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ABSTRACTS

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1. The Salt Taste and Angiotensin Type 2 Receptor Polymorphism

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The role of the renin-angiotensin system in the regulation of blood pressure and the pathogenesis of both hypertension and renal complications involves an intricate interplay of genetic and environmental factors. During recent years, polymorphisms of the genes have been studied, with varying outcomes. ACE inhibitors/ARBs are the first choice of therapy in diabetic patients with hypertension. Adverse reactions to these drugs often cause taste disturbance. However, gene-diet interaction and salt taste sensitivity is unclear. 1) To investigate the effects of heredity on body mass index (BMI) and threshold level for salt taste in adolescence students, 39 students, 22 fathers and 33 mothers were estimated. The threshold of salt taste was positively correlated with those in the mother, but not significantly associated with those of the father. 2) The salt intake and the threshold of salt taste in hypertensive male subjects carrying the C allele of angiotensin type 2 receptor (AT2R) C3123A polymorphism were increased compared with those carrying the A allele. 3) To evaluate the activity of the autonomic nervous system, R-R spectral analysis was measured in 53 healthy females. Total power and a low frequency component in subjects carrying the AA genotype were increased compared with those in subjects with the CC or CA genotype. 4) After a 4-month dietary intervention in 29 elderly hypertensive males, decrease in systolic blood pressure was positively associated with the Na/K ratio in subjects carrying the C allele, but it was not associated with those in subjects carrying the A allele. In conclusion, these findings suggest that these polymorphisms are associated with salt intake and the threshold of salt taste.

2. Facilitation of Repetitive Voluntary Swallowing by Input from Pharyngolaryngeal Water Receptors in Humans

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Swallowing can be initiated either voluntarily or by stimulation of oropharyngeal mucosa. In animal experiments, it has been found that excitation of laryngeal water-sensitive receptors (water receptors) elicits swallowing reflex and that 0.3 M NaCl strongly inhibits the excitation of water receptors. In the present study, we investigated how water receptors are involved in the initiation of swallowing in humans. Each subject was instructed to perform repetitive swallowing as fast as possible. Distilled water (water) or 0.05–0.3 M NaCl solution was delivered to the pharyngolaryngeal region through a fine tube at a slow flow rate (0.2 ml/min). EMG activity was recorded from suprahyoid muscles during swallowing. The intervals between two consecutive swallows in a test were measured. The swallowing interval (SI) was shorter in the case of infusion of water than in the case of infusion of 0.3 M NaCl, suggesting that water stimulation facilitates voluntary swallowing. The water effect was determined by subtracting the SI with water infusion from the SI with 0.3 M NaCl infusion in each subject. Values of SI by voluntary swallowing with infusion of 0.3 M NaCl varied in the subjects. The water effect was plotted against SI with 0.3 M NaCl infusion. There was a linear relationship between them: the longer the SI with 0.3 M NaCl infusion was, the stronger was the water effect. We found that resting saliva facilitates voluntary swallowing, suggesting that resting saliva can excite water receptors. We report here the mechanism of facilitation of voluntary swallowing by inputs from pharyngolaryngeal water receptors.

3. Clinical Implications of Histamine Neurons Activation Driven by Mastication and L-histidine: An Efficient Visceral Fat Reduction and Weight Loss

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The present topics aim to demonstrate that mastication and/or L-histidine which activate histamine neurons are effective on weight and visceral fat reduction. Depletion of neuronal histamine from the mesencephalic trigeminal sensory nucleus (Me5), a mastication center, reduced eating speed, but that from the ventromedial hypothalamus (VMH), a satiety center, increased both meal size and its duration leaving eating speed unaffected. Turnover of neuronal histamine in the Me5 elevated at the early phase of feeding and that in the VMH did at the later phase. This elevated turnover was abolished by gastric intubations of an isocaloric liquid diet or an equivolume of water. Mastication-induced activation of histamine neurons suppressed physiological food intake through H_1-receptor in the hypothalamic paraventricular nucleus and the VMH. On the other hand, the histamine neurons activation accelerated lipolysis particularly in the visceral adipose tissues and up-regulated mRNA expression of uncoupling proteins through sympathetic efferent nerve. Mastication thus plays an important role as a potent input signal to activate histamine neurons. In fact, mastication before meal decreased visceral adiposity even in obese patients. Our recent findings show histamine neurons make a negative feedback loop

tightly with leptin signaling system. Based on those series of our studies, L-histidine was proved effective as brain foods because its oral load activated hypothalamic histamine neurons efficiently as well. Practice of mastication at each meal together with increased consumption of L-histidine–containing foods was useful for improvement of and prevention from morbid obesity and visceral fat accumulation.

4. Analysis of Salt Taste Signal Transduction Using Salty Peptides and KT-1 Cells

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Mammals detect salty tastants with two types of receptors; one that is NaCl-specific, and another that does not discriminate NaCl, KCl and NH_4Cl. It has been known that NaCl-specific reception is inhibited by amiloride, but amiloride has broad inhibitory effect on ion channels and transporters. Another analyzing tool besides amiloride has been accordingly required for elucidating a mechanism of NaCl-specific response.

Here we report two analyzing tools; one is a salty peptide, and another is a KT-1 cell line, derived from mouse tongue epithelial cells. The dipeptide, ornithyltaurine, evoked NaCl-specific response at a single fiber of the mouse chorda tympani nerves. NaCl stimulation caused Na^+ influx to the KT-1 cells in a dose dependent manner. Ornithyltaurine also enhanced Na^+ influx in the KT-1 cells. From these results, ornithyltaurine and KT-1 cells can be used for analyzing a mechanism of salt signal transduction.

5. Maillard Reacted Peptide, the Taste Enhancer that Increase the Intensity of Mouthfulness and Continuity in Food

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Long-term ripened miso has a characteristic mouthfulness and continuity of flavour, which is heightened from 11 months of ripening. Changes of protein and sugar were investigated from 10 days up to 20 months of ripening, however, these changes during ripening did not correlate with sensory evaluation results. From visual observation of miso colour and colourimetric analysis of water soluble fraction, it was apparent that the Maillard reaction was occurring during 5–11 months of ripening. The water soluble fractions of each miso sample which had the different ripening period were analyzed by gel filtration HPLC detected by fluorescence for monitoring maillard reaction. The chromatograms exhibited that the signals were gradually larger according to the ripening period. By fractionation analysis and evaluation of umami, mouthfulness and continuity for each fraction, a water soluble fraction of 20 month ripened miso with a molecular weight of 1000–5000 which was coloured and appeared to be a peptide that has undergone the Maillard reaction. Thereafter sensory evaluation of the Maillard reaction products prepared from the MW 1000–5000 fraction of 5 months-ripened miso revealed that obtained products showed higher score for mouthfulness and continuity than non reacted fraction. From these results, the Maillard-reacted peptide was considered to be a key substance which gives the characteristic flavour (mouthfulness and continuity) of long-ripened miso.

6. Establishment of Technology on Miracle Fruit Tablets and Its Applications

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We have made a successful study of pulverizing miracle fruit berries and making miracle fruit tablets. As a result we have developed the technique to mass-produce miracle fruit tablets for the first time in the world, which has enabled us to carry miracle fruit at room temperature instead of freezing them to preserve after harvest. They have become easier to take by removing their seeds. And by growing them outdoors in Taiwan, we have made it possible to supply them steadily. With regard to producing the tablets, we rent a clean room from a medical company and produce them under sanitary conditions. With the aim of improving the Quality of Life (QOL) of the people whose sugar intake is restricted, including diabetics, we have also produced low-calorie and healthy confectionery by using citric acid. Such cakes taste as sweet as the usual ones when eaten after miracle fruit. We are planning to increase the sorts of food which can make use of the properties of miracle fruit.

I would appreciate it if this study could contribute to familiarizing taste modifier substances/plants to more people and their being used widely for the good of society in the future. This is a report on our study and its results.

7. Distribution and Morphological Features of Taste Buds in Medaka, *Oryzias latipes*

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Medaka was used in many fields, such as developmental biology, histology, biochemistry and molecular biology. Recently, Medaka was used in the study of taste, but no details of the distribution and morphological features of taste buds in the animal were reported. In this study, the distribution and morphological features of Medaka taste buds were studied using light and scanning electron microscopy. Medaka, taste buds were distributed widely in the epithelium of the intra-oral cavity, including the upper and lower lips, oral roof (palate), oral floor, including the tongue, gill (gill archs and gill rakers) and pharynx. The greatest number of taste buds was found in the gill region; fewer occurred within the upper and lower lips. No taste buds were seen in the outer skin surface including the fins. Taste buds of Medaka are classified into three types according to their morphological features and degree of elevation from the surface of the epithelium. The first type is mainly situated in the upper and lower valves, oral floor, tongue and gills, and protrudes from the epithelia. The second type is located on the lip and is sunken within the epithelium. The third type of taste bud is of

a larger size than the other two and is located in the pharynx, sunken within the epithelium.

8. Multiple Receptor Sites for Phasic Taste Responses of the Glossopharyngeal Nerve Revealed by Cross-Adaptation Method in the Frog

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Some taste substances applied to the tongue elicit phasic responses in the frog glossopharyngeal nerve. However, it is not known whether there are multiple taste receptor sites in phasic responses. In the present study, cross-adaptation was carried out by bitter substances, some salts and saccharin-Na. The peak magnitude of the integrated responses of the glossopharyngeal nerve to taste stimuli in bullfrogs (*Rana catesbeiana*) were measured. Bitter substances (quinine-HCl, caffeine, denatonium and theophylline) were scarcely affected after adaptation of salts (NaCl, KCl and NH_4Cl) or saccharin-Na. The present results obtained suggest that bitter substances, the salts and saccharin-Na stimulate different receptor sites. After adaptation of NaCl, the response to KCl was scarcely affected, but the response to NH_4Cl was strongly decreased, suggesting that NaCl and KCl stimulate different receptor sites. After adaptation of KCl, the responses to NaCl and NH_4Cl were strongly decreased. After adaptation of NH_4Cl, the responses to NaCl and KCl were strongly decreased. The results suggest that there exist receptor sites stimulated commonly by NaCl, KCl and NH_4Cl, but a part of the receptor site responsible for the NaCl response may be different from the receptor site responsible for the KCl response.

9. Effects of Osmotic Pressure on the Phasic Component of Bullfrog Taste Responses

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Upon taste stimulation, we have to change, at least, two experimental conditions simultaneously, the concentration of taste substances and the osmotic pressure of irrigating solutions on taste receptors. We had investigated the effect of osmotic pressure on bullfrog taste nerve responses to salts where the osmotic pressure was increased by the addition of nontastants such as urea or sucrose to stimulating salt solutions. We had reported that the magnitude of tonic-response to 0.5 M NaCl was increased by 1 M urea, whereas the magnitude of tonic-response to 1 mM $CaCl_2$ was suppressed by urea at concentrations higher than 0.6 M. Moreover, the addition of 1 M urea to various salts differently increased the tonic-response magnitude of the salts. The extent of increase depended on the differences between the mobility of each cation and anion forming the tested salt. In this study, we focused on phasic-responses, and investigated whether they are modified by hypertonic solutions. The addition of 1 M urea had no effects on phasic-responses to 0.5 M NaCl and 1 mM quinine. In contrast, their magnitudes of responses were modified after the adaptation of 1 M urea for 10 to 40 s. That is, the response to 0.5 M NaCl increased by being dependent on adaptation-time of 1 M urea, whereas the response to 1 mM quinine decreased. These results suggested that the osmotic pressure gradually increases the conductance of tight junctions, and enhances diffusion potentials across them, which depolarize or hyperpolarize the basolateral membrane of taste receptor cells and modify the neural responses.

10. Labeling of the Glossopharyngeal Nerve and Taste Disc Cells by DiI in the Frog Taste Organ

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A taste disc is located in the fungiform papilla of frog tongue. The cells in the taste disc are classified into several types on the bases of morphological and electrophysiological features. Strangely, taste responses have not been recorded to date from cells possessing synaptic connections with afferent nerves. Furthermore, the connection between taste disc cells and afferent nerves has not yet been clarified.

The present study was undertaken to clarify the connection between taste disc cells and afferent nerves. Using the carbocyanine fluorescent dye DiI, we observed the innervation of the glossopharyngeal nerve in the fungiform papilla as well as the connection between the taste disc cells and the nerve in the frog *Rana catesbeiana*. A taste disc along with a branch of the glossopharyngeal nerve was dissected from a frog tongue that had been fixed with paraformaldehyde. DiI was applied to the cut stump of the nerve. After a diffusion period of several days, the taste disc and nerve were examined under a fluorescence microscope. Approximately ten nerve fibers were labeled in each fungiform papilla. Those fibers ran along the blood vessels in the fungiform papilla to form a mesh work of fibers beneath the taste disc. Numerous taste receptor-like cells were labeled by transcellular diffusion of the dye in the taste disc. Those cells showed apical processes reaching the disc surface and their cell bodies were located at the middle or lower layers of the taste disc.

11. Properties of Arachidonic Acid-Induced Currents in Frog Taste Disc Cells

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Frog wing (Ib) cells display parabolic inward currents in response to arachidonic acid. In the present study, we investigated the biophysical properties of arachidonic acid-induced currents while recording the whole cell currents of isolated wing cells in bullfrogs. When 50 μM arachidonic acid was applied extracellularly, the wing cells displayed parabolic inward currents. The currents conducted poorly at the membrane potential of −80 mV despite the presence of arachidonic acid, but their conductance increased at a resting potential of −50 mV. Arachidonic acid (50 μM) elicited inward currents of

−40 ± 9 pA (N = 10) at −50 mV. Replacement of external Na^+ with $NMDG^+$ reversed the inward currents to outward currents. Ca^{2+}-free (1 mM EGTA) saline solution did not change the reversal potentials of the currents, but hyperpolarized the peak by 8 mV. The mean reversal potential of arachidonic acid-induced current was about +4mV in the condition with internal CsCl. Linoleic and docosahexaenoic acids induced similar currents in the wing cells. Potency in these fatty acid was linoleic > arachidonic ≥ docosahexaenoic. There was no difference in the reversal potential between the fatty acids. Internal arachidonic (2 cells) and linoleic (4 cells) acids did not induce any response in the wing cells. The results suggest that polyunsaturated fatty acids do not function as intracellular second messengers in frog wing cells, but elicit parabolic inward currents as external ligands.

12. Denatonium-Induced Ca^{2+} Responses in Mouse Taste Cells Expressing G-proteins

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Denatonium, a synthetic bitter substance, is widely used in many studies of bitter signal transduction, and its transduction mechanisms have been studied in detail. Based on previous studies, a possible mechanism shown in a number of studies is that denatonium activates PLC β_2 via G-protein–coupled receptors.

In this study, we measured the denatonium-induced changes in $[Ca^{2+}]_i$ in isolated mouse taste cells using calcium imaging. After that, we examined the expression of G-proteins, α-gustducin and IP_3R3 in the mouse taste cells responding to denatonium by double immunocytochemical staining.

