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ABSTRACTS

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1. A Male-Attracting Pheromone in the Urine of Female Masu Salmon

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Over the last 20 years, it has been speculated that hormonal pheromones (steroids and prostaglandins) are shared among fish. However, the concept of ''hormonal pheromones'' cannot be easily applied to many other species that have own species-specific reproduction manner. Recently, we identified a sex pheromone other than hormonal pheromones. The masu salmon is an important fish found in Far Eastern Asia. In the spawning season, ovulated female salmon releases a sex pheromone in the urine. This pheromone induces sexual excitement and locomotive behavior in spermiating males. Bioassay-guided fractionation of the urine yielded the male-attracting pheromone that was identified as L-kynurenine. L-Kynurenine is a major metabolite of L-tryptophan in vertebrates. We investigated behavioral responses of immature males, spermiating males, and ovulated females when the synthetic pheromone was introduced into the experimental flume. Collaterally, electroolfactograms to the pheromone was also recorded. Behavior to L-kynurenine was observed only in spermiating males, and the behavior was elicited at even picomolar concentrations. Olfactory response thresholds for the pheromone were 10^{-14} M in precocious spermiating males, 10^{-11} M in immature males, 10^{-9} M in sexually regressed males, and 10^{-9} M in ovulated females. These results show that the pheromone is specific for reproductively mature males in behavioral and olfactory responses. HPLC showed that concentrations of L-kynurenine in ovulated female urine (OFU) were 51–112 times higher than those in the urine of immature females and spermiating males. Other salmonid OFU contains little L-kynurenine. Therefore, L-kynurenine is a sex pheromone in masu salmon. This finding will update the concept or the diversity of fish pheromones.

2. Alarm Pheromone in Male Rats

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Chemical communication plays an important role in various social interactions among individuals of the same mammalian species. In contrast to sexual pheromone communication, little progress has been made in understanding the ''alarm pheromone'' communication among rodents. Six years ago, we found that a stressed male Wistar rat released an ''alarm pheromone,'' which enhanced behavioral responses (e.g., increased freezing, sniffing, and walking and decreased resting behavior), and aggravated the stress-induced hyperthermia (SIH) in recipients. Subsequent experiments had revealed that the alarm pheromones can be divided into two categories according to the body region and testosterone dependency of their production and release: the one released testosterone dependently from the whisker pad evokes behavioral responses, whereas the other released from the perianal region in a testosterone-independent manner aggravates autonomic responses. We then focused on the latter because of its ''primer'' pheromone property and found that this pheromone was water soluble and increased the level of anxiety. It seems therefore likely that various physiological as well as behavioral responses evoked by the alarm pheromone, such as aggravated SIH in the home cage, increased defensive and risk assessment behaviors in a modified open-field test, and enhanced acoustic startle responses, are all secondary to the increased anxiety. In addition, the vomeronasal organ-excised (VNX) males fail to show the autonomic response to the pheromone, suggesting that the vomeronasal system is involved in the perception of the alarm pheromone. Now, we are trying to identify the chemical nature of this unique alarm pheromone.

3. Mechanisms for Neurotransmission from Mouse Taste Buds

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Taste buds are aggregates of 50–100 cells, and based on the patterns of gene expression, these cells have been classified into Types I–III. Type II cells express G protein–coupled taste receptors and taste transduction proteins, whereas Type III cells form specialized chemical synapses on sensory fiber and have exocytotic machinery. Thus, we have called these as ''receptor cells'' and ''presynaptic cells,'' respectively, to reflect their functional properties. We have used cellular biosensors to identify the transmitters released from taste buds upon taste stimulation and to study the mechanisms of neurotransmitter secretion. Ca^{2+} imaging of isolated mouse taste cells shows that receptor (Type II) cells respond to bitter and sweet (and presumably umami) tastants, whereas presynaptic (Type III) cells respond to KCl depolarization. Other, unidentified cells are nonresponsive to either of these stimulants. Presynaptic (Type III) cells release 5-HT when stimulated (KCl depolarization). In contrast, receptor (Type II) cells secrete ATP when stimulated (tastants). In isolated intact taste buds, ATP is secreted from

receptor cells via pannexin hemichannels. This ATP activates purinoceptors on presynaptic cells to induce 5-HT release, revealing the existence of cell–cell communication within the taste bud. Our results suggest that ATP and 5-HT are important neurotransmitters in taste buds. One or both may activate sensory afferent nerves.

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4. Flavor Creation Using Optical Imaging

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Near-infrared spectroscopy (NIRS) is a noninvasive, optical technique that continuously monitors cerebral hemodynamics for the assessment of functional activity in the human brain. Using multichannel NIRS, we sought to monitor cortical activity during the sensory evaluation period in order to evaluate the effectiveness of flavorings on taste caused by central integration of flavor. We have sought to improve the taste of artificial sweeteners, having bitterness, astringency and aftertaste, by applying sugar-like flavorings. When a subject drank a conditional sugar solution, then after 60 s drank a test sugar or an artificial sweetener solution, we noted that the conditional sugar solution reduced the amplitude of the response to the test solution. In other words, the cortical response to a test solution showed adaptation by the conditional sugar solution. Sugar–sugar self-adaptation was significantly greater than sugar–artificial sweetener cross-adaptation recorded at specific regions of the temporal and frontal cortex. The sugar–artificial sweetener difference in taste can thus be monitored by the difference between the magnitude of sugar–sugar self-adaptation and that of sugar–artificial sweetener cross-adaptation of cortical responses. Sugar-flavored artificial sweetener cross-adaptation tended to come close to sugar–sugar self-adaptation in the subjects who felt improvement by a flavoring. Therefore, the similarity of the adaptation of cortical responses might be an important indicator for screening effective flavorings in order to improve taste. The method of recording cortical responses to various foods with flavors may help improving their perceptual quality.

5. The Effect of Sensate Chemicals to Fragrance and Flavor

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The role of the flavor or fragrance to foods or cosmetics is to make a contribution to characterization of the product. However, the flavor or fragrance are required the functions and effects but not only the purpose of characterization in various cases, as the preference of consumer is wide varieties and time or cost to apply to product development also are restricted. As a result, it is necessary to increase the kind of material to creation for provision of new flavor or fragrance. The sensate chemicals including menthol are the TAKASAGO speciality products that was developed by technology of chemical synthesis. These chemicals have the cool sensation, but they will have wide varieties of different functions according to their concentration (stimulus intensity). These useful features will be utilized to various products. Our study showed that the fresh feeling cannot be expressed only by the strong cool sensation, but the affinity between a flavor type and the cool stimulus is important in oral care products. Moreover, our research suggested that a weak strength of sensate chemicals, which does not feel cold sensation, affects sense of use or fragrance note of a product. We introduce the sensory, hedonic, and physiological response to sensate chemicals from the development example of oral care and hair care products and consider the potential role of the sensate chemicals.

6. Evaluation of Pungency Threshold of Capsiate and Its Analogues in a Non-pungent Pepper ''CH-19 Sweet''

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Capsaicin, a pungent ingredient of red peppers, has been reported to have various physiological functions, most of which are derived from the stimulation of the sympathetic nervous system by binding to TRPV1 (transient receptor potential vanilloid 1) receptor distributing on the body surface including the gastrointestinal rumen. Strong pungency of capsaicin limits the usage of red peppers as a ''health promoting material.'' Capsiate and its analogues (capsinoids) are ingredients found in a newly bred pepper named ''CH-19 sweet.'' In capsinoids, vanillyl moiety and fatty acid chains are bonded together by the ester bond instead of the amide bond in capsaicin. Similarly, as capsaicin, capsinoids activate TRPV1 and thus enhance energy expenditure. In this study, capsinoids' pungency after oral ingestion was evaluated. Due to lability of the ester bond in capsinoids in aqueous media, we modified the common Scovil method, where edible oil was used instead of aqueous solution for extraction of capsinoids or capsaicin from the fruit. The thresholds to sense the pungency of capsinoids were in the range of 3.0–7.5 g/kg-oil and capsaicin was 0.0041 g/kg-oil. Thus, capsinoids have approximately 1/1000 lower pungency compared with capsaicin. This low pungency will be advantageous to make ''CH19 sweet'' a new health promoting material.

7. *Six1* Gene in the Morphogenesis of Mouse Lingual Papillae

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Six1 gene, which is a mammalian homologue of *Drosophila sine oculis*, is known to function as a transcription factor, and its deficiency leads to abnormal structures of the inner ear and olfactory organ. We examined whether *six1* controls the structure of lingual papillae, in which taste buds are located. Using cRNA probes, we found *six1* mRNA to be expressed in the bottom epithelium of the trench wall of mouse circumvallate and foliate papillae from embryonic day (E)

15 to postnatal day (P) 0 by *in situ* hybridization. From E17.5, *six1* was expressed also in von Ebner's glands. The trench wall of the circumvallate papillae was also immunoreactive towards antibody against *six1*. In the developing fungiform papillae, *six1* was expressed in the dorsal epithelium at E14.5-E19. By scanning electron microscopic observation of *six1* knockout homozyous mice (*six1*−/−) at E19, circumvallate, foliate, and fungiform papillae were smaller in size than those of wild-type mice; fungiform papillae in the posterior region of the tongue showed a stunted morphology. Also, trench grooves of circumvallate and foliate papillae were shorter in length. Proliferating cells (BrdU-immunoreactive) were much smaller in number than those in the normal mice. IGFBP-4, which is involved in the downgrowth of grooves, was weakly expressed in *six1*−/− mice. No von Ebner's glands were observed. PGP9.5-immunoreactive nerves were reduced in number in fungiform, foliate, and circumvallate papillae of *six1*−/− mice. In contrast, the structure of filiform papillae was similar to that of wild type. Our data suggest that *six1* regulates the structures of taste bud–bearing papillae, grooves of circumvallate and foliate papillae, and perhaps the shape of fungiform papillae.

8. Columnar-Like or Patchy Patterns in Macaque Gustatory Cortex

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At primary gustatory cortex of rhesus macaque, we observed a thickly stained band in layer IV and the weak-stained CO-positive band in layer II/III. I Layer II/III CO-positives fluctuated, and they looked as a columnar-like or patchy. There is a weak stained band at layer VI. The distribution of CO-positive cells are of opposite from the distribution of CB-positive cells and neutrophils. These results may suggest a possible existence of functional column in gustatory cortex.

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9. Expression of FXYD6, a Member of Na KATPase Regulator Family in Taste Buds

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Na KATPase is the transport system responsible for the maintenance of the Na^+ and K^+ gradients across the plasma membrane. Na KATPase is present in all cells to ensure basic cellular homeostasis, but it also contributes to specialized tissue functions; neuronal excitability, Na^+ reabsorption, and so on. Various reports have described that the existence of multiple Na KATPase α, β isoforms permits the production of isozymes with different transport properties. Moreover, recent experimental evidence shows that members of the FXYD protein family specifically associate with and modulate the transport properties of Na, KATPase. In this study, we searched our original full-length cDNA library from the epithelium of circumvallate papillae to identify Na KATPase–related genes expressed in taste buds, and discovered that Fxyd6 was expressed in a subset of taste cells of circumvallate papillae. Double in situ hybridization showed that Fxyd6 was coexpressed with Trpm5 and indicated that Fxyd6 might be related to sweet, bitter, and umami taste signal transduction. To obtain more information about Na KATPase in taste buds, RT-PCR and in situ hybridization were carried out using each Na KATPase α and β subunit isoform. In situ hybridization showed that Na KATPase β1 was expressed in a subset of taste cells and was coexpressed with Fxyd6. Moreover, immunohistochemistry study indicated that Na KATPase α1 might be coexpressed with Fxyd6 in a subset of taste cells. These results suggested that the α1–β1 Na KATPase complexes associated with FXYD6 might be necessary to maintain the intracellular Na^+ concentrations in sweet, bitter, and umami-responsive cells.

10. GPR Expression in the Rat Taste Bud Relating to Fatty Acid Sensing

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G protein–coupled receptor GPR40 and GPR120 were reported as fatty acid receptor in the pancreas and gastrointestinal tract, respectively. To investigate the novel fatty acid recognition receptor on the tongue, we examined the expression of GPR40 and GPR120 in rats. A significant expression of GPR120 mRNA in the epithelium of the circumvallate papillae was detected but not in the nonsensory epithelium, while the expression of GPR40 mRNA was undetectable in the sensory papillae by RT-PCR. Western blotting analysis showed a clear band around 40 kD in the circumvallate papillae, which corresponds to the band of GPR120 in the colon sample as a positive control, indicating that this antibody could recognize a native form of GPR120. In support of the western blotting data, immunohistochemistry using anti-GPR120 antibody revealed that some cells within each taste bud of the circumvallate papillae were stained positively with more intense labeling in the apical part of the cell. GPR120-immunoreactive cells were also found in the taste cells of the foliate and fungiform papillae. CD36 is known as a fatty acid translocase expressed in the taste bud cells and is related to fatty acid sensing in the tongue. Double immunostaining of GPR120 and CD36 revealed that majority of GPR120-immunoreactive cells did not express CD36. These results suggested that GPR120 is expressed in the taste cells of the circumvallate papillae to sense dietary fat, like the receptor expressed in the enteroendocrine cells and raise the possibility that a novel pathway of the recognition of dietary fat in the oral cavity.

11. Distribution of Snare-Associated Proteins in Taste Organs of Rat Oral Cavity

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Taste cells are categorized into basal progenitor (type IV) cells, dark (type I) cells, and light cells. Light cells are subdivided into type II and type III cells according with the presence of synapse. Light cells without apparent synapse are defined as type II cells, and those with synaptic vesicles are named type III cells. It is known that many synapse-associated proteins are necessary for neurotransmitter release at presynaptic membrane in taste buds. Previously, we reported that not only type III cells but also major population of type II cells show the immunoreactivity of synapse-associated proteins in circumvallate papilla. But the relationship between synapse-associated proteins and type II or type III cells is still unknown in other gustatory epithelia of oral cavity. In this study, we performed double immunohistochemistry for three synapse-associated proteins [vesicle-associated membrane protein2 (VAMP2), syntaxin, and synaptosomal-associated protein of 25 kDa (SNAP-25)] and markers of type II or type III cells to foliate papillae, nasoincisor ducts, and soft palate to reveal the relationship between two types of light cells. We use phospholipase C β2 (PLCβ2) as a marker for type II cells and neural cell adhesion molecule (NCAM) as a marker for type III cells. Double immunohistochemistry revealed that many PLCβ2-immunoreactive cells (type II cells) express three synapse-associated proteins as well as NCAM-immunoreactive cells (type III cells) in taste buds in all gustatory epithelia examined. These results indicate that synapse-associated proteins are present in both type II and type III cells in all gustatory epithelia of oral region.

