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ABSTRACTS

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An attempt to establish *in vitro* models for analyses of gustatory organs with clonal cell lines derived from taste buds

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Many researchers tried to isolate taste buds and develop *in vitro* systems. In our laboratory, we collected taste buds from a tongue of a *p53*-deficient mouse and established clonal cell lines (TBD cell lines). In this study, we characterized TBD cell lines and tried to analyze gustatory tissue differentiations. Expression patterns of some taste receptors in four TBD cell lines were examined with RT-PCR. These cell lines expressed T1R3 and some of them expressed also T2R8 for bitter taste. In addition, candidates for sour receptors, HCN4, PKD2L1 and PKD1L3 were expressed in some of TBD cell lines. In addition, one of TBD cell lines responded to citric acid, and a transient elevation of intracellular Ca^{2+} was elicited. The result suggests that these TBD cell lines can respond to sour taste stimuli, and could be useful models *in vitro* of taste receptor cells. Next, we tried to culture TBD cell lines three-dimensionally, and analyze the cultured cells. TBD cell lines cultured in collagen gels formed cell clusters that had an internal cavity. Deposition of laminin, one of marker proteins for basal membrane, was immunohistochemically detected at the surface of the clusters. In addition, electron microscopic observation revealed microvillus-like structures at the internal surface of the cavity and tight junction-like intercellular structures in TBD-a5 cell clusters. These results suggest that TBD cells develop 3-dimensinal architectures with a basal membrane-like structure and apical-basal polarity. In conclusion, TBD cell lines are useful models for analyses of gustatory function and taste cell differentiation.

Regulation of type III cell by transcription factors

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The gustatory cells in taste buds have been identified as paraneuron, they possess characteristics of both neuronal and epithelial cells. Like neurons, they form synapses, store and release transmitters, and are capable of generating an action potential. Like epithelial cells, taste cells have a limited life span and are regularly replaced throughout life. However, little is known about the molecular mechanisms that regulate taste cell genesis and differentiation. In the present study, to understand the mechanisms that regulate taste bud cell differentiation, we have investigated the role of *Mash1* in regulating taste bud cell differentiation using *Mash1* KO mice and forced expression of transcription factors in lingual epithelial cells. We found that amino acid decarboxylase-immunoreactive (AADC-IR) cells were not evident in either the circumvallate papilla epithelia or in taste buds in the soft palates of *Mash1* KO mice. However gustducin, a marker of type II taste bud cells, was expressed in taste buds in the soft palates of *Mash1* KO mice. Forced expression of neural-lineage-specific transcription factors in tongue epithelial cells induced neural cell marker expression and neural cell morphology. These results suggest that transcription factors could play an important role of differentiation of taste bud cells from tongue epithelial cells.

Expression of the glucose transporters in mouse circumvallate papillae

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The glucose transporter families SGLT and GLUT are responsible for the absorption of glucose across the small intestine, the reabsorption of glucose from the glomerular filtrate, and the uptake and release of glucose from all cells in the body. These transporter families are known to have distinct regulatory and/or kinetic properties that reflect their specific roles in cellular and whole body glucose homeostasis. However, the functions of these families in mouse taste tissues have not yet been elucidated. We have therefore examined the expression patterns of glucose transporters (GLUT1-4 and SGLT1-3) in mouse gustatory tissues.

Reverse transcription/polymerase chain reaction assays have revealed that GLUT1, 3, 4 and SGLT1 mRNAs are expressed in the circumvallate papillae. In the circumvallate papillae, the antibody against GLUT1 yielded the labeling of a subset of taste bud cells and the trench wall epithelium. Double-labeling experiments have demonstrated that GLUT1 -positive cells coexpress gustducin. These results show that GLUT1 may play a role in the uptake of glucose in mouse circumvallate taste buds.

The histochemical effects of continuous dietary sodium restriction on taste cells

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It is needless to say that correct nutrition intake is very important for development. Sodium chloride is an indispensable nutrient for every organ of a living body to maintain homeostasis, proper function of nerve and muscle and so on. Previous reports showed that continuous dietary sodium restriction (DSR) at early embryonic period brings alterations in central projecting areas of gustatory nerve in nuclei solitary tract. But, most of the things about the effect of DSR on individual cells constructing taste buds is still unknown. In this study, to investigate the effect of DSR through embryonic days to postnatal life on number and distribution of taste cells, we performed immunohistochemistry on taste buds of the rats with treatment of DSR through embryonic day 3 to adulthood. We used some molecules locating in special types of taste cells such as G-α gustducin, PLCβ2 (considered to participate in biter and sweet taste transmission) as candidate markers of type II cells, PKD2L1 (considered as a sour receptor) and NCAM as markers of type III cells. quantitative analysis of imunohistochemistory were carried on postnatal day 60. We could not find significant alteration in the expression of G-α gustducin, PLCβ2 and PKD2L1, but the number of NCAM immunoreactive cells decrease after DSR, and this reduction was partially rescued by sufficient sodium intake after PN day 40. These results suggest that appropriate sodium intake is necessary for the proper development of peripheral taste system and the possibility that continuous DSR make some alteration on the property of type III cells in taste buds.

Expression of Six1 and Six4 in mouse taste buds

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Members of the *Six* gene family are expressed in various tissues including sensory organs, such as the inner ear and olfactory epithelium. We examined the expression of *Six1* and *Six4* mRNAs in mouse taste buds by using *in situ* hybridization. Six1 was detected immunohistochemically in the nuclei of taste bud cells, in a subset of type-II cells, as shown by double-immunolabeling with anti-Six1 together with anti-PLCβ2 or anti-IP_3R3 antibodies. Six1-immunoreactive (IR) nuclei appeared at embryonic day 17.5 in the dorsal epithelium, and in the trench wall epithelium of circumvallate papillae at postnatal day 5. At this stage, Six1-IR nuclei were observed in all newly-formed type-II cells. During postnatal development, type-II cells increased in number, but those with Six1-IR nuclei showed no apparent increase. After transection of the bilateral glossopharyngeal nerve, type-II cells gradually disappeared; but some of them remained in the epithelium even at 11-17 days post-transection. The remaining type-II cells showed Six1-immunoreactivity. At 24 days after nerve transection, regenerating type-II cells appeared; and strong Six1-immunoreactivity was observed in them. Also, enhanced green fluorescent protein-immunoreactivity and β-galactosidase-immunoreactivity, which were indicators for *Six1* transcripts and *Six4* transcripts, respectively, overlapped. These results suggest that *Six1* and *Six4* genes are expressed in the taste bud cells, in newly formed or surviving type-II cells.

Spatiotemporal characteristics of taste-sensitive neurons in the rostral nucleus of the solitary tract in the rat

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The rostral nucleus of the solitary tract (rNST), the first-order taste relay, receives spatially organized projections from plural taste nerves. Here we examined relationships between the temporal characteristics (onset latency and response duration) and localization of rNST taste-sensitive neurons. Multi-barrel glass micropipettes were used to record extracellularly single unit activity under urethane anesthesia. The recording sites marked by dye spots (ejected from the recording electrodes) were reconstructed on the rostrocaudal (RC), mediolateral (ML) and dorsoventral (DV) axes. Taste solutions were applied to the anterior tongue and oral cavity. Forty-eight taste-sensitive neurons were classified into 18 NaCl (N)-best, 14 NH-best, 10 HCl (H)-best, 5 sucrose (S)-best, 1 SH-best and 0 quinine (Q)-best. In N-best neurons, the average duration of responses to the best-stimulus (4.2 s) was significantly longer than that in NH-best (2.1 s) (one-way ANOVA, $p<0.05$, followed by Tukey HSD, $p<0.05$). Most N-best neurons were observed in the rostral half of rNST. In S-best neurons, the average onset latency of response was longer to the best-stimulus (1.6 s) than that in NH-best. S-best neurons were mainly found in the caudal half of rNST apart from N-best neurons. H-best neurons tended to localize more caudally than N-best neurons, but the two distributions overlapped extensively. Distributions of neurons of the different best tastes did not differ markedly on the ML or DV axis. These results suggest that taste qualities may be represented by spatiotemporal patterns of neuronal activities along the RC axis in rNST.

Large bitter response mediated by gustdcuin-independent mechanism in the chorda tympani nerve and its small contribution to avoidance behavior

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Both phasic and tonic responses from the greater superficial petrosal nerve to bitter tastants in gustducin-knockout (GKO) mice were markedly reduced compared to those in wild-type mice (WT). However, phasic responses from the chorda tympani nerve (CT) to quinine-HCl and denatonium benzoate were not affected by GKO, while their tonic responses were reduced. Therefore, transduction mechanisms with and without gustducin are both involved in bitter nerve response, and the extent of contribution of each mechanism to nerve responses differs among taste nerves and among bitter

substances. However, it is unclear if neural responses mediated by each mechanism equally evoke bitter sensation that drives avoidance behavior. To evaluate and compare the relative strength of bitter sensation evoked gustducin-dependenlty and -independently, licking tests were conducted. A series of solusions distilled water (DW), five ascending concentration series of a bitter substances and DW was presented three times to 24 h water-deprived mice. Number of licks for 10 seconds after the first lick was counted. Licking numbers in all series on WT mice were markedly reduced at the highest concentration tested. In GKO mice, licking numbers in the first and second series were not decreased, suggesting the bitter sensation depended on gustducin. In the third series, the licking numbers at the highest concentration were decreased in GKO mice, suggesting the avoidance behavior evoked through gustducin-independent mechanism appeared in GKO mice with decreasing thirst. These results indicated that bitter sensation linked to avoidance behavior was markedly reduced in GKO mice in spite of the nerve responses largely remained in CT.

Epithelial sodium channel (ENaC) is involved in sodium taste: Evidence from tissue-specific conditional ENaCα knockout mice

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Observations that amiloride and other ENaC blockers alter taste responses to sodium salts generated the hypothesis that ENaC is involved in salt taste reception. To directly test this hypothesis, we have generated mice with the ENaCα subunit selectively eliminated in the lingual epithelium using the Cre-*loxP* mediated conditional gene deletion technique. Electrophysiological experiments have shown that mice with the tissue-specific conditional ENaCα deletion lacked the amiloride-sensitive component of chorda tympani nerve responses to lingual application of sodium salts (NaCl, sodium acetate, monosodium glutamate). However, the amiloride-insensitive component of the response to the sodium salts, responses to non-sodium salts (KCl, $CaCl_2$, $MgCl_2$), sweet (sucrose, saccharine) bitter (quinine) and sour (HCl, citric acid) taste stimuli, or responses to irritants (menthol, capsaicin, ethanol) were not affected in these mice. In brief-access tests, ENaCα knockout mice had an attenuated aversion to higher concentrations of NaCl compared with control mice. These data provide direct evidence that ENaC is involved in detecting sodium taste. Our results further demonstrate that there is a significant ENaC-independent component of taste responses to salts.