Of 30 cells responding to denatonium, 18 expressed α-gustducin (60%), and α-gustducin immunoreactive cells were also immunoreactive for IP_3R3 (78%). These data suggest that cells responding to denatonium are possible to have a signaling pathway via G-protein–coupled receptors. Although it was also showed that cells responding to denatonium did not express both α-gustducin and IP_3R3, suggesting a possible G-protein–independent pathway in denatonium signal transduction.

To determine whether intracellular Ca^{2+} increases by release from intracellular stores or by Ca^{2+} influx, we examined responses in the acute absence of extracellular Ca^{2+} and depleted Ca^{2+} stores with 1 μM thapsigargin, an inhibitor of Ca^{2+} transport into intracellular stores.

Our results that the cells did not respond to denatonium in the absence of extracellular Ca^{2+} complemented a G-protein–independent pathway. On the other hand, after thapsigargin treatment, 60% of Ca^{2+} responses were diminished. These data support the notion that denatonium responses involve intracellular Ca^{2+} by release from intracellular stores.

13. Expression of α-gustducin in mouse taste receptor cells that respond to umami stimulus

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A member of Gαi family α-gustducin (Gαgust) is taste tissue specific G protein α-subunit. Recently, it was reported that Gαgust was involved to bitter, sweet, and umami taste sensing. Using anti-Gαgust antibody, we performed immunohistochemistry with TRCs that increased intracellular Ca^{2+} level to umami stimulus (a mixture of 10 mM MSG and 0.5 mM IMP), and investigated the participation of Gαgust to umami transduction. Then, we used the TRCs that isolated from mouse circumvallate and foliate papillae. Thirteen percentage of TRCs (n = 2/15) that responded to mixture stimulus were positive to anti-Gαgust antibody. Eighty-seven percentage of the TRCs (87%, n = 13/15) were negative to anti-Gαgust antibody. Thus, since umami responsive TRCs that expressed Gαgust were few, in the posterior area of tongue, the participation of inhibitory G protein other than Gαgust in umami transduction was suggested.

14. Evaluation of Taste Intensity and Quality of Umami Substances and the Synergistic Effect

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It is thought that a lot of taste substances have an important role in foods. Above all, umami substance is one of the essential elements that contribute flavor of foods. It is known that synergistic effect occurs between L-amino acids and 5′-monophosphate (IMP). We examined taste intensity and quality of umami substances such as L-theanine, which is included in tea, betaine, which is included in marine products, disodium succinate, which is included in shellfish and glutamate salts by psychophysical method using labeled magnitude scale. For all substances, taste intensity and quality changed in various concentrations.

Besides this, we examined synergistic effect of taste intensity and quality when IMP was added to umami substances. For all substances, taste intensity increased and the enhancement of quality was occurred in umami taste in addition of IMP. As for disodium succinate, the degree of synergistic effect was smaller than the other substances.

15. Behavioral Analysis of the Taste of Succinic Acid and Sodium Succinate in C57BL/6J Mice

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It was reported that succinic acid contributes to clam flavor. However, the taste quality of succinic acid (ScA) and its sodium salt (ScA2Na) was unclear. In order to clarify the taste quality of the both two compounds, aversion thresholds and generalization pattern in C57BL/6J mice were examined by using CTA experiments. The trained mice were injected with LiCl after intake of 5mM ScA or 50mM ScA2Na and control mice was injected with LiCl after intake of distilled water. The aversion thresholds for ScA and ScA2Na were estimated as 0.1 mM and 10 mM, respectively. ScA generalized to ScA2Na, 1, 3 mM HCl, 10 mM L-Glutamate. This result suggests that B6 mice perceive 5 mM ScA as HCl-like and L-Glu-like taste (sour). ScA2Na generalized to ScA, Qui, NaCl, MSG, MSG+IMP. It suggests that B6 mice perceive 50 mM ScA2Na as Qui-like (bitter) and Sodium-like (salty) taste, also perceive MSG-like taste. However, it is thought that mice perceive MSG-like taste as salty taste rather than umami taste, because conditioned mice did not avoid MSG mixed with amiloride (blocked salty taste).

16. Change of Preference and Fos-like Immunoreactivity of Umami Taste by Addition of Inosine 5′-Monophosphate in Mice

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Monosodium L-glutamate (MSG) and monopotassium L-glutamate (MPG), both of which are employed as umami substances, have considerably different taste qualities in human, but the taste quality of MPG with inosine 5′-monophosphate (IMP) to be close to that of MSG. We have demonstrated using a two-bottle preference test that mice, whose umami sensitivity is comparable with that of human, preferred MSG and MPG with IMP (MPG + IMP) to MPG, and the mice preferred MSG and MPG + IMP similarly. Then, we examined the distribution pattern of umami taste-stimulated Fos-like immunoreactivity (FLI) in the parabrachial nucleus (PBN) and the nucleus of solitary tract (NTS) in mice. In the PBN, we observed a difference between the distribution pattern of MSG-stimulated FLI and that of MPG-stimulated FLI toward horizontal, vertical, and anteroposterior axes, but it was altered by addition of IMP to be similar pattern of MSG-stimulated FLI. Particularly, in anteroposterior axis, the distribution pattern of MSG or MPG + IMP-stimulated FLI was dominant in anterior part of PBN, but that of MPG-stimulated FLI was dominant in posterior part of PBN. In the NTS, we could not observe any difference in horizontal and vertical axes, but in anteroposterior axis, we found a tendency that is similar to that observed in PBN. These results suggest that IMP-induced taste quality change of MPG is accompanied by alternation of pathways toward anteroposterior axis within NTS of taste, followed by the alternation of pathways toward three-dimensional axes within the PBN.

17. Temperature Dependency of the Chorda Tympani Nerve Response to Sweeteners in C57BL/6N and BALB/c Mice

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It is well known that temperature modulates human perception of different taste modalities. The clearest example is the strong enhancement of perceived sweetness with increasing temperature. In mice, increasing temperature between 15 and 35 °C markedly enhances the CT nerve responses to sweet compounds in wild type but not in TRPM5 knockout mice. TRPM5 is a highly temperature-sensitive, heat-activated channel.

There may exist two different response components of the CT nerve for sweeteners in mice, one is inhibited by gurmarin [gurmarin-sensitive (GS)] and the other is not [gurmarin-insensitive (GI)]. The C57BL/6N mice possess both components in the taste buds innervated by the CT nerve, whereas BALB/c mice almost exclusively have the GI component.

In the current study, therefore, we compared temperature dependency of the CT nerve responses to sweeteners between GS and GI components.

In both C57BL/6N and BALB/c mice, the CT nerve responses to all of sweet compounds significantly increased with increasing temperature from 15 to 35 °C. Comparing the GS and GI components, we found that increase of response of the GI component with increasing temperature from 25 to 35 °C was larger than that of the GS component. In addition, responses to noncaloric sweeteners, such as Saccharin and SC45647 were larger in the GS than the GI component, whereas the reverse was true for sugar responses.

These results suggest that (1) both GS and GI components exhibit the temperature sensitivity, and (2) the temperature sensitivity of the GI component is slightly larger than that of the GS component, and (3) response of GS and GI components differs among sweet compounds.

18. Association between Polymorphism of the Sweet Receptor Gene, Tas1r3 and Gurmarin Sensitivity

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Gurmarin (Gur), a polypeptide isolated from the plant *Gymnema sylvestre*, is known to selectively suppress behavioral and gustatory neural responses to various sweet compounds without affecting responses to salty, sour and bitter substances in rodents. In mice, the sweet-suppressing effect of Gur differs among strains and taste nerves. Responses to sweet compounds in the chorda tympani (CT) nerve innervating the anterior tongue were inhibited to ~ 50% of control by Gur in C57BL/6 but only slightly if at all in BALB/c (BALB) mice. Recently, the taste receptor T1R3 was identified as the gene product of a single locus, *Sac* (*Tas1r3*), on mouse chromosome 4; *Sac* had been shown to influence behavioral and nerve responses to artificial sweeteners, such as saccharin, and to several sugars. It has been proposed that differences between taster (C57BL) and non-taster (BALB, various sublines of the 129 strain etc) strains in behavioral taste thresholds for saccharin and sucrose are caused by polymorphisms in the *Tas1r3* gene (e.g., T55A and I60T). This raises the possibility that strain differences in sensitivity to Gur may also be due to polymorphisms of *Tas1r3*. To investigate this possibility, we examined the effect of Gur in another *Tas1r3* non-taster strain, 129X1/Sv mice. The results indicated that unlike non-taster BALB/c mice but similar to taster C57BL/6 mice, 129X1/Sv mice exhibited significant inhibition of CT responses to various sweet compounds by Gur. This suggests that the mouse strain difference in the Gur inhibition of sweet responses of the CT nerve may not be associated with polymorphisms of *Tas1r3*.

19. Behavioral Analysis of Responses to Sweet Substances in T1R3–KO Mice

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T1R3 is an important taste receptor component that could form a heterodimer with T1R2 or T1R1 protein. In combination with T1R2, it responds to many sweet compounds, while that with T1R1 it does to amino acids including umami compounds. Previous

studies reported that mice lacking T1R3 exhibited largely reduced out not abolished behavioral preferences for sweet substances. This suggests a possibility that there may be T1R3-independent sweet-responsive receptor component in mice. In the present study, to further examine behavioral taste responses of T1R3-KO mice, we used a conditioned taste aversion (CTA) test and measured numbers of licks (/10s) to various taste solutions after conditioning was made to avoid to each of sucrose, glucose, glycine and D-phenylalanine in T1R3-KO mice. We demonstrated that T1R3-KO mice were conditioned to learn to avoid 1.0M sucrose. The aversion generalized to 0.3M sucrose but not to the other various sweet compounds. Avoidance conditioned to 0.5M glucose generalized to 1.0M sucrose, 0.5M maltose, 1.0M sorbitol, 0.1M NaCl and 0.1M MSG, whereas that to 0.3M glycine did to only 0.3M glycine. Conditioned aversion to 0.1M D-phenylalanine was generalized to 0.5M fructose, 1.0M sorbitol, 0.3M glycine and 30mM D-tryptophan. The results indicate that T1R3-KO mice still possess the ability to learn the conditioned avoidance to various sweet compounds with different generalization patterns. This suggests that there may exist multiple T1R3-independent sweet-responsive components in mice.

20. Response Properties of NaCl Responsive Taste Cells in Mouse Fungiform Papillae: Amiloride-Sensitive and Insensitive Taste Cells

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The mouse chorda tympani nerve contains at least two types of NaCl-responsive fibers. N-type fibers respond specifically to NaCl. NaCl responses of N-type fibers are strongly inhibited by amiloride, a blocker of the epithelial sodium channel (ENaC). The other type (E- or H-type) responds to not only NaCl but also other electrolytes such as KCl and HCl and shows almost no amiloride sensitivity. In contrast to nerve fibers, there are little evidences about response properties of NaCl-responsive taste cells. In this study, we examined NaCl responses of mouse fungiform taste cells in isolated taste bud and amiloride sensitivity of them. In our experiments, taste stimuli were applied only to the pore side of an isolated taste bud, and responses of one single cell of the bud to the stimuli were recorded from its basolateral side of the membrane as increase in firing frequency. The response to apical NaCl stimulation was recorded from some fungiform taste cells. These responses were concentration dependent. Amiloride mixed with apical NaCl solution inhibited NaCl responses in some taste cells [amiloride sensitive (AS) cells] but not in others [amiloride insensitive (AI) cells]. AI cells responded to other electrolytes such as KCl and HCl. These results suggest the existence of at least two types of NaCl sensitive cells, AS and AI cells. N- or E-type fiber may selectively innervate AS or AI cells, respectively.

21. Relation of Polymorphisms of ENaC Subunits to Mouse Strain Differences in Amiloride Sensitive Salt Responses

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Amiloride, a epithelial Na^+ channel blocker, is known to inhibit responses to NaCl of taste cells and the chorda tympani (CT) nerve innervating the anterior tongue in various mammalian species. In mice, amiloride sensitivity varies among strains; C57BL/6 (B6) mice exhibited inhibition of NaCl responses by amiloride to ~50% of control, whereas NaCl responses were only slightly inhibited by amiloride (~20% of control) in 129P3/J (129) mice. The amiloride-sensitive epithelial Na^+ channel (ENaC) expressed in taste cells is a potent candidate to play a role in the salt taste transduction. In this study, using amiloride-sensitive B6, amiloride-weakly sensitive 129 strains and their F_2 hybrids, we investigated possible relationships of the amiloride sensitivity with single nucleotide polymorphisms (SNPs) and mRNA expression levels of three subunits of ENaC (α, β, γ) in the anterior tongue. The results showed that sequencing detected a SNP resulted in an amino acid substitution, R616W in α-subunit. No SNP was found in β- and γ-subunits. F_2 hybrid mice were divided into 3 groups according to their αENaC R616W genotypes (129/129, B6/129 and B6/B6). Responses of the CT nerve to 0.03~0.3 M NaCl decreased after amiloride treatment in B6 and F_2 (B6/129 and B6/B6), whereas only weak inhibition was evident in 129 and F_2 (129/129). No significant difference in the expression levels of ENaC subunits between B6 and 129 was observed. These results suggest that R616W of ENaC α-subunit may be one of factors responsible for mouse strain differences in amiloride sensitive salt responses.

22. Behavioral and Electrophysiological Analysis of Salty Substitute Candidateaste

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Some salty substitutes except KCl have been developing, but there is little information about their gustatory properties. We tried to clarify the information by mice behavioral and electrophysiological studies. We use synthetic glycine ethyl ester, which has been reported as one of the salty enhancers in soy sauce. Behavioral studies with conditioned taste aversion assay showed that glycine ethyl ester solution was partially generalized into NaCl solution. Neural recording from chorda tympani showed that the response to glycine ethyl ester solution was not suppressed by amiloride, which suppress the component of gustatory response via N-type nerves. These results suggest that the saltiness of this compound dose is not caused by the enhancement of Na+ inward to taste cells.

23. Acute Blockade, but Not Genetic Deficiency, of c-fos Gene Expression Impairs Long-Term Memory in Taste Aversion Learning

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Roles of c-*fos* gene expression and its protein product, Fos, in conditioned taste aversion (CTA) learning remains unclear. To address the issue, we examined the effect of inhibition of Fos protein synthesis on CTA formation using the antisense oligodeoxynucleotide (ODN) method in rats and in mice carrying c-*fos* gene deficiency. Infusion of antisense ODN (AS-ODN) directed against c-*fos* mRNA into the parabrachial nucleus (PBN), but not into the amygdala or insular cortex (IC), impaired the acquisition, while infusion of randomized and inverted control ODNs had no effect. Suppression of Fos synthesis in the amygdala or IC impaired the retention, but not the acquisition, of CTA. Simultaneous infusions of AS-ODN both into the amygdala and IC significantly attenuated the acquisition. Retrieval of an acquired CTA was not impaired by AS-ODN infusion into the PBN or amygdala. In contrast, mice carrying c-*fos* gene deficiency showed normal acquisition and retention. Suppression of the expression of another immediate-early gene, *zif268*, using AS-ODN technology in the PBN or AMY did not impair the acquisition and retention of CTA. The present results suggest that the Fos-mediated signals in the PBN, amygdala, or IC play key roles in the acquisition and/or consolidation, but not the retrieval, of long-term CTA memory.