12. Apoptotic Cells in Rat Circumvallate Papillae Following Nerve Injury

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We examined apoptotic cells in degenerating rat circumvallate papillae after glossopharyngeal nerve injury. We detected apoptotic cells by single-stranded DNA (ssDNA) immunohistochemistry and double labeling with α-gustducin; phospholipase Cβ2 (PLCβ2); markers for type II cells; neural cell adhesion molecule (NCAM), a marker for type III cells; and Jacalin, a marker for type IV cells. In normal animals, about 20% of ssDNA-positive cells were labeled by PLCβ2 and α-gustducin but none with NCAM or Jacalin. The number of ssDNA-positive cells reached at maximal level at postoperative day 1. At that time, in addition to type II cells, some type III and type IV cells were also labeled with ssDNA. The present results show that apoptosis occurs at type II cells, and probably, type I cells in normal animals and that all types of taste bud cells die by apoptosis during degeneration.

13. Expression of Taste Receptor Gene in Rat Circumvallate Papillae

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When taste receptor genes express remains unclear, although taste buds with gustatory pore are found after birth in rat circumvallate papillae (CVP). We reported about expression of taste receptor gene in CVP of prenatal rats. In the present study, the posterior portion of the tongue was removed at postoperative days (PO) 6, 9, 12, and 15 after glossopharyngeal nerve injury and gustatory and nongustatory epithelia were dissected from CVP using Laser Micro Dissection System (Leica), and we examine the expression of taste receptor gene in the CVP by RT-PCR. Although taste buds disappear following glossopharyngeal nerve injury, RNAs for α-gustducin and mGluR1 were detected in the trench wall of CVP. These results indicate that similar to the development, taste receptors are expressed in denervated gustatory epithelium even when there are no apparent taste buds. Rats without matured taste bud may receive all kinds of gustatory stimulation, acid, salt, bitter, sweet, and umami.

14. Dopamine β-Hydroxylase Immunoreactivity in Type II and III Cells of the Frog Taste Disc

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We immunohistochemically investigated the existence of dopamine β-hydroxylase (DBH), a noradrenalin-synthesizing enzyme from dopamine, in taste disc cells of the frog, *Rana catesbeiana*. A large number of DBH-like–immunoreactive cells were observed in the taste disc. Those cells all possessed a cell body located in the intermediate layer of the disc, a thick or thin apical process reaching the free surface of the disc, and a single or several basal processes, all characteristics of type II and III cells. The present observations support the argument that noradrenalin may work as a chemical transmitter in the frog taste organ. In the frog taste disc, type III cells are reported to have basal processes that form afferent synapses with the nerve, while the existence of basal processes have not been reported in type II cells. In the present study, we found that type II–like cells possessed a long basal process extending towards the basal lamina. We also found that some apical processes of type II– and type III–like cells ran close together and those cells seemed to form groups. It would be of interest to further clarify whether the long basal processes of type II–like cells form synapses with afferent nerves and to clarify the roles of the group formation of taste cells.

15. Neuroethological Study of Taste Reception in the Axolotl (*Ambystoma Mexicanum*) with Bitter-Masking Materials

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Our previous experiments suggested that the aquatic amphibian, axolotl, prefers salty taste at the lower concentration and hates sour and bitter tastes and that the salt taste transduction is not primarily mediated by amiloride-sensitive sodium channels in the axolotl. In the present study, we examined the mechanism of bitter taste reception by using bitter-masking materials ''BENECOAT BMI-40, BMI-40L (Kao Corporation, Japan)'' that selectively suppressed the bitter taste sensation in humans and bullfrogs. The bitter taste sensation of axolotl was quantified by the rejection ratio [rejection/(rejection + swallowing)] after snapping the gel pellets containing a bitter substance (quinine hydrochloride, denatonium benzoate,

or caffeine). When 3.0% BMI-40 was added to a bitter gel pellet, the rejection ratio was significantly decreased at 1–10 mM quinine hydrochloride and 0.001–0.01 mM denatonium benzoate. On the other hand, addition of BMI-40 to a sour gel pellet showed no effect at 0.01–0.15 M citric acid. These results suggest that the taste masking effect of BMI-40 is selective to bitter taste sensation in the axolotl. Therefore, the mechanism of bitter taste reception in the axolotl may be similar to those in humans and bullfrogs. In addition to behavioral studies, electrophysiological analyses of the bitter taste–masking effect are ongoing. In our preliminary experiments with black-spotted pond frogs (*Rana nigromaculata*), we observed that the bitter taste responses of glossopharyngeal nerve to 10 mM quinine hydrochloride, 10 mM denatonium benzoate, and 100 mM caffeine were suppressed by the pretreatment of BMI-40L (1 ml) on the tongue, confirming the bitter-masking effect on the nerve response level.

16. Phasic Responses of Single Units of the Frog Glossopharyngeal Nerve to Quinine and Chloride Salts: Initial Process of Taste Reception

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Quinine-sensitive units (Q-units) of the frog glossopharyngeal nerve respond to Cl-salts such as NaCl and choline Cl (Ch–Cl). Quinine–HCl (Q) and Cl-salts are quite different taste substances. Q-units show only a phasic response. In the present study, we investigated the specificity of and similarities between taste reception of Q and that of Cl-salts. Bullfrogs (*Rana catesbeiana*) were anesthetized with urethane. Unitary impulses were recorded from a single fungiform papilla using a suction electrode. An electrical surge (onset of stimulus) appeared when the first drop of stimulant reached the tongue surface. The time between the onset of stimulus and appearance of the first impulse were measured as the latency of taste response. The latency decreased and the frequency of impulses increased with increasing concentrations of Q or Cl-salts (NaCl, KCl, LiCl, Ch–Cl). In each Q-unit, the relation between the latency and the frequency of impulses elicit by Q at 0.001–0.5 mM are similar to that elicited by Cl-salts at 1–500 mM, suggesting that single taste cells respond to both Q and Cl-salts. Cross-adaptation experiments between Q and Cl-salts in the present study revealed that Q and Cl-salts interact with different receptor sites. Furthermore, the relation between the latency and the impulse frequency in the response to the stimulus applied secondarily after the response to the first stimulus had declined was identical to that to the first stimulus. The present results suggest that single taste cells have multiple receptor sites and that taste adaptation occur in receptor sites in the surface membrane of the taste cell but not the taste cell soma.

17. Changes in Membrane Currents and Intracellular Calcium of Morphologically Identified Cells of the Frog Taste Disc Elicited by Quinine–HCL Stimulation

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The frog fungiform papilla (taste disc) contains morphologically distinct cells, type I through type III. By using slice preparation of the frog taste disc without enzyme treatment, we have demonstrated that there is a good correlation between electrophysiological characteristics and cell morphotypes in three types of cells (type Ib, type II, and type III cells). Knowing the changes in membrane currents and intracellular Ca^{2+} in taste cells elicited by taste stimulation is important to understand the mechanism of taste reception. In this study, we could record simultaneously changes in membrane currents and intracellular Ca^{2+} of identified taste cells in the bullfrog (*Rana catesbeiana*) taste disc elicited by taste stimulation of quinine–HCl. We used patch electrodes containing calcium green-1 dextran for labeling cells and Ca^{2+} imaging. Under the laser scanning confocal microscope, responses of three types of cells to 10 mM quinine–HCl applied focally to the apical ends of taste cells were recorded. Of the 11 type Ib cells tested, eight cells showed transient inward current and intracellular Ca^{2+} increase. Of the 11 type II cells tested, two cells showed transient inward current and intracellular Ca^{2+} increase. Of the eight type III cells tested, none of the type III cells showed detectable current and intracellular Ca^{2+} responses. From these results, it appeared that type Ib and/or II cells are bitter receptor cells.

18. Analysis of Bitter Binding Protein on Quinine

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Quinine is one of alkaloids, strong bitter taste, has fluorescency under UV radiation, which is useful for the analyses of behavior of bitter compounds in saliva. We have reported that Histatin 3, 5, and 6 and PRP-PE in saliva might bind quinine. The purpose of this study was to examine the dynamic effect of the synthetic peptide of Histatin 5 and PRP-PE to quinine sulfuric acid with the agarose gel electrophoresis method. The degree of the movement of quinine with those chemicals depends on the concentration of these peptides electrophoretically. This result suggested that these two proteins had interactions with quinine. In addition, the relationship between the bitter taste threshold and Histatin 5 concentration in parotid saliva was clarified, where according to bitter taste thresholds the 10 subjects were classified into two groups, one was a high threshold group and the other was low threshold one. Histatin 5 concentrations in parotid saliva of every subjects were measured by ELISA. The concentration in the subjects with low bitter taste threshold for quinine was significantly lower than that of high threshold ones. This result suggests that Histatin 5 in parotid saliva might be a carrier protein of bitter substance.

19. Taste Nerve Responses in Zebrafish

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The stimulatory effectiveness of amino acids, sugars, IMP, and bitter compounds on the external gustatory receptors of the zebrafish (*Danio rerio*) was investigated with the extracellular facial nerve recordings using a glass suction electrode. The taste nerve of the fish responded strongly to L-Ala and L-Pro; moderately to L-Ser, L-Tyr, L-CysH, Gly, denatonium, and quinine; and hardly to other amino acids (L-Arg, L-His, L-Glu-Na, L-Lys, L-Leu, L-Asp-Na, L-Val, D-Ala), betaine, IMP, and sugars. Amino acids were highly effective stimuli for zebrafish gustatory system, as well as for the other fish. The magnitude of taste response to L-Ala was dose dependent in the range of 10–10 mM, with an EC_{50} of 17 μM. The threshold for denatonium, one of the bitter compounds, ranged between 0.1 and 1 mM in this species. We also investigated the response to 8 binary mixtures between each of L-Ala, L-Pro, L-Ser, and L-CysH and either IMP or betaine, which are known to enhance the taste response to amino acids in mammals and fish, respectively. However, it was revealed from the present experiments that IMP and betaine did not have synergistic effect on the taste system of zebrafish. Cross-adaptation study by continuously applying adapting solution (1 mM L-Ala) depress the taste responses to 1 mM L-Pro, L-Ser, L-Tyr, L-CysH, and Gly. These results indicated that those amino acids might share a certain common transduction mechanism.

20. Maternal Zinc Deficiency during Lactation Period Modify the Regulation System of NaCl Preference of Their Developed Pups

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It has been well known that maternal dietary NaCl intake influences weaning rats' salt preferences, and brief exposure to NaCl during early postnatal development enhances adult intake of sweet and salty compounds. However, few data have been published concerning about the maternal milk nutrients such as zinc level and later NaCl preference in their developed pups. We have already shown that short-term zinc deficiency clearly causes the increase of NaCl preference, so we demonstrated whether or not the maternal zinc deficiency during lactation period cause grown rats' NaCl preference using this zinc-deficient system with SD/Slc rats. Zinc deficient (0.7 mg Zn/Kg), low zinc (4.0 mg Zn/Kg), and zinc sufficient as a control (33.7 mg Zn/Kg) diets were fed to the lactating mother during lactation period only (for 3 weeks after birth), and zinc-sufficient diet was fed to the all group's rats after weaning. With water and 0.5 M NaCl solution, 2-bottle preference experiment was undertaken and was shown that maternal low-zinc diets during lactation period caused the increased 0.5-M NaCl preference in their developing pups up to 11 weeks old (after 8 weeks from weaning), even though after their recovery from zinc deficiency. After weaning, significant increases of norepinephrine (NE) and epinephrine (E) in central nucleus of amygdala (CeA) and decreased plasma oxytocin concentration were observed in low-Zn group. However, it was confirmed that oxytocin secretion ability after NaCl loading was not impaired in the low-Zn groups, whereas the NE and E secretion signaling mechanism should be further clarified.

21. Neurophysiological Study on the Mechanism Involved in the Astringent Taste Formation by Acidified Soy Protein

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Human evaluation studies suggest that astringency perceived in the mouth upon ingestion of tannic acid and other substances are generally regarded as dry, puckering sensations, which seem more closely allied to the tactile than to the gustatory sense. On the other hand, perceptual assessments of astringency may be closely linked to bitterness in human tasting study, and most of the electrophysiological studies by experimental animals have shown that the chorda tympani and glossopharyngeal taste nerves, instead of lingual trigeminal nerve, transmit the astringency signal. Therefore, we attempted to show the role of salivary component participation in the occurrence of the astringency of acidified glycinin (one of the isolated soy protein) solution by recording from the rat taste nerve response. Adult female Wistar rats were used for the chorda tympani nerve responses to various acidified glycinin solutions. We showed at first that the human saliva can be used for this analysis and found out that especially the $NaHCO_3$ component had a big influence on the taste nerve activity caused by acidified glycinin solution, instead of the precipitation formation itself (acidified glycinin solution makes the precipitation after alkalization by salivary component around the isoelectric point [pI = pH 6.3]) by the interaction of acidified glycinin and salivary alkali components. Further detailed clarification from the chemical structure–activity relationship must be required.

22. Behavioral Estimation of Enhancing Effects in Taste Enhancer

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Glycine ethyl ester (GOE) is one of the salty taste enhancers. To estimate the enhancing effects, various mixture of NaCl and GOE were offered to mice one after another, and their licking behavior was analyzed. After the conditioned taste aversion (CTA) against NaCl, their licking behavior was reanalyzed. The addition of 0.1 M GOE seemed to boost saltiness same level of 0.1 M NaCl before CTA, but the boosting effects of 0.1 M GOE increased to the level of 0.15 M NaCl after CTA. This result suggests that the enhancing effects of GOE cannot be explained with simple additive logic or multiplicative logic. To make the mice increasing the desire for saltiness, they were fed with diuretic diet containing such as KCl or Spironolactone, for more than 3 weeks. Short-term two-bottle preference tests revealed that the mice fed with diuretic materials showed high distinctiveness for the concentration of NaCl solutions, but they did not have considerable interest in the salty taste enhanced by GOE. These results might suggest that the salty taste enhanced by GOE gives less satisfaction for the people who want to take some salty food physiologically, such as postexercise.