Analysis of caffeine transduction mechanism in different type of mouse taste cells

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Animals avoid bitter taste because most of bitter substances is harmful. But, humans enjoy bitterness of caffeine at appropriate concentration. Thus there may be different transduction mechanism between high and low concentration of caffeine. In addition, there are a lot of unsolved issues about the pathway from taste cells to taste cells or taste nerves. Therefore, we analyzed the caffeine transduction mechanism of mouse using behavioral tests [two-bottle preference (TBP) and conditioned taste aversion (CTA) tests], calcium imaging, and immunocytochemistry. In the TBP test, C57BL/6J mice significantly avoided over 10 mM caffeine, whereas in the CTA test they exhibited generalized aversion to 3 mM caffeine after aversion conditioned to 1 mM quinine. This suggests that 3 mM caffeine may elicit taste that is at least partly similar to quinine, but is not aversive to mice. In contrast, when the responsiveness of taste cells to caffeine were examined by calcium imaging followed by immunocytochemistry to detect expression of G_α-gustducin and IP_3R3, type II cell markers, we found that both 3 and 10 mM caffeine provoked substantial responses of not only type II cells but a small population of other type of taste cells as well. This suggests no clear difference in response patterns for 3 and 10 mM caffeine at the taste cell level. Differential behavioral responsiveness between 3 and 10 mM caffeine may be due to the action of the peripheral and central neural systems. In the other aspect, during the course of experiments, we found that a part of type II cells, like type III cells, was depolarized by high concentration of K^+, and taste cells responding to both caffeine or high K^+ existed. Also, ATP stimulates either type II or other type of cells. These findings are partially different from those previously reported. Further experiments are needed to clarify these discrepancies.

Involvement of the opioidergic system in the impaired CTA retrieval induced by systemic administration of midazolam

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In a conditioned taste aversion (CTA) paradigm, a palatability shift of the taste (conditioned stimulus: CS) is one of factors for the subjects to reject the CS after conditioning. Midazolam (MDZ), a benzodiazepine agonist, has a unique feature to facilitate ingestion of palatable, but not unpalatable, foods and fluids. It has been reported that a systemic administration of MDZ impairs retrieval of CTAs to palatable, but not to unpalatable, CSs. We hypothesized that the MDZ-induced impairment of CTA retrieval is mediated by enhanced palatability of a preferred CS through activation of the opioidergic system. To elucidate the issue, we examined the effects of naloxone (NAL), an oioidergic reseptor antagonist, on the MDZ-induced impairment of CTA retrieval. Systemic preinjections of NLA significantly blocked MDZ-induced impairment of CTA retrieval in dose-dependent manner in mice. The present findings suggest that MDZ enhances rewarding or preferred properties of palatable CSs via activation of the opioidergic signaling, and that the activated opioidergic signaling results in masking and/or antagonizing conditioned aversive shift of palatability at CTA retrieval.

Substance P-immunoreactive nerve fibers in the frog fungiform papilla: origin and coexistence with certain neurochemical markers

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Although the presence of substance P (SP) immunoreactive (IR) nerve fibers has been reported in the frog fungiform papilla, the functional roles of these fibers remain to be elucidated. To clarify the roles of the SP-IR fibers, we immunohistochemically examined the coexistence of SP and other neurochemical markers such as synaptosome-associated protein of 25 kDa (Snap 25), vasoactive intestinal peptide (VIP), tyrosine hydroxylase (TH) and phospholipase Cβ2 (PLCβ2) in the fungiform papilla of the frog, *Rana catesbeiana*. SP-IR nerve fibers ascended around blood vessels. Some SP-IR fibers appeared to reach the free surface of the taste disc and some formed a meshwork surrounding the taste disc. Approximately ten SP-IR nerve fibers were observed in a single fungiform papilla. Double-labeling immunohistochemistry showed thin nerve fibers that were Snap 25-IR colocalized SP, whereas thick fibers that were Snap 25-IR did not. Both thick and thin Snap 25-IR fibers innervated the taste disc. Nerve fibers that were VIP-IR ascended in the taste disc and VIP-IR neuronal cell bodies were observed in the glossopharyngeal nerve branches in the tongue. Although TH-IR fibers formed a plexus in the subepithelial tissue and reached the bottom of the taste disc, there were no TH-IR fibers observed within the taste disc. Nerve fibers that were SP-IR did not colocalize either VIP or TH. Although some SP-IR neuronal cell bodies were observed in the jugular ganglion, which includes the glossopharyngeal nerve ganglion in the present report, no SP-IR cell bodies were observed in the tongue. Above results suggest that nerve fibers that were SP-IR are unmyelinated sensory nerves. Since the fibers that were SP-IR colocalized PLCβ2, a signal element in the taste transduction cascade in mammalian taste cells, nerve fibers that were SP-IR might be involved in taste reception in some way.

Effect of stimulation of the tongue with taste stimuli on voluntary swallowing in humans

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Mechanical and chemical stimulation of the laryngopharynx (LP) can evoke reflex swallowing. Taste stimuli applied to the tongue, however, does not elicit swallowing reflex. Thus, there is a fundamental difference between sensory inputs from receptors in the LP and tongue for initiation of the swallowing reflex. Swallowing can be initiated voluntarily. Mechanical and chemical stimulation of the LP facilitates voluntary swallowing in humans. In this study, we examined whether stimulation of the tongue with taste stimuli affect voluntary swallowing. Healthy adult volunteers were instructed to perform repetitive swallowing as quickly as possible during the infusion of taste stimuli to the tongue. Water and taste solutions (0.3 M sucrose, 0.2 M acetic acid, 5 mM Q-HCl and a mixture of 40 mM MSG and 40 mM IMP) dissolved in water were used. We measured swallowing intervals (SIs) in repetitive swallowing. SI was shorter in the case of infusion of taste stimuli than in the case of infusion of water. That is, both pleasant (sweet and umami taste) and unpleasant taste (sour, salty and bitter taste) stimuli similarly facilitated voluntary swallowing. This implies that sensory inputs from gustatory receptors in the tongue appear to be potent for triggering swallowing when a swallow is initiated voluntarily. However, the central mechanism is unknown. Facilitation of voluntary swallowing by infusion of the taste solutions to the tongue was identical to that by stimulation of the LP. Therefore, gustatory inputs are important for performing voluntary swallowing smoothly.

Effect of amiloride on sodium taste in Japanese population: Analysis with a description method

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Amiloride, a blocker of epithelial sodium channel (ENaC), attenuates gustatory responses to lingual NaCl in many species. Recently it was demonstrated that ENaC functions as the sodium taste receptor in mice. However, psychophysical experiments in humans remain conflicting and several studies, using Caucasians as research subjects, have failed to substantiate a significant effect for amiloride in the perception of saltiness. In mice, strain differences in amiloride-sensitivity on salt taste responses have been reported and it was proposed that single nucleotide polymorphisms of ENaC may control the amiloride-sensitivity. It is possible that genetic diversity among humans may lie behind the conflicting results. In the present study, we investigated the effect of amiloride on salt taste in Japanese population, using a description method. Subjects were told to describe changes in taste of a test-solution after a lingual treatment with amiloride. Amiloride altered taste quality of only sodium containing solutions (250 mM NaCl, 800 mM Na-acetate) significantly but not 50 mM citric acid and 10 mM Acesulfame K. Changes in the taste quality of the sodium containing solutions were described as becoming 'mild', 'less sharp' or 'not stimulating'. These results suggest that amiloride block ENaC and alter the taste of sodium salts in Japanese as well as in other species, but the change of the taste is not necessarily perceived as a reduction of saltiness.

Involvement of GABA receptors in the basolateral amygdala on the retrieval of conditioned taste aversion in rats

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The basolateral amygdala (BLA) is known to be one of the key regions in the central nervous system for learning and memory. It has been shown that gamma-aminobutyric acid (GABA) functions in the BLA on acquisition, retention and extinction of conditioned taste aversion. However, it has not yet been examined on

blockade of $GABA_A$ receptor on the retrieval of conditioned aversion learning. To elucidate the role of BLA on the retrieval of conditioned taste aversion, we investigated the effect of microinjections of muscimol, a $GABA_A$ receptor agonist, on the intake of conditioned stimulus (CS) on the retrieval test. On the conditioning day, rats were presented with 5 mM saccharin as CS followed by intraperitoneal administration of 0.15 M lithium chloride (unconditioned stimulus, US). Two days after the conditioning, muscimol (50 ng) or vehicle was bilaterally infused into the BLA just before the presentation of the CS and intakes of the CS were measured. The muscimol-injected group had significantly high consumption of CS than the vehicle-injected group. There was no aversive behavior detected while testing for the experimentals, whereas the vehicle group only took a sip and avoided the CS. These results suggest that the facilitation of $GABA_A$ receptors in the BLA disrupts the retrieval of CTA. This might be due to decreased aversion for the CS or blockade of the aversive memory retrieval.

The brain mechanisms of taste reactivity responses by microinjection of $GABA_A$ receptors agonist into the ventral pallidum in rats

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We previously showed that microinjections of muscimol, a $GABA_A$ receptor agonist, into the ventral pallidum (VP) evoke taste reactivity responses. Low dose (10 ng) of muscimol induces ingestive responses, while high dose (100 ng) causes aversive ones. To elucidate the neural mechanisms responsible for the stimulation of $GABA_A$ receptors in the VP inducing the taste reactivities, we examined the effects of application of muscimol into the VP on the activities of other brain regions, using Fos-like immunoreactivities (FLI). Rats were implanted with guide cannulae in the VP. After the surgery, they received bilateral injections of 0, 10 or 100 ng muscimol into the VP. The rats were perfused 120 min after the muscimol injections, then Fos-positive neurons were detected. In this experiment, we analyzed the rostral and caudal parts of the basolateral (BLA) and central (CeA) nuclei of amygdala. Microinjections of vehicle (0 ng) or 10 ng muscimol into the VP produced few of FLI in both subnuclei of amygdala. Higher dose (100 ng) of muscimol induced larger number of the FLI in the CeA but, on the contrary, no or less in the BLA. These results indicate that the stimulation of $GABA_A$ receptors in the VP causes inhibition of the BLA neurons and excitation of the CeA neurons. Since these amygdaloid subnuclei are known to be involved in the retrieval of CTA, it is suggested that the inputs from the VP to amygdala may play a role in behavioral response to learned aversive taste stimulus in CTA.