24. Mesolimbic and Amygdalar Neuronal Activities during Ingestion of Taste Solutions in Rats

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The mesolimbic system and its related structures are considered to be important for ingestive behavior. However, the processing of taste information in palatability-induced intake of food and fluid is still unclear. To obtain further information concerning the role of these structures in ingestion of taste stimuli, we recorded single neuron activities in the nucleus accumbens shell (NAc), ventral tegmental area (VTA), ventral pallidum (VP), and central (CeA) and basolateral (BLA) nuclei of the amygdala during ingestion of taste solutions in freely behaving rats. In the experimental chamber, the rats were previously trained to lick distilled water from each spout of the bottles through a small hole on the chamber's wall. In the recording session, the rats were presented with distilled water and various taste stimuli at each trial in pseudorandom order. After a 2.5 kHz cue tone presentation for 2.5 s, rats were allowed 5 s to access the spout. Increased (20%) and decreased (25%) firing rates were observed in 60 VTA units during licking behavior independent of the taste presented at each trial. Twenty-eight percent of VTA units increased their activities just before the start of licking. These firing patterns were also observed in the NAc and VP. A small number of units in the CeA and BLA selectively responded to hedonically positive or negative taste stimuli. These results suggest that the reward system including the VTA, NAc and VP is likely to make use of the information about hedonic value of taste from the amygdala to regulate ingestive behavior.

25. The Involvement of GABAergic System of the Ventral Pallidum in the Retrieval of Conditioned Taste Aversion in Rats

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We previously reported that the microinjections of $GABA_A$ receptor antagonists bicuculline into the ventral pallidum (VP) increased the intake of conditioned stimulus (CS) and changed hedonic response to it from aversive to ingestive on the retrieval of conditioned taste aversion (CTA). These results suggested that the GABAergic system in the VP were concerned with the expression of aversive responses and reduction of CS intake. Therefore, we examined the extracellular GABA release in the VP on the retrieval of CTA using *in vivo* microdialysis in freely behaving rats. Rats were presented with sapid solution with a single-bottle (Bottle group) or an intra-oral infusion method (IO group). On the conditioning day, rats were presented with 5 mM saccharin as CS, followed by an i.p. injection of 0.15 M LiCl as unconditioned stimulus. On the retrieval test, the IO group increased the extracellular release of GABA in the VP, but not the Bottle group. The difference in the GABA level may be due to the number of aversive responses during the reexposure to CS. Since the IO group was forced to experience the reexposure of the CS, it showed a number of aversive responses. On the other hand, since the Bottle group could freely access to the CS, it avoided to intake CS and showed a few aversive responses. Therefore, it is suggested that the increase of the extracellular GABA release is involved in the expression of aversive responses to CS on the retrieval test.

26. The Effect of Taste Palatability on Peripheral Blood Cytokine in Rats

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It is known that taste plays roles in appetite, food choices and nutrient intake. Besides these functions, we examined the effect of taste on the immunity in rats. They were randomly divided into 3 groups: each group was trained to eat a mash made up with powdered food and a liquid [distilled water, Control mash; 0.05 M saccharin, Saccharin mash; 0.01 M quinine, Quinine mash]. On the 7^{th} day (test day), the blood was collected under anesthesia, before and 30 min after eating the mash. Using the enzyme-linked immunosorbent assay (ELISA), we measured the level of serum interleukin-1β (IL-1β), interleukin-2 (IL-2) and interleukin-12 (IL-12) to examine the possible effect of taste on peripheral blood cytokine as a marker of immunity. The levels of serum IL-2 and IL-12 were not significantly different among the 3 groups. The level of IL-1β in rats that were trained to eat a saccharin mash was significantly increased, whereas that of IL-1β in rats that were trained to eat a quinine mash was decreased both before and after its eating. In the next experiment, rats were conditioned to acquire taste aversion to a saccharin mash. The conditioned rats showed decrease in the IL-1β level to the saccharin mash just like rats trained to eat a quinine mash. These results show that palatable taste increases, but aversive taste decreases the level of serum IL-1β.

27. The Role of Serotonin in the Intake of Taste Solutions in Rats

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It is known that serotonin is involved in feeding behavior. However, the role of serotonin in taste hedonics is not clear. Therefore, the present study examined the effects of administration of a serotonin synthesis inhibitor, para-chlorophenylalanine (PCPA) and an inhibitor of reuptake and breakdown of serotonin, 6-Methyoxy-tetrahydro-beta-carboline (6-MeO-THBC) on the intake of taste solutions. Rats were trained to drink water for 4 h in a day. After stabilization of water intake, rats received i.p. injection of PCPA (50, 100 or 500 mg/kg) 19 h before the test or 6-MeO-THBC (5, 10 or 50 mg/kg) 30 min before the test. On the test day, rats were presented with taste solution (5 mM saccharin, 0.1, 0.3 M sucrose, 0.1 M sodium chloride, 0.3 mM quinine hydrochloride, 0.3% xanthan gum or mixture of 0.3% xanthan gum and 5% corn oil) or distilled water for 4 h with a one-bottle method. After completion of behavioral experiments, serotonin level in the dorsal raphe nucleus was examined using immunohistochemical techniques. Depletion of serotonin by PCPA increased the intake of 5 mM saccharin, 0.1 M sucrose, 0.1 M sodium chloride and 0.3 mM quinine hydrochloride, but not distilled water, 0.3 M sucrose, 0.3% xanthan gum and mixture of 0.3% xanthan gum and 5% corn oil. On the other hand, increase of serotonin by 6-MeO-THBC decreased the intake of 5 mM saccharin and distilled water. These results suggest that the serotonin may be involved in the intake behavior of taste solutions.

28. Effect of Zinc Deficiency on Umami Taste Preference and Monoamines Levels in Hypothalamus in SD Rats

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We have demonstrated that the short-term zinc deficient signal after 4 days of the feeding increases the NaCl preference in rats significantly, but few data have been reported concerning about the umami taste preference in this model. Therefore, the present study was performed to elucidate the effect of zinc deficiency on the regulation of monosodium glutamate (MSG) appetite with respect to the monoamine levels in hypothalamus of zinc deficient rats model. Four weeks old male SD rats were fed the zinc deficient (0.7 mg Zn/Kg, Zn-Def), low zinc (4.0 mg Zn/Kg, Low-Zn), and pair-fed control (33.7 mg Zn/Kg, Zn-Suf) diets for 6 weeks, and two-bottle of water and MSG solutions (30 mM) preference test was undertaken in the first experiment, the Zn-Def rats showed the increased preference for MSG solution only after 2 days of the feeding, and Low-Zn rats showed the intermediate preference for MSG solution throughout the experimental period. However, the preference pattern of MSG solution was different from that of NaCl solution. The long-term intake of the MSG solution as a drinking solution in the second experiment (4 weeks or longer feeding of Zn-Def diet), MSG solution ingestion caused obvious decrease of hypothalamus epinephrine levels in all groups. Zinc deficiency caused decreased serotonin levels in the hypothalamus of both Water and MSG groups, and decreased norepinephrine levels in dorsal hypothalamus of the MSG groups only. It is therefore suggested that hypothalamus monoamines must be involved in the MSG preference mechanisms in the Zn-deficient rats.

29. Maternal Zinc Deficiency during Lactation Period Modify the Regulation System of NaCl Preference of Their Developing 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 developing 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 causes weaning and grown rats' NaCl preference using this zinc deficient system with SD/Slc rats. Zinc deficient (0.7 mg Zn/Kg), Low zinc (4.0 mg Zn/Kg), and Zinc sufficient 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 all the group rats after weaning. Two-bottle preference tests of 0.15M NaCl versus water showed that maternal zinc-deficient and low-zinc diets during lactation period caused the decreased NaCl preference in their developing pups, even though after their recovery from zinc deficiency. After weaning, significantly increased norepinephrine and epinephrine in central nucleus of amygdale (CeA) and decreased plasma oxytocin concentration were observed in Low-Zn group. Moreover, decreased chorda tympani nerve response to NaCl, and NaCl response suppression rate after amiloride treatment were also observed in Low-Zn group. These results suggest that not only central nervous system but also peripheral mechanism of taste reception were strongly involved in the developing pups' taste preference by early zinc deficiency during lactation period.

30. Dietary Zinc Deficiency Induces Reduced Acceptance of Calcium Chloride in C57BL Mice

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Our previous study showed that zinc-deficient mice had lower lick rates for 10–30 mM calcium chloride ($CaCl_2$) than did control mice. In this study, we investigated the effects of repletion with dietary zinc and of dissection of the chorda tympani and glossopharyngeal nerves on the ingestion of $CaCl_2$ and NaCl solutions in zinc-deprived mice. Behavioral responses were examined by using a 10-s lick test and a 48-h two-bottle choice test between a solution and water. Relative to control mice fed the zinc-replete diet, those fed the zinc-deficient diet had decreased intakes of 0.01 M $CaCl_2$ in both the 10-s and 48-h tests. On the other hand, dietary zinc deficiency increased the intakes of 0.1 M NaCl in the 48-h tests, but not in the 10-s tests. When the zinc-deficient mice were fed a zinc-replete

diet for ~2 weeks, reduced intakes of $CaCl_2$ and increased intakes of NaCl disappeared. Both the taste denervated and sham-operated mice, fed a zinc-deficient diet, had lower intakes of 0.01 M $CaCl_2$ and higher intakes of 0.1 M NaCl than control mice with intact taste nerves. Thus, the results suggest that unusual intakes of $CaCl_2$ and NaCl by the deficient mice are recovered by repletion with dietary zinc for ~2 weeks, and that taste information conveyed by the taste nerves does not play an essential role in reduced acceptance of $CaCl_2$ in zinc-deficient mice.

31. Discrimination of Taste Qualities of the 4 Basic Tastes in Zinc Deficient Rats

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Some clinical reports demonstrated that zinc deficiency brings about taste disorder. But there is very little report about the gustatory behavior of zinc deficient animals. In the present study, therefore, we examined whether or not zinc deficient rats could discriminate qualities of the four basic taste stimuli by using the conditioned taste aversion paradigm. As the zinc deficient animals, male Wistar rats fed zinc deficient diet for 5 weeks after the weaning period were used. When the zinc deficient rats were subjected to aversive condition to one of the basic taste stimuli, any animals could acquire the conditioned taste aversion. The zinc deficient rats as well as control rats never generalized to other for basic taste stimuli. These results suggest that zinc deficient rats have the same degree of the ability for the discrimination of the taste qualities as control rats.

32. Salt Preference and a Brain Hormone in Zinc-Deficient Rats

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Zinc deficient rats are known to drink 0.3 M NaCl, avoided by normal animals, in preference to water. We have indicated that serum sodium has a tendency to decrease and circulating aldosterone significantly increased in the deficient rats, and suggested that such abnormal salt intake reflects increases in sodium appetite. In order to confirm this view, we analyzed angiotensin II in cerebrospinal fluid and serum calcium (involved in the generation of salt appetite) in zinc deficient rats. Animals were given access to either a zinc deficient diet (group 1) or its normal control diet (group 2) ad libitum for one or four weeks. A group 3 was pair-fed with the group 1. Although aldosterone levels were higher in the group 1 than in the group 3, as described in our previous report, angiotensin II levels of both serum and cerebrospinal fluid varied little between these two groups. On the other hand, with the progress of the deficiency, serum calcium concentrations decreased to levels near those of rickets animals. However, there was no significant difference in the calcium levels between the groups 1 and 3 because of the variance within the group 1. These results suggest that unusual salt intakes of zinc deficient rats are mainly due to salt appetite stimulated by a combination of low calcium and high aldosterone in blood. Serum and brain angiotensin II seem not to contribute to the generation of salt appetite of zinc deficient rats.

33. Enhancement of Preference to Sodium in Rats Fed Zinc Deficient Diet for 1 Week

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Our previous study demonstrated that the rats fed zinc deficient diet for 5 weeks enhanced their preference to sodium by their post-ingestive effect. In the present study, we investigated whether or not the rats fed zinc deficient diet for only 1 week also enhanced their preference to sodium by their post-ingestive effect. As experimental animals, Wistar male rats were used. In the 48 h two-bottle preference test, preference percents for 0.1 and 0.3 M NaCl in the rats fed zinc deficient diet for 1 week were higher than those in the rats fed normal diet. However, in the 10 min two-bottle preference test, there was no significant difference between zinc deficient rats and control rats. These results suggest that the enhancement of preference to sodium is brought about by the post-ingestive effect in the zinc deficient rats for 1 week as well as 5 weeks.

34. Effects of Monosodium L-Glutamate (MSG) Ingestion on Diet-Induced Obesity in Rats

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Monosodium L-glutamate (MSG) elicits umami taste and is used as a flavor enhancer in various cuisines in world-wide. In the present study, we tested the hypothesis that ingestion of MSG suppresses weight gain and obesity in a diet-induced obesity model. Effects of voluntary ingestion of 1% MSG solution with water on weight gain, food intake, fluid intake, blood biomakers, and abdominal (intra-abdominal and subcutaneous) fat depositions were investigated in male Sprague–Dawley rats that fed either low fat (LF, 10% fat), high fat (HF, 45% fat), ultra-high fat (UHF, 60% fat), or high sucrose (HS, 67% sucrose) diet. Rats with free access to water but not MSG acted as controls. Rats had high (93–97%) preference for MSG in all four diet groups. Ingestion of MSG significantly suppressed weight gain, plasma leptin levels, and abdominal fat volumes relative to control animals. Caloric intake, blood pressure, blood glucose, and plasma levels of insulin, triglyceride, total cholesterol, and albumin were unchanged by MSG ingestion. The results suggest that free access to MSG suppresses obesity, fat deposition, and hyperleptinemia, and may also suppress the development of leptin resistance in rats with diet-induced obesity. Having umami-rich foods (e.g., typical Japanese cuisines) rather than high fat, high calorie foods could reduce the incidence of obesity in humans as well.

