of transcriptional regulation of the mouse T1R3 gene have not been elucidated. In this study, we examined the promoter region of mouse T1R3 gene using the luciferase reporter assay in the T1R3 expressing cell line, GLUTag.

Sequence comparison between the human and mouse T1R3 upstream region revealed high levels of conservation at the C/EBP binding site and TATA box. The luciferase reporter assay showed that the promoter region of mouse T1R3 gene was located between -242 and -1 bp relative to the start codon in GLUTag. Deletion analysis of the T1R3 promoter showed that the putative C/EBP binding site might represent a binding site recognized by the specific positive regulatory element. These results show that the member of C/EBP family may play a role as the transcription factor regulating mouse T1R3 promoter activity in GLUTag.

## 7. Binding site for gymnemic acid at the sweet receptor hT1R2/hT1R3

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Gymnemic acid (GA), a triterpen glycoside isolated from the plant *Gymnema sylvestre*, is known to selectively suppress taste responses to various sweet compounds without affecting responses to salty, sour and bitter substances. Sweet-suppressing effect of GA is specific to humans and chimpanzees, but not to rodents. The effect of GA is normally long-lasting but rapidly disappears after rinsing the tongue with  $\gamma$ -cyclodextrin (CD) in humans. In order to examine whether GA directly interact with T1R2+T1R3, we used the sweet receptor T1R2+T1R3 assay in transiently transfected HEK293 cells. Similar to previous studies in humans and mice, GA (0.1 mg/ml) inhibited the  $[Ca^{2+}]_i$  responses of HEK293 cells heterologously expressing hT1R2+hT1R3 to SC45647, saccharin and D-tryptophan. The effect of GA was diminished by rinsing the cells with 1%  $\gamma$ -CD. The mouse pair (mT1R2+mT1R3) did not show the sensitivity to GA. One mismatched pair (hT1R2+mT1R3) behaved like the fully mouse heterodimer, showing no sensitivity to GA. These results demonstrate that hT1R3 is required for GA

sensitivity. To identify the binding site for GA, we examined the responses of the mouse/human chimeras of T1R2 and T1R3. The results suggest that the sensitivity to GA depends mainly on the transmembrane domain of human T1R3. Using directed mutagenesis, we identified several amino acid residues within the transmembrane domain of T1R3 that determine differential sensitivity to GA.

## 8. Fibroblast growth factor (Fgf) and its receptor (Fgfr) expression in mouse taste buds analyzed by RT-PCR

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Taste buds are maintained by continuous cell renewal throughout the mammalian lifespan. Taste bud cells which arise from local epithelial cells have an average lifetime of about 10-14 days. While the turnover of taste buds cells is assumed to be regulated by various signaling pathways activated in a highly coordinate manner, information concerning the specific signaling molecules is limited. Fibroblast growth factor (Fgf) signaling is required for a variety of regulatory processes in cell growth, differentiation and functional regulation. Currently twenty-two Fgfs and seven Fgf receptors (Fgfrs) have been identified in mouse. As for the taste buds, Fgf8 was reported to be expressed transiently in embryonic taste placodes on the anterior tongue, and Fgfr1 expression was observed in circumvallate papillae on the posterior tongue of adult mouse. However, expressions of other Fgfs and Fgfrs in taste buds are not reported. We examined expression of Fgf and Fgfr genes by RT-PCR for taste buds in adult mice. The tongue epithelium was peeled off and divided into two groups: (T) the taste bud region containing the circumvallate and foliate papillae, and (NT) the no taste bud region surrounding the circumvallate papillae. RT-PCR for 22 Fgfs and 8 Fgfrs in each group revealed that: 1) 2Fgfs were specifically expressed in T, 2) 3Fgfs were more intensely expressed in T than in NT, 3) 10Fgfs were almost equally expressed in both T and NT, 4) 5Fgfrs were expressed in both T and NT. These results suggest that Fgf signaling may play an important role in cell growth and differentiation in the adult mouse taste buds.

## 9. Regulatory role of *Six1* in the development of taste papillae

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The *Six* family genes encode homeobox transcription factors and their deficiency leads to abnormal sensory organ structures. We analyzed the expression patterns of *Six1* and the role in morphogenesis of taste bud-bearing lingual papillae during mouse embryonic development. *Six1* was expressed in the mesenchyme of the tongue at embryonic day (E) 11.5 and in the epithelium of fungiform papillae during E14.5-E17.5. In the posterior region of the tongue, *Six1* expression appeared in the epithelial thickenings of the center

of dorsal surface at E13.5-E14.5 and was observed in the trench wall of circumvallate and foliate papillae at E15.5- postnatal day 0. *Six1*-deficient mice (*Six1*-/-) showed distinct morphological changes: the fungiform papillae were increased in size and number, and their distribution in the tongue differed from that of wild-type mice. The number of primordia of fungiform papillae marked by *Shh* and *Wnt10b* were increased in *Six1*-/. As for the circumvallate papillae, more extensive invagination of epithelial thickenings, earlier formation of papillae, and stunted trenches were noted in *Six1*-/. The expression of *Shh* was altered, consistent with these anomalies. The foliate papillae were elevated initially and showed stunted trenches in *Six1*-/. These findings suggest modulation of signaling events in *Six1*-/, leading to earlier formation and abnormal distribution of fungiform papillae, and to earlier formation and malformed trenches of circumvallate and foliate papillae.

## 10. Effect of continuous dietary sodium restriction on salt perception and properties of taste cells

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It is needless to say that proper nutrition intake is necessary for correct development. Previously, it has been reported that continuous dietary sodium restriction (DSR) at early embryonic period causes alterations in central projecting areas of gustatory nerve, as well as in taste sensitivity especially for salty taste. But little is known on the effect of DSR on expression of molecules related to taste perception. In this study, we design to investigate the effect of continuous dietary sodium restriction (DSR) through embryonic days to postnatal life on number and distribution of the molecules related with taste perception in taste cells by quantitative analysis of immunohistochemistry on postnatal day 60. The molecules examined are G- $\alpha$  gustducin, PLC $\beta$ 2, candidate molecules relating sweet and bitter taste pathway and also considered as typical markers of type II cells in taste buds, PKD2L1 considered as a sour receptor and NCAM, markers of type III cells. We could not find significant change in the expression of G- $\alpha$  gustducin, PLC $\beta$ 2 and PKD2L1, but NCAM immunoreactivity in taste buds decrease after treatment of continuous DSR, and this reduction is rescued by sodium intake after PN day 40 in a part. These results suggest that appropriate sodium intake is necessary for the proper development of peripheral taste system and the possibility that continuous DSR make some alteration on the property of type III cells in taste buds.

## 11. Morphological characteristics of cells involved in glutamate signaling in the stomach

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Physiologic studies conducted in rats have demonstrated that afferent fibers of the gastric branch of the vagus nerve increase their firing rate with the intragastric administration of the amino acid glutamate. However, the cell types involved in glutamate signaling have not yet been identified. In this report, we examined the distribution and morphological characteristics of serotonin (5-HT) and serotonin type-3 receptor (SR3)-containing cells by immunohistochemical procedures. We demonstrated that 5-HT-immunoreactive (ir) cells are localized in the surface epithelium and in the base of fundic glands. Their density was greater in the former. In contrast to flask-shaped 5-HT-ir cells in the duodenum, those in the stomach are pleomorphic. Some 5-HT-ir cells were flask-shaped, but others were ovoid- or spindle-shaped. SR3-ir cells were present in the lamina propria, muscularis mucosae, and muscle coat. In the lamina propria, SR3-ir cells with long processes that ran parallel to the basement membrane of the fundic glands and those with short process whose somata were located in its deeper region close to the muscularis mucosae were remarkably seen. A subpopulation of SR3-ir cells was immunoreactive for protein gene product 9.5 and microtubule-associated protein 1b that was general marker for neurons and endocrine cells. In addition, a few endocrine cells located in the fundic glands exhibited SR3 immunoreactivity. These results suggest that 5-HT released by 5-HT-containing cells make biological effects on SR3 containing cells located in the lamina propria, leading to the initiation of excitation of gastric branch of the vagus nerve.

## 12. Coexpression pattern of T1R3 and gastrointestinal hormones in duodenum

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Recent studies have demonstrated that taste receptor T1Rs and T2Rs are expressed not only in taste buds but also in gut. In duodenum it is thought that secretin (S cells) and somatostatin (D cells) are secreted by acid sensing gut cells. Gastrin (G cells) and CCK (I cells) are secreted by protein and amino acid sensing gut cells.

We examined the expression of T1R3 in duodenum by reverse transcription polymerase chain reaction (RT-PCR) and *in situ* hybridization. Double-label studies showed that approximately 50% of secretin positive cells (S cells) were coexpressed with T1R3. On the other hand, T1R3 never colocalized with somatostatin (D cells), gastrin (G cells), and CCK (I cells). These results suggest that S cells may regulate secretion of secretin not only by acid but also by sweet substance and/or amino acid. Our results of double staining with T1R3 and gastrointestinal hormones suggest that G and I cells may express other receptors for amino acids like metabotropic glutamate receptors (mGluRs).

## 13. Molecular and functional analysis of gustatory sugar receptor genes in *Drosophila simulans*

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Polymorphism of Ala218Thr in Gr5a, a sweet-taste receptor gene in *D. melanogaster* wild populations has been known to be responsible for the behavioral preference for trehalose solutions. In contrast, the corresponding amino residue of *D. simulans*, a closely related species of *D. m.*, is not polymorphic and is always Thr, expecting to be the low sensitivity allele of the gene. Nevertheless we found that wild *D. s.* strains (6 strains; A~F) are divergent for the trehalose / sucrose gustatory preference.

We analyzed the preference between water and low concentrations of sucrose and found that trehalose-preference strains have high threshold of sucrose and sucrose-preference strains have low threshold of sucrose.

Gr64a is known to be a receptor for sucrose in *D. m.*. Therefore we examined the nucleotide sequences of Gr64a in these *D. s.* strains. We found 8 amino-acid substitutive mutations in them. But as the result of preference test, we cannot find correlations between the threshold of sucrose and the amino-acid substitutions.

Then we examined the nucleotide sequences of all other sweet receptors (Gr61a, Gr64b~f) in the 3rd Chromosome of these *D. s.* strains. We found 44 amino-acid substitutive mutations and 2 frame-shift mutations. But no mutations correlated with the threshold of sucrose.

We are under analyzing the threshold of other sugars in these strains. We found that the thresholds in some sugars are significantly different among strains, and that some strains are more sensitive to certain sugar whereas less sensitive to other sugars. Therefore the sugar sensitivity of the fly must not be controlled by a simple mechanism.