23. Spatiotemporal Coding of Taste Quality and Intensity in Hindbrain

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Taste, besides smell, contributes to our experience of environment, from the pleasure of eating to the formation of childhood memories. How are the information about taste qualities (e.g., salty, sour, sweet, and bitter) and intensity represented by activity in the nervous system? This question lies at the center of a long-standing debate in the field of gustatory neurobiology. In that time, two major theories have emerged that have dominated the literatures. There are the labeled-line theory and the across-neuron-pattern theory. However, it is not yet clear how the nervous systems process the information about taste quality and intensity. The peripheral taste nerves code taste qualities based on spatiotemporal activity patterns across the nerves. The gustatory information transmitted from the peripheral nerves is processed in hindbrain based on the neuron types, which have multiple sensitivities to taste qualities. In order to study how the gustatory information is encoded in the hindbrain receiving the temporal patterns of peripheral nerve activities, we propose a neural network model of the hindbrain. We show that by short-term synaptic plasticity, the temporal pattern of peripheral nerves is separated to phasic and tonic activities of hindbrain neurons and then both the activities are bound in a higher region to form a dynamical attractor representing a memory of taste quality. The firing pattern elicited by the phasic responses may contribute to fast perception of taste qualities, whereas that elicited by the tonic response may serve for further analysis of taste quality and intensity.

24. The Taste Difference in Kanto and Kansai Area People of Around 20 Years Old by Marked Expression Characteristics of Taste Receptors

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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 and THTRs by RT-PCR (SCREP method). In this study, expression characteristics of taste receptor in Kanto and Kansai areas were measured by SCREP method. The subject, normal person that the people were not noticed of dysgeusia, 18- to 25-years old, Kanto that the people lived in around Tokyo, and Kansai that the people lived in Osaka and Okayama were recruited by Showa Women's University. The expression characteristics of taste receptors were measured by SCREP method about these subjects. As a result, the taste receptors as hTAS2R9, 10, 16, and 48 were expressed over 45% subject lived in Kanto and Kansai areas. These were marked no difference between Kanto and Kansai areas. It is suggested that these receptors could potentially be viewed as common taste. In contrast, hTAS2R1, 7, 45, and 49 marked difference in expression characteristics between Kanto and Kansai areas. The expression frequency of these taste receptors were 20–60% in Kanto area, which were higher than Kansai area. It is suggested that these receptors are could potentially be viewed as make difference of Kanto and Kansai taste. At last, hTAS2R3, 4, 8, and 47 were difference between Osaka and Okayama, both in Kansai area. It is suggested that these receptors could potentially be viewed as make the taste of area specify. This study depended on a Grant-in-Aid for Scientific Research subsidy in 2005 and 2006.

25. Intragastric Administration of Glutamate Elicits Activation of the Brain Via Vagus Nerve

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Monosodium L-glutamate (MSG), a sodium salt of amino acid L-glutamate (Glu), elicits umami taste. Recent studies suggest the postoral effects of Glu. Consumption of umami substance is decreased in gastric-vagotomized rats. Gastric vagal afferent fibers specifically respond to the intragastric (luminal) administration of Glu, but not to the other amino acids. These results raised hypotheses that ingested Glu is detected by chemoreceptors in the gastrointestinal (GI) tract and the signal is transmitted to the brain via vagus nerve. The goal of the present study was to demonstrate spatiotemporal activation of the brain following intragastric Glu administration (in the absence of taste) using functional magnetic resonance imaging. Rats were anesthetized with α-chloralose and 60 mM MSG solution was infused in the stomach for 10 min. To exclude effects with sodium ion, NaCl (60 mM) was used as control. Second, we assessed a role of the vagal afferent nerve using rats with subdiaphragmatic total vagotomy (TVX). The results showed that only MSG elicited significant activation on several brain regions including the medial preoptic area, dorthomedial hypothalamus, habenular nucleus, and amygdala. Both MSG and NaCl activated the anterior cingulate, caudate-putamen, insular cortex, and hippocampus. TVX eliminated substantially the MSG-induced activation. Our data are the first evidences demonstrating that postoral Glu can activate the brain via vagus nerve. Glu is an important signal molecule on protein ingestion in the GI tract. The brain activation caused by postoral Glu may link to physiological regulations such as body temperature, food preference, emotion, and memory in food intake.

26. Sodium Appetite in Calcium-Deficient Rats

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Several reports demonstrated that calcium-deficient (CaX) as well as zinc-deficient rats enhanced their preference to high concentration of sodium chloride. In the present study, we conducted behavioral experiments to investigate whether or not taste affected the enhancement of preference for sodium in CaX rats. Results were as follows: in the long-term (48 h) two-bottle preference test, the preference percents for 0.3 and 1.0 M NaCl in the CaX rats were higher than those in the control rats, but in the short-term (10 min) test, there was no significant difference in the preference percents

between CaX and control rats. When the CaX rats transected the taste nerves were used for long-term two-bottle preference test, there was no significant difference between the preference percents for 0.3 and 1.0 M NaCl in the CaX and the sham rats. These results suggest that the enhancement of preference for sodium chloride is caused by the postingestive effect rather than taste effect.

27. Effect of Amiloride on Umami Recognition in Human

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Amiloride is the epithelial sodium channel blocker and some studies have shown a suppression of saltiness by amiloride in various mammalian species including humans. Many umami substances have Na^+, so they have both umami and saltiness. Amiloride is used in many umami studies of animals to suppress Na^+ taste, but very little is known about the effect of amiloride in humans. So we examined the effect of amiloride in humans on umami substances including monosodium glutamate (GluNa), monosodium aspartate (AspNa), and for synergistic effect, GluNa + 5′-monophosphate (IMP), AspNa + IMP. Stimuli included 10 μM amiloride. Participants rated the total taste intensity of the stimuli using labeled magnitude scale. For all stimuli, the addition of amiloride produced only small decreases in rating of total taste intensity. Participants were also asked taste quality. For 100 mM GluNa, 100 mM AspNa, and 100 mM AspNa + IMP, taste quality rate of saltiness decreased and the rate of umami increased by the addition of amiloride. For 100 mM GluNa + IMP, no effect was observed with amiloride. Whereas for 10 mM GluNa, 10 mM AspNa, 10 mM GluNa + IMP, 10 mM AspNa + IMP, as their taste intensity were weak, the effect of amiloride was unclear. It is possible that amiloride was effective for recognition of umami substances. Further experiments will be needed to investigate the saltiness suppression mechanisms of amiloride.

28. Effect of Dietary Phytate to Taste Sensitivity in Mice

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Recently, it has been proposed that chronic taste defect is derived from Zn deficiency. Phytate is known to disturb the absorption of divalent cations and to have an antioxidant and anticancer ability. We assumed that continuous excess intake of phytate from supplement or food additives causes taste defect with Zn deficiency. In Zn deficiency, it is known that blood Zn concentration descends, and subsequently, degradation of weight, intake, and drinking water, gustatory buds defect, taste disorder are caused. So, in this study, we examined the effect of dietary phytate to taste sensitivity, two-bottles selection test (quinine versus water) blood Zn concentration, from and observation of their circumvallate papillae with mice (C57BL/6J, female, 7 weeks) which had taken sodium or calcium phytate (0.35, 0.7, 1.4 wt%) for 4 weeks. There are not large difference in blood Zn concentration between control and phytate-fed groups, and they had grown normally. In two-bottles choice test, just as control groups, the preference rate of quinine chloride of phytate-fed groups declined as the concentration rose, and there are no significant differences ($p < 0.05$). These results suggest that dietary phytate (~1.4 wt%) did not lead to Zn deficiency in adult female mice.

29. The Palatability of Linoleic Acid to Mice as Measured by Licking Tests

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Long-chain fatty acids (LCFAs) were reported to be recognized in the oral cavity and possibly involved in fatty foods recognition. However, dietary oil generally consists mainly of triacylglycerol and a low concentration (less than 1%) of LCFAs. The importance of LCFAs on the tongue has still not been determined. Little is known regarding whether the low concentrations of LCFAs are chemically recognized in the oral cavity and followed by the behavioral response, and whether fatty acids have an impact on animals' behavior or preference. To understand the importance of oil recognition in the oral cavity, we investigated the effect of various concentrations of a fatty acid in a licking test. Linoleic acid (LA), which is a main component of corn oil, was used as a representative LCFA. In the licking test for LA, the mice showed the largest number of initial lickings for the 1% LA, while the licking rates for the high concentration of LA decreased. There was an optimal concentration of LA according to the preferences of mice is a low-range concentration (0.25–1%). However, methyl linoleate, which have the same carbon chain and the same number of double bonds as LA, was not palatable to mice in the licking test. Thus, carboxylate group plays an important role in the recognition of LCFA. These results suggested that LCFAs are recognized chemically, and mice could discriminate the concentration of LA in the oral cavity.

30. Mice Discriminate the Concentration of Dietary Oil in the Oral Cavity

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Animals show a strong preference for dietary oil, which is likely to be elicited by stimulation in the oral cavity. To investigate whether mice can discriminate the concentrations of dietary oil in the oral cavity, a short-term two-bottle choice test and a licking test for corn oil as representative dietary oil were conducted. Serial dilution of corn oil (0, 1, 5, 10, and 100% corn oil) was used in the both tests. In the two-bottle choice test, mice were offered a pair of different concentrations of corn oil for 10 min. The mice consistently selected the higher concentration of corn oil in any cases. In the licking test, mice were offered each concentration of corn oil for 30 min and the licking rates were calculated for 60 s from the first lick. In agreement with the result of the two-bottle choice test, the licking rates for the corn oil were increased in a concentration-dependent manner, and pure (100%) corn oil elicited the strongest responses. These results suggest that mice can discriminate the concentration of dietary oil in the oral cavity, possibly from gustatory cues, and that pure dietary oil is a highly preferable solution in mice.

31. Cannabinoid Type-1 Receptors Expressed in the Nucleus Accumbens Regulate the Intake of Preferred Solutions

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The cannabinoid type 1 (CB1) receptor is known to facilitate feeding behavior, especially of palatable food. However, it is unclear which CB1 receptors in the brain are responsible for the facilitation of feeding behavior. In this study, we investigated the role of CB1 receptors in the nucleus accumbens shell in the palatability-induced ingesting behavior. Male Wistar rats were microinjected with the endogenous cannabinoid anandamide or the CB1 receptor antagonist AM251 into the nucleus accumbens shell bilaterally. After microinjections of drug or vehicle, 5 mM saccharin, 0.3 mM quinine, or distilled water was presented for 180 min and the amounts of each solution consumed were measured. Anandamide (1.0, 2.5 μg) significantly increased the intake of saccharin but had no effect on the intake of distilled water or quinine. On the other hand, the inhibition of CB1 receptors by AM251 (2.5, 5.0 μg) significantly reduced the consumption of saccharin and distilled water but had no effect on the intake of quinine. Neither anandamide nor AM251 had significant effects on the locomotor activity when microinjected into the nucleus accumbens shell. These results suggest that the CB1 receptors in the nucleus accumbens shell selectively facilitates the intake of palatable solutions.

32. Prenatal Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Development of Taste Preference in Rats Offspring

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Since our previous study indicated that prenatal exposure to TCDD affected the development of the limbic system, it might also affect various motivated behaviors controlled by the limbic system. In the present study, we investigated taste preference, especially amino acid taste in rat offspring with prenatal TCDD exposure. On the gestational day 15, the appropriate volume (2–3 ml) of TCDD solution dissolved in corn-oil (1.0μg/ml) was administrated to the dams of the TCDD-exposed group based on body weight (1.0 μg/kg) by single intragastric injection using an oral cannula. The control group received only corn-oil in same way. After weaning, taste preference to sapid solutions were measured in a choice paradigm, in which the rats were free access to six amino acid solutions, saline, and distilled water. The results indicated that the female offspring with prenatal TCDD exposure drank less MSG solution than controls. Furthermore, pharmacological analysis of the rat brain specimens indicated that the level of phosphorylated CaMKIIα in the orbital cortex was increased without significant changes in a ratio of phosphorylated CaMKIIα to total CaMKIIα in the TCDD-exposed female offspring and that a ratio of phosphorylated CaMKIIα to total CaMKIIα was significantly decreased in amygdala of the exposed female offspring. The results suggest that TCDD exposure during pregnancy and lactation disturbs the development of amino acid taste preference in female rat offspring through functional changes in the amygdala and orbital cortex.

33. Central Ghrelin Inhibits Reflex Swallowing Induced by Superior Laryngeal Nerve of the Rat

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The effect of ghrelin on reflex swallowing induced by the electrical stimulation of the superior laryngeal nerve (SLN) was examined in urethane-chloralose–anesthetized rats. The administration of ghrelin into the fourth ventricle decreased the number of swallowing during the electrical stimulation of the SLN. The latency of elicited swallowing significantly increased in the presence of ghrelin. The administration of vehicle did not induce any changes. The administration of ghrelin induced dose-dependent inhibition of swallowing. Simultaneous administration of ghrelin and GHS-R antagonist ([D-Lys^3] GHRP-6) did not change the number of swallowing induced by the electrical stimulation of the SLN. Neither mean blood pressure nor mean heart rate changed after the administration of ghrelin. After sectioning both vagi at the cervical level below where the SLN entered the vagal trunk, the inhibitory response of swallowing induced by ghrelin was still observed. Microinjection of ghrelin into the vicinity of the solitary tract produced marked decrease in swallowing number induced by the SLN stimulation. The latency of elicited swallowing significantly increased after the injection of ghrelin. The injection of vehicle (Ringer solution) did not change the number of swallowing. The administration of orexin-A into the fourth ventricle decreased the number of swallowing during the electrical stimulation of the SLN. The latency of elicited swallowing significantly increased in the presence of orexin-A. These results suggest that ghrelin inhibits reflex swallowing modifying the neural activities of the dorsal medulla where swallowing center is present.

34. Promoter Analysis of Human Umami Receptor, T1R1 Gene

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The T1R family (T1R1, T1R2, and T1R3 receptors) has a role in the detection of umami and sweet tastes in taste buds. The T1R family is also expressed in small intestine, colon, and liver. However, the mechanisms of transcriptional regulation of the human T1R1 gene (*Tas1r1*) have not been elucidated. In this study, we examined the promoter region of *Tas1r1* using the luciferase reporter assay and the electrophoretic mobility shift analysis in the *Tas1r1*-expressing cell line, HuCCT1 and Caco-2. The luciferase reporter assay showed that the 118-bp region upstream of the translation initiation codon for *Tas1r1* had promoter activity. The putative Sp1 binding

site was identified in the 118-bp region. Site-directed mutagenesis indicated that the putative Sp1 binding site of *Tas1r1* might represent a binding site recognized by the specific positive regulatory element. The supershifting analysis with HuCCT1 nuclear extracts and specific antibodies against Sp1 showed that Sp1 is capable of interacting with the Sp1 binding site of *Tas1r1*. These results show that Sp1 may play a role as the transcription factor regulating *Tas1r1* promoter activity in HuCCT1 and Caco-2 cells.