35. Amino Acid-Sensing by the Vagus in the Rat Alimentary Tract

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Recent advance in molecular biology in the field of taste perception has indicated the possibility for ingested nutrients to be ''tasted'' in the alimentary tract. The vagus nerve is widely distributed throughout the alimentary tract and functions as the primary neuroanatomical circuit in the gut-brain axis to transmit meal-related signals. Here, we tried to identify the amino acid-sensing profiles of the gastric and intestinal mucosa by the vagus. Intraduodenal application of each amino acid all modulated the vagal afferent activities of the rat celiac branch. For instance, the celiac afferents were activated by glutamic acid, tryptophan, and lysine, and suppressed by glycine. However, the afferent fibers of the rat gastric vagus increased their firing rate solely with intragastric application of glutamic acid. Other amino acids failed to have the same effect in the stomach. Gastric afferent activities were modulated by various endogenous mucosal transmitters; especially 5-HT. Vagal response to luminal glutamate was disappeared by the 5-HT depletion or the mucosal anesthesia. These results suggest the existence of a unique sensing system for glutamate in the rat gastric mucosa. Assuming there is a universal coexistence of free glutamate with dietary protein, the glutamate sensing by the gastric vagus might have a new physiological role in the gastric phase of protein digestion via the vagal reflex.

36. Effect of Dietary Free Glutamate on the Postprandial Sensation for Protein-Rich Liquid Diet

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Free glutamate activates taste nerves to elicit a unique taste, 'umami'. It is abundant in protein-rich natural foodstuffs and enhances the secretion of saliva, gastric juice and pancreatic juice. Recently, it was reported that glutamate promotes gastric emptying in adults. In this study, we examined its effect on the postprandial sensation for protein-rich meal in randomized double-blinded test in which 66 healthy adult males were enrolled. After an overnight fasting of 16 h, the subjects were asked to assess the intensity of various symptoms including fullness, stomach heaviness and bloating using a questionnaire with visual analogue scales (VAS) at 15 min intervals for a postprandial period of 240 min. The test liquid meal (400 kcal/400 ml) consisted of 12.5% dextrin and 12.5% casein-calcium. Monosodium L-glutamate (0.5% w/v) was added to a control meal. All meals were flavored with aspartame and plum odor to mask the taste of glutamate. Area under the curve (AUC) of VAS value was calculated as the index for each symptom and analyzed by ANOVA. The VAS values for postprandial symptoms achieved a peak synchronically within 15 min after ingestion and gradually decreased. However, glutamate had no effect on either AUC or peak values for postprandial sensations. The stratified analysis based on age revealed that glutamate had a tendency to diminish fullness (P = 0.202), stomach heaviness (P = 0.369) and bloating (P = 0.438) of the subjects older than 45 years old (n = 14) suggesting the possible effect of glutamate on the postprandial sensations in older age. It may reflect the involvement of the functional loss of GI tract with aging.

37. Taste Properties of L-Glutamate Salts

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We measured the taste properties of the solutions of L-glutamate salts, sodium salt (MSG), potassium salt (MPG), ammonium salt (MAG), calcium salt (MCG) and magnesium salt (MMG) using sensory evaluation methods. The taste qualities of the solutions were measured with labeled magnitude scale. Umami was perceived as the main quality of tastes, and the umami intensities were almost the same among the salts. Saltiness (MSG, MMG, MAG, MCG and MPG), bitterness and/or KCl-like harsh taste (MCG, MMG, MPG and MAG) and sourness (MAG) were also perceived as the side tastes. The threshold concentrations were measured by two-alternative forced choice test. The threshold concentrations for di-glutamate salts (MMG and MCG) were much lower than the half of those for mono-glutamate salts (MPG, MAG and MSG). The magnitude of the synergistic umami enhancement of each salt with 5′-inosine monophosphate (IMP) was compared using a ranking test and there was no consistent difference between salts. The intensities of umami and the enhanced umami by adding IMP depended on the concentrations of glutamate, and did not depend on the species of cation. The qualities of side tastes depended on the species of cation of the salts. The threshold concentrations depend not only on the concentrations of glutamate but also on the species of cation.

38. Comparisons of Long- and Medium Chain Triacylglycerol As an Induces of Reinforcing Effect in Mice

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We investigated the influence of fatty acid chain length of triacylglycerol on reinforcing effect in operant responding under a progressive ratio (PR) schedule ($n \times n/2$ where n = number of reinforcers received) in BALB/c mice. A break-point under the PR schedules has been widely used to quantify the reinforcing effect. We used 100% corn oil as a representative of long chain triacylglycerol (predominantly $C > 16$) (LCT) and tricaprylin as a representative of medium chain triacylglycerol ($C = 8$) (MCT). In experiment using oil-naive mice, both LCT and MCT offered were preferably taken in short-term two bottle choice test when compared with mineral oil which has a dietary oil-like texture. Moreover, in two bottle choice test using oil-naïve mice, LCT and MCT were equally consumed. It suggested that in oil-naive mice, the chain length of fatty acids was irrelevant to selection of fat. On the contrary in the case of mice which habituated to LCT through the training session, the break-point of LCT was significantly higher than MCT, suggesting

that the difference of the chain length of fatty acids was an important factor for the intensity of the reinforcing effect. After repeated one bottle choice test for three days, mice consumed significantly larger amount of LCT than the first test but if mice given only MCT, repeated one bottle choice test did not increase in MCT intake in mice. These results suggested that the chain length of fatty acids of triacylglycerol may function through and after acquisition of the reinforcing behavior, although other studies are required to explain the mechanisms of the postprandial recognitions of fat.

39. The Effect of Naloxone on Licking Behavior of Rat Ingesting Corn Oil Emulsion

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To investigate the relationship between endogenous opioids and hedonic response to fat, we examined the effect of naloxone, a non-selective opioid receptor antagonist, on licking behavior, which used as a barometer of hedonic response, of rats ingesting corn oil emulsion. Rats were trained for 5 days to drink 5% corn oil emulsion in 0.3% xanthan gum solution for 20 min. On Day 6, 1 h prior to the emulsion presentation, one group of rats received intraperitoneal injection of naloxone (1 mg/kg body weight), and the other group of rats received injection of saline. Licking behavior was recorded with lick sensor during the emulsion presentation. The naloxone-treated group showed reduced initial lick rate (1 min) and intake per lick, as compared to the saline-treated group. Latency to the first lick was not affected by naloxone injection, suggesting that the opioid system may be irrelevant to the wanting behavior before introducing fat into the oral cavity. To rule out the side effect of the 1 mg/kg naloxone, licking behavior of 22 h water-deprived rats, which compelled to perform the highest licking rate, was examined under the conditions. No significant differences were observed between naloxone-treated and saline-treated groups in the initial lick rate and intake per lick. These results suggest that the suppression of licking behavior by naloxone injection was due to reduced hedonic impact concerning to the fat in the oral cavity, and not to suppression of motor activity of rat's tongue. We suggest that endogenous opioids contribute to bring about hedonic impact on fat introduced in the oral cavity.

40. Effects of Prenatal Exposure to 2,3,7,8-Tetrachrolodibenzo-p-dioxin (TeCDD) on Motor and Emotional Learning, and on Taste Preference of Rat Offspring in Early Developmental Stage

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Recent studies suggested that prenatal maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TeCDD) affected growth of their offspring. Previous animal studies indicated that prenatal exposure to TeCDD causes diverse changes to reproduction, immunity, learning and behaviors. However, these studies investigated effects of prenatal exposure to TeCDD using only adult offspring, and the effects on neuro-behavioral development remain unclear.

In the present study, to investigate effects of prenatal exposure to TeCDD on development of the rat offspring, TeCDD or corn oil was orally administered to pregnant rats. Both the TeCDD-exposed and control offspring were fed ad libitum with free access to maternal breast milk after birth. The results indicated that TeCDD significantly delayed fetal growth, especially brain growth. In an inclined plane test, TeCDD significantly extended mean latency for the pup offspring to turn up, suggesting delay in motor development. Active avoidance learning was also disturbed in the TeCDD-exposed male rats. In a taste preference test, the rat offspring were fed with lab chow ad libitum in individual cages and daily consumption of 8 solutions [amino acids, monosodium glutamate (MSG), NaCl] was measured. The results indicated that daily total intake was decreased in the TeCDD-treated rats. Furthermore, in the TeCDD-treated female rats, daily intake of MSG was decreased, while that of histidine HCl was increased. The present results demonstrated that prenatal exposure to TeCDD affected fetal growth, motor development, emotional learning and taste preference of the offspring in childhood, and that effects of TeCDD on neuro-behavioral development were sexually dimorphic.

41. Improving the Taste of Artificial Sweeteners Using Flavorings

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In response to recent, markedly growing consumer demand for low-calorie/sugar-free food products containing nonnutritive artificial sweeteners and continuing preference of many consumers for the sweetness of sugar, we have sought to improve the taste of artificial sweeteners, having bitterness, astringency and aftertaste, by applying sugar flavorings. In this study, we tried to estimate cortical responses of the brain during sensory evaluation using near-infrared spectroscopy (NIRS). NIRS can be performed noninvasively to monitor changes of the human cerebral blood flow as an indicator of cortical activation. Our objectives are to detect difference between cortical responses to sugar and artificial sweeteners using NIRS, and to create flavorings that minimize the sugar–artificial sweeteners difference in cortical responses and in sensory evaluation. When a subject drank a conditional sugar solution, then after 60 s drank a test sugar or artificial sweetener solutions, 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 for adding to artificial sweeteners in order to improve the taste.

42. Tastes Production of γ-Amino Acid Introduced Peptides

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Ten kinds of peptides containing γ-amino butyric acid (GABA) or Taurine (Tau) were chemically synthesized and tastes were evaluated. All synthesized peptides produced sweetness and several of them with bulky side chain produced bitterness as well. Shallenberger-Acree-Kier model for sweetness production proposes that sweetness is produced by a proton donor (AH), a proton acceptor (B) and a hydrophobic group (X) and average distances of AH-B, AH-X and X-B are 2.6 Å, 3.5 Å, 5.5 Å, respectively. On γ-amino acid introduced peptides, amino residues on amide bond or amino group corresponding to AH, carboxyl group or sulfonyl group corresponding to B and amino acid's side-chain corresponding to X. On calculated stable structure of these peptides on CAChe WorkSpace, the AH, B and X were located on triangle of the Shallenberger-Acree-Kier model and the Shallenberger-Acree-Kier model was well applicable to their sweetness production. Goodman's theory for sweetness production of dipeptides and related compounds proposes that the sweetness production requires the AH, B and X on the triangle, and the taste are decided the direction of the X. The Goodman's theory was applicable to tastes production of GABA-peptides. Taste production of Tau-peptides may require alternative theory using the AH, B and X, because of their unique straight forms.

43. Oolong Tea Suppresses α-Amylase Activity

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Oolong tea is expected to be a preventive measure in a daily life since some elements in the tea are believed to suppress blood glucose level through inhibition of the α-amylase activity.

In the present study, we measured inhibition level for the α-amylase activity by the oolong tea through measuring of salivary and pancreatic α-amylase activity using iodine–starch reaction *in vitro*.

Five grams of the oolong leaf were enclosed into a tea bag, extracted in 100 ml of hot water for 60 min, and preserved as a stock solution. Within a range of dilution used, from 1/10 to 1/2, the activity of human salivary α-amylase as well as porcine pancreatic α-amylase was suppressed dose dependently. The pH-manipulated oolong tea, which was decreased to 2.0 by adding HCl and then was titrated to restore the original pH, preserved its potential to suppress α-amylase activities. These results suggested the possibility that ingestion of the oolong tea might be to decrease starch digestion to result in reduction of glucose uptake in the intestinal tract. In addition, suppressant(s) action on the α-amylase activity was pH-independent. Suppression rates for the human salivary α-amylase activity versus those for the porcine pancreas were revealed linear relation within the range of oolong tea concentrations used. Besides, within this range of concentration, suppression rate of 60 to 100% for salivary α-amylase corresponded to those of 40 to 80% for pancreatic one.

We concluded that salivary α-amylase activity was a good measure to evaluate pancreatic α-amylase activity, which was expensive and rather difficult to purchase.

44. Sensory Evaluation of Chocolate Using Organic *Matcha* Cultivated with Reduced Organic Fertilizer

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The production of *matcha*—powdered green tea used for a Japanese tea ceremony—has been increasing year by year. *Matcha* is produced from tea leaves grown under a sunshade to decrease the catechins, which are bitter components, and requires the application of a large amount of nitrogen fertilizer to increase the amino acids which are the *umami* and sweet components. However, too much fertilization causes underground water pollution. Therefore, we cultivated *tencha*, which is the material of *matcha*, in the organic tea field, in order to reduce gradually the annual nitrogen input from 635 kgN/ha in 2003 to 435 kgN/ha in 2004, and then to 320 kgN/ha in 2005 for the purpose of reducing the nitrogen eluviations.

It is known that reducing the nitrogen input lowers the quality of *matcha*, because of the reduction of the amino acids. However, we reported last year that the *matcha* produced in 2004 was evaluated relatively valuable as an ingredient for chocolates. We prepared the chocolates, to be which were added the *matcha* samples produced in 2003, 2004 and 2005, and evaluated them by a scoring method. As a result, the *matcha* chocolate produced in 2005 was also evaluated to be as valuable as the *matcha* chocolate produced in 2004 based on color, sweetness, bitterness, etc. This indicated that the *matcha* sample produced in 2005 was suitable as an ingredient of *matcha* chocolates.

45. Facilitation of Repetitive Voluntary Swallowing by Taste Stimulation of Na Salts in Humans

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We have reported that infusion of distilled water (water) into the pharyngolaryngeal region facilitated repetitive voluntary swallowing in humans, but 0.3 M NaCl prolonged the voluntary swallowing. The results suggested that excitation of water receptors in the pharyngolaryngeal region is responsible for facilitation of repetitive voluntary swallowing. However, little is known about the role of taste receptors in swallowing reflex in humans. In this study, each subject

was instructed to perform repetitive swallowing as fast as possible. Taste solutions were delivered to the tongue base or the anterior tongue through a fine tube at a slow flow rate, 0.2 ml/min. The swallowing intervals were measured. In some subjects, the swallowing intervals were shorter in the case of infusion of 0.15 M Na salts (NaCl and Na acetate) than in the case of infusion of water or 0.15 M KCl, suggesting that excitation of Na-taste receptors are responsible for facilitation of repetitive voluntary swallowing. However, some subjects did not show the Na-taste effect. In a given taste stimulation, swallowing intervals varied greatly from subjects to subjects. We found that the longer the swallowing interval was, the stronger was the Na-taste effect. In a previous paper, we have proposed a model that explains facilitation of repetitive voluntary swallowing by sensory inputs from oropharynx in humans. According to this model, the ability of the central pattern generator to perform repetitive swallowing as fast as possible varies in the subjects. It is likely that subjects showing a long swallowing interval in voluntary swallowing show the strong Na-taste effect.

46. The Influence that Maple Sugar Gives to Heart Rate Variation after Step Test

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Recently, the consumption of the maple sugar increases. The reason is that it has natural taste, desirable fragrance, etc. The aim is examining the influence that maple sugar gives to heart rate variation after step test.