Modulation of histaminergic activity by umami solution

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Sodium salts of L-glutamate elicit a unique taste called umami which does not belong to the four basic tastes. Recent study indicates that spontaneous ingestion of 1% monosodium L-glutamate (MSG) can increase energy expenditure resulting in reduction of weight gain and body fat mass in rats. Since intra-gastric administration of L-glutamate (0.06 M) is shown to activate the medial preoptic area and the dorthomedial hypothalamus by functional magnetic resonance imaging study, it is postulated that these areas are involved in MSG-induced thermogenesis. However, neurochemical information of the transmitters associated with this mechanism is still lacking. The histaminergic system is known to be influenced by taste information, and take part in thermoregulation in the medial preoptic area. Thus, in the present study, we examined the effect of umami solution on histamine release in the medial preoptic area by *in vivo* microdialysis using urethane-anesthetized rats.

We used two types of umami solution: 0.06 M MSG and 0.1 M monopotassium L-glutamate (MPG), because NaCl solution (i.e., sodium ion) induced large responses of hypothalamic histamine release in our previous study. Histamine release was not altered by intraoral stimulation of both of the solutions. Previous study revealed that 0.1 M MPG did not enhance responses of the chorda tympani, thus the present result is in line with that study.

By contrast, histamine release was significantly decreased by intra-gastric administration of both of the solutions. These findings indicate that umami solutions modulate histaminergic activity in the medial preoptic area *via* visceral pathways.

Gustatory sensation and autonomic nerve activity 1. Reactions to 4 basic tastes

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The autonomic nervous system functions to maintain homeostasis of the body against internal and external environmental changes. In response to gustatory stimulation, reflex changes in expression, adjustment of digestive and absorptive activities via autonomic nerves, and expressions of emotion, such as comfortable and uncomfortable feelings, and accompanying reactions occur. The autonomic nerve system is considered to be influenced by olfaction, but the details remain unclarified. Basic studies in ophthalmology have revealed that autonomic nerve functions can be evaluated using pupillary reactions as an index, and the influences of hot spring bathing, occlusal interference, and a special sensation, olfaction, on autonomic nerves have been reported. We analyzed pupillary reactions to basic tastes (sweet, sour, salty, and bitter) to qualitatively clarify autonomic nerve reactions to gustatory stimulation. In experiments, filters soaked with sweet (80%), salty (20%), sour (8%), and bitter (4%) tastes and water were placed on the tongue tip of resting subjects and pupillary reactions were measured. For analytical parameters, the pupillary area and maximum miotic rate were employed. The stimulations were loaded in a random order, but the bitter taste was given last. The pupillary areas were significantly greater after gustatory stimulation with salty and bitter tastes compared to those before stimulations. Regarding the maximum miotic rate, no significant differences were noted in responses

to any gustatory stimulation, but it tended to be increased by sweet sensation. Sympathetic nerves were significantly excited by salty and bitter tastes, and parasympathetic nerves tended to be excited by sweet taste.

Gustatory sensation and autonomic nerve activity 2. Reaction to stepwise gustatory stimulation

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Various human reactions are induced by external stimulation. Although it is considered that adjustment of digestive and absorptive activities via autonomic nerves and expressions of emotions, such as comfortable and uncomfortable feelings, and accompanying reactions occur in response to gustatory stimulation, details of the relationship between gustatory stimulation and autonomic nerve activity remain unclarified. Since autonomic nerve activity can be analyzed based on pupillary reactions of miosis and mydriasis, we qualitatively analyzed autonomic reactions to 4 basic tastes using pupillary reactions as an index. Gustatory stimulations were loaded at stepwise-increasing concentrations, and pupillary reactions were analyzed to qualitatively investigate autonomic reactions to stimulation. In experiments, 1 ml of a bitter solution (quinine hydrochloride) was dripped into the oral cavity at threshold or higher levels in resting subjects, and pupillary reactions were measured. As an analytical parameter, the pupillary reaction rate (size of the pupillary diameter after bitter taste stimulation relative to that before stimulation while resting) was calculated. Eight-step bitter taste concentrations were set: 0.00156, 0.0031, 0.0063, 0.0125, 0.025, 0.05, 0.1, and 0.2%, and solutions were randomly dripped. Subjects sufficiently gurgled between stimulations. Pupillary reaction rates were influenced by the concentration, and a significant increase was observed as concentrations rose. Since dilator and sphincter muscles of pupils are mainly controlled by autonomic nerves, pupillary dilation and miosis are induced by sympathetic and parasympathetic excitement, respectively. It was suggested that sympathetic nerves are excited by bitter tastes, and activity is enhanced with the increasing concentration of bitter solutions.

The effect of umami taste on saliva secretion

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Because saliva contains various components for the maintenance of oral mucosa and tooth, hyposalivation sometimes causes oral dysfunction. We have recently found that umami taste induced sustained salivary secretion. In this study, we examined two distinct methods for taste stimulation on saliva secretion using fifteen healthy adults aged from 20's to 50's as subjects. We compared the following two methods; swirling the taste solution in the mouth (whole mouth method), and spraying the solution into the mouth (spray method). Taste stimuli were: 100 mM MSG (umami), 3.8 mM citric acid (sour) and 440 mM xylitol (sweet). Each concentration was adjusted to have 'moderate' taste intensity evaluated by a labeled magnitude scale. For the whole mouth method, each subject were administered 3 ml of the taste solution into the mouth with a pipette. For the spray method, individuals were sprayed the taste solution (approx. 0.25 ml) into the mouth using a spray bottle. After holding the taste solution for 30 seconds, whole saliva was collected into individual cups every 30 second for 10 minutes. Then, the salivary flow per minute, depending on different stimulation, was calculated by the weight of saliva. Salivary flow without taste stimuli (''no stimuli'') was also measured. In the whole mouth method, all taste solution showed significantly larger amount of saliva as compared to ''no stimuli''. However, in the spray method, only MSG showed elevated amount of saliva than ''no stimuli''. In both methods, MSG had prolonged salivary flow rate than citric acid and xylitol. In conclusion, a small amount of the umami stimulation could result in sustained salivary secretion.

Receptor subtype specific activation of the celiac vagal afferent fibers to serotonin in rats

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Serotonin (5-HT) has widespread actions within the gastrointestinal tract that effect on both neural and non-neural tissues. Previously, it was reported that 5-HT affected on mesenteric afferent nerves in rats. However, it is still unclear what receptor subtype of 5-HT activates on vagal celiac afferent activity. In the present study, we examined the effects of various 5-HT receptor subtype-specific agents on vagal celiac afferent activity and identified pharmacological characteristics involved in the activation of vagal celiac afferent fibers by 5-HT. Systemic administration (i.v.) of 5-HT evoked both transient and sustained activities of the vagal afferent. The transient vagal afferent activity was mimicked by 5-HT_3 receptor agonist 1-phenylbiguanide and blocked by 5-HT_3 antagonist granisetron. The sustained vagal afferent activity was mimicked by 5-HT_2 agonist alpha-methyl-5-hydroxytryptamine, but 5-$HT_{2B/2C}$ agonist *m*-CPP had no effect on the vagal afferent activity. In addition, 5-HT_{2A} antagonist ketanserin inhibited the sustained vagal afferent activity. The inhibition was more profound when 5-$HT_{1/2}$ antagonist methysergide was applied to the system. These results suggest that the increase in vagal celiac afferent activity represents the excitatory effects of 5-HT_3, 5-HT_1 and 5-HT_{2A} receptors.

Mechanisms of neural response to the ingested nutrients

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The gut-brain axis, which transmits nutrient information from the gastrointestinal tract to the brain, is important for perceiving the ingested dietary nutrients. There are two major pathways for this signal transmission; neural pathway (mainly vagus nerve) and humoral factors (e.g. insulin, GLP-1, ghrelin, and leptin). Recently, it has been shown that the information of the ingested foods is processed in the several forebrain regions, including the hypothalamus, limbic system, and the prefrontal cortex, where modulation of the next feeding behavior occurs. We investigated the mechanisms of this post-ingestive effect by behavioral and functional magnetic

resonance imaging (fMRI) studies in rats. First, we investigated the contribution of the vagus nerve by comparing the changes of blood oxygenation level-dependent (BOLD) signals between intact and total vagotomized rats in response to intragastric load of glucose or L-glutamate. Plasma insulin, L-glutamate, and blood glucose levels were separately measured to assess the correlation with BOLD signals. Intragastric administration of L-glutamate or glucose induced activation in distinct forebrain regions with different timings. L-glutamate-induced activation of the forebrain strongly suppressed by the abdominal vagotomy. In contrast, the vagotomized rat did not show significant difference in brain activation induced by glucose. Instead, signals in the nucleus accumbens and amygdala in response to gut glucose varied with fluctuation of plasma insulin. These results indicate that distinct activation pattern is due to the internal signals (vagus nerve or insulin) to convey the information of dietary nutrient from the gut to the brain.

Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes melanocortin-dependent anorexia

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Nesfatin-1, a recently discovered anorectic molecule, is localized in the hypothalamic paraventricular nucleus (PVN). However, anorectic neural pathway of nesfatin-1 remains unknown. This study was aimed to clarify anorectic neural mechanisms of nesfatin-1. Central injection of nesfatin-1 induced c-FOS expression in the PVN and brain stem nucleus tractus solitarius (NTS) and intra-PVN injection of nesfatin-1 decreased food intake, suggesting that PVN is one of the action sites for anorectic nesfatin-1 action.

Nesfatin-1 increased c-FOS expression in PVN oxytocin (Oxt) neurons of both magnocellular and parvocellular regions. Nesfatin-1 directly interacted with PVN Oxt neurons and increased cytosolic calcium concentration ($[Ca^{2+}]_i$). Nesfatin-1-induced anorexia was abolished by H4928, an Oxt receptor antagonist. Central injection of Oxt decreased cumulative food intake while blockade of Oxt receptor by H4928 increased food intake.