35. Mash1 Regulates Taste Bud Cells Differentiation

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The Notch pathway is involved in determining cell fate within the nervous system and in various sensory organs. For example, *Mash1* is expressed in subsets of neuronal precursors in both the central nervous system (CNS) and the peripheral nervous system (PNS). Disruption of the Mash1 gene in mice results in the elimination of most olfactory and autonomic neurons, showing a role for Mash1 in the development of particular neural lineages. In addition, Mash1 promotes differentiation during retinal development and is essential for proper ratios of neural cell types. *Mash1* is expressed in subsets of neuronal precursors in both CNS and PNS. Recently, *Mash1* is expressed in cells of the taste bud lineage and that the expression of *Mash1* in rat taste buds is dependent upon gustatory. However, involvement of the Notch signaling pathway, except for *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 in lingual epithelia, we have investigated the role of *Mash1* in regulating taste bud cell differentiation using forced expression of *Mash1* in lingual epithelia. Forced expression of *Mash1* in tongue epithelial cells induced type III cell markers (AADC and GAD67) expression. These results suggest Mash1 play an important role for differentiation of type III cell in taste buds.

36. Sweet-Suppressing Effect of Gymnemic Acid Examined by Using hT1R2+hT1R3 Assay in Transiently Transfected HEK293 Cells

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Gymnemic acid (GA), triterpene 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 in humans and chimpanzees. In order to examine whether GA directly inhibits the human sweetener receptor, we used the human sweet receptor hT1R2+hT1R3 assay in transiently transfected HEK293 cells. Similar to psychophysical studies in humans, GA inhibited the $[Ca^{2+}]_i$ responses of cells heterologously expressing hT1R2+hThR3 to 0.3 mM SC45647, 10 mM D-tryptophan, and 10 mM saccharin. It has also been shown that in human psychophysical study, the sweet-suppressing effect of GA is diminished by rinsing the tongue with γ-cyclodextrin (CD). So we examined the restorative effect of (α, β, and γ) CDs in vitro. After application of GA followed by each of CDs, only γ-CD showed restorative effect in HEK293T cells expressing hT1R2+hT1R3, though after treatment with α-CD and β-CD, HEK293T cells expressing hT1R2+hT1R3 did not show $[Ca^{2+}]_i$ increase to various sweeteners. Our present study confirmed the previous finding in human psychophysical study that GA suppresses sweet responses and γ-CD diminishes sweet-suppressing effect of GA and also demonstrated that GA directly interacts with hT1R2+hT1R3 and this interaction is inhibited by forming inclusion complex between γ-CD and GA.

37. Temperature Dependency of Two Different Sweet Response Components of the Chorda Tympani Nerve in TRPM5 Knock-Out Mice

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Our previous studies demonstrated that responses of the mouse chorda tympani (CT) nerve to sweet substances have two different components, one is inhibited by gurmarin (Gur) (Gur-sensitive: GS) and the other is not (Gur-insensitive: GI). Two components exhibited temperature-dependent increase (TDI) from 15 to 35 °C. TRPM5 channels, involved in taste transduction for sweet taste, has recently been shown as sensors for temperature from 15 to 35 °C. The TDI of sweet responses, thus, may be occurred through TRPM5. In the current study, we examined TRPM5 dependency of TDI of GS and GI components by comparing the CT nerve responses to sweet compounds before and after the treatment with Gur at three different temperatures (15, 25, and 35 °C) in TRPM5 knock-out mice. We found that responses to glucose (Glc) before and after Gur exhibited TDI. No such TDI was evident in responses to sucrose (Suc) and saccharin (Sac). In addition, responses to Glc were significantly suppressed by Gur at 15, 25, and 35 °C, although responses to Suc and Sac were not significantly suppressed by Gur. These results suggest that (1) there are TRPM5-dependent and -independent pathways involved in the occurrence of GS and GI components and (2) it is possible that unknown pathway other than TRPM5-dependent one may be involved in the TDI of sweet responses, and (3) contribution of TRPM5 in occurrence of response and TDI differs among GS and GI components to different sweet compounds.

38. Responses to Acids in the Mouse Chorda Tympani, Glossopharyngeal, and Superior Laryngeal Nerve

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Human sensory evaluation tests indicated that acetic acid provokes characteristic strong sensation around throat as compared to other

organic acids. In this study, to investigate the neural basis for the characteristic phenomenon, we compared electrophysiological responses to various acids (acetic acid, citric acid, and HCl) among the mouse chorda tympani (CT), glossopharyngeal (GL), and superior laryngeal nerve (SLN). The results indicated that the mouse CT and GL nerves produced responses to all three acids with clear concentration dependencies, whereas the SLN innervating epithelia around throat exhibited such concentration-dependent responses only to acetic acid. These neural response data are comparable with the phenomena observed in human sensory evaluation tests and suggest that there is a possibility that characteristic strong sensation provoked by acetic acid in humans may be due to information derived from the SLN.

39. ATP Release from Mouse Fungiform Taste Cells with Action Potentials

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ATP has been proposed as a key neurotransmitter released from taste cells to gustatory nerve fibers by some recent reports. However, the mechanism has not been elucidated. In the present study, we measured tastant-evoked ATP release from single taste cells with action potentials of mouse fungiform papillae. The recording electrode was positioned on the basolateral membrane of a single taste cell. Just after an increase in the action potentials was observed in response to a taste compound, the electrode solution was collected and applied for luciferase assay. The detection limit of ATP in the luciferase assay was 40 pM. When taste cells showed an increase in the firing rate in response to saccharin, ATP was able to be detected in the electrode solution. The amount of ATP increased in a dose- and firing rate-dependent manner. The results indicate that sweet sensitive taste cells release ATP by taste stimulation and that the ATP release is triggered by the action potentials of taste cells.

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40. Analysis of Taste Responsiveness of Subsets of Receptor Cells in the Mouse Single Fungiform Taste Bud

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A single taste bud contains various types of cells, such as receptor cells, supporting cells, and precursor cells. Among these cells, we have recently shown that taste receptor cells generating action potentials are the cells that transmit major taste information to gustatory nerve fibers. The plural numbers of taste receptor cells with action potentials may exist in a single taste bud. However, it is unclear whether these cells in a bud would possess different taste sensitivity or they would share the same taste sensitivity. In this study, therefore, by using a loose patch recording technique, we recorded action potentials from two different taste cells within a single taste bud separately or simultaneously and compared their taste selectivity to conventional taste stimuli (NaCl, HCl saccharin, quinine, and MSG). In 6 cases, maximum response of each cell was evoked by similar taste stimulation. In 4 of 6 cases, two cells responded to same set of taste stimuli. Whereas in 8 cases, maximum response of each cell was evoked by different classes of taste stimuli, such as saccharin vs. quinine, saccharin vs. NaCl, etc. In 5 of 8 cases, each cells responded to completely different set of taste stimuli. These results suggest that a single taste bud may contain the plural numbers of taste receptor cells having similar and different taste responsiveness.

41. Timing of Differentiation and Innervation of the Basal Cells of Taste Buds during Mouse Embryogenesis

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Taste bud cells are constantly differentiated from the basal cells. We have examined expression patterns of Shh, Prox1, and Mash1 as basal cell markers in the soft palatal region and the anterior tongue of mouse embryo. In situ hybridization analysis demonstrated that Prox1 was coexpressed with Shh from the beginning of Shh expression in a patchy pattern at E14.5 in the soft palatal region and at E12.5 in the anterior tongue. Mash1 expression lagged Shh and Prox1 expression and was initiated at E15.5 in the soft palatal region and at E14.5 in the anterior tongue. The number of Shh-expressing spots on the soft palatal region reached a peak level at E15.5 that was almost equal to the number of the island-shaped clusters of taste buds. Each cluster housed one to four taste buds in adult soft palate. These data suggest that the differentiation of basal cells of taste buds synchronously occurs with the patterning of Shh spots and that multiple taste buds housed in an island in adult soft palate are generated from a single Shh spot in the soft palatal region of embryos. Immunohistochemistry of PGP9.5 and Shh revealed that Mash1 began to express almost simultaneously or slightly after the nerve reached the basement membrane of epithelium where Shh was expressed. These results raise the possibility that the differentiation of Mash1-expressing cells begins nerve dependently, although the differentiation of the basal cells begins nerve independently.

42. Gustatory Response from the Soft Palate in Gustducin-KO Mice

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Gustducin is a taste cell-specific G-protein and involved in the transduction of sweet and bitter tastes. Gustducin is predominantly coexpressed with a bitter taste receptor (T2r) in the circumvallate papillae (CV) and with a sweet taste receptor (T1r) in the fungiform

papillae (FF), indicating a regional difference in gustducin function. In consistent with the expression patterns, attenuation of the gustatory responses in the gustducin-knock out (KO) mouse indicated that gustducin is primarily involved in bitter taste in the CV and in sweet taste in the FF. Type III IP3 receptor (IP3R3) is one of the common critical calcium-signaling molecules for sweet, umami, and bitter signal transduction in the taste cell. IP3R3-expressing cell population includes all cell populations expressing taste receptors for these three tastes. We recently reported molecular evidence suggesting that gustducin is involved in all the three tastes in the soft palate (SP): gustducin was expressed in 96.7% of IP3R3-expressing cells in the rat SP. In the present report, we examined the coexpression pattern of gustducin and IP3R3 in the mouse SP and the neurophysiological responses in gustducin-KO mice from the greater superficial petrosal nerve (GSP) innervating the SP in comparison with the responses from the chorda tympani nerve (CT) innervating the FF. Gustducin was expressed in 91.1% of IP3R3-expressing cells in the mouse SP, and gustducin KO resulted in the reduction of neurophysiological responses to both bitter and sweet stimuli in the GSP. In contrast, 59.6% of IPR3-expressing cells expressed gustducin in the FF, and the significant reduction of responses from the CT was observed only for sweet but not for bitter stimuli. These results support our hypothesis of the SP-specific function of gustducin.

43. Involvement of Lipid Mediators in Extracellular Calcium Sensing of Frog Parathyroid Cells

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Extracellular Ca^{2+} concentrations in vertebrates are maintained constant by the actions of the parathyroid hormone (PTH), 1,25 dihydroxycholecalciferol ($1,25(OH)_2D_3$) and calcitonin (CT). Parathyroid cells containing PTH directly monitor extracellular Ca^{2+} concentration using Ca receptors (CaR). In isolated frog parathyroid cells, the intracellular Ca^{2+} concentration increases in response to high extracellular Ca^{2+} concentrations. High extracellular Ca^{2+} concentration also induces a large increase in membrane conductance. In the present study, we investigated how extracellular Ca^{2+} increases parathyroid intracellular Ca^{2+} concentration while recording the whole cell current. Extracellular Ca^{2+} induced an increase of the inward current at −50 mV in a dose-dependent manner. The apparent EC_{50} for Ca^{2+} was about 6 mM. When 10 μM U73122 (an inhibitor of PLC) was applied extracellularly, the magnitude of 6 mM Ca^{2+}-induced current at −50 mV decreased to 35% of the controls. Internal dialysis of 10 μM U73122 also decreased the response to 33% of the controls, but inactive 10 μM U73343 did not affect the response. Tetrahydrolipstatin (10 μM, an inhibitor of DAG lipase) and methyl arachidonyl fluorophosphonate (1 μM, an inhibitor of MAG lipase) decreased the 6 mM Ca^{2+}-induced current to 10–6% of the controls, respectively. Internal dialysis of 50 μM 2-arachidonyl glycerol (2-AG) induced a Gd^{3+}-sensitive inward current in 3 of 9 cells, but internal 50 μM 2-AG ether did not elicit any response (6 cells). Arachidonic acid also induced the Gd^{3+}-sensitive inward current. The EC_{50} for arachidonic acid was about 14 μM. The results suggest that the arachidonic acid cascade is involved in the extracellular Ca^{2+} sensing of frog parathyroid cells.

44. Effects of Chemical Stimulation of the Amygdala on the Spontaneous Discharge in the Insular Cortex Neurons in Rats

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It has been shown that the amygdala has a major role for conditioning taste aversion. The insular cortex is also demonstrated that this gustatory cortex has an important role for neuronal mechanism in taste learning. Anatomically, there are reciprocal connections between the insular cortex and the amygdala. However, a few electrophysiological studies on the neuronal networks between of these nucleuses have been done. In the present study, we investigated the effect of microinjection of glutamate (Glu) or gamma-aminobutyric acid (GABA) into the amygdala on the spontaneous discharge of the insular cortex neurons in anesthetized rats. Extracellular neuronal activity was recorded by using glass microelectrodes. Pico-Pump was used for pressure microinjection of chemicals. In most neurons, spontaneous discharge was decreased after microinjection of Glu. On the other hand, GABA injection into the amygdala was no effect on the spontaneous discharge. Injection sites were histologically ascertained to be located in the basolateral and around the nucleus of the amygdala. Furthermore, we investigated the changes in spontaneous discharge after electrical train stimulation of the amygdala for 2 s (100 Hz). The spontaneous discharge was decreased after electrical stimulation, similarly as in the case of Glu injection. In the present experiment, no neurons of the insular cortex showed antidromic spikes following electrical stimulation of the amygdala. These results indicate that output from the amygdala may suppress the neuronal activity of the insular cortex neurons via GABAergic neuron.

45. Searching for Distinction of Sweet Expression Group of Monatin-Related Compounds

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Monatin[(2*S*,4*S*)-4-hydroxy-4-(indol-3-ylmethyl)-glutamic acid] is a natural sweet unusual amino acid isolated from *Schlerochiton ilicifolius* in 1992. The sweetness is about 1000–1400 times stronger than sucrose on a weight basis. In 2000, we firstly synthesized monatin. Since then, we have synthesized 20 monatin-related compounds and have studied the taste expression. In order to decide a required conformation for the sweet expression, optimized geometries of monatin and monatin-related compounds were prepared using computer modeling. At the beginning, we calculated their stable conformations and then each global minimum conformation in aqueous medium was obtained by the AM1 semi-empirical method and the conductor-like screening model (COSMO) method in MOPAC module through the energy minimization of the molecule performed by molecular mechanics (CONFLEX/MM2). To search the allocation of functional groups for the sweet expression, monatin-related compounds were superimposed of monatin(2*S*,4*S*) based on hydrophobic group, and every functional group on them close to

that on monatin(2*S*,4*S*) was picked up. Results indicated that allocation of functional groups of some low or no-intensity sweet monatin-related compounds were similar to that of monatin-related compounds with high sweet intensities and suggested that allocation of functional groups was not the only factor to express the sweetness. And so, we considered contribution of reactivity of each functional group for the sweetness expression. Each functional group was classified into two types, low or high reactivity, using nucleophilic/electrophilic superdelocalizability, and their reactivities on high-sweet monatin-related compounds were significantly higher than those on lower ones. Hence, electrophilic/nucleophilic reactivity of the functional group was considered to be one of necessary factors besides proper allocation of functional groups for the sweet expression.