Subjects of 7 young men drank 200 cc (5% solution) of maple sugar and refined sugar. In the experiment, changes of heart rates were compared. Exercises load of step test are about 1.5 times of normal activity. After 30 min (preparation time), the experiment is started. After 5 min (the rest time, subjects sitting on the chair), subjects drank solution. After 9 min rest, the step test of 3 min was done. The recovery period of the heart rate was 17 min (subjects sitting on the chair). Sixteen experiments for each subject were done and 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)) were analyzed. The difference between the value after for drinking at the standby time and the value testing the step was great, and the significant relation between the values of both was seen. Moreover, it has been understood that the sympathetic nerve works while testing the step. Partially of the Lorenz plot, the case where the heart rate decreases slightly after for drinking the maple sugar was admitted.

47. Estimation of Familiarity for Sweeteners

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We expected mere exposure effect for taste and odor as it showed in general vision. Why we have more maple sugar? This reason should be explained by mere exposure effect. We carried out the experiment estimated the familiarity for maple sugar and as the reference white sugar using 1% cider vinegar. Subject made the choice of acceptable one from two samples (maple sugar and white sugar), after they were done preceding presentation (4 times) for maple sugar. These results suggested that the choice of all subjects made maple sugar. Subjects done 7-order evaluation fitting from very likes to very dislikes at the same time. The familiarity was estimated by the statistical treatment using these evaluations, and the result was suggested that maple sugar was better than white sugar.

The factors of this familiarity were given by ''fresh,'' ''mild,'' ''natural'' and ''likes'' from the sensory evaluation.

48. Prefrontal Activity during Intentional Encoding of Taste: fNIRS Study

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Taste remains one of the least-explored human senses, especially regarding the neural bases of its cognitive processing. To facilitate its understanding, it is worth applying to taste the psychological paradigms that have been used with other extensively studied senses. Thus, using multichannel functional near-infrared spectroscopy (fNIRS), we examined the lateral prefrontal cortex (LPFC)'s of healthy volunteers (N = 18) during the intentional memorization of a basic taste. In order to minimize the confounding effects of verbal processes that are known to employ the left LPFC, we used quaternary taste mixtures that were difficult to verbalize, and confined analysis to those who did not use a verbal strategy during memorization (N = 10). By contrasting the cortical activation under encoding conditions with that under control conditions without memory requirement, we found activation in the bilateral ventro-LPFC and the right posterior portion of the LPFC. We compared the location of current activation foci with data in literature, by probabilistically estimating and anatomically labeling the location of fNIRS measurement points in the Montreal Neurological Institute (MNI) standard brain coordinate space. The spatial pattern of activation foci was consistent with previous studies on the encoding of nonverbal materials using other senses. This suggested an amodal role of LPFC in intentional encoding, at least at a macro structural level. The current study also demonstrates that, by using fNIRS, LPFC functions on taste can be examined with experimental paradigms relevant to those used for other senses. This experimental system will aid in further exploration of the neural bases of taste cognitive process.

49. Location of Area G in Lefty Handed Subjects—Relationship between Dominant Hand and Location of Area G

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Previous studies with MEG and fMRI identified the human area G one of the primary gustatory cortices, at both buried parietal operculum and posterior superior insula in right handed subjects. Then we noticed area G more posteriorly on the left than on the right hemisphere. The present study was aimed to study whether area G locates more posteriorly on the dominant than on the nondominant hemisphere. A total of 27 neurologically healthy volunteers (19–38 years old), 15 right-handed and 12 left-handed, participated in the study. While repetitively stimulating the tongue tip with a short pulse of 1 M NaCl, we detected BOLD signals by 1.5T MRI scanner (Magnetom Vision, Siemans) and analyzed them by SPM99. Single subject analysis, disclosed activations at area G in 9 right-handed and in 8 left-handed subjects (threshold at $P < 0.05$, FDR, corrected across entire volume). Averaged Y value of area G in MNI coordinate was more posterior on the dominant than on the nondominant hemisphere in either group. Group analysis with random effect models revealed activation at area G on both hemisphere of either group ($P < 0.01$ FWE corrected, ROI analysis), and confirmed the findings on the Y coordinate of area G with single subject analysis.

50. The Pictures of Fruits Affect Flavor Perception of Fruit Juices

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We humans use vision as a primary source of information about external world. We know that we also use vision in eating behavior: When we select and evaluate foods, we fully use a vision. There are many anecdotes about that, however, not so many academic studies studying about that. In this study, participants were required to drink four kinds of juices (i.e. apple, peach, orange and grape juices) under three conditions. Participants were asked to put an apparatus like mask, which blindfolded participants and gave them a computer screen. In no image conditions, they drank a cup of juice without any visual images of fruits. In appropriate conditions, they drank a cup of juices with appropriate pictures of the juices. In inappropriate conditions, they drank with inappropriate pictures, e.g., participants drank apple juice with a picture of orange. As a result, it was found that these visual stimuli enhanced flavor intensities and preferences when they were appropriate to the flavor. These results are compatible with those of preceding studies, which investigated the interactions of olfaction with gustation and/or vision and suggested that these interactions occur in cognitive level.

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51. Comparison of Pleasantness for Salty Taste Among the Family Constituents

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The taste hedonic tone and taste intensity of the salty taste was evaluated in the healthy university students (n = 96), their parents (n = 192), and their siblings (n = 74). The materials tested were 0.6%, 0.9%, and 1.4% NaCl, and 0.6%, 0.9%, and 1.4% salty concentration of miso soup.

Pleasantness for salty taste of the children (male) was significantly correlated with that of their mother (0.6% NaCl, 0.9% miso-soup). Pleasantness for salty taste of the children (female) was significantly correlated with that of their mother (0.6%, 0.9%, 1.4% NaCl, 0.9% miso-soup). Pleasantness for salty taste of the children (female) was significantly correlated with that of their father (0.6%, 0.9%, 1.4% NaCl, 0.6%, 0.9% miso-soup). The higher salty concentration of home-made miso-soup was pleasantness of higher concentration of miso-soup.

These findings suggested that pleasantness for salty taste among the family constituents might be similar and that the higher salty concentration of home-made miso-soup might be pleasantness of higher concentration of miso-soup.

52. Relationship between Serum Leptin Level and Sweet Taste Changes in Pregnant Women

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Objectives: We studied the sweet taste change during pregnancy and investigated the relationship between serum leptin level and sweet taste in normal pregnant women.

Methods: Thirty-nine normal pregnant female were recruited to this study. Gustatory test was performed with the filter paper disk method. The five graded concentrations (%) of solutions 0.3, 2.5, 10, 20 and 80 were made from sucrose and distilled water, respectively. The serum leptin was measured by a RIA assay. Body Mass Index (BMI) was calculated by weight (kg)/(height (cm))^2. Statistical analysis was performed by Spearman Rank correlation coefficient test and Mann–Whitney U test.

Results: The threshold of sweet taste was significantly higher in the first trimester compared with second or third trimesters and decreased according to the progress of pregnancy. The threshold of sweet taste in normal pregnant female was inverse correlated with their BMI during pregnancy. The threshold of sweet taste in normal pregnant female was inverse correlated with their serum leptin concentration during pregnancy and serum leptin concentration was correlated with their BMI in pregnant female.

Conclusions: It is suggested that the change of sweet taste was related to the physiological weight gain such as increased need of fat according to the progress of normal pregnancy. Inverse correlation between the serum leptin concentration and the threshold of sweet taste may give suggestions that pregnant women have a different mechanism for lipids metabolism from nonpregnant women. The sweet taste may be related to physiological demands for fetal nutrition during pregnancy and may be an indicator of weight gain for pregnant women.

53. Oral Squamous Cell Carcinoma with Hypercalcemia and Gastrointestinal Chemical Sensors: Case Report Providing a Perspective on Palliative Medicine

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Malignancy associated hypercalcemia of oral squamous cell carcinoma is a late manifestation and terminal condition. In the literature, patients survival is reported to be 1.5–2 months or less. Nausea and vomiting, constipation, anorexia and malaise, complicated by appetite loss are the main important clinical symptoms of malignancy associated hypercalcemia. We encountered a case of oral squamous cell carcinoma with hypercalcemia showing prolonged survival for more than 6 months. Case presentation: A 70-year-old male with recurrent lower gingival carcinoma showed a total course (initial treatment to end point) of 3 years and 4 months. The patient received stomach tube nutrition due to swallowing disorder for 293 days prior to the end point. Hypercalcemia developed 209 days before the end (plasma calcium value of 11.3 mg/dl and PTHr-P intact value 1.8 pmol/l), and he received bisphosphonate i.v. at 2-week intervals, which improved the main symptoms. Furthermore, nutrition through a feeding tube could be continued up until the end point. Meals were prepared by his wife, based on his preference for seasonable fish, fluid and vegetable. Individual preferences were considered to affect the reflex effects of gastrointestinal sensors on vagal nerve activities, which induced positive emotions and maintained good nutritional condition. It was suggested that in malignancy associated hypercalcemia, it should be considered necessary not only to control the plasma calcium level but also to improve the nutritional condition based on patient's individual food preferences even in patients receiving nutrition through a stomach tube.

54. Palliative Medicine and Morphology of Tongue Mucosa and Taste Cells—Autopsy Study

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Case: A 70-year-old male with recurrent lower gingival carcinoma received palliative medicine from winter to autumn 2005 (total of 293 days of until death). He received nutrition through a stomach tube due to swallowing disorder and meals were based on his preference for seasonable fish, fluid and vegetable. In addition Oral care included scrubbing with a cotton swab wetted with green tea, seasoned with pickled ume. Main autopsy findings were pneumonia and pulmonary congestion and pulmonary metastasis. Pathologically, respiratory failure was considered the cause of death. These findings are ordinary end points of terminal oral cancer patients. Autopsy findings also showed normal structures for the tongue mucosa and papillae, and taste buds in the epithelial layer of the circumvallate papillae were clearly shown despite more than 6 months of nutrition through a feeding. Excluding tumor invasion, there were no signs of dry mouth, mucositis, candidosis or other oral pathological conditions usually seen in terminal patients. It was concluded that oral chemical sensors can be maintained until death. That in terminal patients, oral taste sensors can be preserved by oral care with taste stimulant, good oral hygiene and respect for personal food nutrition preference, even in patients received through a gastric tube nutrition. Oral and gastrointestinal chemical senses that are considered to induce good psychoneurological conditions are important during the care of terminal cancer patient.

55. Effect of Mental Stress on Histatin and PRP-PE Contents in Saliva

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Objectives: It was reported that taste perception may depend on physiological and psychological conditions. We reported two peptides, Histatin 3, 5, or 6 and the basic Proline-rich peptide P-E were detected with partial sequence analysis as bitter binding proteins, utilizing quinine sulfate which shows fluorescence under UV radiation. Thus, this investigation intends to establish the clinical concentrations for Histatin and PRP-PE in saliva, and a comparison of the two peptides in parotid saliva of healthy subjects before and after mental stress.

Methods: Stimulated parotid saliva was obtained from 10 healthy normal subjects before and after the administration of a Kraepelin psychodiagnostic test under conditions of a loud sound (5,000 Hz with 100 dB) for 30 min. The LF/HF (using power spectral analysis of heart rate variability) ratio, an index of sympathetic nervous activity was analyzed with ECG during these procedures. The concentration of Histatin and PRP-PE in saliva was quantified by ELISA using the primary antibody (anti-Histatin5 monoclonal antibody and anti-PRP-PE monoclonal antibody) against the synthetic peptides designed for Histatin and PRP-PE. The salivary cortisol concentration was assayed using the SALIVARY CORTISOL ENZYME IMMUNOASSAY KIT (SALIMETRICS). Wilcoxon t-test and Mann–Whitney U-test were used, and significance was set up at $P < 0.05$.

Results: For the group a significant increase in salivary cortisol levels resulted after the Kraepelin psychodiagnostic test. The increase in LF/HF ratio was observed as well. Following the Kraepelin psychodiagnostic test, subjects showed a tendency towards sympathetic hyperfunction response, and Histatin levels of ELISA was significantly reduced. In contrast, this effect was not observed in PRP-PE levels.

Conclusion: These findings suggest that the decrease of Histatin by mental stress might be a natural stress response.

56. The Taste of Saliva in the Taste Disorder Patients (Spontaneous Abnormal Taste Sensation): An Investigation Using a Taste Sensor

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Objective: Recently, the number of patients with taste disorder has steadily increased in Japan. To clarify the cause of taste disorder

(spontaneous abnormal taste sensation), saliva of patients with taste disorder was evaluated using a taste sensor.

Subjects: Patients who visited the outpatient clinic for taste disorder of the Niigata University Medical and Dental Hospital: The patients with taste disorder were divided into two groups: group A, without spontaneous abnormal taste sensation (2 males, 8 females, age: 62.3 ± 11.7), and group B, with spontaneous abnormal taste sensation (2 males, 7 females, age: 63.6 ± 15.0).

Methods: 1) Filter disc method (sweet, salty, sour, bitter) was used for the taste examination. 2) Taste information in saliva were measured using a taste sensor.

Results: 1) Taste examination: Patients ID1, ID2 and ID4 were diagnosed as abnormal. (ID1 for bitter and sour, ID2 for bitter and ID4 for sweet, salty, sour, and bitter). The other patients were diagnosed as normal. 2) The taste information in saliva using a taste sensor: Only ID2 patient had a significant variation compared to the other patients. This saliva reacted strongly to astringency and weakly to saltiness.

Conclusion: These results suggest that this taste sensor can sufficiently assess human tastes perception. Therefore, this taste sensor enabled the evaluation of the taste in saliva. Moreover, it was found that human saliva varies in taste qualities. Therefore, the differences in the taste of human saliva may be influenced by individual perceptions of taste.

57. Usefulness of a Whole Mouth Method of Taste Examination to Follow and Evaluate Recovery from Taste Disorders

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In Japan, a filter paper disc (FDP) method (Tomita et al., 1986) has frequently been used to examine recognition threshold of taste disorder patients to basic tastants, while a whole mouth method (WM) is often used only at the initial consultation, especially when the threshold is too high to measure with the FDP method. We measured the taste recognition thresholds of eleven patients using a previously published concentration series of tastants (Yamauchi et al., 1995) along the course of treatment with zinc supplement (35 mg/day as polaprezinc) to evaluate the treatment. Five of the eleven patients had decreased threshold to some or all of the four basic tastants in 4–8 weeks in response to the treatment, four did not show any improvement in WM recognition thresholds until 8–12 weeks, and the remaining two returned to the hospital they previously consulted to. Although we have limited experience in taste clinic, we found that the WM method is better in the following two points than the FDP method: (1) the WM method gave threshold values to patients which encouraged them to take zinc supplement, and (2) the WM method is much more timesaving than the filter paper method.