Moreover in the PVN, nesfatin-1 directly targeted nesfatin-1 neurons themselves and the neurons containing both Oxt and nesfatin-1, and stimulated Oxt release. Immunoelectron micrographs revealed nesfatin-1 specifically in the secretory vesicles of PVN neurons. Exogenous nesfatin-1 increased and immuno-neutralization of endogenous nesfatin-1 suppressed Oxt release in the PVN slices, suggesting paracrine/autocrine actions of nesfatin-1 in the PVN.

Oxt immunoreactive terminals were closely associated with proopiomelanocortin (POMC) neurons in the NTS, and Oxt increased $[Ca^{2+}]_i$ in NTS POMC neurons. Oxt-induced anorexia was blocked by SHU9119, a melanocortin 3/4 receptor antagonist.

These results demonstrate that the nesfatin-1-operative oxytocinergic signaling in the PVN causes melanocortin-mediated anorexia.

Gene expression of androgen receptor at the brain region related to extinction memory of conditioned taste aversion in mice

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We examined the sex difference of the extinction memory retention in the conditioned taste aversion (CTA). Mice acquire CTA memory (CTAM) by application of a novel taste, sodium saccharin (Sac), as a conditioned stimulus (CS) followed by an i.p.injection of LiCl, as an unconditioned stimulus (US). However, when mice undergo repeated presentation of CS without US, they become to recognize this Sac as a safe taste and to drink Sac again. This process is called the extinction of CTA. Recent studies have demonstrated that the extinction is a process of relearning, where mice acquire the extinction memory of CTA. Premature male and female mice (C57BL/6) underwent the conditioned period followed by the extinction period. After a month, the retrieval test of the extinction memory showed that both sexes represented only weak the extinction memory retention. In contrast, after the sexual maturation, male mice presented significantly higher the extinction memory retention than that of female. We chronically administrated an androgenic hormone, testosterone, into the castrated mice in prematuration period, resulting in an enhancement of the extinction memory retention by testosterone in both sexes. We investigated the gene expression of androgen receptor (AR) in the ventral medial prefrontal cortex (vmPFC) and amygdala, which have been reported to play an important role in extinction of CTA. There was a tendency of the age-dependent difference between prematuration and maturation in the expression level of AR gene. These results suggest that the sex difference of the extinction memory retention is caused by the effect of testosterone on vmPFC and amygdala during maturation period.

Conditioned taste aversion in the lactating mice and effect of oxytocin-injection

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We examined whether conditioned taste aversion (CTA), a kind of the emotional learning, is inhibited during the lactation period or not. The acquisitions of CTA of mice during lactating period were suppressed more than those of the virgin mice. Oxytocin (OT), which is produced in the hypothalamus and secreted from the posterior pituitary gland during the late pregnancy or lactation period, is thought to contribute to the social behavior such as maternal behavior. Therefore, we examined the effect of the OT on CTA by OT-injection to the lateral ventricle (central

OT) for 20 days. The CTA memory in the OT-injected mice was markedly enhanced compared to those of the sham-operated mice. This result suggests that central OT may not be involved in the suppression of the acquisition of CTA in the lactating mice. On the other hand, mice during the lactation period preferred water rather than high concentration of saccharin, whereas virgin mice preferred saccharin to all the concentration tested. These results indicate a possibility that peripheral OT affects suppression of CTA of mice during the lactating period.

Conditioned Flavor Preference in Weanling and Adult Rats

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Few studies have examined whether weanling animals acquire associative memory for reward. The present study tested 3- and 8-week-old rats on a conditioned flavor preference task. Half of the rats in each group received an unsweetened grape-flavored solution (CS-) on odd-numbered days and a sweetened (32% sucrose) cherry-flavored solution (CS+) on even-numbered days. The remaining rats received a sweetened (32% sucrose) grape-flavored solution (CS+) on odd-numbered days and an unsweetened cherry-flavored solution (CS-) on even-numbered days. During the acquisition phase of testing, the designated solution (CS+ or CS-) was presented to each rat for 15 min daily across 6 consecutive days. On the preference phase, each rat received unsweetened cherry- and unsweetened grape-flavored solutions simultaneously for 15 min daily across 4 consecutive days. The 8-week-old rats showed a significant preference for the flavor in CS+ compared to that in CS-. In contrast, the 3-week-old rats showed a significant aversion for the flavor in CS+. When 2% sucrose was used in the CS+, the 3-week-old rats showed a significant preference for the flavor in CS+, suggesting a hedonic shift from positive to negative with increasing the concentration of sucrose. The associative learning acquired at the age of 3 weeks was preserved when retested at the age of 20 weeks. These results suggest that weanling experience of food is important in the formation of feeding behavior in adulthood.

Molecular cloning of umami receptor gene, T1R1, from carnivorous animals

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Most of mammals, such as carnivorous and herbivorous animals have strong food preference. The question is why carnivorous animals do eat only meat. The simple answer is because they think it tastes good. To investigate functional difference of the *T1R1* genes between carnivorous and herbivorous animals, genomic DNAs from carnivorous animals, such as *Panthera pardus*, *Panthera leo*, *Felis concolor* and *Panthera tigris*, have been purified and PCR amplified. Primers for PCR were designed in region conserved by both *Canis lupus familiaris* and *Felis catus.* Using degenerated PCR method, exon 3 and exon 6 of the *T1R1* gene from each animal DNA were successfully amplified and sequenced. Comparison of sequences obtained revealed the existence of conserved nucleotides, resulting in the existence of conserved amino acid residues between carnivorous animals. Interestingly these conserved amino acid residues are also conserved for *Ailurus fulgens styani,* but not for *Ailuropoda melanoleuca* which eat only bamboo, although they belong to Carnivora. This observation strongly suggested that *A. fulgens styani* still have trait as carnivorous animal. This corresponds with the fact that *A. fulgens styani* sometimes eat small animals and insects, but *A. melanoleuca* do not.

An exploring study about the effect of social context on taste perception

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This study was constituted by analysis of recordings of daily eating behavior and comparison of taste perception of confectionery between in laboratory settings and in home use tests. Analysis of recordings of daily eating behavior revealed that eating foods with someone (family members, close friends and acquaintance) makes foods more palatable and more fulfilled than eating alone. This tendency was supported by an experiment. In the experiment, participants were asked to evaluate palatability and sweetness for the chocolate and cookies first in a laboratory setting (eating them alone in a laboratory) and then in a home use test setting (eating them with someone in a home). Both evaluations were higher in a home use test setting. These results suggest that human perceptions of taste and food palatability were affected not only by foods but also by contextual and social variables.

Can we get accustomed to consuming diluted miso- soup?

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In our study, 11 healthy university students were requested to consume 150 ml of miso (fermented soybean) - soup that was diluted to 70% of the usual concentration. They were also requested to take it once a day for 30 days (experimental group). The remaining 11 students were asked to consume 150 ml of undiluted miso-soup similarly (control group). The taste hedonic tone and taste intensity of the miso- soup of 0-90 dilution were evaluated at 3 points: before intake, immediately after intake, and after 30 days.

There was no between- group difference in the taste hedonic tone for any dilution at all the 3 points. In the experimental group, however, the taste intensity of miso- soup of the 10% dilution was higher after intake than before intake. These results suggest that we can get accustomed to consuming diluted miso- soup.

Mental stress caused by solving difficult puzzles affects taste perception

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In our everyday life, we are exposed to many stressors that induce mental stresses, and many people suffer from having mental illness, such as depression, bipolar mood disorders, and so on. Although there are many studies showing that eating and drinking something is useful for easing these mental stresses, there are almost no researches that investigates changes in taste under mental stress. In this study, two stimuli, coffee and chocolate, were presented to participants during exposure to the stressors. Evaluations for taste (sweetness and bitterness) and palatability were compared before and after stressors. Seventy-two participants were asked to rate their mood on the state-trait anxiety inventory (STAI), and to work out puzzle (SUDOKU), and to rate their mood on STAI again. Working out SUDOKU made participants stressful. Drinking bottled coffee or eating chocolate did not suppress the mental stresses significantly. Also there are no significant differences in taste evaluations between before and after solving a SUDOKU puzzle. A preceding study showed that mental stresses caused by Uchida-Krepelin test (simple summation test) decreased bitter sensitivity. Disagreement between results of this study and that of preceding study should be cleared by future studies.

Activation of efferent connections of the basolateral nucleus of amygdala on the retrieval of conditioned taste aversion memory: manganese-enhanced MRI study

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It is well known that the amygdala plays a critical role in conditioned taste aversion (CTA). The amygdala consists of several regions including basolateral, central, lateral and medial subnuclei. Although previous studies suggest the involvement of the basolateral nucleus of amygdala (BLA) in CTA, the role of the output pathways from the BLA are still unclear. Therefore, in this study, we investigated the activities of the efferent neuronal projections from the BLA, using a manganese-enhanced magnetic resonance imaging (MEMRI) technique. All rats were implanted with a guide cannula and an intraoral cannula. After the surgery, they received a pairing of 5 mM saccharin (conditioned stimulus, CS) with an i.p. injection of 0.15 M LiCl (CTA group) or saline (control group) as unconditioned stimulus. Two days after the conditioning, rats were microinjected with a 50 nl of 40 mM manganese chloride into the BLA. Thirty min after the manganese injection, rats were presented with CS. One and two hours after the manganese injection, the T1-weighted MR images were acquired by an 11.7 T MRI. In the CTA group, the signal intensity of the central nucleus of amygdala (CeA) in the 2-hour later MR image was stronger than that in the 1-hour later one. On the other hand, the control group did not show apparent time-dependent change in the signal intensity of the CeA. These results indicate that the neural pathways from the BLA to CeA are activated by the presentation of learned aversive taste stimulus. It is suggested that the intraamygdaloid connections play a role in the retrieval of CTA memory.

Changes in preference for bitter amino acids by addition of glycogen in C57BL/6 mice

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A lot of the edible parts of shellfish and sea urchins contain glycogen and if its content is high, glycogen is thought to enhance the deliciousness. Although it is tasteless and does not directly contribute to taste, it may have various interactive effects on the taste of amino acids. In this study, I investigated changes in preference for bitter amino acids by addition of glycogen in C57BL/6 mice using the 2-bottle preference test. No significant preference for 0.1% glycogen alone was observed. The total intake for 50 mM valine (Val) and methionine (Met, 50 and 100 mM) was not significantly different to distilled water (DW), while that for 100 mM isoleucine (Ile) was significantly less and 100 mM Val and 100 mM leucine (Leu) was significantly more than DW. After addition of glycogen, the total intake for Met changed to being less than DW and for Ile showed no difference to DW. However, preference for Val and Ile significantly increased after addition of glycogen. On the other hand, preference for Met and Leu did not significantly change after addition of glycogen. These results suggest that addition of glycogen has various effects on the taste of bitter amino acids.