46. Behavioral Analysis of the Taste of Taurine in C57BL/6J Mice

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It was reported that taurine (Tau) contributes to clam flavor (Fuke and Konosu, 1991). However, the taste quality and effect on taste of Tau, especially 50 mM (similar concentration to extract of clam), were unclear. In order to clarify the taste quality and any effect on the taste of Tau, C57BL/6J mice were examined by using CTA experiments. The trained mice were injected with LiCl after intake of 50 mM Tau, MSG + IMP +Ami (presented as a mixture of 50 mM MSG, 2.5 mM IMP, and 30 μM amiloride added to block sodium taste), and 50 mM sodium succinate (ScA2Na), and control mice was injected with LiCl after intake of distilled water. The mice conditioned with 50 mM Tau avoided 50 mM Tau, but did not avoid any basic tastants. This result suggests that B6 mice perceive 50 mM Tau as no basic taste, but any other taste. An aversion to MSG + IMP + Ami generalized to 50 mM Tau + 100 mM ScA2Na, but not generalized to 100 mM SCA2Na. It suggests that taste quality of 100 mM ScA2Na changed to MSG-like (umami) taste after addition of 50 mM Tau.

47. Evaluation of Chocolates Using Organic *Matchas* Cultivated during the 3-Year Phased Fertilizer Reduction

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In an organic tea field, we underwent an annual nitrogen input (N-input) reduction from 635 kg N/ha in 2003 to 435 kg N/ha in 2004, 320 kg N/ha in 2005, and then to 280 kg N/ha in 2006 because too much fertilization is not good for the quality of the underground water. As reported in a previous paper, although the *matcha* (435 kg N/ha, 2004) was not highly evaluated for drinks used in a tea ceremony, it was evaluated to be relatively valuable as an ingredient for *matcha* chocolate. Moreover, as reported last year, the *matcha* (320 kg N/ha, 2005) was also evaluated to be as valuable as the *matcha* chocolate (435 kg N/ha, 2004). Based on these results, we tried to evaluate the *matcha* chocolates to which were added the low N-input *matchas* ((320 kg N/ha, 2005) and (280 kg N/ha, 2006)), along with the *matcha*, which was cultivated in 2006, by application of the average amount of fertilizer as a control. As a result, although the *matcha* chocolate (280 kg N/ha, 2006) was comprehensively less evaluated than the *matcha* chocolate (320 kg N/ha, 2005), it was also evaluated to be nearly as valuable as the *matcha* chocolate (control, 2006). Also, the *matcha* (320 kg N/ha, 2005) was evaluated to be nearly as valuable as the *matcha* chocolate (control, 2006). This indicated that the *matcha* (280 kg N/ha, 2006) was suitable as an ingredient of the *matcha* chocolate, while the *matcha* (320 kg N/ha, 2005) was the most suitable of all the *matchas* as an ingredient of the *matcha* chocolates which were produced during the annual N-input reduction.

48. Contribution of GABA to Taste Sensation Evaluated by Taste Test and Effect of Compounds in Spices on the Activity of GABA Synthetic Enzyme

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GABA synthetic enzyme, glutamate decarboxylase (GAD), is expressed in the type III taste bud by using knock-in mice (Nakamura, et al. 2006. Ajitonioigakkaisi. 13:547). This early study showed the presence of $GABA_A$ and $GABA_B$ receptors by RT-PCR study to suggest that GABA and/or GAD may be involved in the taste signal transduction mechanism. In order to clarify the roles of GABA and/or GAD, we have examined if GABA influences on the human taste sensation and how food components affect the GAD activities. GABA taste tests were performed on healthy young women. It was found that GABA was not tasteless; actually, the majority indicated sour and bitter tastes. In the presence of GABA, umami and salty tastes were enhanced most significantly and then sour taste followed. Since GABA can influence the taste sensation, we have examined the effect of several food components on GABA production by incubating GAD with glutamate and extracts of the food components and the produced GABA was analyzed quantitatively. We have found that most of the examined spices and tea extracts strongly inhibited the GAD activity. On the other hand, fungi and algae extracts showed little effects on the GAD activity. Our present results suggest that GABA is involved in the taste mechanism and its production can be influenced by daily food intake. It is highly probable that some food components may alter the taste sensations via GAD inhibition as well as the traditional way via taste receptors.

49. Characterization of Riboflavin-Binding Protein as a Novel Bitter Inhibitor

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Riboflavin-binding protein (RBP) from chicken egg-white exhibits selective suppression toward protein sweetness such as monellin,

thaumatin, and lysozyme. Since it was found that RBP also suppress bitterness, we studied the effect of RBP on the bitterness of quinine–HCl, naringin, theobromine, denatonium benzoate, caffeine, and glycyl–L-phenylalanine (Gly-Phe). RBP was purified as riboflavin-free form from chicken egg-white by ammonium sulfate fractionation, ion exchange, and gel chromatography. In order to evaluate the bitter inhibition of RBP on various tastants at same levels of bitterness, the point of subjective equality (PSE) of each bitter tastants for quinine was determined. RBP elicited broadly tuned inhibition toward various bitter substances including quinine–HCl, naringin, theobromine, caffeine, Gly-Phe, and denatonium benzoate, whereas other proteins such as ovalbumin and β-lactoglobulin did not alter bitterness. Both bitter tastes of quinine and caffeine decreased after prerinse of mouth with RBP. It was found that RBP binds to quinine but not to caffeine, theobromine, naringin, and Gly-Phe. However, the binding of RBP to quinine seemed not to be responsible for the bitter inhibition because ovalbumin bound to quinine as well as RBP. Considering from these results, it was more likely that the bitter-inhibitory effect of RBP would be due to interaction with taste receptors rather than with bitter tastants.

50. Utilization of L-Leucine Can Reduce the Salt Content in Food

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To find compounds strengthening saltiness, we investigated the effect of bitter substances on saltiness of NaCl solution. The addition of 0.38% L-leucine to 0.8% NaCl solution was shown to strengthen the saltiness of NaCl solution significantly. The addition of L-isoleucine also showed the effect strengthening saltiness. However, L-valine, L-phenylalanine, quinine hydrochloride, and caffeine did not possess the same effect. The addition of 0.38% L-leucine to 0.8% NaCl/0.2% KCl solution was also shown to strengthen the saltiness of its NaCl/KCl solution significantly. This result indicates that the addition of 0.38% L-leucine strengthened the saltiness even if KCl existed in NaCl solution. The saltiness strength of the 0.8% NaCl solution containing 0.38% L-leucine has been shown to be same as that of 0.9% NaCl solution. There was no bitterness in 0.8% NaCl solution containing 0.38% L-leucine. So, we investigated the effect of the addition of L-leucine to consommé soup in order to produce the foods containing low salt content. The following two soups were prepared: soup A (consommé soup containing 0.9% NaCl) and soup B (consommé soup containing 0.8% NaCl and 0.38% L-leucine). The sensory evaluation showed that there were no significant differences in saltiness and bitterness between both soups A and B. From these results, we concluded that the utilization of L-leucine was able to reduce the salt content in foods.

51. Umami Sensitivity of Elderly Females—Comparison with Middle-Aged Females

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We established an easy procedure to measure the threshold value of monosodium L-glutamate (MSG) using rice gruel as a base medium which is familiar for the elderly. The subjects were two groups, elderly females (n = 39, mean age of 84.3 ± 6.1) and middle-aged females (n = 40, 49.6 ± 5.6). Subjects tasted pairs of the control sample (rice gruel containing 0.2% NaCl) and the test sample with MSG (0.063, 0.125, 0.25, 0.5, or 1.0%), and then chose the stronger taste one and the preferable one. The threshold value was 0.5% for the elderly and lower than 0.063% for the middle-aged, respectively. Then we divided the middle-aged into two groups, the 40's (n = 20, mean age of 44.8 ± 2.8) and the 50's (n = 20, 54.3 ± 3.1), and analyzed individual true threshold value which was the lowest MSG concentration of consecutive correct answers of a subject. True threshold values of both the middle-aged were significantly lower than that of the elderly ($p < 0.01$: Mann–Whitney's U-test). There was difference between the distribution patterns of the true threshold values of the 40's and the 50's of the middle-aged group; 40's showed unimodal distribution, while 50's showed bimodal distribution similar to the elderly. Most preferable concentration of MSG was near the threshold value. Most preferable concentration of MSG of the elderly subjects was higher than those of the middle-aged subjects. In summary, as MSG threshold concentration elevated after 50's, preferable concentration of MSG elevated.

52. Sodium Reduction by Umami Taste without Decreasing Palatability—The Effect Of Monosodium Glutamate (MSG) in Miso Soup

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The purpose of this study was to investigate sodium-lowering effect of monosodium glutamate (MSG) in miso soup which is a traditional Japanese soup and to consider how the sensory attributes contribute salt reduction when it takes effect by adding MSG. We made questionnaire that consisted of 19 attributes (7-point category scale) for the evaluation and conducted sensory evaluation of the miso soups containing varied concentrations of NaCl and MSG determined from central composite design. First, we estimated the sodium-lowering effect of MSG from the equation predicting the palatability from NaCl and MSG concentrations. The equation obtained from the data using response surface method was $z = -8.482 + 17.749x + 8.947y - 10.011x^2 - 2.787xy - 10.524y^2$, where x, NaCl (%); y, MSG (%); and z, palatability score. From the equation, the z score of a 0.53% NaCl at 0.26% MSG soup was calculated as high as a 0.88% NaCl without MSG soup. This equality of palatability was also supported by paired comparison. We indicated that sodium content in miso soup can be reduced approximately 30% by adding MSG without decreasing palatability. Next, factor analysis (FA) revealed that the sensory attributes we used had three factors: "total taste intensity," "taste strength," and "brothy taste." These factors were used as the latent factors in SEM. The model obtained from SEM suggested that "saltiness" and "umami" contributed to discrete latent factors. We also indicated that umami increases the palatability of low-sodium miso soup by affecting not "taste strength" but "total taste density" directly when it works effectively for sodium reduction.

53. Estimation of Umami Intensity of Miso Soup Served at Hospitals and Residences for the Elderly

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The threshold value for an umami compound, monosodium L-glutamate (MSG), of the elderly was significantly higher than the middle-aged (Hayakawa *et al*., JASTS. 2007). It should be taken into consideration when middle-aged or young people cook meals for the elderly that there lies the difference in umami sensitivity between them. In this experiment, to estimate the umami intensities of the miso soups which were served at hospitals and residences for the elderly in Tokyo, we collected 30 samples of miso soup from 10 faculties and analyzed their concentrations of umami compounds. Miso soup is a traditional Japanese savory soup and served frequently in such facilities in Japan. Supernatant fraction of each soup was prepared, and then free L-glutamate (Glu) was analyzed with an amino acid analyzer L-8800 (HITACHI) and 5′-purinemononucleotides were analyzed with HPLC using anion-exchange column. The concentrations of Glu ranged from 0.03 to 0.66% (w/v) and those of nucleotides from 0 to 0.03% (w/v), respectively. In several facilities, the Glu concentrations were far below than the optimum Glu concentration of miso soup derived from sensory evaluation with young and middle-aged subjects (Imada et al. 2007. JASTS). In such facilities, umami intensities of the soups were estimated to be too weak for the elderly and the dietitians of such facilities actually reported that the elderly complained about weak umami of the soups. Since every facility has its own recipe to cook miso soup in each region of Japan, we should collect and analyze miso soups around the country to know the trend of their umami intensity accurately.

54. Local Clearance of Gastric Mucosal Serotonin Released by Luminal-Free Glutamate

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More than 90% of 5-HT in the body is localized in the gastrointestinal mucosa. With pharmacological studies, we recently reported that luminal glutamate might stimulate the gastric vagal afferent nerves via mucosal serotonin mobilization and proposed a hypothesis that the mucosal serotonin is used as a paracrine substance to recognize specifically a luminal amino acid (glutamate) by the vagal afferents, like reported in the duodenal glucose sensing. In the present study, we tried to confirm whether serotonin could be produced in the gastric mucosa in response to luminal glutamate or not. Specially modified microdialysis tube was implanted into the rat gastric mucosa and serotonin content in the perfusate was measured with a conventional HPLC/ECD system. Plasma serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the portal vein were also measured. Consequently, mucosal serotonin content could not be significantly changed by intragastric administration of glutamate (50, 150 mmol/L). Plasma serotonin content in the portal vein was not changed even by intragastric administration of 450 mmol/L glutamate as well. However, the content of plasma 5-HIAA in the portal vein was increased in response to luminal glutamate (450 mmol/L). These date strongly indicate that 1) luminal glutamate produced serotonin in the gastric mucosa and 2) the locally produced serotonin was metabolized to 5-HIAA within the mucosa. It was revealed that the rat stomach had a potent clearance mechanism for a luminal nutrient (glutamate)-stimulated serotonin production within the mucosa.

55. Pungency of a ''Non-pungent'' Pepper, ''CH-19 Sweet''

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Pungent red peppers have incorporated into traditional foods worldwide. In addition to the usage of spice, associating various physiological functions derived from capsaicin have been considered to have some health promoting action. Strong pungency of capsaicin, however, limits the usage of red peppers in this context. ''Non-pungent'' pepper, ''CH-19 sweet'' is newly bred pepper that contained ingredients of capsiate and its analogues (capsinoids). Capsinoids have shown to have similar physiological functions as capsaicin. By estimating the oral sensing threshold of each pungent entity, we found that each capsinoid had approximately 1000 times lower pungency than capsaicin. Because CH-19 sweet fruits were known to contain a slight amount of capsaicin, the whole fruits were subjected to the estimation of pungency in this study for its future usage for food. The pungency of CH-19 sweet fruit was evaluated by 2 trials. In the first, the degree of pungency of the fruit was evaluated by a modified Scovil method, the most common method to evaluate the ''hot taste.'' Due to capsinoids' lability in aqueous media, we used edible oil solution instead of aqueous one in our modified method. The pungency of CH-19 sweet was found to be of 1/150 lower pungency compared with Takanotsume. In the next trial, the degree of pungency of CH-19 sweet was separately calculated and summed up based on the ratio of capsaicin and capsinoids contents in fruits. The threshold of pungency was estimated as approximately 1/300 lower pungency compared with Takanotsume. This low pungency checked by the 2 trials will be advantageous to make CH-19 sweet fruit a new health promoting food.