## 14. Expression patterns of T1r/T2r, gustducin and their functions on gustatory neural responses in the soft palate of mice

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Gustducin is a taste-specific G-protein mediating bitter, sweet and umami tastes. The function of gustducin was implicated primarily in bitter taste in circumvallate (CV) papillae and in sweet taste in fungiform (FF) papillae. Gustducin knockout mice showed a major reduction in nerve responses to bitter compounds from the CV and to sweet compounds from the FF papillae. However, the functions of gustducin in the soft palate (SP) are unknown. To clarify the role of gustducin in the SP, nerve response properties of the greater superficial petrosal (GSP) and the chorda tympani (CT) nerve in gustducin-KO and C57BL mice were recorded to determine the effect of gustducin-KO on sweet and bitter responses of the two nerves. Also, the coexpression patterns of taste receptors and gustducin in the SP and FF of C57BL/6J mice were examined using double-labeled *in situ* hybridization. Results revealed that nerve responses to both sweet and bitter stimuli were markedly reduced in the GSP of

gustducin-KO mice, while the CT produced reduced responses to sweet, but not to quinine-HCl or denatonium. *In situ* hybridization showed that most T1r2, T1r3 and T2r-positive cells were included among the gustducin positive cells both in the SP and FF. These results suggest that another taste signal transduction mechanism without involving gustducin may exist in the FF.

### 15. Taste responses of mouse fungiform taste cells to multiple taste compounds that elicit similar taste sensation

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Various taste compounds are perceived as similar taste quality. Our previous studies demonstrated that majority of mouse fungiform taste cells expressing gustducin (Type II) or GAD67 (Type III) responded specifically to one of 5 basic taste qualities (sweet, bitter, umami, sour and salty). However we did not test multiple taste compounds eliciting same taste sensation. In this study, we examined whether bitter sensitive taste cells expressing gustducin and sour sensitive taste cells expressing GAD67 responded to multiple bitter or sour taste compounds, respectively. Five bitter compounds [20 mM quinine, 20 mM denatonium, 0.1 mM cyclohexamide, 10 mM caffeine, 0.5 mM sucrose octaacetate (SOA)] and three sour compounds (10 mM HCl, 10 mM citric acid, 10 mM acetic acid) were tested in bitter or sour sensitive taste cells. We found that a large majority of bitter and sour sensitive taste cells responded to multiple bitter or sour compounds although all bitter sensitive cells tested did not respond to SOA and caffeine. These results suggest that taste receptor cells may detect multiple taste compounds that elicit same taste sensation. Supported by KAKENHI 18077004, 18109013 (YN) and 21791808 (RY).

### 16. GABA functions as neurotransmitter in the mice taste buds

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Taste bud cells (TBCs) communicate with sensory afferent fibers and may also exchange information with adjacent cells. Recently,  $\gamma$ -aminobutyric acid (GABA) has been proposed as a candidate neurotransmitter or neuromodulator in mammalian taste buds. However the precise role of GABA in the taste buds remains unclear. In this study, we examined possible expression of GABA receptor subunits in taste cells by using RT-PCR and potential effect of basolateral GABA application to single fungiform TBCs on their electrical activities. The results indicated that TBCs expressed GABA type A receptor (GABA<sub>A</sub> receptor) subunits,  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 5$ ,  $\beta 2$ ,  $\beta 3$ ,  $\gamma 2$ ,  $\delta$ ,  $\pi$ , and GABA type B receptor (GABA<sub>B</sub> receptor) R1, R2, but not expressed GABA<sub>A</sub> receptor subunits  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 6$ ,  $\beta 1$ ,  $\epsilon$  and  $\theta$ . Some TBCs were evoked action potentials by applica-

tion of GABA, while some of the other TBCs were inhibited by GABA. Furthermore, Cl<sup>-</sup> transporters Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter (NKCC1) and K<sup>+</sup>-Cl<sup>-</sup> cotransporter (KCC2) that mediated GABA actions were detected in TBCs by using RT-PCR. These results suggest that GABA may participate in modulation of spontaneous firing rate in taste bud. The Cl<sup>-</sup> cotransporters may be involved in the GABAergic signaling.

### 17. Enhancement of the chorda tympani nerve responses to mixtures of sweet and salt compounds in mice

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Ordinary gustatory experiences, which are usually evoked by taste mixtures, are determined by multiple interactions between different taste stimuli. Although human psychophysical responses to taste mixtures have been investigated extensively, there have been few reports on the neurophysiological coding of taste mixtures in the mammalian gustatory system. In the present study, we investigated the role of the peripheral gustatory system in these physiological interactions by measuring the effects of sweet-salt mixtures on responses of the chorda tympani (CT) nerve in the C57BL/6 mice. The CT nerve responses to sweet compounds (sugars and sugar alcohols) increased when they were mixed with NaCl (over the sum of responses to each component). Enhancing effects of sweet responses by addition of NaCl with or without amiloride (Na<sup>+</sup> channel blocker) were also evident after treatment with gurmarin (sweet inhibitor). These results suggested that (1) gustatory mixture interactions are initiated at the level of the taste bud or peripheral nerve, and (2) the enhancement of the CT nerve responses to mixture of sweet compounds and NaCl may occur through the amiloride-insensitive and gurmarin-insensitive pathways.

### 18. Neuronal response latency in the rostral nucleus of the solitary tract, the parabrachial nucleus and the gustatory cortex in the rat: Roles of GABAergic inhibition

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Although the firing frequency and temporal pattern of neuronal activity have been examined in different taste-related regions of the brain, direct comparisons of taste response characteristics among these regions remain to be performed. We investigated here temporal characteristics (onset latency and response duration) of neuronal responses in the rostral nucleus of the solitary tract (rNST), parabrachial nucleus (PBN) and gustatory cortex (GC) in the rat. Multi-barrel glass micropipettes were used to record extracellular single unit activity under urethane anesthesia. The onset latency of a neuronal response was measured as the time interval between the stimulus onset and the time firstly exceeding the activity (mean + 2SD) during the pre-stimulus period. The response duration was

calculated as the entire period where the activity remained above this level. The mean onset latency in the rNST was significantly longer than that in PBN or GC (one-way ANOVA,  $p < 0.001$ ). The smaller coefficient of variation of taste response magnitudes (i.e., more stable responses) in the PBN may be partly due to abundant convergences from the rNST. Taste responses were examined in a subset of rNST neurons before and after the local iontophoretic application of GABA<sub>A</sub> receptor antagonist gabazine. Gabazine increased mainly taste responses to the best-taste, and shortened the onset latency, indicating that taste responses in the rNST units were partly modulated by GABAergic inhibition. These results suggest that GABAergic inhibition may play a role in determining differential taste responses in the rNST, PBN and GC.

## 19. The neural mechanisms of aversive taste reactivity by stimulation of GABA<sub>A</sub> receptors in the ventral pallidum in rats

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We previously showed that the higher GABA level in the rat ventral pallidum (VP) induces aversive taste reactivity. To elucidate the neural mechanisms responsible for the VP GABA-induced aversive taste reactivity, we examined the effects of microinjections of GABA<sub>A</sub> agonist muscimol into the VP on animal's behaviors and the Fos-like immunoreactivities (FLI) in other brain regions. Rats were implanted with guide cannulae in the VP. After the surgery, they received bilateral injections of 0, 10 or 100 ng muscimol into the VP. The rat's behaviors were videotaped in the observation chamber for 1 min at 0, 30, 60, 90 and 120 min after the drug injections. One week after the behavioral tests, the rats were perfused 120 min after the muscimol (200 ng) injections, then Fos-positive neurons were detected. Frame-by-frame video analyses revealed that the 10 ng muscimol increased ingestive taste reactivity. Although the 100 ng muscimol-injected rats also showed ingestive taste reactivity immediately after the drug injection, their behaviors shifted to aversive taste reactivity, especially chin rubbing and paw treading, at 30-120 min after injections. The microinjections of muscimol into the VP induced FLI in the rostral region of nucleus solitary tract, pontine parabrachial nucleus, central nucleus of amygdala and gustatory cortex. These results indicate that intense stimulation of VP GABA<sub>A</sub> receptors induces aversive taste reactivity, whereas weak stimulation causes ingestive taste reactivity. It is suggested that the aversive taste reactivity by facilitation of the VP GABA receptors is due to the activation of the gustatory-related brain regions.

## 20. Role of the medial prefrontal cortex in extinction of conditioned taste aversion in mice

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Conditioned taste aversion (CTA) is an associative learning that is established by pairing(s) of a novel taste (conditioned stimulus, CS) with visceral illness (unconditioned stimulus, US). Repetition of CS

presentation without US (extinction procedure) after CTA training gradually extinguishes CTA behavior (it means the experimental extinction of CTA). Although the prefrontal cortex is suggested to be involved in CTA extinction, its role remains unsolved. We examined the role of the medial prefrontal cortex (mPFC) in CTA extinction in C57BL/6J mice. First, lesions of the mPFC with an excitotoxin significantly disrupt CTA extinction in one-bottle drinking test. Second, the same mPFC lesions enhanced conditioned disgust (aversive) reactions in the taste reactivity test. Third, intra-mPFC infusions of a GABA<sub>A</sub> receptor agonist muscimol also strengthen the expression of conditioned disgust reactions. These results suggest that activation of the mPFC play a role to suppress learned aversive behavior to the CS and thereby contribute to facilitate CTA extinction.

## 21. Overconsumption of a sucrose solution and brain reward system in mice

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Palatability of fluids and food is a crucial factor to elicit hedonically motivated eating that is mediated by the brain reward system including the ventral tegmental area (VTA). It still remains not fully understood how palatability of food affect the VTA function to develop binge-type overconsumption of highly palatable food. To investigate the issue, we developed a mouse model for binge-type overconsumption with limited access schedule to a 0.5 M sucrose solution for only 4h a day under food deprivation. Proportion of caloric intake derived from sucrose in total daily caloric intake was increased upto 44% after 30-day training without change of body weight. Next, we examined whether ghrelin-related signaling in the VTA play a role in binge-type overconsumption. Ingestion of normal chow induced by intra-VTA infusions of ghrelin in binge-trained was significantly greater than that in non-trained control mice, suggesting that binge training enhances the sensitivity of ghrelin receptors in the VTA. These findings suggest that the enhanced ghrelin signaling in the ventral midbrain may greatly activate the neural mechanisms of hedonically motivated eating to develop binge-type overconsumption of the highly palatable sweet solution.

## 22. Pupil response of elderly for taste stimulation

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Generally, the gustatory threshold is considered to rise, and gustatory responses to decline, with aging, but the details remain unclear. In this study, using infrared videopupillography, which is useful for the objective evaluation of preferences and autonomic nerve function, we compared the pupillary light reflex and pupillary responses to gustatory stimulation between young and elderly subjects to clarify the response characteristics of the elderly to gustatory stimulation. The subjects were 10 elderly and 10 young people with normal gustatory and visual functions. First, the light reflex was measured in a resting state. Then, gustatory stimulation (water or a bitter solution of 0.25% quinine hydrochloride) was given, and pupillary

responses were compared. For the resting light reflex, both the initial and minimum pupillary diameters were significantly smaller in the elderly than young subjects, but no difference was noted in the reaction time. During stimulation with the bitter solution, the pupillary response was more marked than during stimulation with water in both the young and elderly subjects, and did not differ between them. The reaction time to light did not differ between the young and elderly subjects, but that bitter solution administration was significantly longer in the elderly. Therefore, the delay in the response to gustatory simulation in the elderly is considered to be primarily due to delays in information processing involving gustatory receptors and the cerebrum.