Influence of "Umami" on human appetite as determined using functional magnetic resonance imaging

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Monosodium glutamate (MSG), which is a component of "Umami", is well known to affect the appetite. In addition, brain responses from visceral afferents of the vagus nerve are detected after intragastric infusion of MSG in rats, and it has been suggested that induced injection with MSG may be related to appetite regulation in behavioral experiments with rats.

We examined the influence of MSG intake on feeding desire afterwards in humans using functional magnetic resonance imaging (f-MRI). The purposes of this study were to clarify the areas that are active in the human brain after MSG intake, to determine the mechanism of the effects of MSG, and to examine the relationship between the desire to eat visualized in the imaging data and that from the visual analog scale (VAS) data obtained from psychological evaluations performed during the imaging experiments.

Three healthy men (mean age, 22.3 years; all right-handed) participated. Informed consent to the experiments and appetite regulation was obtained in accordance with the recommendations of the ethics committees of Tokyo Denki University and Chiba University. The experiments were conducted using four different conditions (hunger-no hunger, and intake-no intake of MSG when drinking a soup). Brain activity was measured by f-MRI during an event-related task using randomly chosen food-related photos

and non-food photos (e.g., landscape photos). The VAS data was obtained before intake and at constant intervals after intake of soup.

The f-MRI results suggest that the intake of MSG may control our appetitive behavior as a result of activity near the insula cortex, subcallosal areas and orbitofrontal cortex (OFC), may change the regulation of recognition of food-related photos and may also influence the olfactory senses. In addition, the VAS data suggests that MSG may control our appetitive behavior and feeding desire by causing a sensing of satiety from the implied meaning of the terms ''hunger'', ''no hunger'', and ''appetite''.

Selectivity in Bitterness-Reducing Activity of Riboflavin-Binding Protein

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Riboflavin-binding protein (RBP), which is a monomeric phosphorylated glycoprotein with a molecular weight of 35 kDa contained in chicken egg white has been reported to have bitterness-reducing activity. In this study, the bitterness-reduction modality was compared with that of sodium chloride (NaCl). Bitterness intensity scored by labeled magnitude scale and bitterness threshold measured by triangle test were used to evaluate the bitterness-reducing activity. NaCl reduced the bitterness intensities and raised the bitterness thresholds of quinine, glycyl-phenylalanine (Gly-Phe) and protein hydrolysates but did not affect the bitternss of naringin, theobromine, salicin and caffeine. On the other hand, RBP reduced the bitterness intensities and raised the bitterness thresholds of many bitter substances including quinine, Gly-Phe, naringin, theobromine, caffeine, protein hydrolysates and PROP but did not affect the bitterness of salicin. The result that bitterness-reducing activities of RBP and NaCl were not effective to all bitter substances would mean that there is a selectivity in each bitterness-reduction of RBP and NaCl. Moreover, the difference of selectivity toward bitter substances in bitterness-reduction between RBP and NaCl indicated that RBP elicits bitterness reduction by different mechanism from that of NaCl.

Relationship Between Structure and Bitterness-Reducing Activity of Riboflavin-Binding Protein

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We previously reported that chicken riboflavin-binding protein (cRBP) is a novel bitter inhibitor which has broadly tuned inhibition toward various bitter substances. The bitterness-reducing activity of cRBP considerably decreased when cRBP was treated at 110 °C for 60 min, whereas it did not change after treated at 80 °C. From circular dichroism spectra analysis, it was presumed that the heat treatment at 110 °C caused a conformational change of cRBP. Quail RBP (qRBP) purified from egg white elicited bitterness-reduction as well as cRBP. The complete amino acid sequence was deduced from its nucleotide sequence of qRBP gene and it was found qRBP has 88% similarity to cRBP, suggesting that the common structure might associate with the bitterness-reduction. Both digests of cRBP and qRBP by lysyl-endopeptidase (LEP) or V8 protease (V8) showed different SDS-PAGE patterns. Whereas LEP digests of cRBP and qRBP caused loss of their bitterness-reducing activities, digests of cRBP and qRBP by V8 still elicited bitterness-reducing activities. These results indicated that the structure essential for bitterness-reducing activity in RBP would be altered by LEP digestion but not by V8. Amino acid sequencing for N-termini of the cRBP- and qRBP-V8 fragments predicted the region for bitterness-reducing activity common to both RBPs.

Study on Oral Fat Sensations: Rinsing Effects of Oolong Tea

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Oral fat sensations can be removed by taking oolong tea. Many Japaneses drink oolong tea during and/or shortly water taking fatty Chinese dishes. We focused our study on the subjective sensation of the greasy which is caused by several edible oils and compared the mouth feeling-refreshing effects of several teas.

Oolong, green, and roasted green tea samples were used and compared with water (control) for the rinsing effect. Forty-two male and forty female Japanese subjects, aged 20-40 years, were tested by sensory evaluation. Each subject took 5 g of commercial available whipped cream as oral fat sensation, and then drank 20 ml of tea samples. Shortly after, they were asked about the extents of taste sensations of creamy, fattiness, greasy, and lubricity by 10 point scales.

The results showed that oolong tea reduced sensations of cream and fattiness than water did. Physicochemically, the interfacial tension of oolong tea was significantly smaller than that of water. Oolong tea may thus remove oral fat sensation by washing out oils from the mouth due to its larger oil-emulsifying activity. The results provide useful information about why people prefer oolong tea to water while taking fatty foods.

Maternal low-zinc nutrition during lactation affects the pups' sodium preference-controlling system after the maturation to adulthood in SD rats

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It has been well known that maternal malnutrition during gestation or lactation influences grown and matured children's dietary habits including un-balanced dietary preference which causes metabolic syndrome in their later life. However, little is known of the effect of the shortage of trace elements, except for iron, in maternal milk on the grown rats' taste preference behavior or dietary habits. Therefore, we carried out a maternal low-zinc nutrition experiment. Low zinc (4.0 mg Zn/Kg) or sufficient zinc (33.7 mg Zn/Kg) diet was fed only to lactating mothers for 3 weeks after birth; and sufficient zinc diet was fed to all the rats after weanling. We undertook a taste preference behavior study with water and 0.5 M NaCl solution in

the 2-bottle preference system, and found that maternal low-zinc diet during lactation caused the increased NaCl preference in their matured, up to 11-week-old offspring. Our novel observation by real-time microdialysis-HPLC system was that a significant increase in norepinephrine (NE) secretion occurred after the 15 to 45 minutes oral 0.5 M NaCl stimulation on the tongue in both the lateral- and paraventricular nucleus of hypothalamus in offsprings of low-zinc diet fed mothers. Therefore, it is suggested that maternal low-zinc nutrition during lactation increased the matured pups' salt taste preference through the hypothalamus controlling system which has been influenced on their babyhood.

Effects on the taste modification of potassium gluconate and calcium gluconate for sweet tastants and bitter tastants

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Artificial sweetener has bitter taste and unpleasant after taste together with sweet taste. The reducing these unpleasant side tastes is required for commercially usage in the food industry. Previous studies showed that gluconate salts reduced the taste intensities of bitter and acid substances. In this study, potential effects of potassium gluconate and calcium gluconate on taste of an artificial sweetener, saccharin, were examined by human sensory evaluation tests with a paired difference test, a paired preference test and a perceived intensity test with a 100 mm labeled magnitude scale, and by recordings of chorda tympani nerve responses from C57BL/6J female mice.

The human sensory evaluation tests revealed that the sweetness intensity of saccharin when mixed with potassium gluconate was stronger than the saccharin alone, whereas the reverse was true for the bitterness intensity of saccharin. Calcium gluconate tended to show similar differential effects on sweetness and bitterness of saccharin. Surprisingly, both gluconates reduced the intensity of bitterness of quinine hydrochloride, although they did not significantly affect the sweetness of sucrose. Whole nerve recordings showed that these gluconates significantly suppressed responses to quinine hydrochloride without affecting responses to saccharin and sucrose.

In conclusion, these findings suggest that the gluconate salts, especially potassium gluconate, improve the taste of artificial sweetener and bitter substances. Further study is needed to find out the mechanism of these effects.

Flavor modifying effect of Theanine

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Theanine, a unique amino acid present in the green tea, gives a characteristic umami and sweet tastes of green tea, and possesses various psychological effects. Theanine may improve unpleasant taste such as bitterness and sourness of food. In order to entertain a new availability of theanine, we investigated the flavor modifying effect by theanine. The estimation in paired preference test for taste modifying activity of theanine to citric acid or denatonium benzoate was. carried out with 20 healthy human volunteers (23±1.9 years olds). The results showed the reduction of sourness by addition of theanine but no effects on bitterness. Next, we investigated the modifying mechanism with the 48-h two bottle preference test using C57BL/6J mice. The preference ratio to citric acid was increased with the concentration of theanine. Furthermore, a taste sensor instrument (TS-500Z) revealed that the addition of theanine decreased the intensity of output signal of sour sensor, slightly increased the intensity of signal from umami sensor and unchanged intensity of signal from bitter, sour and astringency. Finally, we measured the pH of the solutions. The pH was slightly increased by the addition of theanine to citric acid. The improvement to sourness by the addition of theanine may have been by the increase of pH, and the umami taste could be arised from theanine.

Effect of Maillard reaction products flavors on umami preference in mice

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Maillard reaction participates not only in the formation of color but also in aroma generation during cooking and thermal processing in the food industry. In this study, we aimed to investigate the effect of furanones and pyridines as one of the Maillard reaction products flavors on uammi taste preference in non-deprived mice. 4-hydroxy-2,5-dimethyl-3(2H)-furanone; furaneol (Fu1), 4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one; sotolon (Fu2), 2,3-diethyl-5-methyl pyrazine (Py1) and 2-ethyl-3,5-dimethyl pyrazine (Py2) Food-Grade Certified Products, SAFC Supply Solutions, USA, were chosen as the Maillard-borne odorants. In the short time one-bottle test, 0.1 and 10 mg/kg Fu1 flavored umami solution containing 10 mM monosodium glutamate (MSG) tended to be consumed more than 10 mM MSG solution. 100 mg/kg Fu2 + MSG was significantly consumed more than MSG. 1 μg/kg Py1 and 1 μg/kg Py2 + MSG tended to be consumed more than MSG. Moreover, in the short time two-bottle test, 0.003 μg/kg Fu2 + MSG tented to be consumed more than MSG, however, there was no difference between 0.03 μg/ kg Fu2 and water. These results suggest that sotolon flavor itself was not preferred but sotolon odor induced enhancement of short time intakes of umami solution. Supported by Society for Research on Umami Taste and KAKENHI (22780127).