56. Identification of Novel Unique Flavor Compounds Derived from Nelson Sauvin Hop and Development of a New Product Using This Hop

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Nelson Sauvin (NS) is a unique hop variety that was bred and grown in New Zealand. This hop gives a specific flavor (exotic fruit-like, white wine-like) to finished beers. However, the key compounds of this flavor have not yet been identified. We have attempted to

identify the specific flavor compounds derived from NS. We first compared the GC–MS peaks between the products made from NS (NS product) and those from typical aroma hop and discovered three specific peaks from the NS products. These peaks were identified as isobutyl isobutyrate, isoamyl isobutyrate, and 2-methylbutyl isobutyrate. These compounds had a floral flavor like green apple. We think that these isobutyric esters contribute to part of the NS-specific flavor because of their amount in the NS products near their thresholds and their fruity flavors. However, the flavor characters of these compounds were different from the total flavor impression of the NS products. We next focused on certain volatile thiols that are well known to contribute to wine flavors, especially Sauvignon Blanc. The NS product lost its specific flavor by contact with copper. Copper is well known as an absorber of thiols in the field of wine flavor investigations. Therefore, it might point to the existence of thiols. We analyzed the NS product by GC–FPD, GC–olfactometry, and GC–MS and identified some volatile thiols having a grapefruit-like odor, similar to that of Sauvignon Blanc. The volatile thiols would explain the specific odor of beers produced with NS.

57. Impact of Subthreshold Carboxylic Acids on Human Perception of Aroma

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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 some research suggests that the addition of a subthreshold odor can enhance the rated intensity of a suprathreshold mixture. Such studies lend to reports of professionals, for example, chefs and flavorists, who suggest that adding seemingly insignificant amounts of ingredients can sometimes have a noticeable impact on aroma or flavor. We investigated whether adding low levels of carboxylic acids affects on psychometric functions for an aroma compound. Concentration and duration of stimuli were tightly controlled via automated olfactometry. In Experiment, ''subthreshold'' concentrations of carboxylic acids were added to concentrations of the aroma compound that spanned the range from above chance-level detection to below perfect detection. Additivity of detectability was assessed with respect to response-addition (independent processing of mixture-components). Overall, mixture-detection exceeded additivity, except at the lowest concentration. These results suggest that there was some evidence of concentration dependence; the overall picture is detection that exceeds independence, that is, synergy. These studies show that ''subthreshold,'' carboxylic acids can have a statistically significant impact on the perception of aroma.

58. Study on Evaluation of Skim Milk and Reconstituted Skim Milks by Sensor Analysis

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For the purpose of evaluating objectively flavor and taste in skim milk, concentrated skim milk and skim milk powder, skim milk and those two kinds of reconstituted skim milk were evaluated by several methods. Each of them was 10% non-fat milk solids and processed with plate-type pilot UHT (ultra-high-temperature) plant. The first method is sensory evaluation performed with profile tests by a panel of experts, the second is analysis using gas chromatography–mass spectrometry (GC-MS), and the third is the method using sensors such as odor sensor and taste sensor. As to the sensory evaluation, sweetness in skim milk was stronger than reconstituted skim milks, while reconstituted skim milks showed to be slighter in body than skim milk. As to GC–MS analysis in headspace, the amount of acids in skim milk was larger than reconstituted skim milks. It was guessed that acids were involved in sweetness. As to sensor analyses, in odor sensor, skim milk and reconstituted skim milks were discriminated. The result suggested that the amount of acids was involved. However, the difference between concentrated skim milk and skim milk powder could not be discriminated. Using the taste sensor, on the other hand, those three kinds of milks were discriminated clearly. The data obtained by the taste sensor correlated well with the sensory evaluation. It was concluded that analyses by taste and odor sensors were effective as methods evaluating objectively flavor and taste in skim milk, concentrated skim milk, and skim milk powder.

59. The Structure Analysis of *Kansei* Evaluation on Meat Product for Flavoring

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When meat products will be flavored, it is difficult to identify a pair of spice matching a meat, and it is necessary to consider about the synergy or offset effects that these spice brings. In this research, 4 spices were expected effects of meat products is added to a spararib on Kansei evaluation. These samples did not change in physical chemistry. Panel evaluated and compared a sample added other spice with a sample added pepper. The methods of analyzing for Kansei evaluation are principal component analysis and graphical modeling. As a result, it is clear the synergy effects of a spice by the structure analysis eating quality of matched spices. The structure of evaluation in samples which are added a identify spice is formed the interaction in complex evaluations as eating quality. Meat products can be felt ''more delicious'' and ''more desirability'' are brought to realization by a pair of these spices matching a meat.

60. Odor Responses of Descending Neurons and Thoracic Motor Neurons in Male Cockroaches

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In the insect, descending neurons project from the brain to the thoracic motor systems through ventral nerve cord, which carry final information processed in the brain and involve in initiation of

behavior by olfactory cues. In this study, spike responses of the descending and motor neurons to odor stimuli (1-hexanol, volatiles of artificial diet, Periplanone-B) were examined using extracellular recordings in male cockroach (*Periplaneta americana*). Recordings were made from the descending neurons of both side connectives close to the prothoracic ganglion and the motor neurons from both side nerves (nerves 5 and 6) of the mesothoracic ganglion. The motor neurons in nerves 5 and 6 mainly innervate extensor and flexor muscles in the mesothoracic leg, respectively. The descending neurons typically showed odor-dependent phasic excitatory spike responses and significant greater firing rates to the stimulations of the antenna ipsilateral to the recording site. The increase of spike number of descending neurons was dose dependent. The motor neuron activities, synchronized with the descending neuron activities, were similar to the initiation of spontaneous rhythmic leg movements in intact male cockroach. More than half of the descending and motor neurons responded to more than one odor. The results show that the males can make spatial comparisons between their two antennae, these males could be able maintain position themselves by the activities of the descending neurons and the motor neurons to odor sources. The results also indicate that some functional overlapping among the descending and motor neurons involved in odor information carrying to leg muscles for initiation of behavior.

61. Effects of Gonadotropin-Releasing Hormone Antagonist on the Sex Pheromone Sensitivity of Mouse Vomeronasal Receptor Neurons

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The nervus terminalis, a nerve containing gonadotropin-releasing hormone (GnRH) projects to the vomeronasal nervous system, and GnRH receptors are expressed in the vomeronasal mucosa. So, GnRH may have some roles on the vomeronasal sensory system. Our previous report showed that the castrated mice reduced sensitivity of vomeronasal receptor cells to urinary pheromones and that GnRH injection to those mice restored the sensitivity. In order to confirm the direct effects of GnRH on vomeronasal neurons, we introduced GnRH antagonist, cetrorelix acetate and agonist, nafarelin acetate alternately to mice and EOG responses to sex pheromones were recorded. At 40 min after the intraperitoneal administration of GnRH antagonist, EOGs' amplitude in responses to mouse urine were reduced compared to those of nontreated controls. Then 2 h later, GnRH agonist was injected, and after this procedure, the EOGs' amplitude was increased larger than those of controls. While relatively small responses to isoamyl acetate vapor were not affected by these ligands. These results suggest that GnRH could directly enhance the sensitivity of vomeronasal neurons to pheromone odors.

62. Relationship between Receptor Code and Odor Quality in Twelve Odorants

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Different odorants are most likely recognized by different combinations of activated olfactory receptors. However, it is little known whether or not odorants activating a similar subset of receptors present a similar odor quality. Therefore, we compared similarities between 12 odorants both in receptor code and in perceived odor quality. Responsiveness to 12 odorants was recorded in isolated murine olfactory sensory neurons (OSNs) by calcium imaging. Out of 1143 OSNs, 55 were responsive to subsets of the test odorants, and 40 different response profiles were obtained. Similarities of receptor code were estimated from the overlap of OSNs responsiveness in all odorant combinations and allowed 12 odorants to be arranged in 2D-plot using multidimensional scaling (MDS). Similarities of odor quality were evaluated by human sensory evaluation and also allowed 12 odorants to be arranged in 2D-plot. We compared the 2D-plot of receptor code similarity with that of odor quality similarity. The position of each odorant seemed similar in both 2D-plots. This result suggests that odorants sharing higher overlap of OSNs responsiveness present similar odor quality.

63. A Study of Active Regions in the Brain on Fruit Odors Revealed by Functional MRI

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The purpose of this study is to find out the active regions in the brain and evaluating the specific differences between brain responses activated by three kinds of fruit odors using functional MRI. The odorant gas of a fruit obtained by carrying out babbling with the no-odor air which was prepared by 0.5% of concentration (% of the weight) and which humidified (i) n-pentyl acetate (banana odor) (ii) gamma-undecalactone (peach odor) (iii) citral (lemon odor) was given, respectively, using the single reagent which presents a fruit odor as an olfactory stimulus. The odorless liquid paraffin was used as a solvent for manufacture. After buffering an odorant gas of a few fruits in a scent bag, the fruit odor gas which synchronized with breathing was led to the nose mask by the sniffing method. During this fMRI experiment on the olfaction, a subject was obligated so as to recall what the fruit odor is. From the results of fMRI analysis, it was shown that all fruit odors activated commonly the following brain regions such as inferior frontal gyrus, premotor area, and cingulate gyrus. In contrast, the different fruit odors activated the different regions in the brain, for example, orbital gyrus and thalamus were activated for the odor of n-pentyl acetate; on the other hand, amygdaloid and precuneus were activated for gamma-undecalactone. Especially, primary sensory area was only activated for the odor of citral. It was suggested that the activations of entate gyrus, parahippocampal gyrus, and caudate nucleus were accompanied with the task reminding odor name.

64. The Sniffing Effects on Odor Behavior

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Many animals, such as horses, sheep, dogs, cats, rats, and mice, commonly exhibit the sniffing behavior when they are searching the foods or the odors (pheromone). It is believed that sniffing is one of the important behaviors in their life. One of the possibilities is that changes in odor sampling behavior, sniff, strengthen the olfactory receptor cell responses and alter higher order coding. Standard respiration rate in rats is 1–2 Hz but sniffing varies between 4–12 Hz. Olfactory receptor cells are elicited the responses by the absorption of the chemical substances. We used continuously or intermittent (mimicked the sniffing respiration pattern) application of odor stimulation methods and have investigated that changes in sniff frequency could change the level of odor behavior or not, and how sniffing alters odor responses during the different sniffing behavior.

65. Regeneration of Olfactory Nerve after Mild and Severe Injury and Efficacy of Dexamethazone

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The olfactory system has a remarkable capacity for neural regeneration following injury. To investigate factors that influence the degree of recovery after olfactory nerve transection, we studied two injury models using transgenic (OMP-tau-lacZ) mice. Flexible Teflon blade and curved stainless steel blade were used for mild and severe injury nerve transections, respectively. Histological assessment of recovery in the olfactory bulb was made at 5, 14, and 42 days after injury using X-gal staining to label olfactory marker protein (OMP) in the olfactory nerve fibers. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) and CD68 were used as a measure of injury-associated changes in astrocytes and histiocytes. Dexamethazone sodium phosphate (DXM) was injected for 5 days after severe injury nerve transection. With mild injury, there was less injury-associated tissue present between the olfactory bulb, and the cribriform plate of the ethmoid bone and more regenerated olfactory nerves were observed reaching the glomerular layer of the olfactory bulb. Astrocytes and histiocytes with severe injury nerve transection were more than those with mild injury at day 42. DMX-injected animals showed less injury-associated tissue, better olfactory nerve recovery, and less astrocytes and histiocytes. These results suggest that optimum regeneration and recovery in the olfactory system is likely to occur with mild injury and reduced injury-associated tissue formation with less astrocytes and histiocytes.

66. Flavor Creation Using Optical Imaging: Adaptation Phenomenon and Pattern Analysis

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In order to create flavorings, we have recorded changes of the human cerebral blood flow during sensory evaluation, as an indicator of cortical activation using multichannel near-infrared spectroscopy (NIRS). The magnitude of the adaptation of the cortical responses recorded at specific regions of the temporal and frontal cortexes might be important indicators to evaluate the effectiveness of the added flavorings. Stepwise discriminant analysis was performed to classify the sweeteners samples using the cortical responses to the samples. The amplitudes of the responses to the test sweeteners solutions and those to the conditional sugar solutions before and after drinking each test solution, recorded at both left and right cortical regions were effective to divide the sweeteners into two categories. The difference in the two categories could be regarded as significant and this discriminant function was useful for discriminating between the sugar and the sweetener category. In the flavor evaluation, each flavored sweetener sample was classified into one of the two categories in accordance with the effectiveness of the flavoring. According to these findings, the observed difference in adaptations of cortical regions was thought to reflect the difference in taste of sweeteners samples. The method of recording cortical responses to various foods with flavorings may help in improving their perceptual quality.

67. An Improved Gustatory Stimulus-Presenting Apparatus for Measuring Evoked Brain Activity

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To measure the gustatory-evoked brain activity with a higher S/N ratio, we improved a gustatory stimulator by using two optical sensors placed upstream and downstream of the stimulus-presenting hole. The results showed that the onset time of gustatory stimulus was 48.5 ms later than the onset time assumed using the one-sensor apparatus.

68. Prospect of Olfactory Disturbance Screening with a Single Smell of Curry

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The odor stick identification test for Japanese (OSIT-J) has been shown to be useful for detecting and evaluating olfactory disturbance in Japanese people. We show the useful smell of OSIT-J

for olfactory disturbance screening in Japanese. We studied the olfactory disturbance screening with OSIT-J in 83 Japanese participants (49 male, 34 female; average age, 49.7 years old) of the executive checkup at NTT West Kanazawa Hospital in Japan with agreement of participation in this study. Olfactory function was evaluated with three smells of OSIT-J (rose, curry, and sweaty smelling clothes). The three smells of OSIT-J had been shown to have a significant relationship with the Japanese standard olfactory test (T&T olfactometer). Participants with not-full scores of three smells test were evaluated with other 9 smells of OSIT-J. Score eight or less in 12 smells test were regarded as lower olfactory ability. The olfactory ability was self-reported on a grade scale with self-questionnaire. In 38 participants with not-full scores of three smells test, the identification of curry smell was significantly correlated with the olfactory ability in 12 smells test ($P < 0.005$). The identification of rose or sweaty smelling clothes was not significantly correlated with the olfactory ability. Self-reported olfactory ability was significantly correlated with the olfactory ability in 12 smells test in 33 participants with a correct answer for curry smell ($P < 0.05$). Olfactory disturbance screening with curry smell of OSIT-J and self-questionnaire is possibly useful in executive checkup for Japanese.