### 23. The visualization of the efferent neuronal projection in the nucleus accumbens on the retrieval of conditioned taste aversion memory using manganese-enhanced MRI

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We previously found that the nucleus accumbens (NAc) and ventral pallidum (VP) play a role in the retrieval of conditioned taste aversion (CTA). Neuroanatomical studies showed the dense projections from the NAc to VP. To elucidate the involvement of the NAc-VP projection neurons in the retrieval of CTA, we visualized the activities of the efferent neuronal projections from the NAc, using manganese-enhanced magnetic resonance imaging (MEMRI) technique. All rats were implanted with a guide cannula and an intraoral cannula. After the surgery, they received a pairing of 5 mM saccharin (conditioned stimulus, CS) with an i.p. injection of 0.15 M LiCl (CTA group) or saline (control group) as unconditioned stimulus. Two days after the conditioning, rats were microinjected with a 50 nL of 40 mM MnCl<sub>2</sub> into the NAc. Thirty min after the MnCl<sub>2</sub> injection, rats were presented with CS (CTA-CS and control-CS groups) or DW (CTA-DW and control-DW groups). Sixty min after the MnCl<sub>2</sub> injection, we evaluated the T1-weighted MR images of the anesthetized rats by 11.7 T MRI. We analyzed two horizontal images which were acquired with a 1 hour interval. The 2nd horizontal image showed that manganese was transported from the NAc to VP, since the signal intensity of the VP area in the 2nd horizontal image was stronger than that of the 1st. The increase in the signal intensity in the VP in the CTA-CS group was higher than other groups. These results suggest that the greater activation of the NAc-VP projection neurons play an important role in the retrieval of CTA memory.

### 24. The effect of umami taste on saliva secretion. -Concentrarioin dependency and synergistic effect of umami compounds

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We reported that the temporal properties of salivary secretion caused with the taste stimulation. Sustained salivary secretion was observed by the umami stimulation, monosodium glutamate (MSG) (Hayakawa et al., *Jpn. J. Taste Smell Res.* 15, 367-370, 2008). In this study, the influences of the concentration of MSG and the synergistic effect of MSG and disodium 5'-inosinate (IMP) on salivary secretion were measured. Twenty-two healthy adults participated in the study as subject. They held 3 ml of each aqueous taste solution for 30 sec without swallowing, and spit whole saliva into cups at every 30 sec for 10 min. The weight of saliva per min was assumed to be saliva flow per min. There was a concentration dependency in the effect of MSG (10 - 300 mM) on the saliva secretion. On the other hand, in the cases of equi-concentration mixture solutions of MSG and IMP (0.65 mM MSG + 0.23 mM IMP - 3.6 mM + 1.3 mM), there was a weak concentration dependency. When compared between solutions of MSG and MSG and IMP at the same umami intensity, MSG stimulated larger amount of saliva secretion than the mixture solution. Saltiness of MSG single solution at higher concentration might stimulate saliva secretion.

### 25. ATP, via the activation of P2X and P2Y receptors, affected on vagal celiac afferent nerves in the rat

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Recently, it is reported that the presence of taste receptors is not only in the oral cavity but also in the gastrointestinal (GI) tract. In the GI tract, it is known that 5-HT and CCK have a role in mediating the nutrient information to vagal afferent nerves. In the oral cavity, the taste information is transmitted from taste receptor cell to gustatory nerve via ATP. Within the GI tract, there is also abundant evidence that ATP acts as a neurotransmitter, being released from either extrinsic sympathetic efferent nerves or from intrinsic enteric neurons. However, there is no evidence for the presence of P2X and P2Y receptors on vagal celiac afferent nerves. In the present study, we examined the effects of ATP and the P2X receptor agonist  $\alpha,\beta$ -methylene-ATP and the P2Y receptor agonist ADP and the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) on the activity of vagal celiac afferent nerves in the rat. ATP (i.v.) and  $\alpha\beta$ -methylene-ATP (i.v.) and ADP (i.v.) each induced dose-dependent increases in afferent nerve discharge. PPADS (i.v.) antagonized the increases in afferent nerves discharge elicited by  $\alpha\beta$ -methylene-ATP and ADP. These results suggest that the increase in vagal celiac afferent activity represents the effect on nerve ending, mediated by P2X and P2Y receptors.

### 26. Relationship between concentration of human salivary Histatin and taste sensitivity to Quinine sulfate

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Histatins are salivary polypeptides that are characterized by a high content of histidine residues. We have reported that Histatin 3, 5, 6 and PRP-PE in saliva electrophoretically showed the same movement with quinine. But the functions of these peptides to bitter substances have not yet been identified. The aim of this study was to investigate the relation between the concentration of human salivary Histatin 5 and bitter taste sensitivity. Furthermore binding of human salivary Histatin with Quinine sulfate was also examined. Fifty six subjects were divided into two groups according to the results of taste sensitivity test to Quinine sulfate; high threshold group (n=26) and normal threshold group (n=30). Stimulus parotid saliva was obtained from all the subjects. The concentrations of Histatin 5 in parotid saliva were quantified by ELISA test. Quinine sulfate solution and Histatin 5 peptide were mixed and filtered with centrifugal devices to measure the binding condition of them.

Binding of Quinine sulfate and Histatin 5 was confirmed by binding assay. Histatin 5 concentration of high threshold group was significantly lower than that of normal subjects ( $p < 0.01$ , two tailed). These results indicated that human salivary Histatin 5 functioned as carrier of bitter substances: Quinine sulfate.

## 27. Effects of inactivation of the central and basolateral nucleus of the amygdala on the voluntary intake of palatable liquid food

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We have recently found that the integrity of the central nucleus of the amygdala (CeA) is required for the voluntary intake of the highly palatable liquid food. However, the role of the basolateral nucleus (BLA; another subnucleus of the amygdala) is remained unclear. In the present study, we investigated the effects of inactivation of the BLA on the palatability-induced voluntary drinking and the expression of c-Fos proteins in the central nervous system. We micro-injected a GABA<sub>A</sub> receptor agonist muscimol (0, 5, or 20 ng, 0.25  $\mu$ l/ side) into the BLA of rats bilaterally and measured the consumption of a highly palatable solution, Ensure Liquid, and observed overt behavior during 10-min drinking. The rats consumed 10.1 g of Ensure Liquid when injected with vehicle, and almost the same amount when injected with muscimol (10.8 g with 5 ng, 8.9 g with 20 ng). The defensive-like forepaw treading, frequently observed after microinjections of muscimol into the CeA, was not observed after muscimol injections into the BLA. Moreover, inactivation of BLA had no characteristic effects on the expression of the c-Fos protein compared with inactivation of CeA. Together with the previous study, it was suggested the central but not basolateral nucleus of the amygdala is selectively involved in the palatability-induced voluntary drinking.

## 28. Taste discrimination among the mixed taste solutions

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The taste hedonic tone and taste intensity were evaluated in the healthy university students (n=47). The taste solutions tested were sucrose (sweet), NaCl (salty), tartaric acid (sour), quinine-sulfate (bitter), monosodium glutamate (umami), and tannic acid (astringent). The three mixed taste solutions of them were examined.

Those who answered two tastes were the most, whereas those who answered more than 4 tastes were a few. The higher rate of correct answer was one or two tastes. The intensity of sucrose, NaCl and quinine-sulfate was lower than that of the other taste solutions. The correct rates were higher in the order of sucrose, tannic acid, and NaCl. The correct rates for sweet and salty tastes were higher in spite of the intensity.

## 29. Comparison of the effects of taste and oral somatic sensation on the activation of the central histaminergic system

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The central histaminergic system is known to be involved in various physiological functions. We previously reported that both feeding behavior and taste information activate the histaminergic system. We also revealed that palatability of tastants influences the histaminergic system; palatable taste decreases the activity of the histaminergic system while aversive taste increases it, suggesting that the histaminergic system has a key role in linking gustatory sensation to feeding behavior. From these findings, we hypothesized that other oral somatic sensation, such as hardness of food, may affect the histaminergic system. In the present study, we examined the effect of hardness of food on the amygdalar histamine release using *in vivo* microdialysis, when rats consumed either of two types of pellets composed of similar ingredients but having different degrees of hardness: hard and soft pellets.

Histamine release was significantly increased in the rats fed with hard pellets. By contrast, histamine release was not enhanced in soft pellets-fed rats. Since there were no significant differences between the hard and soft pellet intakes during the experimental period, the amount of food intake is not a factor which affects histamine level, and post-ingestive effect on the activation of the histaminergic system seems to be negligible. When rats acquired a conditioned aversion to soft pellets, histamine release was increased during feeding, in sharp contrast to no change of histamine release pattern seen during unconditioned soft pellet intake. These observations indicate that the histaminergic system is modulated by oral somatic sensation of food related to palatability as well as gustatory sensation.

## 30. The effect of dietary free glutamate on dessert intake

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It is known that free glutamate makes soup more satisfying and that its preload does not affect meal intake. Dessert intake has a different mechanism in appetite regulation than main course intake has; we tend to eat dessert even when in a satiated state. If this craving is in order to seek satisfaction, a glutamate-enriched satisfying soup might substitute and reduce dessert intake. The purpose of this study was to investigate the effect of free glutamate in chicken soup on dessert intake while in a satiated state. Fifteen healthy subjects were recruited to participate in three experimental sessions. In the first session, the subjects joined an ad libitum food intake study to calculate the amount of food that they usually eat. In the remaining sessions, we conducted randomized cross-over tests to measure dessert intake (ad libitum cake intake) in a satiated state, after the consumption of chicken soup (150 ml) with or without 0.5 wt% of monosodium glutamate (MSG). To reach a state of satiation, the subjects were made to eat the same amount of food that was eaten in first session. In all of the sessions, the subjects assessed subjective satiety and appetite ratings using a visual analog scale represented on a portable computer system (with an originally developed program). As a result, soup with MSG decreased subsequent dessert intake significantly ( $227 \pm 95.2$  kcal vs.  $276 \pm 108$  kcal,  $p=0.037$ , paired-t test). Subjective satiety and appetite ratings were not significantly differed by MSG-enrichment. These findings suggest a need for further investigation of the postprandial efficacy of glutamate, umami taste, and satisfaction from food palatability.

### 31. Packages of chocolate affect taste perceptions and palatability of chocolate

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Eighty female university students participated in this study. They were divided into 4 groups randomly. The first two groups (Group A and Group B) were received three kinds of chocolate (black, milk and hi-milk) and asked to evaluate their sweetness, bitterness and palatability with visual analogue scale presented onto the head mount display. Participants in Group A started evaluating from black chocolate, and the others (Group B) started from hi-milk chocolate. The results showed that participants evaluated black chocolate as least sweet and bitterest, and hi-milk chocolate as sweetest and least bitter. The latter two groups (Group C and Group D) were received two kinds of chocolate out of three chocolates with two kinds of packages. Participants in Group C were received black chocolate with package of black chocolate (B-B) at first, and then received milk chocolate with package of hi-milk chocolate (M-H). Participants in Group D were received hi-milk chocolate with package of hi-milk chocolate (H-H) at first, and then received milk chocolate with package of black chocolate (M-B). The results showed that M-H was evaluated sweeter and less bitter than M-B. These results supported that the visual stimuli, such as package and commercial messages, affects taste perception by top-down processing (Sakai, 2009).