Effect of Maillard reaction products flavors on umami sensitivity in human

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Maillard reaction participates not only in the formation of color but also in aroma generation during cooking and thermal processing in the food industry. It is reported that some aroma compounds enhanced or decreased taste rating. In this study, we aimed to investigate the effect of furanones as one of the Maillard reaction products flavors on uammi taste intensity and threshold in humans. 4-hydroxy-2,5-dimethyl-3(2H)-furanone; furaneol and 4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one; sotolon were chosen as the Maillard-borne odorants. Fifteen untrained female students between 18 and 19 years old were recruited for this study. In the umami taste

strength test, 10 mM MSG solution with 0, 0.001, 0.01, 0.05 mg/kg furaneol and 10 mM MSG solution with 0, 0.001, 0.01, 0.1 μg/kg sotolon were prepared. For sensory evaluation, a visual analogue scale whose extremities were defined as "not intense" and "extremely intense" was used. In the umami taste threshold test, 0.1, 1, 2, 3, 5 mM MSG solution with 0.01 mg/kg furaneol or 0.01 μg/kg sotolon were prepared. The lowest concentration that the subject identified as umami taste was defined as umami threshold. Furaneol flavor tended to enhance umami taste strength in a dose dependent manner. Sotolon showed a significant enhancement of umami taste in a dose dependent manner. In addition, furaneol has the downward trend of umami taste threshold, and sotolon significantly lowered umami taste threshold. These results suggested that furanones-odor induced enhancement of umami taste was observed in humans.

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Effect of freezing condition on melting property of ice cream

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Melting is one of the major physical properties for ice cream product. This property is determined mainly by freezing process since ice cream is a complex multi-phase food product and its structure is constructed during the freezing process. Therefore, understanding the relationship between melting property and freezing process condition is profitable to make ice cream with high quality. The objective of this study is to design statistical models that show the effect of continuous freezer parameters (mix flow, overrun, drawing temperature, cylinder pressure and dasher speed) on melting property of ice cream using response surface methodology. According to a central composite face-centered design (design of experiment), thirty-one combinations of the five above-mentioned freezer conditions were designed, and ice cream samples were manufactured. Two indexes measured by a melt-down test were adapted for estimation of melting property; the time when first drop of melted ice cream sample falls down through a wire mesh screen, and the melting rate, which is the percentage of melted ice cream of the initial when held for 70 minutes at room temperature. Measured melting indexes were fitted to a second-order polynomial model, and by eliminating unnecessary terms for simplification, two statistical models were developed. These statistically significant models show that the overrun and drawing temperature most strongly affect the melting property. Higher overrun and lower drawing temperature, effectively restrained the melting of ice cream.

The Effect of Previous Experience of Tasting Carp as a Tradition Ingredient of Foods in Shinshu

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The meat of carp, *Cyprinus carpio L.,* has been used as an ingredient of foods in a formal event of Saku City, Nagano. In our previous study, we reported on the instrumental analysis and sensory tests of carp taste and odor.

In this study, a questionnaire about the food experience and preference of carp was asked to high school students, junior collage students and dieticians in Nagano.

The respondents with fathers or mothers from Nagano had a significantly higher frequency of eating carp than other respondents ($p<0.0001$). Middle- or advanced-aged respondents had a significantly higher frequency of eating carp than young respondents ($p<0.0001$). Respondents from a three generation family also had a significantly higher frequency of eating carp than those from a two generation family ($p=0.0215$). Those who have experienced eating carp at a young age had a significantly higher frequency of eating carp than other respondents ($p<0.0001$). The respondents which ate the carp once a year had a significantly higher frequency of eating carp than the respondents which ate it not so often ($p<0.0001$). The carp-liking respondents want to keep eating it in the future ($p<0.0001$). Three factors influenced the respondents' early introduction to eating carp: being female ($p=0.0001$), being advanced age ($p=0.0331$), or having a father from Nagano ($p=0.0054$).

The results of this study show that people who begin to eat carp in early childhood tend to eat it as repeaters.

Zinc-binding protein in saliva (gustin) of the taste dysfunction patients before and after zinc oral administration

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

Parotid saliva was obtained from 11 patients of taste dysfunction. The concentration of CAVI in saliva was quantified by the enzyme-linked immunosorbent assay (ELISA) using the polyclonal antibody against the synthetic peptide designed from human CAVI. ELISA plates were coated by saliva diluted with coating buffer (50 mM carbonate), followed by the ABC method, which were measured in a microplate reader at 405 nm. CAVI titers were calculated by reference to the standard curve of the synthetic peptide.

The tendency of increase in serum zinc value and CAVI in parotid saliva after medication of zinc was observed. In addition, the taste threshold decreased after zinc administration.

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

Influence of Smoking on the Expression of Taste Receptors hTAS2Rs

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Despite the evidence on the medical risks of smoking, the large majority of smoking cessation subjects remain addicted to cigarettes. Against this backdrop, we focused on a study to clarify the relationship between smoking and taste sense.

We investigated the influence of smoking on the expression of hTAS2Rs to aim at confirming whether smoking injures the sense of taste.

Smokers (n=22) and healthy non-smokers (n=91), were recruited as subjects. Two subjects having abstained for 30 and 10 years were also recruited. They were asked to complete self-report questionnaires and to receive semi-structured interviews to explain their smoking history. Smears were sampled from the tongue of each subject to investigate the expression of hTAS2Rs.

The numbers of expressed hTAS2Rs in the smoking group, the healthy control, and the abstinence group were 2.1, 14.0, and 5.5, respectively. Even though this result suggests the influence of smoking on taste sense, all the subjects of the smoking group answered that they tasted the ordinary meal well. The suppressed expression of hTAS2Rs may thus partially recover during abstinence.

Smoking history possibly deteriorates the sense of taste, although smokers do not recognize the deterioration.

Isolation of taste receptor cells of the blow fly *Phormia regina* and identification of their modality by the whole cell clamp experiments

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The five receptor cells, namely, the sugar receptor cell, the salt receptor cell, the deterious receptor cell, the water receptor cell and the mechano receptor cell, are housed in the chemosensillum of the fly, *Phormia regna*. They were isolated from the labellar sensillum by the enzymatic and mechanical treatments. To identify the sugar and salt receptor cell, we examined sensory responses by applying sucrose solution, NaCl solution, and physiological stimulant respectively to the isolated cells in the whole cell clamp experiments. With repeated such experiments, we obtained the clues that showed they have a round shape in common, but that the sugar receptor cell was usually much smaller than the salt receptor cell in size.

Attempting to evaluate the quality of milk coffee by its buffer capacity

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A validity of simple instrumental method for measuring the buffer capacity of coffee beverages with different milk ratios was studied. Model beverages made of soluble coffee and whole milk powder were analyzed using a buffer capacity-measuring device as a combination of automatic titration system and personal computer. A regression equation was obtained from the instrumental data on various milk-coffee samples. Three canned beverage samples with unknown milk-coffee ratios were submitted to evaluation for estimation of the ratios with considerable accuracy from their buffer capacity measurements. The results suggest that the instrumental analysis of buffer capacity is useful as a simple method for quality evaluation of given milk coffee samples.

Identification of metabolites specific to axillary odor types by metabolome analysis of human axillary sweat

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Human axillary odor develops as a consequence of bacterial metabolism of secretions from sweat and sebaceous glands of skin. Although previous reports suggested that various metabolites that lead to many volatile compounds are involved in axillary odor, the metabolic pathways and key molecules remain to be fully elucidated. Since our past study showed that human axillary odor of Japanese male subjects can be classified into several odor types, the axillary sweat were collected according to odor type and analyzed to elucidate the mechanism of odor development. Axillary sweat extract was concentrated and metabolome analysis was performed by CE-TOFMS, a metabolomics platform that has advantages of extremely high resolution and high precision. We detected 326 charged metabolites and identified 122 compounds in human axillary sweat. A Kruskal-Wallis test of metabolite peak areas revealed that contents of 35 compounds in the extract of axillary odor type C were significantly higher than those of other odor types. Similarly, contents of 4 compounds in the type A extract were significantly higher. A decision tree method classified axillary odor types accurately. Specific metabolite abundance could characterize odor types whereby the classification pattern was consistent with the olfactory evaluation results. These results indicate that specific metabolites are probably the key components of individual axillary odor types. This study is the first application of the metabolome technology with CE-TOFMS to the global analysis for the charged metabolites on human skin surface, and shows that CE-TOFMS serves as a powerful tool for the analysis of human skin surface metabolites.

Expression patterns of G protein alpha subunits in the olfactory system of *Xenopus laevis*

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The olfactory organ of the African clawed frog, *Xenopus laevis*, is housed in three separate nasal chambers: the principal chamber, the middle chamber and the vomeronasal organ. They are lined with the olfactory epithelium (OE), middle chamber epithelium (ME) and vomeronasal epithelium (VNE), respectively. The ME contains two types of olfactory receptor cells (ciliated and

microvillous olfactory receptor cells). Their axons project to the ventral region of the main olfactory bulb (MOB). The ventral region of the MOB can be subdivided into some compartments by several markers, although the significance of the subdivision is unclear. In this study, we investigated the expression patterns of G protein alpha subunits by immunohistochemistry in the olfactory organs and olfactory bulbs of *Xenopus laevis*. In the ME, ciliated olfactory receptor cells and microvillous olfactory receptor cells were immunopositive for Golf and Go, respectively. The cell bodies of the Golf-immunopositive cells were situated in the upper layer of the ME and those of the Go-immunopositive cells in the lower layer. The ventral region of the MOB was subdivided into two compartments, rostromedial and caudolateral, depending on the expression of Golf and Go. The rostromedial compartment was immunopositive for Golf and immunonegative for Go, whereas the caudolateral compartment was immunopositive for Go and immunonegative for Golf. These results suggest that the Golf-immunopositive receptor cells and Go-immunopositive receptor cells were situated in the upper layer and lower layer of the ME of *Xenopus laevis*, and project their axons to the rostromedial and caudolateral compartments in the ventral region of the MOB, respectively.