69. Signal Propagation between the Piriform Cortex and the Endopiriform Nucleus in the Rat Horizontal Slice Preparation

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We have demonstrated by optical imaging of intrinsic signals a possible code for odor concentration in the anterior piriform cortex (PC) (Sugai et al., 2005). Lower concentrations activated the rostral region of the dorsal part of the anterior PC, whereas higher ones generated caudally spreading activation, suggesting an important role of a rostro-caudal gradient in odor sensitivity among cortical neurons. In this study, we made optical recordings from horizontal slice preparations including the dorsal part of the PC and the endopiriform nucleus (EPN) to investigate signal propagation between these two regions. Optical imaging using a voltage-sensitive dye revealed that electrical stimulation of EPN evoked an excitation that propagated slowly to both rostral and caudal directions from the stimulation site in the EPN. On the other hand, stimulation of the PC induced signal propagation from the PC to the EPN. Excitation in the EPN, however, propagated slowly from rostral to caudal, whereas the propagation to the opposite rostral direction was weak. Application of bicuculline produced long-lasting excitation not only in the PC but also in the EPN. These results thus seem to suggest that slow excitation propagation caudally in the EPN is involved in further processing of information from the anterior PC which may be associated with odor concentration.

70. Analysis of Aroma Compounds from Fresh Leaves of Mint and Lavender and the Effects of These Compounds on Autonomous Nerve Activity

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In order to extend the degree of utilization of horticultural plants, we are trying to explore the physiological functions of plants aroma. In most of research reports on physiological functions of aroma compounds from plant resources, extracted oils have been used as samples. In this study, aroma compounds from fresh leaves of Spearmint (*Mentha spicata*) and English lavender (*Lavandula angustifolia*) were analyzed and compared to those of extracted oils. The effects of aroma from leaves on autonomic nerve system were also investigated. Aroma compounds were collected by the adsorption to SPME fiber and subjected to GCMS analysis. Heart rate variability power spectral analysis was adopted for investigating the effects of leaves aroma on the autonomic nerve system. The composition of aroma compounds from mint leaves was almost in agreement with that of mint oil. The major compounds of mint aroma were limonene and carvone. Whereas lavender aroma from leaves contained camphor as one of the major compounds and little linalool, the major aroma compound of lavender oil was linalool, indicating the different composition between leaves and extracted oils. The flavor of mint and lavender leaves showed the tendency to cause the increase of sympathetic nerve activity but no effect on parasympathetic nerve activity. Such results about the autonomic nerve system were discussed relating to those of GCMS analysis of mint and lavender leaves.

71. Major Urinary Proteins Increase the Number of Nestin-Positive Cells in Mouse Vomeronasal Cell Cultures

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The vomeronasal organ (VNO) retains a stem cell population, which continually generates new neurons throughout life. Stowers *et al*. reported that the vomeronasal neuron (VSN) in the transient receptor potential (TRP) C2 channel$^{-/-}$ mouse not only fails to signal but also fails to regenerate VSNs. These findings suggest that the regeneration of VSNs is activity dependently regulated by pheromonal signaling. However, the precise mechanism for the regeneration of VSNs is largely unclear. To address this issue, we examined the effect of natural stimuli on cell proliferation and/or survival using the mouse VNO cell culture, which was prepared from newborn female mice. A lot of mammalian pheromones are secreted in urine. Major urinary proteins (MUPs) are members of the lipocalins family of pheromone-binding proteins and included at high levels in urine. In addition, MUPs are highly polymorphic proteins to code for individuality. Stripped and ligand-bound MUPs and their bound ligands were purified separately from urine of female and male Balb/c mice as natural stimulants. Immunocytochemical staining showed that when MUPs plus their ligands derived from female urine were added to the mouse VNO cell culture, the number of nestin-positive VNO stem cells significantly increased after 2 weeks. Similarly, stripped MUPs derived from male urine caused a significant increase in the number of nestin-immunopositive cells. Some of nestin-positive cells also expressed β-tubulin (class III), a marker for neurons. These results support our hypothesis that MUPs and/or their bound ligands enhance the proliferation of VNO stem cells and the regeneration of VSNs.

72. A New Molecule Subserving Pheromonal Learning: Oxytocin

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The neuropeptide oxytocin (OT) has been implicated in a number of social behaviors, including maternal care, affiliation, and social attachment. We have shown that the mitral to granule cell synapse in the accessory olfactory bulb (AOB) is critical site for olfactory learning in mice, in which a female forms a memory to the pheromonal signal of the male that mates with her. The formation of this memory depends on mating-induced synaptic plasticity (long-term potentiation: LTP) at the mitral to granule cell synapse. Vaginocervical stimulation increases OT in the olfactory bulb. Moreover, the oxytocin receptor is expressed in the AOB. These findings raise the possibility that OT is a critical determinant of the olfactory learning. To test this possibility, we analyzed lateral olfactory tract (LOT)–evoked excitatory synaptic transmission at the mitral to granule cell synapse and its synaptic plasticity in slice preparation of the mouse AOB by field potential recording. OT (2 μM) induced robust LTP at the mitral to granule cell synapse by pairing with subthreshold stimulation (100 Hz, 100 pulses × 2) that only produced short-term potentiation.

73. Effects of Essential Oils on the Proliferation and Degeneration of Immortalized Hypothalamic Neurons

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Aromatherapy has been widely used for the treatment of physical and mental disorders. We have demonstrated that the essential oil of geranium (*Pelagonium graveolens*) was effective in the treatment for premenstrual syndrome in adolescent women and suggested that the geranium oil was implicated in the regulation of hormonal pathways. However, it remains elusive. Here, we observed effects of the essential oils on the proliferation and death of immortalized hypothalamic neurons, which possess the neuronal characteristics and the ability to secrete gonadotropin-releasing hormone (GnRH). GT1-7 cells possess estrogen receptors, and 17beta-estradiol (E2) caused the proliferation of, namely, the increase of cell viability and the extension of neurites. We found that the exposure to essential oils of lavender, mandarin, grapefruit, and geranium caused the increase of cell viability. However, the exposure to the geranium oil with E2 cancelled the effects of E2-induced cell proliferation and exhibited the lower cell viability. Meanwhile, other oils did not influence E2-induced cell proliferation. Furthermore, the geranium oil enhanced the toxicity of tamoxifen, an antagonist of estrogen receptor. These results suggest the possible association of the geranium oil and estrogen receptors. Our developed method which combines in vitro cellular experiments and in vivo clinical tests will be useful in the investigation for the physiological mechanism of essential oils.

74. Synaptic Characterization of Accessory Olfactory Bulb Neurons in Coculture with Vomeronasal Neurons

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To investigate morphological changes of accessory olfactory bulb (AOB) neurons arising from pheromonal stimulation, we have established a coculture system of AOB neurons and vomeronasal (VN) neurons. Recently, our study indicates that the dendritic morphology of the cocultured AOB neurons was different from that of single cultured AOB neurons; the numerical density of dendritic spines in coculture is less than in single culture, whereas the volume of spine head is larger in coculture than in single culture. In the present study, we identified morphological characterization of synapses in coculture using an electron microscope. The 4-weeks–cultured AOB neurons were fixed, scraped out, and centrifuged. The pellets were postfixed and embedded. A variety of synapses were recognized in coculture and single culture, and they were categorized into four synaptic types according to the difference of postsynaptic structure, asymmetrical synapse on dendritic shaft, asymmetrical synapse on dendritic spine, symmetrical synapse on dendritic shaft, and symmetrical synapse on dendritic spine. The rate of synapses on the dendritic spines was higher in coculture than in single culture. In contrast, the rate of synapses on the dendritic shafts was higher in single culture than in coculture. The size of postsynaptic density was measured in each type of synapse. Only asymmetrical synapses on dendritic spine were significantly larger in coculture than in single culture. Some reciprocal synapses were found only in coculture. These results suggest that the synaptic maturation of AOB neurons is induced by coculture with VN neurons as well as dendritic morphology.

75. Noradrenergic Enhancement of Morphological Changes in Cultured Accessory Olfactory Bulb Neurons

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Noradrenergic input to accessory olfactory bulb (AOB) is thought to be critical for modification of dendrodendritic synapses between mitral-tufted (MT) cells and granule (Gr) cells during pheromonal imprinting. However, little is known about the mechanism of noradrenergic action on individual AOB neurons in detail. In present study, we analyzed the noradrenergic effect on dendritic morphology of Gr cells using primary cultured AOB neurons. The bath application of noradrenalin (NA) in AOB culture containing a few GFP-expressing neurons was performed, and the cells were fixed. After immunofluorescent staining, the dendritic protrusions, including filopodia and spines, of GFP-positive Gr cells were observed using confocal fluorescence microscope. The number of filopodia was significantly increased by incubation with 30 or 50 μM NA at 30 min; however, at more than 1 h, there is no significant difference. Small spines, having a spine head less than 1 μm, were also increased at 30 min. In contrast, the number of large spines, having a spine head larger than 1 μM, was markedly increased

by incubation with 50 μM NA only at 2 h. These results suggested that morphological change of dendritic protrusions on Gr cells is enhanced in short time by NA treatment.

76. Generation of Transgenic Rats That Overexpress BDNF in Their Olfactory Receptor Cells

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We have demonstrated that brain-derived neurotrophic factor (BDNF) was expressed by both olfactory receptor cells (ORCs) and sustentacular cells in rat olfactory epithelium. To clarify functional roles of BDNF secreted from ORCs, we generated transgenic (Tg) rats in which BDNF was overexpressed by olfactory marker protein (OMP)-expressing ORCs. In brief, a rat BAC clone (pTARBAC2.1) that contains promoter, coding, and enhancer regions for rat OMP was chosen as a backbone of ''expressing cassette.'' The coding region for OMP was replaced by human BDNFcDNA and cDNA of c-Myc (an antigenic peptide located near the carboxyl terminus of human myc protein) using Red/ET technology. This recombinant clone, named OMP-BDNF-myc RecBAC (size: about 200 kb), was inserted as the expressing cassette into fertilized eggs of Wister rats by ''semi-knock-in'' technology." These eggs were implanted into rat uteri and founders in which the OMP-BDNF-myc RecBAC was inserted into their genomes were determined using southern hybridization technique. Then, the F1 rats of the founders were analyzed immunohistochemically using antibodies to c-Myc and OMP. In transgenic (Tg) F1 rats in which the OMP-BDNF-myc RecBAC was present in their tail cells, OMP-immunoreactive ORCs contained c-Myc immunoreactivity in the supranuclear region of ORC somata. By contrast, c-Myc immunoreactivity was not detected in nasal respiratory epithelium of Tg rats. Thus, we conclude that Tg rats in which human BDNF in addition to rat BDNF was expressed by ORCs were established. Analyses for determining biological effects of overexpressing BDNF by ORCs are underway in our laboratory.

77. Relationship between the Bitterness for Green Leaves Juice and the Feeling for Health—Comparison of the Young and Their Parents Generation

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The taste hedonic tone, taste intensity, and feeling for health were evaluated in the healthy university students (n = 47) and their parents (n = 53). The materials tested were green leaves juice and orange juice, added by 0, 0.16, and 0.32% caffeine. The young group like thinner bitterness for green leaves juice, while in parents group the level of pleasantness for thick bitterness as well as thin one. In both groups, the more pleasant juice was the more useful for health. Whereas the young group, who has more confident for health, thought to be the better for health in orange juice, and the parents group, who has more confident for health, thought to be the better for health in green leaves juice. These findings suggested that feeling for health about green leaves juice might be different between young and parents generation.

78. Influence of the Taste of Preference of the Drink on Alpha and Beta Waves of Electroencephalogram (EEG) and Heart Rate Variation

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Recently, various kinds of drinks are sold. We want to analyze the method of comparatively simple criterion when we take a method to switch a feeling by the drink. Four kinds of drinks (coffee, sports drink, tomato juice, and the maple sugar drink) are tested. Subjects of 4 men and 1 woman drank 60 cc per each drink. After 15 min (preparation time), the experiment is started. After 3 min (rest time, subjects sitting on the chair), subjects drink solution for 30 s. The recovery time is 3 min. Four experiments for each subject are done. We analyzed physiologic influence by drinking and evaluated the taste of the drink with an analog scale. Physiology analyses are EEG, heart rate variation, blood flow, and thermography. ECG and the R-R time interval were analyzed in FFT. HF and LF of the power spectrum were obtained. The change of parasympathetic index value (HF/(HF+LF)) and the sympathetic index value (LF/(HF+LF)) was analyzed. The results are as follows: after drinking, vasodilation is done. Velocity of blood flow level is down, but mass is up. It is supplied with blood in body. In thermography of nose, skin temperature is rose. When individual EEG analysis is done, comfort vote is not there just before and after drinking. Result of Lorenz plot, heart rates increase after drinking.

79. The Effect of TV Commercial on Perception of Bottled Green Tea

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In our daily lives, we are exposed to many commercial messages (CMs) about foods and beverages and use this information to purchase these products. In Japan, we have a trend toward to purchase bottled green tea. The bottled green tea has no strong flavor; thus, differences in sensory properties among these products are not so much. However, these products show a big differential in sales. In this presentation, we would like to show an experiment approaching this phenomenon. In this study, three kinds of bottled green tea and three kinds of CMs were used. At first, participants were presented a CM projected on a screen by a PC and were asked to evaluate expectations for intensity of and preference for the flavor of bottled green tea appeared in the CM. Then they were presented a combination of a glass of bottled green tea and a CM and asked to evaluate intensity of and preference for the tea. All participants received three combinations of tea and CM. The main effects of CMs in preference ratings were significant. That is, preference ratings for green tea were highest when they were presented with CM evoked most

preferable expectation for the flavor. The results seemed to indicate that visual stimulus had evoked objects' image, and which evoked expectation for the following flavor, and then the expectation affect perceived flavor palatability. Preceding related studies suggest that association between expectation and flavor (taste and odor) is developed by the experience through our daily lives and that this association makes objects' image in our brain.