#### Reference:

Sakai N: Cognitive and contextual factors affecting olfactory and gustatory perception and palatability of beverages. *ChemoSense*, 11(3), 1-2, 4-6 (2009)

### 32. Simple evaluation of powdery instant soup by measurement of buffer capacity

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To explore the substitute for organoleptic method to evaluate the taste of powdery instant soup, we investigated validity of simple instrumental methods with titration and/or electrode. The taste of sixteen kinds of samples on the market was analyzed by using high performance liquid chromatography, taste sensor and buffer capacity measuring device and then compared with the sensory evaluation using the trained panels. The instrument for measuring buffer capacity was simply made of the combination of automatic titration system and personal computer. The regression equations were calculated between these instrumental data and the average panel taste score. The "umami" and "body" score by the panels were shown to be predicted significantly from the values by measuring buffer capacity. These results suggest that the instrumental analysis of buffer capacity is substitutive for organoleptic taste evaluation method of powdery instant soup.

### 33. Association of the expression of taste receptors to dietary intakes

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We reported the expression characteristics of taste receptor of Japanese. In this study, the association of the expression of taste receptors to dietary intake was examined. Subjects were 8 normal female university students aged 18 - 20. The 7-day diet record (Estimated recording method) with photograph was used this study for energy and each nutrition intakes during the entire investigation. The expression of taste receptors (hTAS2Rs) was adopted to as marker of taste. The expression of taste receptors was measured by following method. Cells were collected from foliate papillae parts by the scrapping at the end of diet record period. And we measured these expressions by RT-PCR (SCREP method). We examined the expression of hTAS2Rs correlated with each nutrient intake by Pearson's product-moment correlation coefficient. As the results that hTAS2R3 and hTAS2R4 correlated with fat. Specifically these receptors correlated with number of saturated fatty acids and some unsaturated fatty acids. However, n-3 polyunsaturated fatty acids did not. The expression of hTAS2R3 and hTAS2R4 might be influenced by fat intake, especially saturated fatty acid intake. hTAS2R9 correlated with arachidonic acid. And the expression of hTAS2R39 is reported to relate to meat intake. Therefore these facts might be exhibit that expression of hTAS2Rs of each related to part of fatty acids. And hTAS2R16 correlate with carbohydrates. The expression of hTAS2R16 might be related to carbohydrates intake that containing bitter compounds. This study depended on a Grant in Aid for Scientific Research subsidy in 2008, 2009 and Nihon

University President's Grant for Specified Multidisciplinary Research in 2007, 2008.

### 34. Analysis of a food intake factor contributing to expression of hTAS2Rs

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We reported possibility of the taste evaluation that used cells from foliate papilla part by scraping, and measurement of expression characteristics of hTAS2Rs by RT-PCR (SCREP method). In this study, we investigated relations of expression characteristics of taste receptor and food intake. The normal subjects that were not noticed of dysgeusia, 18-21 years old were recruited by Showa Women's University. The expression characteristics of taste receptors were measured by SCREP method about these subjects. Food intake was investigated using Food Frequency Questionnaire based on food group (FFQW65) that can calculate an estimate total energy intake value, an estimate protein intake value, an estimate lipid intake value, an estimate carbohydrate intake value and intake frequency and semi-quantity of 65 kinds of food/food group. The subjects who expressed more than six kinds of hTAS2Rs, a intake value of the food/food group kind correlated with kinds of expression of hTAS2Rs. In contrast, total energy intake value, protein/lipid/carbohydrate intake value had no correlation with kind of expression of hTAS2Rs. As a result of having analyzed all subjects, intake of food/food group characteristics and expression of hTAS2Rs, it is suggested that an intake frequency and value of soy bean related with expression of hTAS2R5, 16, 40, 43. As for chicken intake related to expression of hTAS2R39, peanut/nut group intake related with expression of hTAS2R16. This study depended on a Grant in Aid for Scientific Research subsidy in 2008, 2009 and Nihon University President's Grant for Specified Multidisciplinary Research in 2007, 2008.

### 35. Effect of bittern ingredients on the taste nerve responses to sodium chloride

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Bittern is a residual component after the extraction of table salts from seawater in the production process, and this includes minerals such as magnesium salts as a major component. Recently, bittern has been used for the supplement for the protection of heart disease and so on, however, scientific research such as safety of this utilization and the taste modification activity have not fully been investigated. So, we especially focused on the taste modification activity of bittern by recording the taste nerve response to sodium chloride in adult female SD rats. Two kinds of standard artificial bittern, i.e., ion exchange-concentrated type and seawater evaporated type of bittern that were provided from the Salt Science Foundation (Tokyo, Japan), and were used for the recording of the chorda tym-

pani nerve responses to sodium chloride solution with or without bittern (or bittern component = MgCl<sub>2</sub>, MgSO<sub>4</sub>, CaCl<sub>2</sub> and KCl). We found out that the chorda tympani nerve response to sodium chloride solution was decreased by an addition of 50-1000 fold diluted ion exchange-concentrated type of bittern, but not by seawater-evaporated type of bittern. Furthermore, the addition of bittern component such as, MgCl<sub>2</sub>, CaCl<sub>2</sub> and KCl to high concentration of NaCl solution caused significant decrease of NaCl response, but MgSO<sub>4</sub> addition did not cause any change of NaCl response. These results suggest that the addition of chloride ion may reduce the chorda tympani nerve response to NaCl solution. We further analyzed the effect of bittern component on the chorda tympani nerve fiber (N-fiber or E-fiber) response to NaCl solution.

### 36. Behavioral estimation of salty enhancing effects of glycine ethyl ester

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Glycine ethyl ester was reported to act as one of the salty enhancers. Though salty enhancing effect of the ester was observed by human sensory evaluations, it has not been drawn an attention because of its small effects and unstable character. To estimate salty enhancing effects, we have performed a brief access test with the sodium-deprived C57BL/6 mice, which were fed on a NaCl-deficient diet containing mineral-corticoid receptor antagonist spironolactone. Under our experimental conditions, the mice showed linear correlation between lick number and saltiness among 0 – 0.06 M NaCl. The licking rate to NaCl solutions was increased by 0.03 M or 0.045 M glycine ethyl ester addition. These results suggest that the ester could enhance saltiness or salty first impression, and that the taste is somehow acceptable. The enhancing saltiness by 0.045 M glycine ethyl ester addition corresponded to that of 0.007 – 0.012 M NaCl.

### 37. Taste alteration effect of spice extracts: Evaluation of the relationship between *in vitro* GAD activity test and salt taste sensation test on human subjects

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Glutamate decarboxylase, GAD, is an enzyme that catalyzes a production of GABA from L-glutamate. We have reported that GAD67, a metabolic isoform of GAD, is specifically expressed in the type III taste buds by using genetically altered GAD67/GFP knock-in mouse and suggested that GABA may play some role(s) in the taste signal transduction [Nakamura et. al., *Japanese J. Taste & Smell Res.*, 13:547 (2006)]. Last year, we reported the significant contribution of GABA to *in vivo* taste sensation [Hisaki et. al., *Japanese J. Taste & Smell Res.*, 14:435 (2007)]. In this study, we have focused on a relationship between GAD activity and taste, particularly salt taste. The effects of spice extracts are examined on both GAD activity and taste. The spice extract treated GAD activity was examined by using the recombinant enzyme. The taste sensation tests were conducted on healthy young women who were briefly exposed to the spice extract since those effects are difficult to examine quantitatively with animal models. The results of the effects of the

spice extracts on the GAD activity and the salt sensation were reported. The effects were also graded and compared with the degree of inhibition on GAD activity. A direct correlation between the effects on the GAD activity and the degree of salt taste alteration was observed; however, there was no direct correlation between the GAD activity and sweetness.

### 38. Effect of miracle fruit on the five basic taste and electrical gustatory threshold

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The taste-modifying protein, miraculin, has the unusual property of modifying a sour taste into a sweet taste. The modification in taste occurs when the miraculin protein firmly binds to sweet receptor cells in a person's tongue when sour substances are present. We investigated whether miracle fruit has the effect of taste modifying to taste substances except for the sour taste. We used freeze-dry miracle fruit.

We evaluated gustatory sensitivity in young females while assessing taste threshold. Gustatory threshold was measured by electro-gustometry and by the sensory evaluation. One region of the tongue was stimulated: the left or right region 2 cm away from the tip of the tongue, where fungiform papillae are situated. Electrogustometry showed no difference in electrical gustatory threshold after miraculin treatment in fungiform papillae. Consequently, this result suggested that miraculin treatment had not inhibited effect in the electrical gustatory threshold.

The sensory evaluation using a scoring method showed the significant difference after miraculin treatment in the sour and salty tastes. Furthermore, we investigated the thresholds of these two tastes by using three point discrimination. Concerning the salty and sour tastes in whole mouth gustatory test, the detection and recognition thresholds increased significantly after miraculin treatment ( $p < 0.01$ ), but those thresholds were no change after miraculin treatment in other taste substances.

In this study, the significant difference of the detection and recognition thresholds were recognized in sour and salty tastes.

### 39. Concentration of Val, Met and IMP in gonads of seven edible species of sea urchin in Japan

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Valine (Val) and Methionine (Met) have a bitter and slightly sweet taste for humans and contribute to the taste of sea urchins (Komata, 1964). In a previous CTA experiment with C57BL/6J mice, the results suggested that the taste quality of Val and Met changed to have umami after mixing with IMP. Also the concentration of Val in *Hemicentrotus pulcherrimus* gonads was examined. In Japan, there are 7 species of sea urchins that are mainly eaten. The taste of gonads differs among these species. However there are no scientific data to prove it. In order to clarify the cause of this difference we investigated the concentration of Val, Met and IMP in gonads from

these 7 species of sea urchin. The content of Val and Met ranged 7~70 mg/100g and 2~26 mg/100g, respectively, and accounted for under 5% of total amino acids in the gonads of each species. The content of IMP was the same level as Met, ranging 1.2~26 mg/100g. These data suggest that the small content of Val and Met may contribute to a pleasant bitterness and small content of IMP may change the taste quality of amino acids in the gonads of each species of edible sea urchin.

### 40. Objective evaluation of the 'mildness' in the soy sauce made of whole soybeans

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The soy sauce made of whole soybeans (WS type soy sauce) has been believed to taste milder than that made of defatted soybeans (DFS type soy sauce). In this study, we aimed to evaluate the 'mildness' of the WS type soy sauce and to analyze what the mildness is derived from. The sensory test and the principle component analysis suggested that some fermented aromas similar to those produced by Japanese pickles as well as saltiness were sensed stronger in the DFS type, whereas aftertasted umami, soybean flavor, smoothness, and sweetness were perceived stronger in the WS type, which appeared to be the causes of the 'mildness' in the WS type soy sauce. Comparative chemical analysis of ingredients and the correlation analysis between individual components and the 'mildness' revealed that glycerine and organic acids except lactic acid and acetic acid were positively correlated, and that aromas and flavors showed a negative correlation. The multiple regression analysis led to the identification of key components likely causing the mildness: the mildness in the WS type soy sauce can be evaluated by an equation such that  $= 1.06 \times \text{glycerine} + \alpha\text{-ketoglutaric acid} - 1.04 \times \text{citric acid}$ . Although this equation remains to be corrected as to more comprehensive analyses in the future, this study is the first to provide a chemical basis for the 'mildness' in the WS type soy sauce.