58. Effects of Intraventricular Angiotensin II on NaCl–Water Discrimination Threshold in Monkeys

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Effects of intraventricular angiotensin II (AII), a dipsogen, on NaCl discrimination threshold was measured in two Japanese monkeys during a NaCl–water discrimination GO/NOGO task. In response to NaCl (GO cue), monkeys had to press a lever within 2 s for a reward (GO task). Against water (NOGO cue) given, they had to not press the lever for the reward (NOGO task). Reaction time (RT) from the GO cue to lever pressing was measured. Physiological saline (vehicle, 20–40 μl) was intraventricularly administered into the lateral ventricle through a cannula. Both monkeys responded to a 0.01 to 0.1 M NaCl with % correction performance (%CP) of >90%, but to 0.003 M at a chance level. The threshold was at around 0.003 M in the control. The RT varied from 700 ms to 1,050 ms depending on NaCl concentration. Neither the vehicle nor AII affected the %CP and prolonged the RT dose dependently, although the vehicle affected them in a reversed way. AII increases NaCl discrimination threshold dose dependently. Effects of AII on the %CP and RT lasted for a shorter time than those on water intake. It is suggested that different neuronal mechanisms are involved in increased NaCl–water discrimination threshold and water intake.

59. Central Ghrelin Induces Gastric Relaxation of the Proximal Stomach in Rats

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Ghrelin is a recently identified endogenous legand for the growth hormone secretagogue receptor (GHS-R). It was originally isolated from the stomach, but has also shown to be present in the central nervous system. In addition to GH-releasing activity, ghrelin has orexigenic effect. In our previous study, orexin-A and neuropeptide Y, which are potent orexigenic peptides, induced relaxation of the proximal stomach. This relaxation is considered as the compensatory response of hyperphagia. Up to the present, the effect of ghrelin on the proximal stomach motility is not known. In the present study, the effect of ghrelin on motility of the proximal stomach was examined in anaesthetized rats. Intragastric pressure (IGP) was measured using a balloon situated in the proximal part of the stomach. The administration of ghrelin into the fourth ventricle induced relaxation of the proximal stomach in a dose-dependent manner. Significant reduction in IGP was observed at the dose of 3, 10 or 30 pmol. Simultaneous administration of ghrelin (10 pmol) and GHS-R antagonist ([D-Lys^3] GHRP-6; 1 nmol) did not induce significant change in IGP. The administration of [D-Lys^3] GHRP-6 did not induce significant change in IGP. These results indicate that ghrelin induces the relaxation of the proximal stomach via the GHS-R situated in the dorsal vagal complex. Since both the bilateral sectioning of the vagi at the cervical level abolished the relaxation induced by the administration of NPY into the fourth ventricle, the relaxation induced by ghrelin is mediated by vagal preganglionic neurons.

60. Sweet Taste Sensing with Various Measurement Methods—Development of Taste Sensing with Electric and Optical Measurements

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A taste sensor using lipid/polymer membranes could not have high sensitivity to natural sweet taste substances such as nonelectrolytic glucides. As a result, it was found that the electric admittance and the near-infrared absorption of aqueous glucide solution were decreased with increasing its concentration. The sweet taste substances used in this experiment were sucrose, glucose, xylitol, lactose and raffinose. The both orders of the decreasing values of admittance and optical absorption of these sweet substances with several concentrations accorded almost to the order of sensory test. Because the taste sensor with positively and negatively charged lipid/polymer membranes responds highly to electrolytes as sour and salty substances, and the optical absorption at 950 nm has been affected in the case of only the presence of sweet and umami substances, we can propose a sweet sensing system with these electrical and optical measurements.

61. Study on Evaluation of UHT Milk Processed with Indirect and Direct Heating Methods by Sensor Analysis

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For the purpose of evaluating objectively flavor and taste in UHT (ultra-high-temperature) milk processed with plate-type pasteurization, which is one of indirect heating methods, and steam-infusion pasteurization, which is one of direct heating methods, those two kinds of UHT milk were evaluated by several methods. The first one is sensory evaluation made by a panel of experts, the second is analysis using gas chromatography (GC) and gas chromatography (GC-MS), and the third is the method using sensors such as an odor sensor and a taste sensor. As to the sensory evaluation, cooked flavor and milk flavor in infusion-type UHT milk was slighter than plate-type. Infusion-type UHT milk was judged to be slight in body. During storage at 10 °C, the change of flavor and taste in infusion-type UHT milk tended to be less than plate-type. As to GC analysis in headspace, the amount of hydrogen sulfide (H_2S) in plate-type UHT milk was remarkably larger than infusion-type. The amount of ketones in infusion-type UHT milk was less than plate-type. Therefore, it was guessed that the change of aroma in infusion-type UHT milk was less than the plate-type during storage. As to sensor analyses, those UHT milks were discriminated clearly. It was suggested that the flavor and taste of those UHT milks were different. Data by odor sensor was correlative with sensory evaluation and GC data. In the case of taste sensor, data showed a high correlation with the sensory evaluation, too. In this work, it was concluded that analyses by odor sensor and taste sensor were as effective as sensory evaluation and GC analysis for flavor and taste of milk.

62. Odor Measurement Using Artificial Olfactory Epithelium

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To quantify the senses, it is required the interpretation of biological recognition process. In smell senses, odor molecules are dissolved into nasal mucus; then, they are detected by odor receptors on the olfactory epithelium in nasal mucus with recognizing common properties of odor molecules; i.e. odotope hypothesis. As the brain can recognize the odor from the combination of electrical signals transported from the receptors, it can be said that the odor is determined by the combination of the activated receptors. In this way, the recognition of odor molecules is very significant to quantify odor. The aim of this study is to develop an artificial olfactory epithelium, and also to attempt to detect and evaluate a wide variety of odor molecules by resolving into several molecular properties.

In the development of the sensor, molecular substructures are utilized as the detection target, and we have attempted to evaluate the odor of aromatic alcohol by its substructures which are hydroxyl group and aromatic ring. This system was duplicated by the electrochemical cell with a water membrane which is corresponded to artificial nasal mucus. In this system, there are developed channels which have the response specificity to peculiar molecular substructures with changing the composition of the water membrane and surface modification technology. As a result, we could obtain the results the channels responded to the specific molecular substructures. Using those results, the molecular information could be quantified; then, the odor quality and intensity of aromatic alcohol can be evaluated based on it.

63. Odor Coding and Synthesis Based on Molecular Informatics

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Odor quality was represented using substructure information of odor molecules. Some simple odorants having a single substructure were chosen as elemental odorants to synthesize quality of a target odorant. An apparatus which works as an odor synthesizing generator by mixing elemental odorants was fabricated. The apparatus displays odor by means of vaporization of mixture of odor water solution. Elemental odorants were selected on molecular informatics. Odor substances which were candidates for elemental odorants were analyzed to evaluate similarities of shape and electrical potential distribution on the molecular surfaces between elemental and target odorants by molecular modeling. QSAR (quantitative structure activity relationship) analysis was carried out to choose

candidates of elemental odorants and to adjust mixing ratio of elemental odorants. The way of making candidates of the elemental odorants is similar to molecular modification in medicinal chemistry. Thus, the odor is represented by combination of simple elemental odorants and odor strength of each chemical. The present information to represent odor quality can be used for odor code, which is utilized in biological olfaction. Obtained odor code was examined using the fabricated odor generator and evaluated by human panels. As a result, mixed elemental odorants had closer odor quality to a target odorant than each single elemental odorant. Consequently, odor coding using substructure unit can be applied for description of odor quality, odor sensing, and odor communication.

64. Development of an Odor Tracking Robot with an Array-Type Semiconductor Gas Sensor

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Odor tracking behavior has been investigated in literatures, such as in the biology of several insects or animals. The artificial sensor systems to identify the location of the odorants have also been studied, and the systems have been expected that it can be applicable to the many problems, for example, early fire detection, a localization of gas leakage point, etc.

In this paper, the odor tracking system using the mobile robot with an array-type semiconductor gas sensor is developed. The sensor array consisted of the identical type of gas sensors tracks the gradient of the concentration of the plume which is generated by the fan and alcohol is used as the odor source in this experiment. To lead the mobile robot to the odor source, steering the nose of the robot by balancing the outputs of sensor pair which is located on left and right sides, the robot can move along the orthogonal direction of the contour line of the concentration of the plume and also can track the gradient ascend direction of the gas concentration. Then we study some experiments to locate the odor to show efficiency of the proposed system.

65. Diversity of Mechanisms for Umami Transduction in Taste Cells

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Umami taste is elicited by L-glutamate (L-Glu), which is found in protein-rich food. Several candidates have been proposed for umami receptors, including NMDA-R, taste-mGluR4 and T1R1+T1R3 heterodimer. However, the response properties of each receptor type have not been directly compared to the responses of native taste cells. We investigated umami taste responses in mouse circumvallate taste buds in a semi-intact slice preparation. We loaded Calcium Green-1 dextran into mouse taste cells in lingual tissue slices and viewed with confocal microscopy for functional imaging. Focal application of L-Glu (30 to 500 mM) at the taste pore induced $[Ca^{2+}]_i$ responses in <5% of taste cells. These cells did not respond to KCl (50 mM) depolarization. In the absence of extracellular Ca^{2+}, glutamate responses were only slightly decreased relative to those recorded in normal Tyrode's medium. Depletion of Ca^{2+} stores by thapsigargin (1 μM) or treatment with U73122 (10 μM), a phospholipase inhibitor, abolished L-Glu-induced responses. Importantly, we also observed L-Glu responses in taste cells from mutant mice lacking T1R3. Further, separate cell populations responded to L-Glu and to L-AP4. And many L-Glu-responsive cells did not respond to other amino acids. These observations indicate that L-Glu stimulation of taste cells triggers phosphoinositide-mediated Ca^{2+} release from intracellular stores. However, because the umami responses of native taste cells are distinct from those of each proposed receptor, detection of umami compounds likely involves multiple receptors with partially overlapping ligand sensitivities.

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66. Species Specificity of Gustatory Responses from the Soft Palate and the Tongue in Rodents

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Various concentrations of NaCl, HCl, quinine- HCl (QHCl), sucrose (Suc) were applied to the soft palate or tip of the tongue in mice, rats, and hamsters. Taste stimuli and rinsing water (DW) was switched by an electromagnetic valve. Integrated neural responses from the greater superficial petrosal (GSP) and the chorda tympani (CT) nerve to various taste stimuli were recorded. Phasic response to 0.1 M NaCl was used as a standard. The height of the peak of the initial phasic response to each stimuli and the height of the tonic response at 10 s after stimulus onset were measured. To compare the magnitude of responses between the GSP and CT, and among three species, summation of all mean responses to NaCl, HCl, QHCl, and sucrose in the same concentration range were calculated to obtain total response magnitudes. Then, each response magnitudes was expressed as a proportion of the TRM for each nerve. QHCl produced robust phasic and tonic responses from the GSP in mice. Amiloride strongly inhibited tonic responses to 0.1 M Na-acetate in the GSP of mice similarly to that in the CT. Taste response characteristics of mice GSP were extremely different from those in the rat and hamster. The effect of the GSP + GL transection on aversive licking behavior for QHCl was larger by up to 75% than that of the CT transection. These results suggest functional differences of soft palate taste buds among rodents.

67. Differentiation of the Taste Buds on the Soft Palate—Expression of IP3R3 and Gustducin

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Taste buds in mammals are located on the tongue in fungiform (FF), foliate and circumvallate (CV) papillae and also on the soft

palate (SP). Since palatal taste buds develop earlier than those on the tongue, they are assumed to have an important role in the early phase of the growth process. Recordings to sweet stimuli from the taste nerve (greater superficial petrosal nerve) innervating the soft palate in rats were of a larger magnitude than those from the chorda tympani or glossopharyngeal nerves innervating taste buds on the tongue. A similar case occurs for bitter stimuli in mice. These results suggest a functional importance of palatal taste in adult rodents. However, the molecular characteristics of the palatal taste buds remain unclear. We compared the mean number of gustducin- and IP3R3-expressing cells in individual taste buds of the soft palate, fungiform and circumvallate papillae. Immunohistochemical analysis showed that IP3R3-expressing cells were mostly gustducin-positive in the soft palate, indicating that cell differentiation of the taste buds on the soft palate is different from those on the tongue.

68. Distribution Pattern and Development of Basal Cells of Taste Buds during Mouse Embryogenesis

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In adult mice, Prox1 was expressed strongly in basal cells of taste buds where the expression of Sonic hedgehog (Shh) and Prox1 completely overlapped. Mash1 was observed in a subset of basal cells with Shh and Prox1 expression as well as in fusiform taste cells. During development, the onset of expression of these basal cell-marker genes is assumed to indicate the differentiation of the basal cells of the taste buds. We have previously reported (JASTS, 2005) that not only Shh but also Mash1 and Prox1 were expressed in tongue and soft palate in mouse embryo. These expressions suggested that the differentiation of basal cells began during embryogenesis; however, the precise localization pattern of the basal cell marker genes remains unclear. We further examined the expression of these genes by whole mount in situ hybridization.

69. GABA is Produced in Taste Bud

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γ-Aminobutyrate (GABA) is synthesized from L-glutamate by glutamate decarboxylase (GAD). GABA is a major inhibitory neurotransmitter in the adult mammalian brain. GABA is a multifunctional molecule since it exhibits different functions in the central and peripheral nervous systems and in some non-neuronal tissues. The GAD67-GFP knock-in mouse is a powerful model for studying the distribution and morphology of GABAergic neurons in the brain. We have examined the tongues of GAD67-GFP knock-in mice by fluorescence microscopy and found cells with strong green fluorescent protein (GFP) signal in the taste bud. Immunohistochemical analyses showed that GFP-positive cells expressed GAD67 and GABA. RT-PCR analyses showed that both of GAD isoforms (GAD67 and GAD65) were expressed in the circumvallate papilla. RT-PCR analyses also revealed that mRNAs encoding GABAA and GABAB receptor subunits were expressed in the circumvallate papilla. Furthermore, immunohistochemical analyses with cell markers, such as gustdusin and PGP 9.5 for Type II cells, and PGP 9.5 and 5-HT for type III cells, have revealed that GABAergic cells in the taste bud were identified as type III cells. Since glutamate, a known umami component, is a substrate for GAD, we believe that GABA-production system in the taste bud may play an important role(s) as modulator and/or regulator of taste sense.

70. Developmental Expression of T1R Genes in Mouse Circumvallate Papilla

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Morphological studies have shown that mature taste buds, i.e. taste buds with apparent taste pores, appear immediately after birth. Recent advances in molecular biology have revealed the presence of taste receptors for five basic tastes; salty, sour, bitter, sweet and umami. It is unknown, however, whether all taste receptors function immediately after maturation of the taste buds. In the present study, therefore, we investigate the developmental expression of T1R genes which contribute to the perception of sweet and umami tastes in mouse circumvallate papillae by RT-PCR analysis. Epithelium of circumvallate papillae of C57BL/6J mice from embryonic day (E) 16 was isolated after injection of 2% collagenase into tongue musculature, and RT-PCR for T1R1, T1R2 and T1R3 was performed. Expression of mRNA for α-gustducin, a taste-related molecule participating bitter perception, was also examined. Both mRNAs for T1R1 and T1R3 were detected in epithelium of circumvallate papillae at E18, while mRNA for T1R2 first detected at postnatal day 1. Alpha-gustducin mRNA was recognized at E18. Since sweet receptor and umami receptor consist of combination of T1R2 and T1R3, and T1R1 and T1R3, respectively, the present results indicate the possibility that umami receptor establishes earlier than sweet receptors.