80. Mood Effects of Exposure to Androstadienone in Lavender Oil

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Mood effects of androstadienone (4,16-androstadien-3-one) were examined in 10 healthy male and 10 healthy female Japanese university students (average age: 21 years old). The physiological measures used were pulse rate, blood pressure, and amylase activities in saliva. Mood changes were evaluated by Profile of Mood Scale (POMS), State-Traits Anxiety Inventory (STAI), and visual analogue scales (VAS) on additional adjectives which describe mood. After the baseline measurement, half of the subjects (5 males and 5 females) sniffed lavender oil (1% v/v; diluted with propylene glycol; L) and the other half sniffed lavender oil added with androstadienone at 250 μM (L+A) under normal respiration conditions. The test was repeated after 20 min with the conditions reversed. Positive mood changes such as reductions in Tension-Anxiety and Anger-Hostility scores of POMS were observed after L in males and females, respectively. These effects were lessened with L+A in both males and females, while the state anxiety score of STAI increased in males. No appreciable change in physiological measures was observed in both males and females. The effect of androstadienone alone, as observed by subtracting the values of L from those of L+A, was an increase in ''happiness'' in females.

81. Enhanced Olfactory Sensitivity by Repeated Exposure

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Our previous experiment with young healthy women revealed that their olfactory sensitivity to coprin (vaginal odor) improves significantly with experience but is independent of their menstrual cycle, while that to androstenone (male hormone) sharpens significantly during their ovulatory phase. In the present study, olfactory sensitivity enhancement by repeated exposure was examined with 46 healthy female volunteers of age 19–23 years by introducing two patterns of learning scheme, that is, repeated 4-time exposure either daily or weekly and two sexually neutral odorants, that is, rose-flavored phenylethyl alcohol (PEA) and lemon as a control. Prior to the experiment, individual olfactory perception level was judged by the three-way choice-bottle test of sensitivity to a dilution series of odorants. Additionally, subjects' hedonic preference was measured by a nine-point line scale. It turned out that 1) the olfactory sensitivity to coprin and lemon improved significantly by the daily exposure, but not by the weekly exposure, 2) while neither daily nor weekly exposure to androstenone and PEA did not improve sensitivity. Hedonic preference to coprin, lemon and androstenone and PEA was respectively extremely bad, good, and neutral. It can be concluded that olfactory sensitivity is more easily enhanced 1) by characteristic smells, either very unpleasant or pleasant than by complacent ones; 2) by complex substances as lemon and coprin than by single ones; and 3) by short-interval exposure than by long-interval ones.

82. Study on Psychological Influence with Essential Oils' Odor: An Insight into the Minds Reaction to Aroma Therapeutic Scents

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This study examined whether differentiation of experiences influenced our cognition (pleasant or unpleasant) and psychological levels (change of mood, feeling, and emotion). The odorants were 9 essential oils; geranium oil, rosemary oil, ylangylang oil, palmarosa oil, lavender oil, bergamot oil, mandarin oil, frankincense oil, cinnamon oil and 48 participants smelled 9 odorants at random, then after they estimated for odors. Forty eight participants were divided between 4 conditions: 1) have no experience and information about essential oil, 2) have no experience and information about essential oil, 3) have experienced (aroma-therapist) but have no information about essential oil, and 4) have experience (aroma-therapist) and information about essential oil. Sense of smell is the most unique of the five senses and depends heavily upon the experiences of the individual (Ayabe, Saito, and Kikuchi 2002). Exposure to certain odors varies from person to person, namely I question whether there are differences between experiences of odor and inexperiences of odor. The odorants in question evoked various mental changes depending on the note of odor (on the field in perfume; top-note, middle-note, base-note) present, namely speed that odor fade out, after each odor was examined emotion and pleasure were estimated. Results reflected that essential oils evoked positive emotion, regardless whether the individual had been exposed to the odor before or not. The emotion of pleasure seems to correlate well with the odor, so when the odor fades so does the positive emotions as well. In addition, without regards to information about essential oil, it did not show that the estimation of odor could effect the essential oil's action.

83. Odors Can Increase Placebo Effects: A Study with Female Students

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The placebo effects of a substance (odorless squalene extracted from olive oil) with or without an odor (neroli) on physical states, emotional states, and task performance were explored with 82 female undergraduate students. Changes of subjective ratings on physical and emotional states were obtained, before and after either of two kind placebo explanations (relaxing or stimulating) and breathing with the substance for 3 min. The change of ratings, task performance after breathing with the substance, and subjective

effectiveness of the substance on the performance were compared among four independent conditions, namely 2 (with or without odor) × 2 (instructions on relaxing or stimulating effects). Analysis indicated that physical and emotional states were influenced by placebo explanations. Relaxing instruction decreased activeness more than stimulating instruction, as predicted. An interaction between two factors was observed on the task performance. Relaxing instruction decreased task performance in the condition with the odor, compared with the condition without the odor. However, such difference was not found in stimulating instruction conditions. Subjective effectiveness of the substance on the task performance was also revealed to be related with the presence of the odor. When the substance has the odor, the number of participants who reported effectiveness was increased. These data suggested that placebo effects may be increased when the substance is accompanied with an odor.

84. Comparison of Olfactory Sensibility of T&T Olfactometer Odors—Between Korean and Japanese University Students

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The objectives of this study were to examine effects of the cross-cultural aspect among various factors related to olfactory sensibility. We carried out olfactory sensibility evaluation of five odor samples on each 50 (male: 38, female: 12) Korean and Japanese university students. For this study, we used semantic differential scale which comprised 25 paired adjectives related to olfactory sensibility. Although differences of olfactory sensibility were not dramatically high, some olfactory sensibilities were significantly different between Korean and Japanese university students. In addition, these differences affected the olfactory structure, and ''esthetic sense,'' ''intensity,'' and ''activity'' were common factors but ''clearness'' was a unique sensibility factor of Japanese university students. In conclusion, olfactory sensibility was affected from culture and country characteristics. Therefore, we need to consider cultural sensibility of country in planning production and publicity activity of smell and fragrance products.

85. Association between Serum Leptin and Taste in Elderly

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Leptin is an adipocyte-derived hormone, involved in a variety of biological processes, including energy expenditure, food intake, and energy balance. A few reports have found that elevated leptin influenced the responses to sweet substances in mice, though no known epidemiological study of that association has been reported. We investigated the association between serum leptin and taste sensitivity in community-dwelling elderly subjects (n = 393; 177 males, 216 females; mean age 61.6 years). A taste test, which included four taste qualities (sweet, salty, sour, and bitter), was given to the subjects and blood samples were collected. In females, higher serum leptin levels tended to be associated with lower sensitivity to sweet taste, while there were no clear associations between the other taste qualities and leptin level. In an analysis of subjects with normal BMI (18.5∼22.9), a stronger association was found between sweet taste sensitivity and leptin level in females (P = 0.017). In contrast, leptin levels were lower in subjects with a lower sensitivity to sour taste. In the male subjects, a similar trend between sweet taste sensitivity and serum leptin level was found, though the association was not statistically significant. These results suggest a reverse relationship between sweet taste sensitivity and serum leptin level in elderly females. This is the first known epidemiological study that supports previous experimental findings that response to sweet taste is influenced by elevated leptin.

86. A Therapy for Taste Disorder with Ocuvite®

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Objective: Ocuvite® was developed for an age-related macular degeneration. This supplement contains many elements and vitamins, for example, zinc, copper, selenium, manganese, vitamin B2, vitamin C and so on. In Japan, we often use zinc for therapy of a taste disorder. So, this supplement was hoped for a good effectiveness in a treatment of a taste disorder.

Method: We used Ocuvite® for 10 patients with a taste disorder. A subjective symptom was checked by a visual analogue scale (VAS). Taste was measured with electric gustometer (EGM), and serum zinc was checked before and after.

Results: VAS and serum zinc were increased, but in EGM, taste was not improved.

Conclusion: Subjective symptom was improved, but taste was not improved. It was not cleared which factor improved subjective symptom. It was thought that each elements or vitamins should be studied for a treatment of taste disorder separately.

87. Detection of Gustin by ELISA in Saliva of Taste Dysfunction

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It is known that more than 60% 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. Thus, this investigation was conducted to compare CAVI levels in parotid saliva of healthy subjects with that of taste dysfunction patients and clarify the relationship between zinc concentration

in serum and CAVI levels. Parotid saliva was obtained from 43 healthy subjects and 38 patients of taste dysfunction, respectively. 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 taste dysfunction patients was significantly lower than that of healthy subjects ($p < 0.01$). Middle-class equilateral correlation was recognized between the serum zinc values and CAVI levels (gustin). These results suggest 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.

88. Oral Cancer Patients and Taste Disorder—Including Terminal Patients

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We clinically examined oral cancer and taste disorder. Ten oral squamous cell carcinoma patients with good oral care and oral hygiene were examined with respect to personal food preference. Subjects consisted of 4 males and 6 females, with mean age of 66.5 years (range 35–87 years). Nine cases of squamous cell carcinoma and one case of adenoid cystic carcinoma were demonstrated by histological examination, and the primary sites were the tongue in 7, retromolar in 1, maxillary gingival in 1, and multiple oral cancer in 1 (tongue, lower gingiva, and palate). Subjects were divided into two groups, terminal patients showing progressive disease (6) and outpatients treated with oral anti-tumor drug administration combined with i.v. chemotherapy continuously for than 1 year (4). Taste disorder was judged from the patient's complaints of taste disorder (+ or −) and personal pleasure sensation from intake of food and/or drink based on the respective clinical records and compared to findings in terminal patients determined before the phase of tube feeding (mean duration of 39.2 days before end point). Only one patient complained of taste disorder 1 week post chemotherapy (used TXT+CDDP), but the complaint was limited to one time only. Nine patients, who had a clearer pleasure response, showed a good response to cancer treatment and/or palliative therapy. In oral cancer patients, serious taste disorders could be prevented by oral care, good oral hygiene, and respect for personal food preference, even in terminal patients.

89. Oral Cancer Patient and Taste Disorder and/or Oral Functional Disturbance—Perspective of Patient's Psychological Background

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Many oral cancer patients appear to have psychosocial disturbances and impairments in such basic functions as speech and eating after undergoing radical and/or more aggressive surgery. Six cases of oral squamous cell carcinoma patients, who refused radical surgery, are presented and the significance of taste sensation in personal life is discussed. The initial primary sites were tongue (6), cheek mucosa (1), maxillay gingiva (1), and mandibular gingiva (1). There were 3 males and 3 females, with a mean age of 64.6 (from 35 to 87). Three patients including 87-year-old female were advised to undergo wide resection at another hospital (the estimated survival rates presented to these patients were under 50∼70%). These patients refused such radical procedures that would deprive them of taste function and prevent the pleasure sensations derived from food. All six patients preferred conservative therapy and were treated mainly with oral administration of the anti-tumor drug TS-1. Excluding the severely progressive tongue cancer patient, these patients were treated as outpatients, continuously receiving medication for one more year after our initial examination, and the pleasure sensation of consuming food and drink at supper at home was maintained with their families' support. Oral cancer particularly in elderly patients requires an understanding of the patient's individual dietary preferences (pleasure sensation) and the relation to their maxillo-oral function in order to provide effective anti-tumor therapy. Conservative therapy was considered often more excellent than wide resection of oral and maxillo-facial tissues, which would adversely affect the quality of human life, psychoneuroimmunology, and function of human's chemical senses.

90. Thallium Transport and the Evaluation of Olfactory Nerve Connectivity between the Nasal Cavity and Olfactory Bulb

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Little is known regarding how alkali metal ions are transported in the olfactory nerve following their intranasal administration. The olfactory receptor neurons are bipolar cells with axons extending toward the olfactory bulb. In this study, we show that an analogue of K^+ ion, thallium, is transported in the olfactory nerve fibers to the olfactory bulb in mice. The olfactory nerve fibers of mice (ICR, male, 8W) were transected on both sides of the body under anesthesia. A $^{201}TlCl/^{54}MnCl_2$ solution was administered into the intranasal cavity the next day. Radioactivity in the olfactory bulb and nasal turbinate was analyzed with gamma-ray spectrometry. Auto radiographic images were obtained from coronal slices of frozen heads. The transection of the olfactory nerve fibers was confirmed with a neuronal tracer (fluoro-ruby). The transport of intranasal administered thallium/manganese to the olfactory bulb was significantly reduced by the transection of olfactory nerve fibers. The olfactory nerve transection also significantly inhibited the accumulation of fluoro-ruby in the olfactory bulb. Findings indicate that thallium is transported by the olfactory nerve fibers to the olfactory bulb in mice. The assessment of thallium transport following head injury may provide a new diagnostic method for the evaluation of olfactory nerve injury.

91. Evaluation of the Taste of Japanese Sake Using Surface Plasmon Resonance Chemical Sensor

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Taste sensor utilizing the surface plasmon resonance (SPR) phenomenon was demonstrated for the taste evaluation of Japanese sake. The discrimination of sticky and dry of Japanese sake is possible by analyzing SPR signal for eight kinds of Japanese sakes. It was found from analyzing SPR signal for man-made synthetic Japanese sake that glucose content and alcohol concentration in Japanese sake is effective to discrimination of sticky and dry.

92. Mobile Robot with Early Fire Detection System Using Odor Sensor Array

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Early fire detection using a mobile robot with a MOS-type gas sensor array is demonstrated. It is confirmed that the early fire detection under disturbed odors can be possible by analyzing the outputs of the gas sensor array which consist of four kinds of gas sensors such as CH-E2 type (sensitive to various odors), CH-N type (sensitive to ammonia gas), CH-A type (sensitive to H_2 and various gases), and CH-H type (sensitive to H_2 gas).

93. Development of Lipid/Polymer Membrane for Detecting Sweet Taste Substances

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In this study, we investigated the sweet-sensitivity sensor with lipid/polymer membranes. The sensitivity for sweetness was not adequate for detecting nonelectrolytes such as glucose, fructose, and sucrose because a potentiometric measurement used in the taste sensor. In our previous study, we found that the lipid/polymer membranes composed of tetradodecylammonium bromide, dioctyl phenylphosphonate, and polyvinyl chloride had the sensitivity to sweeteners. The surface of the membrane was modified with phenolic compounds in order to enhance the electric response to sugars. In this study, we examined the potential change and structural activity of the phenolic compounds with different number and the location of phenolic OH groups.

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