### 41. The taste characteristics of carp sashimi from two producing districts

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Carp meat has been used as an event food on formal occasions in Nagano Prefecture, especially, Saku City. Local people say that the crunchy meat is very delicious due to the cold winter in Saku City. In our previous study, however, younger generations claimed the carp meat had a muddy odor or fishy smell. Therefore, the carp meat's taste and smell are weak-points.

In this study, we considered the taste of carp sashimi from 2 producing districts (Saku City, Nagano prefecture and Kooriyama City, Fukushima Prefecture) with a sensory evaluation and instrumental analysis (Hitachi L-8800 Amino Acid Analyzer). The carp sashimi was subjected to sensory evaluations of taste by 42 trained panelists (average age was 22.9).

As a result, the carp sashimi of Kooriyama City had a stronger umami taste than the Saku carp. On the instrumental analysis, the

carp sashimi of Kooriyama City had significantly more amount of tasty free amino acids, e.g. Tau, His, Thr and Ser, than Saku's carp.

We already reported the result of the physical properties measurement that the carp sashimi of Saku City was harder than the carp from Kooriyama. The carp sashimi of Saku tended to be rougher than the carp of Kooriyama in this study. The roughness of the carp sashimi from Saku is possibly related to hardness.

As above, there was a difference in taste of the carp sashimi from the 2 producing districts.

## 42. The influence of flavor on the sweet and sour tastes

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To examine possible influence of flavor on taste, we performed sensory evaluation tests with 13 human subjects for sweet and sour taste solutions applied with maple, vanilla, cinnamon or jasmine flavors (made in TAKASAGO). We used 3% sugar solution without flavors as the standard for the sweetness evaluation. The sweetness estimated by the subjects for 3-9% sugar solution was significantly increased when the solution was applied with maple, vanilla or cinnamon flavor (t-test,  $p < 0.05$ ), but not with jasmine flavor ( $p > 0.05$ ).

Estimated sweetness for mixture of 5% sugar solution with sour taste solution (1% malic acid) was significantly increased when applied with all 4 flavors ( $p < 0.05$ ), whereas estimated sourness for the mixture was significantly decreased with all 4 flavors ( $p < 0.05$ ). The sweetness-increasing effect of 3 flavors may be due to the sweet image for the flavors that the subjects had learned through their food history. But this may not be the case for jasmine flavor. One of possible reasons for the decreased sourness by 4 flavors may be increased familiarity of the flavor and tastes during repeated trials, as suggested by Wilson and Stevenson (2006).

Wilson DA and Stevenson RJ: Learning to Smell, Johns Hopkins University press, Baltimore (2006)

## 43. The influence of prolonged aerobic exercise on bitter taste sensitivity

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In order to determine the influence of prolonged aerobic exercise on bitter taste sensitivity, we investigated the relationship between physical fatigue and taste intensity and/or palatability by performing sensory evaluation. We used 0.01 and 0.03 mM quinine-HCl solutions as test samples. Twelve subjects performed aerobic exercise—a full-length hike of 36 km for about 12 hours. Measurement of taste sensitivity was performed at 4 points (0, 16, 25, and 36 km). The average energy consumption of the subjects was  $4468 \pm 243$  kJ at the final point. The physical fatigue increased significantly according to increase of the walking distance. There was no significant change in the subjects' perception of taste intensity and pal-

atability for quinine-HCl solutions recorded at the 4 measurement points. However, the fluctuating values were observed in each measurement points. In comparison with the start point, the taste intensity at the 16 km point slightly decreased and increased at the 25 km point. It decreased again at the 36 km point. And, in the points where the taste intensity was high, the palatability tended to become weak. This tendency was observed with both 0.01 and 0.03 mM. This result shows the possibility that bitter taste sensitivity changes by the physical fatigue.

## 44. Examination of influence that monosodium glutamate intake gives to feeding desire afterwards -a fMRI study-

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It is well known that brain responses from visceral afferents of the vagus nerve are found after intragastric infusion of monosodium-L glutamate (MSG) in rats. In addition, it has been further suggested that induced injection of MSG may relate to appetite regulation in the behavioral experiments of rats.

Therefore, we have examined the influence of monosodium glutamate intake on the feeding desire afterwards in humans using functional magnetic resonance (fMRI) and evaluation of the visual analogue scale (VAS), which is often used in psychological experiments. In our experiments two healthy men (18-25 ages; right-handed) were used and informed consent and their agreement to participate in this experiment with appetite regulation was received prior to carrying out the experiment based on the recommendation from both ethical committees at Tokyo Denki Univ. and Chiba Univ.

The experiments were completed under the pre-text of a total of four different conditions (hunger-not hunger, and intake-not intake of MSG when drinking the soup). Brain activities in the fMRI experiment were measured with event-related tasks using food related photos and non-food related photos (mainly landscape photos) at random. The VAS data was evaluated before intake and at constant time periods after drinking the soup.

From the results of fMRI in this experiment, brain activities related to the feeding desire after intake of MSG in humans were mainly found near the insula cortex and subcallosal area and so on. VAS data have also suggested the capability of MSG to control our appetite from the tendency of the terms, such as: "hunger", "not hunger", and "appetite".

## 45. Diagnosis of taste dysfunction using zinc-binding protein in saliva (gustin)

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It is known that more than 70% of taste dysfunction patients have zinc deficiency. Carbonic anhydrase (CA) VI being identical with gustin which is a zinc metallo-protein in human saliva with molecular weight of 37,000 has significant relation to taste dysfunction. This investigation was conducted to examine CAVI levels in parotid saliva of taste dysfunction patients in connection with zinc concentration in serum.

Parotid saliva was obtained from 81 patients of taste dysfunction. According to zinc concentration in serum, subjects were divided into 2 groups, i.e., normal group and zinc deficient group. The concentration of CAVI in saliva was quantified by the enzyme-linked immunosorbent assay (ELISA) using the polyclonal antibody against the synthetic peptide designed from human CAVI. ELISA plates were coated by saliva diluted with coating buffer (50 mM carbonate), followed by the ABC method, which were measured in a microplate reader at 405 nm. CAVI titers were calculated by reference to the standard curve of the synthetic peptide.

The concentration of CAVI in parotid saliva of zinc deficient group was significantly lower than that of normal group ( $p < 0.01$ ).

This result suggests that the ELISA using this antibody can be a probe for the quantitative measurement of CAVI, which may be useful to diagnose taste dysfunction caused by zinc deficiency.

#### 46. Involvement of masticatory stimuli in the induction of rat salivary cystatins in response to dietary quinine or tannic acid

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We previously demonstrated the existence of some deleterious substances in food induced cystatin S in the submandibular saliva of rats. To explore the mechanism underlying induction of this salivary protein by dietary constituents, we compared its induction characteristics in response to dietary quinine or tannic acid. In this experiment, some animals' molar teeth were extracted on the bilateral upper jaws or put on cannula for perfusion of the oral cavity. Detection of cystatin S was performed by SDS-PAGE after an affinity chromatography on a papain column. The addition of 0.3 % quinine or 5 % tannic acid to an ordinary powdered chow increased the weight of the submandibular gland and induced cystatin S in its secretion in normal intact rats. Liquefaction of these diets with distilled water, however, markedly reduced such effects of tannin, suggesting that masticatory stimuli are important for increases in the gland weight and induction of cystatin S. This view was confirmed by experiments using animals with tooth-extraction and with oral perfusion of a solution of 0.3 % quinine or 5 % tannic acid where increases in the gland weight and induction of cystatin S were recognized. Possibly, neither chemical stimuli nor mechanical stimuli individually increase full production of cystatin S and some level of integration between the sensations of both stimuli are required for its full production.

#### 47. Creation of flavor database including the calculated molecular features

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It is important to consider the chemical features of the molecules in the research related to sense of smell. The 83 flavor molecules used commercially in Japan were selected for this study. The physical property values such as a boiling point and lipophilicity, and the description of flavor, for the 83 molecules, were collected from the three published books and the Aldrich's catalog. The other chemical features such as flexibility of molecule were calculated and analyzed using a computer. As a result of the conformational analysis of the 83 molecules using CONFLEX (ver. 6), the 8 molecules, including acetophenone (CAS 98-86-2), 1,8-cineol (CAS 470-82-6), and benzaldehyde (CAS 100-52-7), were found to be the most rigid. It is suggested that the rigid 8 molecules are suitable chemicals for study of olfaction, because the rigid molecules may pass through the simpler olfactory reception mechanism. Furthermore, the optimized structures, the ChelpG charges which reflect electrostatic potentials, the infrared absorption (IR) spectra, and the Raman spectra were calculated by B3LYP/6-31G+(d, p) using Gaussian 03W(ver. 6.1). The collected data from the publications and the calculated data in this study were accumulated in the database, "Flavor Chemicals Database". This database is now open to the public at the website "<http://homepage2.nifty.com/odour/>". This is good and new in the points that the molecular flexibility, the optimized structure, and the electronic structure of each flavor molecule are visualized. This website is expected to give some hints and the insights into the other researchers by further supplementation and completion of the contents.

#### 48. Procedures for observation of the pheromone receptive region by scanning electron microscopy

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The mammalian vomeronasal organ (VNO) is a chemosensory apparatus that detects pheromones. The pheromone receptor molecules are located in the microvillus membrane of vomeronasal receptor cells (VRC) that are housed in the VNO. The vomeronasal sensory epithelium (VNSE) is covered with microvilli of the VRC and supporting cell. Therefore, it is difficult to perform a morphologic analysis of the surface of the VRCs. In the present study, samples for the observation with the scanning electron microscope (SEM) were prepared with the KOH treatment method and the sonication method to perform morphologic analysis of the pheromone receptive sites. Using the KOH treatment method, the degree of removal of the microvillar plexus was inconsistent and VRC-DEs were often destroyed. In case of the sonication method, we succeeded in removing plexus microvilli of supporting cells, which allowed us to observe individual VRC-DEs and surrounding structures. The present study suggests that the sonication method is superior to the KOH method to analyze individual VRC-DEs and their microvilli. Using the sonication method, it is likely to obtain ultrastructural evidence for "ultrasensitive" VRC by analyzing numbers and density and other morphological characteristics of microvilli from VRC-DEs.

## 49. Histological properties of the nasal cavity and the olfactory bulb in brown-eared bulbul *Hypsipetes amaurotis*

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The brown-eared bulbul (*Hypsipetes amaurotis*) is commonly found in Japan where it is regarded as a harmful bird that causes damage to agricultural products. Few studies have investigated the sensory apparatus of this bird, and consequently little is known of sensory modalities it uses. Here, we analyzed the anatomical and histological properties of the nasal cavity and olfactory bulb (OB) of the bulbul in order to investigate the functional level of olfaction in this species. Although both anterior and maxillary conchae were observed in the bulbul nasal cavity, there was no structure equivalent to the posterior concha. The OB located on the ventral side of the anterior extremity of the cerebrum and the ratio of the olfactory bulb size to that of the cerebral hemisphere was very small. Interestingly, the left and right OBs were completely fused at the midline of the cerebrum. Furthermore, certain types of lectins that bind to the olfactory nerve of vertebrates with a well-developed sense of smell also bound positively to the olfactory nerve and glomerular layers of the bulbul OB. These findings suggest that the brown-eared bulbul has an anatomically and functionally less well developed sense of smell compared with other avian species. Although the molecular and developmental mechanisms underlying the fusion of the OB are still unknown, we suggest that the fused OB may offer a unique model for studying the evolution and development of the central olfactory nervous system of vertebrates.