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71. Apoptotic Cells in Rat Circumvallate Papillae

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Traditionally, cells constructing taste buds are categorized as at least three types, spindle-shaped dark (type I), light (type II and III), and rounded basal (type IV) cells. There are major two hypotheses about cell lineage, i.e. single cell line theory and multiple cell line theory. The relationship between type II and III cells is, however, still unknown. It is well known that the life span of cells constructing taste buds is about 10 days and they die by apoptosis. But it is unknown which type(s) of cells undergo apoptosis. In this study we perform double immunohistochemistry for single stranded DNA (ssDNA) and markers for type II and III cells to reveal which

type(s) of cells die by apoptosis. We use G α-gustducin (Gust) and phospholipase C β2 (PLCβ2) as markers for type II cells and neural cell adhesion molecule (NCAM) as a marker for type III cells. We found some ssDNA immunoreactive (IR) nuclei in Gust and PLCβ2 IR cells, but rarely in NCAM IR cells. We also observed that ssDNA IR nuclei are observed in Gust IR cells and PLCβ2 IR cells at almost same ratio in spite of the fact that PLCβ2 presents in all type II cells, but approximately two thirds of type II cells lack Gust IR. These results suggest that type II cells are the conceivable cells that will be going to die by apoptosis, and that Type III cells possibly differentiate into type II cells, additionally, that Gust IR appear in old stage of PLCβ2 IR cells.

72. Lectin Histochemistry in the Gustatory Epithelium of the Various Mammals

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Mammalian taste buds contain 50–80 elongated epithelial cells (taste cells) as well as proliferative basal cell (progenitor cells). Elongated cells are divided into 3 types according to their morphology. Many histochemical studies have been performed to distinguish cell types in taste buds. Lectin histochemistry is a useful method to characterize the properties of epithelial and mesenchymal cells. We previously reported lectin binding patterns in rat gustatory epithelium, but little is known on other mammals. In the present study, we examined mice, guinea pigs and rabbits, compared with rats. Paraffin sections of circumvallate papilla of rat and mouse, and foliate papilla of guinea pig and rabbit were prepared, and incubated with either FITC-conjugated Jacalin, or FITC-conjugated Concanavalin A (ConA), or FITC-conjugated Wheat Germ Agglutinin (WGA) or FITC-conjugated succinylated WGA (sWGA) for 14–16 h at room temperature. Jacalin, which binds to basal cells (type IV cells) of the rat taste buds, also labeled rounded cells of mouse taste buds. Labeling of Jacalin could not be detected in guinea pig and rabbit. ConA bounds the membrane of some elongated taste cells of all species examined. Both WGA and sWGA labeled intragemmal spindle-shaped cells of mouse, rat and rabbit, but not of guinea pig. The present results indicate that lectin binding patterns are varied among species, probably is due to the difference in their dietary behavior.

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73. Jacalin in Developing, Degenerating and Regenerating Circumvallate Papilla of the Rat

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Previously we reported characteristics of rat taste bud cells by lectin histochemistry. Jacalin, a specific lection for galactosyl (β-1, 3) galNac, binds rounded cells at the basal portion (type IV cells) and spindle-shaped cells within rat taste buds. In the present study, we further examine Jacalin binding pattern during development and degeneration/regeneration following glossopharyngeal nerve injury. During development of rat circumvallate papilla, Jacalin bounds rounded cells at the basal portion of the taste buds as well as cell membrane from basal to granular cell layer of surrounding epithelium. At postnatal day 5, its binding pattern became almost identical to that of the adult. During degeneration caused by bilateral crush injury of glossopharyngeal nerve, number and size of taste buds cells decreased gradually. Jacalin was found at the membrane of rounded cells, and of some spindle-shaped cells. At regeneration process, many Jacalin-binding rounded cells were observed. The present results indicate that Jacalin is a reliable marker for basal cells (type IV cells) of the rat taste buds during development, degeneration and regeneration.

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74. Cloning and Characterization of Sex Pheromone Receptor Genes in Four Moth Species

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Many sex pheromones have been identified in various insect species. However, the receptors for sex pheromone have not been identified except silk moth. Identification of sex pheromone receptors of various moth species is important in order to aid in the control of the agricultural pests and to elucidate the evolution of sex pheromone receptors. Here we report on the cloning of sex pheromone receptor genes of *Plutella xylostella*, *Samia cynthia*, *Mythimna separata*, and *Diaphania indica*. By using degenerate primers based on the sex pheromone receptor of *B. mori* we cloned one candidate sex pheromone receptor gene from each of the moth species and named *PxOR1*, *ScrOR1*, *MsOR1*, and *DiOR1*, respectively. RT-PCR analysis showed that the transcripts of these genes were restricted in the male antennae of each moth. Double-labeling *in situ* hybridization with OR1 and pheromone binding protein (PBP) RNA probes, OR1 expressing cells are surrounded by PBP expressing cells, showing that these OR1 genes are expressed in the olfactory receptor neurons that are responsible for sex pheromone. In addition, the *Xenopus* oocytes co-injected with *PxOR1* and *OR83b* orthologous gene cRNA specifically and dose-dependently responded to a component, Z11-hexadecenyl acetate, among three components of the *P. xylostella* sex pheromone. To summarize these results, we concluded that PxOR1 is a sex pheromone receptor of *P. xylostella*.

75. Specific Expression of *Xenopus* Vomeronasal Receptor Genes (V1R) in Main Olfactory Epithelium

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Most vertebrates possess two anatomically distinct olfactory organs; the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). In rodent, 100–200 vomeronasal receptor genes (V1Rs) are specifically expressed in the VNO and are considered to be responsible for pheromone reception. Recently, a single V1R gene was identified in fishes and was expressed in olfactory epithelium (OE). Phylogenetically, VNOs first appeared in amphibians. To examine when the vertebrate V1R repertoire expands and functions as pheromone receptors in VNO during the course of evolution, we analyzed the genomic database of amphibian *Xenopus tropicalis* and identified 21 intact V1R sequences. We cloned all of these sequences from the genome and analyzed the expression of these genes.

We will present the results and discuss about the possibility that pheromonal information detected in the MOE is transmitted and processed via the main olfactory system.

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76. Effects of Gonadotropin Releasing Hormone on the Sex Pheromone Sensitivity of Mouse Vomeronasal Receptor Neurons

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The nervous terminalis, a nerve containing gonadotropin releasing hormone (GnRH) projects to the vomeronasal nervous system and GnRH receptors are expressed in the vomeronasal mucosa. The role of GnRH in modulation of sex pheromone responses in mouse vomeronasal neurons was examined using NBT labeling which visualizes activated neurons. GnRH was administrated 30 min before the pheromone odor supply. In intact animals, GnRH administration did not affect the vomeronasal receptor sensitivity to sex pheromones. In castrated males and females, the number of responded vomeronasal receptor cells to urinary pheromones was reduced compared to those observed in castrated animals, but GnRH administrations restored the number of responded receptors. This suggests that GnRH directly enhances vomeronasal neuron responses to pheromone odors and that the reduced sensitivity in castrated animals may be resulted by depletion of GnRH caused by a deficit of sex steroids positive feedback.

77. Expression of a Putative Pheromone Receptor Gene and G Proteins in Goat Olfactory Receptor Neurons

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Most mammals have two distinct nasal epithelia (olfactory epithelium (OE) and vomeronasal epithelium (VNE)). Olfactory receptor neurons (ORNs) in the OE express olfactory receptors (ORs) whereas vomeronasal receptor neurons (VRNs) in the VNE express two subtypes of pheromone receptors (V1r and V2rs) in rodents. ORs, V1rs and V2rs are coupled with Golf, Gi2 and Go, respectively. In previous study, goat pheromone receptor gene (gV1ra1) is expressed in not only VRNs but also ORNs. In this study, we investigate whether goat OE has the ability to detect pheromones. Double-labeled in situ hybridization revealed that Gi2 and V1ra1 were expressed in the same ORN. We also analyzed the expression of olfactory marker protein (OMP) and growth association protein 43 (GAP43) in gV1ra1-Gi2 expressing ORNs. V1ra1-Gi2 expressing ORNs do not express OMP and GAP43. In fish, OMP negative ORNs function as chemosensory cells, suggesting that gV1ra1-Gi2 expressing ORNs serve as chemoreceptor cells in the goat OE. We identified other subtype of gV1ra1 expressing ORNs from the goat OE. This type of gV1ra1 expressing ORNs expresses Golf and OMP but not Gi2 and GAP43, indicating that gV1ra1-Golf expressing ORNs are functional ORNs in goat OE. Although we need to investigate the biological meanings why two types of gV1ra1 expressing ORNs exist in goat, our present results indicate that a pheromone is detected in goat OE as well as VNE.

78. Two Types of Periglomerular Cells (PG1 and PC2) in the Main Olfactory Bulb of Mice and Other Mammals

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Glomeruli of the main olfactory bulb (MOB) are now considered to serve as functional units in the olfactory information processing. In the rat MOB we have found that each glomerulus consists of two compartments, olfactory nerve (ON) compartment and non-ON compartment; in the ON compartment ON terminals make synaptic contacts with dendritic tufts of both projection neurons and type 1 periglomerular cells (PG1), whereas they do not contact with dendritic processes of another type of PG cells (PG2). In this study, we investigated the structural organization of glomeruli with confocal laser scanning microscope (CLSM) in mice and other mammals, especially Madagascan lesser hedgehog tenrecs (*Echinops telfairi*). In mice, PG1 included GABA positive neurons and tyrosine hydroxylase-immunoreactive (TH-IR) neurons, whereas PG2 apparently included calbindin (CB)-IR and calretinin (CR)-IR neurons, resembling those in rat MOB. However, in contrast with rat PG2 which were not GABA positive, not only all TH-IR neurons but also about 70% of CR-IR neurons and all CB-IR neurons were also GABA positive. TH-IR, CR-IR and CB-IR neurons were 26%, 38% and 10% in GABAergic somata, respectively. Thus two types of PG cells were commonly present but their chemical properties could be different among animals.

Furthermore, in tenrec MOB, the compartmental organization of glomeruli and two types of PG cells we proposed as the common organizational principles were also recognized. The colocalization relationship of chemical substances of PG1 and PG2 was similar to that in the musk shrew, an insectivore.

79. Facilitatory Effects of Oxytocin on Olfactory Learning and Synaptic Plasticity in the Olfactory Bulb in Young Rats

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Oxytocin (OT) within the olfactory bulb (OB) has been reported to be important for the induction of maternal behavior and recognition of offspring. The activity of mitral cells, olfactory relay neurons in the OB is inhibited by granule cells via reciprocal dendrodendritic synapses, underlying adaptation or lateral inhibition of olfactory information. In vivo and in vitro electrophysiological studies have revealed that OT modulates mitral cell activity by acting on mitral and granule cells. In a classical conditioning paradigm, postnatal day (PND)-12 rats show aversion to the odor that has been paired with foot shock in a 30-min training session on PND-11. Our studies have shown that plasticity in the OB is critical for this olfactory learning. Therefore, we examined the involvement of OT in olfactory learning using behavioral and electrophysiological analyses. Pups that received OT infusion into the OB in the presence of citral odor developed an aversion to the odor without shock. Coinfusion of OT antagonist with OT agonist failed to induce aversive responses. It is suggested that OT infusion has a facilitatory effect on olfactory learning. OT antagonist infusion, however, did not prevent establishment of aversive olfactory learning after odor exposure paired with shock. Using OB slices of PND-11 rats, long-term potentiation (LTP) was induced in field EPSPs recorded in the granule cell layer in the OB by tetanic stimulation of the lateral olfactory tract. OT administration also facilitated LTP. These results demonstrate that OT is involved in olfactory learning in young rats.

80. Modulatory Effects of the Accessory Olfactory Bulb on V2R Family Pheromone Receptor Expression in Cultured Vomeronasal Neurons

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Pheromones are detected by unique receptors in the vomeronasal organ (VNO), and then affect endocrinal status and behavior in various kinds of animals. Although it is well known that the VNO mediates sexually and developmentally different behavioral and neuroendocrine responses, the cellular mechanisms regulating these functional differences are not much understood. The differences in pheromonal effects might be in part due to differential expression of pheromone receptors. To characterize the receptor expression, we examined two representatives (VR1 and VR4) from the V2R family of pheromone receptors using cultured vomeronasal neurons (VRNs). Previously, we established the VNO organ culture, which form a spherical structure with a central cavity, and reported the maturation of each VRN was induced by coculture with accessory olfactory bulb (AOB) neurons. A western blotting analysis showed the expression of VR1 and VR4 in the VNO was increased with days in coculture, an effect which was not observed in the VNO-alone culture. Moreover, we applied charged compounds in mouse urine iontophoretically into the cavity of VNO using a 3-barreled-microelectrode and analyzed VNO response using a Ca^{2+} imaging method with or without cultured AOB cells. When urine compounds were ejected into the VNO cocultured with AOB cells with a negative current, subpopulation of VRNs clearly showed long-lasting Ca^{2+} increases. Injections of a same current alone had no effect. Such Ca^{2+} increases were not observed without AOB cells. These functional results support that VRNs result in expressing pheromone receptors by interacting with AOB neurons.

81. Volatile and Nonvolatile Urine Responses and Functional Subdivisions in the Rat Accessory Olfactory Bulb

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To investigate how pheromonal information is processed in the rat accessory olfactory bulb (AOB), we optically imaged intrinsic signals to obtain high-resolution maps of activation induced by urinary stimulation. Application of volatile components in male rat urine mainly induced activation in the anterior AOB (aAOB), irrespective of gender, whereas volatile female rat urine elicited activation not only in the aAOB but also to some extent in the caudal part of the posterior AOB (pAOB) of male, but not female, rats. Nonvolatile components of both male and female rat urine induced activation mainly in the rostral part of the pAOB and to a lesser extent in the aAOB, irrespective of gender. Further, single-unit recordings were carried out in the active region where optical response was observed. We analyzed the firing activity of 46 aAOB neurons in nine animals. Of 13 aAOB neurons that responded to volatile stimulation (5% male urine, 5% female urine or 0.3% 2-heptanone) by increasing their firing rate significantly, 11 neurons responded to one type of stimulation and 2 neurons responded to both female urine and 2-heptanone. From these results obtained from single-unit recordings, it was confirmed that volatile urinary components activate at least the aAOB.