Noradrenergic modulation of dendritic morphology and synaptic plasticity in accessory olfactory bulb neurons

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The mammalian accessory olfactory bulb (AOB) receives a remarkable noradrenergic input from the locus ceruleus. Noradrenergic fibers terminate densely in mitral/tufted cell layer and granule cell (GrC) layer. It is postulated that noradrenalin modulates dendrodendritic reciprocal synapses between the dendritic shaft of mitral/tufted cell and the dendritic spine of GrC. These synapses play a key role in the formation of pheromonal memory. However, the mechanisms by which this neuromodulater cause synaptic changes remain unclear. In this study, we investigated noradrenergic modulation in GrCs by using a vomeronasal organ–AOB coculture system.

Many small interneurons containing GrCs were identified though the expression of fluorescent proteins in the coculture. GrCs were categorized into 3 subtypes, depending on their dendritic morphology: typical GrC (Ty-GrC), spiny GrC (S-GrC) and aspiny GrC (A-GrC). Ty-GrC dendrites have large spines, the so-called ''gemmule''. S-GrC dendrites have numerous small spines, whereas A-GrCs have a few spines.

We analyzed Ca^{2+} responses of GrCs induced by addition of NA (2.5 μM) to the coculture.

NA-induced Ca^{2+} transients were observed in most A-GrCs and some Ty-GrCs. However, NA-induced Ca^{2+} responses were not detected in the majority of S-GrCs. Therefore, we analyzed NA-induced chronological changes in the dendritic spines of the GrCs by NA using time-lapse photomicrography. Only in the A-GrCs, spine motility increased during the first 1 hour.

Because spine motility is predicted to influence synaptic formation and maintenance, we postulated that the increase in spine motility through NA-induced Ca^{2+} transients in the A-GrCs is a basis for the synaptic plasticity of pheromonal memory.

Sex steroid-metabolizing enzymes in the rodent olfactory mucosa

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It is known that the sex steroids influence the olfactory system and vice-versa. However, little is known about the cellular and biological bases of their interaction. Thus, we examined the physiological roles of sex steroids in the olfactory system, along with immunolocalization and gene expression of some sex steroid-metabolizing enzymes in the olfactory mucosa of rats. The enzymes we examined were, the steroid side chain-cleavage enzyme (P450scc), 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD-1), and 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2).

The mRNAs of all the above-mentioned enzymes were detected in the olfactory mucosa. Western Blot analyses also demonstrated presence of these enzymes in the olfactory mucosa. Immunohistochemically, the immunoreactivity for all enzymes apart from aromatase was observed in the supporting cells of the olfactory epithelium and in the Bowman glandular cells. At the electron microscopic level, immunoreactivity for 17β-HSD-1 was localized in well-developed smooth endoplasmic reticulum (SER) of the supporting cells. The steroid-producing cells in the gonads possess a well-developed SER in where 17β-HSD-1 and 17β-HSD-2 are localized. We demonstrated that the olfactory supporting cells play a role similar to the gonadal steroid-producing cells. Our ongoing study has demonstrated that cholesterol receptor immunoreacrtivity was localized in the olfactory supporting cells and that the estradiol-17β (E2) receptor was localized in olfactory receptor cells (ORCs). Thus, the following working hypothesis proposes that the olfactory supporting cells in rats take up cholesterol via capillaries located just beneath the olfactory epithelium and biosynthesize E2, which exerts physiological effects on ORCs.

Presence of steroid-biosynthesizing enzymes in the primary vomeronasal system of rodents

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It has been reported that the vomeronasal system is influenced by sex steroids during the pre and postnatal development as well as adult stages of rodents. However, our recent research has demonstrated that the sensory organ of the vomeronasal system, the vomeronasal organ (VNO), *per se* is involved in the biosynthesis and/or metabolism of sex steroids. Some of our recent data and a working hypothesis highlighting biological significance of sex-steroids in the vomeronasal system will be presented in the symposium entitled ''Olfactory system and steroids.''

As an experimental model, Sprague-Dawley rats have been used in our laboratory. The first and last enzymes involved in sex steroid-biosynthesis pathway, the P450scc steroid side chain-cleavage enzyme, and aromatase respectively, are not expressed in the rat VNO. However, 17*β*-hydroxysteroid dehydrogenase type

1 (17β-HSD-1), which catalyzes the activation of androgens and estrogens from their inactive forms, is expressed in vomeronasal receptor cells (VRCs), and localized in their smooth endoplasmic reticulum (SER). Furthermore, 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2), which catalyzes the conversion of active androgens and estrogens into their inactive forms, exhibits a similar expression and localization pattern as that of 17β-HSD-1. Real-time PCR analyses demonstrated that the amount of 17β-HSD-2 mRNA in the VNO of adult rats was much higher than that of 17β-HSD-1, including ovary and testis. This result suggests that sexually matured rats tend to inactivate sex steroids rather than activating them.

Together with the specific histological property of rodents that VNOs contain intraepithelial capillaries, we concluded that testosterone and estradiol produced by the gonads reach the VRCs via these capillaries. We hypothesize that VRCs are direct and active targets for gonadal steroids as our resent study strongly suggests that rat VRCs express androgen and estrogen receptors,

Inhibitory effects of topical anesthetics cocaine on voltage-gated currents in olfactory receptor cells: Analysis of outward K^+ currents and the reversible action of high concentrations of cocaine

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Cocaine is commonly used as a topical anesthetic in otorhinolaryngeal nasal cavity surgery. It has been reported that some patients complain of olfactory deficits after surgery, and decreased olfaction is found in cocaine abusers. The primary effect of 1-5% cocaine is analgesia during surgery, but the effects on olfactory receptor cells (ORCs) are unknown. To examine this question, newt ORCs were isolated from nasal tissue as previous reported. ORCs were examined using an inverted microscope and identified morphologically (i.e., cilia and/or bipolar-shape). In this study, we focused on the effect of high concentrations of cocaine and the dose-suppression curve on isolated outward currents. With the whole cell voltage clamp method, the membrane potential was depolarized from a holding potential of -100 mV stepwise between -90 and +40 mV and voltage-gated currents were recorded. The air puff application of 5% cocaine reduced significantly both inward and outward voltage-gated currents, which recovered completely after wash-out. The dose-suppression curves of cocaine for voltage-gated potassium currents were fitted by the Hill equation. The half-blocking concentration was 54 μM, and the Hill coefficient was 0.9. These findings are similar to previous data on sodium currents, suggesting that the inhibitory action of cocaine on ORCs is caused by blocking ion channels. Recovery from suppression after treatments with 10 mM cocaine perfusion as well as 5% cocaine puff application implies that cocaine does not permanently alter olfactory function after high dose topical treatments during nasal cavity surgery.

Odor responses of descending interneurons and thoracic leg muscles in the male cockroach

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In insects, odor-evoked behavior is thought to be activated by brain descending signals originating from the higher brain center (protocerebrum). However, the activation mechanism of the descending signals for odor-evoked locomotor behavior remains largely unknown. In order to examine the mechanisms underlying odor-evoked locomotor behavior, we simultaneously recorded responses to odor stimuli from brain descending neurons (BDNs) in both side connectives of the ventral nerve cord and from one thoracic middle leg muscles (depressor, levator and extensor) of the male cockroach, *P. americana*. Each pair of silver wires was positioned the connectives and the leg muscles. The BDNs responded to different odor stimuli in a different manner. Contralateral antennal odor stimuli to the recording site typically induced significant high-frequency responses in the BDNs. Odor-evoked sustained small-amplitude responses followed by large-amplitude burst responses were recorded from each of the leg muscles. Depending on different odor stimuli and which side antennae stimulated, these two type muscle responses showed distinct characteristics in their response onset timing and duration. Responses of the BDNs to odor stimuli always preceded the onset of the leg muscle responses. These results suggest that the BDNs might feed the odor-evoked command signals to the thoracic motor center, so that control the spatio-temporal firing of motor neurons and leg muscles is selected and activated for an odor specific behavior.

Sustained expression of V2R family vomeronasal receptors by the interaction between vomeronasal and accessory olfactory bulb neurons

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Many mammals detect pheromones by a unique sensory organ, the vomeronasal organ (VNO). Previously, we reported that cocultures of VNOs with accessory olfactory bulb (AOB) neurons resulted in the maturation of vomeronasal sensory neurons (VSNs) and a greater expression of V2R family vomeronasal receptors than VNO cultures alone using immunoblot and immunocytochemical analyses. To further characterize the V2R expression, we investigated the time course of the expression of V2R mRNA in the presence or absence of AOB neurons using RT-PCR analysis. The expression of V2R mRNA was already detectable not only in the VNO cocultured with AOB neurons at 3 days in coculture (DICC) but also in the VNO cultured alone at the same culture period. However, the expression of V2R mRNA in the VNO cultured alone was remarkably decreased over the additional culture period, although that in the cocultured VNO was equally sustained. Moreover, the application of 2 μM TTX to the cocultured VNO resulted in a marked decrease in the V2R mRNA expression to the level equal to the VNO cultured alone at 14 DICC.

Our previous working hypothesis was that the expression of vomeronasal receptors in the VSNs was induced by interacting with AOB neurons. However, the present results suggest that the receptor expression in the VSNs is independent of the interaction with AOB neurons in the early developmental stage, but it is sustained by the active interaction with AOB neurons.

Propylene glycol, a volatile chemical, elicits Ca^{2+} transients from rat vomeronasal receptor neurons

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Propylene glycol (PG) has been recognized as safe and considered to be odorless for humans and other animals so that it is utilized as a solvent for odorous chemicals employed in some odor-related experiments. If laboratory rats, however, can detect the vapor of PG and if exposure to PG induces some psychogenic effects on their behaviors, such effects might confound data obtained from experiments exposing conscious rats to odorants dissolved in PG. We, therefore, examined the effect of PG on biological responses of male Wistar rats by using the acoustic startle reflex (ASR) as an index. A habituation/dishabituation test was also conducted to assess the ability of rats to detect the vapor of PG. The sensitivity of the vomeronasal neurons (VNs) in slice preparations was measured by using confocal Ca^{2+}-imaging approach as well.