## 50. Synaptic configuration of mitral/tufted cells in accessory olfactory bulb by three-dimensional ultrastructural reconstruction

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Mitral / tufted (MT) cells, the projection neurons in the accessory olfactory bulb (AOB), have several primary dendrites receiving input from vomeronasal neurons in the glomeruli, and form the dendrodendritic reciprocal synapses (RSs, consisting an asymmetrical (excitatory) synapse (AS) and a symmetrical (inhibitory) synapse (SS)) between granule cells in the MT cell layer (MTCL). The RSs have important roles for pheromonal recognition and memory. However, synaptic pattern and distribution on the MT primary dendrites are unknown. To understand the microcircuit in the AOB, we performed the three-dimensional (3D) reconstruction of the primary dendritic region, labeled by biocytin using whole-cell patch-clamp method, from serial-section electron micrographs.

On the primary dendrites of MT cells, RSs between dendritic spines of Gr cells were observed ( $1.54 \pm 0.47$  per  $10 \mu\text{m}$ ,  $n=4$ ). In some cases, RSs were consisted of an AS and two SSs on large dendritic spines,

so-called gemmules, of the Gr cells. On the other hand, AS presynaptic regions contacted with small spines were founded ( $2.11 \pm 0.64$  per  $10 \mu\text{m}$ ,  $n=4$ ). Inhibitory inputs from dendritic spines and shafts were received ( $1.16 \pm 0.50$  per  $10 \mu\text{m}$ ,  $n=4$ ). Excitatory inputs to MT cells were not identified on the primary dendritic shafts in the MTCL. ASs and SSs in MT cell layer were not defined as the synapses between MT cells and Gr cells. Recently, existence of a few small inter neurons in the MTCL was reported. Some efferent feedback input into MTCL from limbic system was founded. In future, 3D reconstruction of dendrite will be performed not only on MT cells more in detail but also Gr cells to study microcircuit in the AOB.

## 51. Pharmacological effects of cocaine hydrochloride on voltage-gated sodium currents in the olfactory receptor cells

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Cocaine hydrochloride is commonly used as a local anesthetic for the otorhinolaryngeal surgery of the nasal cavity. The main effect of cocaine is analgesia during the surgery of the nasal cavity, but the effects on the olfactory receptor cells are unknown. Olfactory receptor cells of the newt were isolated from the nasal tissue, after the 5 min incubation in 0.1% collagenase solution as has been reported previously (Kurahashi, 1989). The culture dish containing isolated cells was set on the inverted microscope equipped with a patch-clamp recording system. Cells were identified with the existence of the cilia and from their bipolar morphology. Under using the whole cell version of the voltage clamp mode, the membrane potential was depolarized from a holding potential (V<sub>h</sub>) of -100 mV in a step wise between -90 mV and +40 mV, and voltage-gated currents was recorded. When cocaine hydrochloride was applied to the recorded cell, the voltage-gated currents, including inward and outward components, were significantly reduced. The dose-suppression curves of cocaine for sodium current components could be fitted by the Hill equation. Half-blocking concentrations were 0.043 mM, and Hill coefficient was 1.06. These phenomena are similar to the suppressive effects of local anesthetics on I<sub>Na</sub> in various preparations, suggesting that both types of suppression are caused by the same mechanism. Recovery from the suppression was confirmed even after the treatments with 10 mM cocaine hydrochloride. This fact implies that cocaine hydrochloride does not affect olfactory ability after locally-high dose treatments of nasal cavity in surgical operation.

## 52. Distribution of Fos-immunoreactive cells in the olfactory bulb of urethane-anesthetized rats

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Neural activity in sensory systems has often been studied using anesthetized animals. It is known, however, that neural activity

in an anesthetized state is not necessary the same to that in an awake state, and that odor-induced mitral cell activity in the olfactory bulb is augmented by anesthesia. To clarify the effect of anesthesia on neural activity in the olfactory system, distribution of Fos-immunoreactive cells in the olfactory bulb was compared between awake and urethane-anesthetized rats placed in a controlled odor environment. In awake animals stimulated with butyric acid vapor, most of Fos-immunoreactive cells were distributed to sparse glomeruli and to the part of the mitral and granule cell layer associated with the positive glomeruli. Spatial pattern of the immunoreactive cells within each layer was similar to some extent, and showed a weak but significant correlation. On the other hand, in urethane-anesthetized animals stimulated with the odorant or even with filtered air, a number of Fos-immunoreactive mitral/tufted cells appeared, in addition to cells in the glomerular and granule cell layers. These mitral/tufted cells lacked apparent topographic organization, and were not always localized to regions beneath the glomeruli delineated with Fos-immunoreactive juxtaglomerular cells, decreasing correlation observed in awake animals. These results suggest that activity of bulbar neurons, particularly that of mitral/tufted cells, is altered by urethane-anesthesia, and that the activity is more selective in awake animals.

### 53. A mechanism of action of group II metabotropic glutamate receptors suppressing dendrodendritic inhibition in the mouse accessory olfactory bulb

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Female mice develop a long-lasting olfactory recognition memory of a partner male. Neural changes underlying this memory occur at the accessory olfactory bulb (AOB), the first relay in the vomeronasal system. The goal of our research is to understand the mechanism of synaptic transmission in the AOB. Recent researches including molecular biological, pharmacological, electronmicroscopic and electrophysiological studies revealed that reciprocal synaptic events in the AOB are critical to memory formation for male pheromones. Relatively little is known, however, about the mechanism of synaptic transmission between dendrites in the AOB.

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 the present study, in order to conduct further investigation on the role of mGluR2 in the reciprocal synaptic transmission, AOB slices were prepared from 23- to 35-day-old Balb/c mice. Miniature EPSCs (mEPSCs) were recorded from granule cells in slice preparations with the patch-clamp technique in whole-cell configuration (holding potential,  $-70$  mV) in the presence of an antagonist for GABAergic transmission, picrotoxin ( $50$   $\mu$ M). An extracellular application of DCG-IV reduced the frequency of mEPSCs with slight decrease in the amplitudes. The present results suggest that mGluR2/mGluR3 can modulate the synaptic transmission from mitral to granule cells through both presynaptic and postsynaptic mechanisms.

### 54. Olfactory responses of descending interneurons and thoracic motors in the male cockroach

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In insect central nervous system, brain descending interneurons (DINs) carry the final information from brain to thoracic motor systems to initiate specific patterns of behavior. Using extracellular recording method, we examined how the DINs form their commands that influence thoracic activity and leg turning movement in male cockroach (*P. americana*) to olfactory stimuli to antennae. Depressor (Ds) and levator (Ls) trochanter motor neurons of the middle leg responded in a set of activity to a pheromone to each antenna. The response characteristics of latency and duration changed depending on which side of the antennae stimulated. The stimulus induced a set of sequential activity of the Ds and slow extensor of the tibia (SETi), and the Ls in the middle leg. Onset timing of activity of the middle leg muscles was different depending on which side of antennae stimulated. 1-Hexanol induced activities of the Ds and Ls, and the middle leg muscles in a different manner. The DINs responded to left-right antennal odor stimuli in a different manner. The DINs activities induced correlated changes of activity of the Ds and Ls motor neurons and the leg muscles (Ds, SETi and Ls). The results suggest that the activities of DINs may correspond to command signals for turning behavior and the commands impart changes to thoracic motor activity.

### 55. Reconstruction of functional expression of a vomeronasal receptor by using adenovirus vectors

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Many mammals, especially rodents, have a specialized chemosensory system for pheromones, the vomeronasal system. Pheromones convey various information about species, age, reproductive stage, social status and individual identity for mammals. They have two kinds of dramatic effects, *i.e.* a releaser effect and a primer effect. Releaser pheromones trigger behavioral changes, *e.g.* fighting and marking, *via* the direct action on the central nerve system. On the other hand, primer pheromones elicit endocrine effects including the induction of estrus and pregnancy block *via* the neuroendocrine system. Pheromones are bound by vomeronasal receptors in the vomeronasal sensory neurons which project their axons to the accessory olfactory bulb (AOB). Vomeronasal sensory neurons express two types of vomeronasal receptors, V1Rs and V2Rs. In rodents, about 150 repertoires for each receptor type have been identified. Moreover, V1Rs detect volatile pheromones, whereas V2Rs detect non-volatile pheromones like peptides and proteins. However, it is largely unknown what compound acts as a pheromone and which receptor detects the pheromone. For elucidation

of pheromone-receptor pair, it is important to the system that can express only one receptor and easily detect its response to a specific pheromone. Therefore, cultured vomeronasal cells were infected with the adenovirus vector carrying a V1R gene and receptor expression and pheromonal response were evaluated. We found that a pheromone-specific response was observed only in vomeronasal cells co-cultured with AOB neurons.

## 56. Effects of major urinary proteins (MUP) on the proliferation and/or survival of mouse vomeronasal stem cells

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The vomeronasal organ (VNO) retains a stem cell population, which continually generates new neurons throughout life. The regeneration of vomeronasal neurons (VSNs) may be activity-dependently regulated by pheromonal signaling, as suggested by the findings in TRPC2-deficient mice. However, the precise mechanism for the regeneration of VSNs is largely unclear. To address this issue, we examined the effect of natural stimuli on the proliferation and/or survival of cultured mouse VNO cells. Major urinary proteins (MUPs) are members of the lipocalin family of pheromone-binding polymorphic proteins and included at high levels in urine. Stripped and ligand-bound MUPs, and their bound-ligands were purified separately from urine of female and male Balb/c mice as natural stimulants. Then, the change in numbers of nestin-immunopositive VNO cells was investigated after adding these stimulants to cultured VNO cells, which were separately prepared from male and female mice. Immunocytochemical staining showed that the number of nestin-positive VNO cells in female-derived cell cultures significantly increased 2 weeks after the application of ligand-bound MUPs derived from female urine. Moreover, stripped as well as ligand-bound MUPs derived from male urine caused a significant increase in the number of female nestin-positive cells. However, in male-derived cell cultures, only ligand-bound MUPs derived from male urine was effective in increasing nestin-positive cells. These results suggest that female VNO cells are more sensitive to MUP stimulation than male VNO cells. MUPs and/or their bound-ligands possibly enhance the proliferation and/or survival of VNO stem cells and the regeneration of VSNs.

## 57. Primer effects in a sex pheromone of masu salmon (*Oncorhynchus masou*)

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Recently, we have reported that ovulated female masu salmon secrete L-kynurenone as a releaser pheromone for spermating male masu salmon parr. In this study, we tested primer effects to conspecific males. Adult masu salmon were catheterized to collect the

urine. Spermating male masu salmon parr were divided into several groups (n=4-7 in each group), exposed to distilled water, mature male urine, preovulated female urine, ovulated female urine, L-kynurenone, coelomic fluid and bile of ovulated females. Time course of the responses and dose-response relationship were also investigated for primer effects of ovulated female urine. Blood sample was taken from the caudal vasculature of each male and the excretable milt was collected before exposure and after exposure. Plasma 17,20 $\beta$ -dihydroxy-4-pregnene-3-one, 11-ketotestosterone and testosterone levels were measured by enzyme-linked immunosorbent assay. Female urine and L-kynurenone induced significant elevations of plasma concentration of steroid hormones 3-5 hours after exposure. Thresholds of primer effects of the urine were as low as behavioral threshold of releaser effects of L-kynurenone. The milt volume, however, the present study showed no increase in any experiment. We suggest that L-kynurenone has both primer and releaser effects. It is necessary to elucidate the detail function of L-kynurenone as a primer pheromone.