82. Odor Responses of Descending Neurons in Male Cockroaches

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It has been thought that olfactory processing function in the insect brain is mediated by dynamic modulation of coherent firing in groups of neurons. However, a lot is still unknown about the functional meaning. In the insect, descending neurons project from the brain to the thoracic ganglion through ventral nerve cord, which carry final information processed in the brain and involve in initiation of behavior by sensory cues. By investigating the relationships between the olfactory processing by a functional neuron group in the brain and odor responses of the descending neurons, it can be understood the neural mechanisms of olfactory information processing in the brain and odor-source searching behavior. In this study, spike responses of the descending neurons to odor stimuli (Periplanone-B, volatiles of artificial diet, 1-hexanol) were examined

using extracellular recording from the ventral nerve cord in the male cockroach (*Periplaneta americana*). The descending neurons showed odor-dependent phasic excitatory spike responses. More than half of them responded to two or more odors. This result indicates some functional overlapping among the descending neurons involved in odor information carrying to thoracic motor systems for initiation of behavior. Our previous work has suggested that a set of coactive neurons in the cockroach's brain encode olfactory information by overlapping groups of neurons. Together these results support the view that odor representation can be accomplished by ensemble networks.

83. A Study for the Distribution of an Estrogen Receptor in the Vomeronasal Organ

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It is known that sex steroids modify physiological activities of vomeronasal receptor cells (VRCs), pheromone-detecting sensory cells as well as bipolar neurons, which are housed in the vomeronasal sensory epithelium (VSE). However, mechanisms underlying the modification of the VRCs by sex steroids remain to be elucidated. We set a hypothesis that this modification of steroids is mediated by steroid receptors, and examined immunohistochemically the presence of estrogen receptor (ER) in the vomeronasal organ (VNO) of adult rats. Using a commercially available antibody to ER, we demonstrated that intense immunoreactivity for ER was remarkably found in the mucomicrovillar complex of the VSE in both male and female rats. As positive control, uterus sections were immunostained as well; intense immunoreactivity for ER was generally seen in cell nuclei of their secretary cells. In contrast, only a subpopulation of cell nuclei of VRCs and other cells within the VSE exhibited moderate ER immunoreactivity. Further analyses using multilabeling fluorescence technique and confocal laser-scanning microscopy demonstrated that immunoreactivity for ER was localized in the apical dendrites and their endings of matured VRCs. Western blot analyses indicated that a clear band for about 66 kDa protein, which corresponds to the molecular weight of ER, was clearly detected from VNO and uterus homogenates. The above results indicate that VRC of rats contain ER molecules, and suggest that some of them are localized in membranes of dendritic endings of VRCs.

84. Effects of Rosemary on Mental Fatigue Measured by the Uchida Kraepelin Test

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This study was conducted to clarify the influence of aromas on mental fatigue. Fifteen healthy young volunteers were subjected to the Uchida Kraepelin Test (UKT), a test involving a continuous series of additive calculations for 12 min with or without aroma stimulation with rosemary fragrance. The rosemary stimulation enhanced significantly ($P < 0.05$) the number of answers in the test. Saliva samples were collected from the subjects before, immediately after, and 10 min after administration of the UKT; and chromogranin A and cortisol, stress markers, and free radical scavenging activity (FRSA) in saliva were then measured. Aromic stimulation was carried out during the test or for 10 min after it. The chromogranin A level was increased significantly ($P < 0.01$) immediately after the test compared with that before it, and the level was reduced significantly ($P < 0.05$) 10 min after the test. The level was even more reduced ($P < 0.01$) by rosemary stimulation for 10 min after the test. When rosemary stimulation was done during the test, the chromogranin A level was not increased. The change in the cortisol level resembled that in chromogranin A. FRSA was increased, not decreased, by the UKT, suggesting that mental fatigue differed quite from physical fatigue. From the above results, we conclude that an aroma such as rosemary decreased the level of mental fatigue and increased the work quantity in the UKT by reducing the level of stress.

85. Effects of Aroma in Woods on Driver

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It is said that the aroma in woods has effects, such as fatigue recovery and cure for drowsiness. The aim of this research is evaluating the effect of the aroma in woods to a driver. An experiment related to driving-task load, using a simple driving task simulator, was devised for this purpose. Eight male subjects aged 23 to 39 participated in the experiment. Subjects were required to perform a dual task. The primary task was to keep the vehicle within a lane on a circular winding course at an automatically controlled steady 40 km/h for 25 min. The secondary task was to press a corresponding switch as quickly as possible when one of two LEDs was turned on. Two kinds of aroma, the aroma containing alpha-pinene and the aroma containing borneol, were used. Four aroma conditions, no aroma air, continuous supply of borneol-aroma, intermittent supply of borneol-aroma, and continuous supply of alpha-pinene-aroma, were carried out for each subject. In the condition of intermittent supply of borneol-aroma, it repeated supplying aroma for 2 min and supplying no aroma air for 4 min. The self-reported adjective moods, the driving performance, and EEG were measured.

In conditions of continuous alpha-pinene-aroma and continuous borneol-aroma, reaction time maintained well, while in conditions of no aroma and intermittent borneol-aroma, reaction time became late as the driving time goes by. It was suggested that the continuously supplied aroma, borneol-aroma or alpha-pinene-aroma, was effective in refreshment, attention and reaction ability.

86. Study on Evaluation Method for Gas Odorants

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Gas odorants are added prior to the gas delivery just in case of gas leak to be detected.

In evaluating the new gas odorants, it is necessary to keep security level of gas delivery as high as the present one when we change the odorants.

Odor quality is one of the most important parameters for gas odorization. For the gas odor to function as a leak detector to

secure the gas utilization, it is essential that not only the customer can notice the smell but also they realize their dangerous situations, and finally they take concentrate actions to make emergency calls to their Gas Company, fire station or police station.

We carried out smell tests conducted by odor bags and questionnaire method. The odor bags were filled with deodorized air, and a given amounts of odorants were precisely added. The questionnaire was consisted of some questions asking the odor strength and the odor quality which was specified to be the odor similarity to the "gas odor image." We attached a unique Yes/No question, asking "Do you make an emergency call to your gas company, fire station or police station when you smell this odor?," to know the tendencies of the realistic behavior of people. We defined the "Positive" rate to this question as an "Emergency Call Ratio" (ECR).

In this study, we investigated the ECR trends of each odorant and made a first attempt to use ECR as a quality of gas odor index.

87. EEG Responses to Comfortable and Uncomfortable Odors for Subjects in Olfactory-Related Occupations

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The purpose of this study is to investigate EEGs responses for subjects in olfactory-related occupations, ten professional perfume researchers and nine perfume salespersons, after inhalation of essential oils and, simultaneously, to observe any effect on subjective assessment. The essential oils, basil, jasmine, lavender, lemon, skatole and ylang-ylang, were presented for 90 s using an olfactometer. The results also showed that the alpha/beta ratio of the professional perfume researcher groups not only suggested that activity in their lobes could be easily distinguished from activity in the left and right hemispheres of the brain stimulation in response to comfortable and uncomfortable odors but also it proves that the frontal lobe is involved in the memory/speculation faculty. However, in the case of the group of salespersons, only the right lobe of their brains responded to both comfortable and uncomfortable odor stimulation. These results indicate that those in the professional perfume researchers group might not be responding to scent through sensory effects but through cerebral effects related to the frontal lobe.

88. Mother's Milk Odors Attenuated Stress Responses to the Heelsticks in Human Infants

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Mother's breast milk odors have attractive effect to their infant, however, little is reported about the effects of the mother's breast milk odors on their infant's stress response. In the present study, we examined whether the mother's breast milk odors affect the stress responses to a capillary puncture on the heel (heelsticks) during routine blood draws to screen for congenital diseases in 5 days-old infants.

Forty eight healthy infants were randomly assigned to the following four groups. In the Control group, infants were exposed to saline odor. In the Own mother's milk group, infants were exposed to their own mother's breast milk odor. In the Other mother's milk group, infants were exposed to other mother's breast milk odor. In the Formula milk group, infants were exposed to the formula milk odor. To assess the infant distress, their facial expression (grimacing) and crying were recorded by video camera and their body movements were recorded by actigraph. After the purpose of the experiment was explained to each infant's parent, the parents gave written informed consent. The protocol observed the tenets of the Helsinki Declaration and was approved by the Ethics Committee at Nagasaki University.

As we expected, infants showed distress during heelsticks in all groups. However, stress responses to the heelsticks were significantly attenuated by their own mother's breast milk odor, but in other mother's breast milk odor or formula milk odor, so that the mother's breast milk odor has a calming effect on her own infant.

89. Changes in Olfactory Sensitivity Round Menstrual Cycle

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Olfactory sensitivity change round the menstrual cycle as well as sensitivity enhancement by learning by repeated exposure were investigated with 30 young, healthy female volunteers of age 21.1 ± 1.2 years with menstrual cycle of 29.6 ± 4.0 days (mean ± SD). Materials used were androstenone and coprin, respectively, representing male hormone and vaginal odor, and rose-flavored phenylethyl alcohol as a neutral control. Personal olfactory perception level was judged by the three-way choice bottle test of sensitivity to a dilution series of respective odorants at each phase of menstrual cycle, i.e. bleeding, follicular, ovulatory and luteal. For androstenone, the subjects were divided into sensitive (n = 17) and anosmic (n = 13) groups according to the existing olfactory standard, whereas no such grouping was made for phenylethyl alcohol and coprin due to lack of such a widely acknowledged standard. The test results were rearranged by increasing number of exposure occasions to examine if experience of the past exposure enhances olfactory sensitivity. It turned out that the olfactory sensitivity 1) to coprin improved significantly with experience but was independent of menstrual cycle, 2) to phenylethyl alcohol was independent of both menstrual cycle and experience, and 3) to androstenone was significantly high during ovulatory phase in the sensitive group but was complacent round the menstrual cycle in the anosmic group. A trend of improving sensitivity to androstenone was also observed in the sensitive group, but its validity is not clear since the number of subjects in ovulatory phase just happened to increase in parallel with exposure repetition in this research.

90. Smell Function by a Familiar Odors Questionnaire

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In Japan, we have a practical test kit for smell function; T&T olfactometer. Although the test kit is a superior examination, it

has not been widely used in clinic because of the smell contamination and complexity of test procedure. Therefore we tried to test an odor function with a familiar odors questionnaire pattern. We choose 20 odors which were familiar for Japanese people. The odors were the bad-smelling socks, curry, India ink, orange, perfume, propane gas, condensed milk, fried garlic, wood, coffee, butter, soy sauce, garbage, sweat, feces, peach, laver, rose, Miso, bread. There are four choices of the answer. Those are ''smell well (point 2),'' ''smell a little (point 1),'' ''do not smell at all (point 0)'' and ''do not understand the odor because I have not smelt it recently (exclusion).'' We evaluated the smell function at the percentage that fell below a point in total with a point of an effective answer in total.

252 cases with olfactory loss were examined with the questionnaire. They were consisted of 89 males and 163 females and the range of age was 13 to 83 years old (mean: 57.1 years old). All the patients were tested with T&T olfactometer and the subjective symptom was calculated by Visual Analogue Scale (VAS).

The relations of VAS versus the questionnaire, T&T olfactometry versus the questionnaire, and T&T olfactometry versus VAS were significantly correlative. The exclusive rate of ''India ink,'' ''condensed milk'' and ''the socks with sweat'' were over 40%.

The questionnaire was effective for evaluation of the smell function, though it was necessary to reconsider some of smell modality.

91. Systematic Structure-Activity Study of Odor–Odor Interactions at Threshold Level

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Odors in the environment rarely originate from single compound. It is necessary to determine the factors or rules that govern the perception of odorants in a mixture. This topic of chemosensory responses to mixtures has relevance not only to understanding chemosensory but also to applying topics such as food aroma or environmental issues. To address this question, we measured psychometric (probability of correct detection vs. concentration) functions for series of aliphatic carboxylic acids and selected binary mixtures. Psychometric functions can provide information on how the senses of smell process mixtures of compounds in detail. Unmixed stimuli included acetic acid (C_2), butyric acid (C_4), hexanoic acid (C_6), and octanoic acid (C_8). Mixtures included C_2+C_4, C_2+C_6, and C_2+C_8. Two psychophysical models of mixture-interaction were applied: response-addition (independent processing of mixture components) and dose-addition (functional equivalence of mixture components). For C_2+C_6 and C_2+C_8, response-addition provided a better description of detection at low levels of performance. At higher levels of performance, dose-addition provided a better description. These results agree well with past findings. In contrast, for C_2+C_4 dose addition provided a better description of detection at low levels of performance. At higher levels of performance, detection fell closer to dose addition, but was much lower than the predictions from either model. These results suggest that interaction among odors in binary mixtures does depend on structural similarity, at least with respect to detection. Future studies can determine if this result is particular to carboxylic acids. The long-term goal is to build a structure-activity model of interactions, which can suggest synergistic or suppress combinations.

92. Mere Exposure Effect of Fragrance (2): Relation between the Preference Change and Impression of Fragrance

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It is known as the mere exposure effect that human comes to form a favorable attitude to the stimuli when one contacts it repeatedly. This examination has done to prove the mere exposure effect of fragrance as olfactory sense stimuli so we analyzed the relation between change in fragrance preference and impression of fragrance. We also studied on change of fragrance preference under the different condition of contact in numbers or in period.

We found some character of fragrance such as ''deep,'' ''heavy'' or ''rich'' would raise fragrance preference by numbers of contact by analyzing the relation between preference change of several types of fragrances by contact and impression.

We obtained the result; as fragrance preference rose by contacting stimuli for plural times; from the plural time contact experiment in a short term in which we changed the advance contact numbers as twice, four times and six times. However, we could not obtain the results that increasing times of contact stimuli was effective to rise preference more though if increasing the numbers of contacting stimuli. From the plural time contact experiment in a long term, we could not obtain the result as rising fragrance preference by continuously contacting stimuli for 4 times every 1week though preference rose by everyday contacting stimuli condition. Thus the mere exposure effect of olfactory sense stimulation was proved in long term contacting case.

93. Axonal Projection of OMP-Immunoreactive Cells in the Anterior Sac of Choana in Tadpoles of the Japanese Toad (*Bufo japonicus*)

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In tadpoles of the Japanese toad at about 10 days after hatching (Iwasawa stage 34 = Gosner stage 27, a feeding and free-swimming tadpole), the rostral portion of choana (internal nostril) is protruded into the buccal cavity and forms the pocket which is referred to as the anterior sac of choana. The anterior sac of choana is lined with ciliated epithelial cells with the apical surface exposed to the buccal cavity. The immunoreactivity of those cells to anti-OMP (olfactory marker protein) antiserum was examined to study whether they are a part of the main olfactory epithelium. Strongly OMP-immunoreactive cells suggesting the olfactory receptor neurons were seen in the anterior sac of choana. The projection of those epithelial cells in the anterior sac of choana was shown by DiI (carbocyanine fluorescent dye) labeling. The axons of DiI-labeled cells in the anterior sac of choana terminated in a posterior ventromedial region of the olfactory bulb. In rodents the posterior ventromedial region of the olfactory bulb is a projection area of the axons from the septal organ of Masera. Therefore, the anterior sac of choana in tadpoles may be an organ homologous to the septal organ of Masera. The anterior sac of choana may serve as an olfactory organ involved in feeding behavior specific to the tadpoles, because they lose this organ when metamorphosed.

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