## 58. An estimation of putative magneto-receptors using classical proboscis extension reflex to a flower odor in honeybees

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Extremely low frequency (ELF) electromagnetic fields show various effects on animals. In ELF electromagnetic fields, waves are much longer than animal size, so electrical components and magnetic components are thought to affect differentially. A simple and typical association learning of a flower odor with proboscis extension reflex to nectar in honeybees was inhibited by exposure to ELF electromagnetic fields, and ELF alternating magnetic fields also yielded same effects. We postulated that the ELF magnetic fields could be detected by magneto-receptors. Honeybees have two types of putative magneto-receptors which contain magnetite, abdominal black short hairs and abdominal trophocystes. We confirmed that the black hairs have a role in magnetoreception and inhibitory action of olfactory learning by magnetic fields. In this work, the role of trophocystes in magnetoreception and inhibitory effect on conditioning learning was investigated. We cut the abdomen longitudinal connective nerve between 3rd ganglion (first ganglion in abdomen) and 4th ganglion because some bundles of axons laterally branched from these abdominal ganglions innervate to trophocystes. An exposure to 100Hz alternating magnetic fields of approximate 45mT also inhibited the performance of conditioning learning in nerve cut bees. So, it was shown that the trophocystes had no role in a sense of magnetoreception which impairs olfactory learning.

## 59. *Shochu* waste stillage as a bait in pot fishing for catching the octopus, *Octopus vulgaris*

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The purpose of this study is to examine the preference of octopuses for bait made of *shochu* waste stillage. Laboratory and field

experiments were conducted to determine whether octopuses were attracted to bait composed of *shochu* waste stillage. The baits made of *shochu* waste stillage were prepared by hardening the ingredients with a solution of agar in water.

In the laboratory experiments, pot containing the bait made of *shochu* waste stillage and pot containing mackerel were placed in the experimental tank, and the behaviors of the octopuses were observed with a digital video camera for 3 hours in each experiment. The experiments were conducted under dark conditions. In the field experiments, 30 pots were placed on the seabed of a fishing ground: 10 pots contained bait made of *shochu* waste stillage; 10 pots contained mackerel; and 10 pots did not contain any bait (control). The pots were hauled up 11 times in the morning and the evening.

In the laboratory experiment, it was observed that the octopuses entered both the pots containing bait made of *shochu* waste stillage and mackerel, but did not enter the pot that did not contain any bait. Similar results were obtained for the field experiment. We observed that octopuses were caught with both the pot containing *shochu* waste stillage and mackerel.

## 60. Proposed method to measure and analyze olfactory response, with the aim of establishing an objective diagnostic method for central olfactory impairment

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Attempts have been made to elucidate the condition and quantify the level of olfactory impairment using diagnostic techniques with non-invasive measurement systems such as electroencephalography (EEG), magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI).

We propose a method for measuring and standardizing uncus and parahippocampal gyrus behavior with fMRI as an objective diagnostic technique for central olfactory impairment.

In this study, to measure brainwave activity accompanying olfactory stimuli, we used a “blast method type odorant stimulator” in conducting oddball tasks of smell with 4 healthy people.

The analysis showed activation in the uncus and parahippocampal gyrus, and fitted responses. Activation was also identified in the cingulate gyrus, middle frontal gyrus, precentral gyrus, postcentral gyrus, superior temporal gyrus, and insula.

The uncus and hippocampus are known to be areas responsible for olfactory impairment in early-stage Alzheimer's disease and other olfactory impairments. Development of the present measurement and analytical methods may thus lead to applications in methods for objective diagnosis in the future. Progress in studies on relationships between olfactory impairment and brain activity will hopefully lead to the early establishment of objective indicators that are useful in diagnosing olfactory impairment.

## 61. The effect of fragrance of fabric softener on touch of towel

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We studied effects of various kinds of fragrance of fabric softener on feelings of touch of towels. Preceding studies showed that towels presented with fragrance are felt softer than no-fragrance towels. To evaluate the relation of odor and touch, excluding the visual information on the color or externals, etc., the examination method using Head Mounted Display was chosen. Participants evaluated the feeling on a visual analog scale, while touching the towel that made softness constant in the draft chamber with which the fragrance was filled, along the instruction displayed in Head Mounted Display. We prepared 9 kinds of fabric softeners, and classified them into 2 fragrance types, fresh green type and sweet floral type. As a result of the evaluation, participants felt softness of towel while smelling of the sweet floral type than fragrance of fresh type. The results of softness evaluations of the towel were correlative with preference ratings of fragrance ( $p < 0.01$ ), but not with strength ratings of fragrance ( $p > 0.29$ ). This tendency was more obvious in the case of the sweet floral type fragrance. These results suggest that there was cross-modal interaction between fragrance and touch, but this phenomenon was depended on fragrance type. We experience this phenomenon in our daily lives. For example, consumer's evaluation in home use test also showed that fragrance of fabric softener emphasizes a sense of touch in accordance with this study.

## 62. Influence of subjectively-chosen odors on task performance

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Several studies have indicated that cognitive task performance was generally improved under odor exposure. However, in these studies odor stimuli were experimentally prepared without concerning individual differences for odor preference and impression. In this study, participants chose an odor according to their preference or feeling of arousal to perform go-nogo task during the odor exposure. In the go-nogo task, one trial consisted of a single figures presentation on the center of computer screen and required a judgment for whether the number was even or odd (Exp.1; low-difficult task). One block included 147 trials and total of 4 blocks was done (588 trials). In Exp.2 (high-difficult task) a single figures was presented as well as Exp.1, the 1st single figures required a same judgment of Exp.1 (one trial), add to this, the 2nd required whether number was multiple of 3 (one trial), and the 3rd required whether number was bigger than the difference between 1st and 2nd single figures (one trial). One block included each of 49 trials of 3 tasks and total of 4 blocks was done (588 trials). Twenty healthy university students participated in Exp.1 and 26 did in Exp.2. As results, refreshing odors reduced errors on low-difficult task and accelerated hit rate on high-difficult task. Furthermore, favorite odors made feeling more relax on low-difficult task and enhanced mood on high-difficult task. These results suggest that a refreshing odor

might suitably keep vigilance and improve task (with even low or high difficulty) performances.

### 63. The impact of sub-threshold odorants on the odor intensity

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In studies of odor-odor interactions, synergy, in which the mixture smells more intense than the sum of the unmixed components, is rare. But creative professionals, e.g., chefs and perfumers, often report that adding seemingly insignificant amounts of key ingredients can have an impact on aroma or flavor. However, there is little published evidence that sub-threshold odors can enhance the rated intensity of odors or tastes of moderate intensity, such as one might commonly experience in foods and beverages. In the current study, experimenters added sub-threshold concentrations of acetic and butyric acids to concentrations three common flavor compounds at concentrations that gave rise to moderately intense odors. Relative to the odor of the pure flavor compounds, sub-threshold acetic acid increased the rated intensity of all compounds tested to a small, but statistically significant degree. This research shows that sub-threshold odors can have a measurable impact on even moderate suprathreshold odors.

### 64. Effect of odors of grapefruit-oil and lavender-oil on physiological and psychological functions

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We studied potential effects of odors of grapefruit-oil and lavender-oil on physiological and psychological functions in human subjects.

By using 17 young female subjects (21 years old), we measured blood pressure, event related potential (ERP)-P300 latency, stress (salivary amylase activity), and temperature of fingertip as parameters for the subjects' physiological function and performed POMS (Profile of Mood States) test, and Kraepelin additional treatment test as psychological functional tests. These tests were carried out before, immediately after, and one hour after odor exposures with grapefruit-oil, lavender-oil or water (control). The results indicate that shortening of ERP-300 latency and increase of fingertip temperature occurred immediately after grapefruit-oil exposure and significant difference in the mood score of tension/anxiety was found immediately and one hour after lavender-oil exposure.

These results suggest a possibility that grapefruit-oil odor may immediately act on physiological function including improvement of brain activity, whereas lavender-oil odor may have long-lasting effect on psychological function including improvement of mood and relaxation.

### 65. Sensory structure on sweet cream Koku

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Dairy products such as sweet cream have a distinct flavor called *koku*. The term *koku* is peculiar to Japan and is used in association

with various concepts; therefore, explaining this term explicitly is difficult. First, the expressions related to sweet cream *koku* were collected from 53 assessors engaged in flavor research while they ate sweet cream. A total of 26 expressions were obtained. The similarity between 27 expressions (26 expressions related to *koku* and *koku* itself) was investigated on a five-point scale. The collected data were arranged and analyzed using cluster analysis and nonmetric multidimensional scaling. On the basis of the results, 12 expressions related to *koku* (*thickness*, *volume*, *creaminess*, *high milk-fat sensation*, *fattiness*, *cohesiveness*, *denseness*, *kotteri*, *nettori*, *deliciousness*, *rich deep taste*, and *aftertaste*) were extracted. Subsequently, the results of factor analysis (FA), which was conducted using principal axis factoring with the 12 expressions, showed that *thickness* and *volume* did not relate well with the other expressions. These 2 expressions were considered higher-order elements. Next, the following 3 factors were extracted by conducting FA with the remaining 10 expressions: *texture* was attributable to *cohesiveness*, *denseness*, *kotteri*, and *nettori*; *richness in milk-fat* was attributable to *creaminess*, *high milk-fat sensation*, and *fattiness*; and *aftertaste* was attributable to *aftertaste*, *rich deep taste*, and *deliciousness*. The model constructed using the FA result was verified by using a structural equation model (SEM), which revealed the following fit indices:  $GFI = 0.883$ ,  $CFI = 1.000$ , and  $RMSEA = 0.000$ . This model was assumed to explain the concept of *koku*. The path diagram shows that 2 parameters—*thickness* and *volume*—and the 3 factors—*texture*, *richness in milk-fat* and *aftertaste*—are closely related.

### 66. Impact of terpene alcohol metabolism to beer flavor –flavor characteristics of Citra hop-

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It is well-known that various beers contain many flavor compounds derived from barley malts, hops and yeast fermentation, and so on. Among these flavor compounds, terpenoids are mainly derived from hops. Beer researchers have regarded hop terpenoids as important components for the flavor characteristics of beers. However, it has been confirmed that most of the hydrophobic terpene hydrocarbons, (myrcene, humulene, etc.), would be lost during beer production from hop cones or pellets to finished beer. Terpene alcohols are more hydrophilic and easier to remain in wort and beer than terpene hydrocarbons. Especially, linalool has been found in various beers, and been regarded as an important factor for a hop-derived beer flavor. Several researchers have reported that monoterpenoid alcohols are bio-transformed by yeast during beer fermentation. Especially, geraniol is mainly transformed to citronellol. Firstly, we compared bio-transformation of monoterpenoid alcohols among various hop cultivars and confirmed that the concentration of citronellol in beer increased depending on the concentration of geraniol in wort. Citra, new hop cultivar bred in US, contained very high concentrations of linalool and geraniol. We found that the beer

brewed from Citra hop contained higher levels of linalool, geraniol and citronellol in comparison with the beer brewed by using other hop cultivars. Secondly, we examined the synergy of monoterpene alcohols and confirmed that the flavors of geraniol and citronellol were enhanced by occurrence of linalool and that the flavor impression of linalool became citrus by occurrence of geraniol and citronellol. From these results, we proposed that the synergy of monoterpene alcohols contribute to the specific 'citrus' flavor of the beer brewed with Citra hop.

## 67. Influence of oil addition on fishy aftertaste perception in wine with seafood pairing

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The typical combination, white wine with fish, is not completely accepted for Japanese consumer because unpleasant fishy aftertaste is sometimes perceived in wine with Japanese-style fish and seafood dishes. We had reported that iron in wine is an essential cause of formation of fishy aftertaste in wine and seafood pairing. However, the consumer perception difference in acceptance is not explained by the content of iron because that is not related to wine type or the country of origin. On the other hand, fat and oil are usually used for fish and seafood dishes in Western cooking though those are rarely used in Japanese-style fish and seafood dishes. We investigated a hypothesis that oil addition to seafood dishes could suppress the perception of fishy aftertaste in wine and seafood pairing. First, the nature of unpleasant fishy aftertaste was evaluated whether it can be attributed to the development of a taste or a retronasal smell by sensory analysis with or without nasal occlusion. The fishy aftertaste sensation was reduced to baseline when the nose was occluded. Second, influence of olive oil addition to dried scallop on fishy aftertaste perception was evaluated by sensory analysis. Third, flavor releases of carbonyl compounds, which are responsible for fishy aftertaste, were evaluated by SPME coupled with GCMS. The fishy aftertaste and the flavor releases of carbonyl compounds were suppressed by the addition of olive oil. These results suggest that oil addition to seafood suppresses the perception of fishy aftertaste that is likely due to development of a retronasal smell.

## 68. The examination of the effective olfactory stimulation for near-infrared spectroscopy as objective olfactory testing

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The objective olfactory testing is not common. Therefore, we repeated the study using a multi-channel near-infrared spectroscopy (MNIRS) to realize of the objective olfactometry. In this study, we examined two effective olfactory stimulations using MNIRS: one is applied to the anterior nasal cavity and the other with an intravenous injection. We used one healthy male subject who had no nasal disease. The result of olfactometry was normal. We employed a 22-

channel near-infrared spectroscopy device with eight light-incident fibers and seven detector fibers, each with an interoptode distance of 2.5 cm on the frontal region. Isovaleric acid was used for stimulation from nasal cavity and Fursultiamine hydrochloride was used for an intravenous injection. Furthermore, a saline intravenous injection was used as a control. We measured the changes in concentrations of oxyhemoglobin [oxyHb], deoxyhemoglobin [deoxyHb] and total hemoglobin [totalHb] from pre-baseline values. Application of Isovaleric acid to the nasal cavity caused increased changes of [oxyHb] and [totalHb]. The intravenous injection of Fursultiamine hydrochloride caused greater increases of [oxyHb] and [totalHb] than those of [oxyHb] and [totalHb] by the application of Isovaleric acid to the nasal cavity. However, the intravenous injection of Fursultiamine hydrochloride caused a pain of vain. As a result, these olfactory stimulations were useful methods, but they also have demerits. Using both methods will be more useful for the objective olfactometry using MNIRS.

## 69. Olfactory screening test on school children

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Pocket Smell Test (PST) was performed on the 403 school children (3<sup>rd</sup>-6<sup>th</sup> grader, 8-12 years old). PST is forced choice examination of smell identification screening test using 3 types of odors (peanuts, mint, and thinner), and full score is 3. The subject is passed screening test when score of PST was 2 or 3. Three hundred and ninety nine children were within normal range but 4 children whose PST score 1 were not passed the test. There was no child whose PST score was 0. Differences among the grades were only significant between 4<sup>th</sup> and 5<sup>th</sup> grade (5%. Tamhane, Dunnett, Games-Howell, and Dunnett C tests). The 4 children who were not passed PST were investigated by cross-cultural smell identify test (CCSIT). The CCSIT scores of all 4 children were 10, 10, 9 and 8 respectively. Therefore, all the children were diagnosed as normal olfaction. Because there is not so often pediatric patient who consult to smell clinic, the present results were agreeable. Importance of olfaction test with school health check program is lesser than eye and/or hearing tests.

## 70. Clinical analysis in patients with unknown origins of olfactory dysfunction

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There are many possible origins of olfactory dysfunction such as sinusitis, common cold, head injury, etc. Some patient cannot be found their origins of olfactory dysfunction. The present study was designed to clarify clinical features and prognostic factors of olfactory dysfunction with unknown origins. We retrospectively analyzed 418 patients presenting to the Mie University Hospital Smell and Taste Clinic over 8.5 years (2000-2009). They underwent clinical examination by interview, endonasal fiberscopy and olfactory function tests (T&T olfactometry) and intravenous olfactory test (the Alinamin<sup>®</sup> test). We diagnosed cases as olfactory dysfunction with an unknown origin by ruling out sinusitis, common cold, head injury, aging,

drug-induced, brain and psychological diseases. Of 418 patients, 45 (11%) were the cases with unknown origins. In those cases, the ratio of male to female in the cases with unknown origins was significantly higher than that in other olfactory dysfunction. Olfactory function tests revealed that the most cases suffered from severe olfactory dysfunction. Two cases got better by treatment mainly with topical steroid. Other 5 cases did not change their olfactory dysfunction and the one got worse mainly with herbal medicine, Tokisyakuykusann. We recommend the topical steroid therapy as the first choice for treatment with olfactory dysfunction with an unknown origin.

## 71. Effect of odor on neocortical responses to taste in fronto-temporal regions of the neocortex

### -Flavor creation using optical imaging-

Ayano Fujiki<sup>1</sup>, Kana Saito<sup>1</sup>, Tomona Matsumoto<sup>1</sup>, Akio Nakamura<sup>1</sup>, Junichi Ide<sup>1</sup>, Masashi Ishikawa<sup>1</sup> and Kensaku Mori<sup>2</sup>

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It has been well known that some odors enhance the perceived intensity of tastes, especially when the odors and the tastes have a common sensory quality. When ethylmaltol, which possesses a sweet odor, is added to sucrose solutions, the perceived intensity of the sweet taste is enhanced. The addition of ethylmaltol to citric acid solutions, however, does not affect the intensity of the sour taste. In other words, the influence of odors on taste intensities depends on the sensory quality of both odor and taste. To evaluate the effect of specific odors on specific tastes intensities induced by the flavor integration mechanism of the brain, we recorded the neocortical responses to the mixtures of odors and tastes during sensory evaluation of the taste intensities using multi-channel near-infrared spectroscopy (NIRS). When ethylmaltol was added to sucrose solutions and when citral was added to citric acid solutions, we observed a statistically significant increase in the amplitude of the responses to the solutions as compared to the control sucrose or citric acid solutions without odor. However, the addition of ethylmaltol to citric acid solutions or citral to sucrose solutions caused no significant change in the amplitude of the responses when compared to the control taste solutions without odor. The results indicate that the specific odors modified the cortical responses to tastes when odor and taste share a common sensory quality. Thus optical imaging of cortical responses provides means to detect the central modification of the taste responses by specific odor.

## 72. Added flavorings enhance neocortical taste responses in the fronto-temporal regions

### -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 foods. Not only the taste but also the aroma components of the broth contribute well to the enhancement. In addition, added flavorings of dried bonito affect the preference as well as the total characteristics of the flavor. To evaluate the effect of flavorings on taste perceived by the brain, we recorded neocortical activities during sensory evaluation using multi-channel near-infrared spectroscopy (NIRS). For this purpose, we compared the cortical responses to the mixture of amino acid-nucleotide solutions without dried bonito flavorings (odorless broth) and the responses to the solutions with the flavorings (flavored broth). Then, we assessed how the flavorings modified the responses to the odorless broth. When subjects tasted an odorless or a flavored broth, distinct cortical responses were observed in specific regions of the frontal and temporal cortices. The regions with significant responses evoked by the drinking of a flavored broth were quite similar to those evoked by an odorless broth. However, we observed a statistically significant increase in the amplitude of the responses to the flavored broth as compared to that of the odorless broth. Among the seven subjects who participated, five subjects (71%) showed significant increases in the amplitude of the responses to the flavored broth as compared to that of the odorless broth. These results indicate that the flavorings modified the cortical responses to the odorless broth. The optical imaging of cortical responses thus provides a means to detect the modification of flavorings on taste responses.

## 73. Odor liking and food preference evoked from fruit flavors

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The food decision making depends on many factors, such as visual information, experience and knowledge of food. Among these factors, organoleptic information also plays an important role in food choice. Since the perceived images for human from the particular flavor are diverse in accordance with countries, cultural background and genomic difference, food preferences evoked by its flavor would also differ in areas.

In this study, fruit preference was investigated using flavors as an only exogenous stimulus and the relationship between fruit preference and odor liking is discussed. The research was conducted in seven large cities in Asia with each 100 human subjects by using blind sensory evaluation test. The test was performed with seven fruit flavors. The questionnaires were as follows; odor liking, odor identification and preference evoked by sniffing fruit flavors.

First, the relationship study between flavor identification and fruit preference showed that those who could identify the appropriate flavor description gave higher fruit preference compared to the incorrect answer group. At the same time, the odor liking was revealed to relate to the fruit preference.

Although many people could not identify the Grapefruit flavor as Grapefruit note, odor liking or fruit preference was rather high. It was suggested that they showed high preference against Grapefruit flavor by regarding it as alternative citrus flavor which they like on a regular basis.

Finally, the pattern of odor liking or fruit preference was found to be varied by areas. This implicates that organoleptic information interacts with many factors on the food decision making.

#### 74. Relationships between odor and free radical scavenging activity

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Free radicals and reactive oxygen are related to a variety of physiological phenomena. It is well known that the free

radical-scavenging activity (FRSA) might be very closely related to stress. The present study was conducted to investigate FRSA elicited by pleasant and unpleasant smells. We collected saliva before and just after and 10 minutes and 20 minutes after odor stimulation (10 minutes) with n-butylic acid (unpleasant odor) and rosemary (pleasant odor). We studied the FRSA, which was measured with the 1,1-diphenyl-2picryhydrazyl (DPPH) method. Here we show one of the possibilities that the stress release from the unpleasant smell increased FRSA rather than the unpleasant smell itself. From our results, a typical pleasant smell, rosemary, slightly increased FRSA in high concentration of an odor, while an unpleasant smell, n-butylic acid, did not increase FRSA just after the stimulation in high concentration of an odor. However, the FRSA value was significantly increased 10 and 20 minutes after completion of unpleasant odor stimulation. Low concentration of pleasant smell did not significantly increase the FRSA, in contrast low concentration of unpleasant smell did remarkably increase FRSA after completion of the odor stimulation.