Pure PG vapor significantly enhanced the ASR at a dose of 100 μM, which was much lower than the dose for efficiently detecting, suggesting that PG vapor acts as an aversive stimulus to rats at the sub-threshold concentration for its detection. The results indicate that we should consider such effect of PG when it is employed as a solvent for odorants in studies using conscious rats. In Ca^{2+} imaging, PG elicited Ca^{2+} transients of VNs in a dose-dependent manner. We found detection thresholds for PG at concentrations near or below 1 μM, supporting the idea that some non-pheromonal volatile odorants may affect animal behavior via the vomeronsal system in rodents.

fMRI brain imaging and sensory tests to evaluate the pleasantness of odors

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One of the most important features of odor perception is the hedonic or emotional component. The purpose of this study was to evaluate the pleasantness of odors using human brain responses measured by functional magnetic resonance imaging (fMRI) and psychological experiments (sensory tests).

The olfactory fMRI responses were analyzed using an event-related MRI method. Each subject was exposed to an odorant pulse gas via a nose mask using air-operated valves which were controlled by electric-valves controlled by the E-prime software package. In this event-related task, two odorants (pleasant odor, amylacetate; unpleasant odor, iso-valeric acid) and two kinds of visual stimuli (pleasant photo, unpleasant photo) were used at random and then two cross-modal sensory interactions were tested by SPM-software for both matching/mismatching conditions and pleasant/unpleasant conditions, respectively.

We detected active areas in the human brain upon cross-modal interactions elicited using odor and visual stimuli. We also performed three psychological odor experiments ([1] visual evaluation test, [2] odor evaluation test, [3] visual and odor evaluation test) using a subjective judgment method for pleasantness/unpleasantness. Based on the fMRI findings and psychological test results, we then attempted to estimate the most suitable evaluating conditions for the pleasantness of odors.

We found that a pleasant odor activated mainly the right cerebrum and the supramarginal gyrus, middle frontal gyrus and amygdale, suggesting that there is a hedonic map of the sense of smell in these brain regions. These results may have implications for understanding emotion-relative evaluation in these brain areas.

Odor Interactions among Ternary Mixture by Human

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Mixture-interactions are a first-order concern in olfaction. At peri-threshold levels, people can often detect a mixture even if they cannot detect any of the individual mixture components when presented alone. The exact rules of this summation remain unclear. We have measured detection of both single chemicals and binary mixtures over a range of concentrations. Significant deviations from additivity occur, and depend on both stimulus concentration and molecular properties. Here, we extend this work to ternary mixtures. We measured detection functions for four homologous carboxylic acids (acetic, butyric, hexanoic, octanoic), and for maple lactone, which is different from the acids in both structure and supra-threshold quality. We also measured detection functions for three ternary mixtures. Analysis of variance showed that mixtures 1 and 3 (the most and least similar) showed approximately additive interactions across the full range of measured concentrations, whereas mixture 2 showed substantial sub-additivity across concentrations. These data suggest that a tendency toward peri-threshold additivity may continue as mixtures become more complex, but that the degree of additivity will depend on the molecules that comprise the mixture. Structure-activity models will require further research.

Dependency on Or83b of olfactory-mediated behavioral responses to propionic, lactic, and pyruvic acids in adult of *Drosophila melanogaster*

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Two types of olfactory sensory neurons (OSNs) in adult of *Drosophila melanogaster*, OrX-Or83b-expressing and IR-expressing neurons, have been reported. Propionic, lactic, and pyruvic acids

strongly elicit the attractive olfactory-mediated behavioral responses even though low vapor pressure of these chemicals. No OrX shows strong excitatory response to these acids. On the other hand, IR (IR75a?) expressing OSNs (ac3A & ac2A) show strong excitatory response to propionic acid although unknown to lactic and pyruvic acids. To examine the main pathway responsible for olfactory reception and perception against these acids, the olfactory behavioral responses of Or83b null mutant (kindly provided by Dr. Vosshall) to these acids were compared with those of wild type flies. The response was evaluated by using relative response index. The response to propionic acid was only slightly low (60 – 80%). The responses to lactic and pyruvic acids were strongly low (20 – 40%). Normal responses to propionic, lactic, and pyruvic acids were restored in Or83b transgenic rescure flies (Or83b-Gal4/UAS-Or83b;Or83b null). These results suggest that IR-expressing OSNs are mainly responsible for olfactory reception and perception to propionic acid, and that OrX-expressing OSNs are necessary for the response to lactic and pyruvic acids.

Sensitivity of the alternating magnetic fields in honeybees from an associative magnetic conditioning of the proboscis extension reflex

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Discrimination experiments indicate that the magnetic sense of the honeybees has its maximum sensitivity below 10 Hz, the sensitivity of the bee magnetoreception system decreases rapidly with increasing frequency of alternating magnetic field and at 60 Hz, alternating field strengths above 100 μT are required to elicit response. This frequency- dependence of response could be a consequence of viscous damping of the motion of the magnetite. But, we found that an associative odor conditioning of the proboscis extension reflex to sucrose in honeybees was inhibited by exposure to extremely low frequency (ELF) electromagnetic field and ELF magnetic field. So, to measure the range of frequencies to which the honeybee magnetoreception system was capable to respond, we trained honeybees to associate magnetic stimuli and a reward of sucrose. In our paradigm, harnessed honeybees learned the elemental association between magnetic stimulation and a reward of sucrose delivered to the proboscis. Thereafter, bees extended heir proboscis to the magnetic stimulation only. Honeybees could readily acquire the magnetic learning at 10 Hz, 50 Hz, and 100 Hz. Reversal intensity-response relations were observed in this magnetic learning. When the intensity was increased from earth's magnetic field to 300 μT, an acquisition of learning was decreased. So intense magnetic field may be unpleasant and have some inhibitory effects.

Interaction between Olfactory and Somatosensory Perception: Do Odors Influence Stiffness and Roughness Perception?

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We acquire many kind of information by sensations (vision, audition, olfaction, somatosensation, gustation) and integrate the information to recognize the outside world. There were many researches on the interaction between olfaction and vision. However, the interaction between olfaction and somatosensation was hardly examined. In this study, we used force-feedback device and Micro-Aroma-Shooter to investigate whether olfactory information (scent) can influence somatosensory (stiffness, roughness) perception or not. In our two experiments, subjects were asked to adjust stiffness (Experiment 1) and roughness (Experiment 2) of two successively presented surfaces (standard and test surface) to the same level. Odors (rose, sandalwood and odorless) were shot toward subjects' nose when standard surface were presented. The result of Experiment 1 showed that in the hard standard surface condition the test surface was perceived harder when sandalwood odor was presented than when either rose odor or odorless air was presented. The result of Experiment 2 showed that in the smooth standard surface condition the test surface was perceived smoother when rose odor was presented than when odorless air was presented. These results suggest the existence of the interaction between olfactory and somatosensory perception. Moreover, it suggests that olfactory perception may have effects on different aspects of somatosensory perception according to types of smells.

Dog's olfaction -training of cancer detector dog

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It is well known that patients have unique smells, for example, diabetic patients emit a sweet body and breath odor. Recently, it has been reported that dogs can detect the odor of cancers. Several researchers have reported that dogs can detect moles or tumors on the basis of odor. The ability of dogs to recognize and cross-match many kinds of odors has been demonstrated in electrophysiological and behavioral experiments. In the present study, we show that dogs can distinguish the breath odor of cancer patients. We trained three female black Labradors to detect a target (breath from a cancer patient) from three other subjects (breath from healthy men). Breath from each participant was collected in Teflon puff bottles. The dog handler and the recorder did not know which of the four subjects the cancer patient was and the investigator was not present during the test. The dog handler puffed air from the cancer patient bottle into the nostrils of a dog for about ten seconds while the dog's behavior and EEG were monitored. The dog then sniffed the four subjects and was asked to sit beside the correct target. Our results indicate that the dogs chose the correct target over 90% of the time. However, this was a pilot study. We can draw no firm conclusions at this time. More experiments need to be performed to confirm that dogs can detect cancer patients by their smell.

The effect of odor in the palatability for Japanese black cattle

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Lolium multiflorum Lam. is commonly known as Italian ryegrass, and is widely cultivated as forage for cattle and other livestock in Japan. Both Italian ryegrass hay and silage produce characteristic odors. By comparing volatile components in hay and silage by GC-MS, higher amounts of low boiling volatile C6 to C9 aldehydes and alcohols were found in hay, which produced a green leaf like aroma. In contrast, the oil of silage was described as a sweet-sour aroma, and was composed of esters (~50%). The blended ratio of esters, lactones, and anhydride appeared to construct the aroma quality of silage. On the other hand, low molecular organic acids (~83%) such as butyric acid were mainly contained in the oil of spoiled silage, which produced an unpleasant and rancid aroma. For the feed preference test, the Japanese black cattle commonly smelled and consumed the hay completely, and then switched to the silage. In contrast, the spoiled silage produced the deterrent effect more than the fresh silage. These results indicated that the green aroma volatiles such as aldehydes and alcohols in the hay, or the low molecular organic acids in the spoiled silage, were effective in the palatability for the cattle.

Bottles and names of fragrance affect recognition of the fragrance

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In this study, effects of verbal label and shapes of bottle on recognition memory of perfume were investigated. Participants in the label group were presented verbal label of the perfume with the perfume, participants in the bottle group were presented the bottle with the perfume and participants in the control group were presented the perfume alone in learning session. Recognition test was repeated twice: 15 min after, and one week after the learning session. All participants showed deterioration of odor recognition memory, but the main effect of group was significant. Participants in control group showed lower odor recognition than the other groups. When the results were compared with those of preceding study by Ayabe et al. (1996), recognition for abstract odors (perfume: in this study) was lower than that for odors experienced in our daily lives (such as odors of soy source, curry, vanilla and so on: in the preceding study).

Feeding experiment on manufactured bait made of waste stillage from *Shochu* (distilled spirits) for the abalone, *Haliotis diversicolor*

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The production of macroalgae, which are the diet of abalones, shows seasonal fluctuations. The discharge of waste stillage from *shochu* distillery increases from the end of summer to winter; waste stillage contains organic compounds such as amino acids, minerals, and vitamins.

Feeding experiments were carried out in order to estimate the possibility of using manufactured bait made of waste stillage obtained from *shochu* distillery instead of using algae. Abalones within a single tank were simultaneously provided 4 types of bait—powdered *wakame* seaweed *Undaria pinnatifida*, sweet potato *shochu* waste stillage, barley *shochu* waste stillage, and seawater. The bait comprising seawater was used as a control.

All the baits used in the tank experiments were prepared by hardening the ingredients with a solution of agar in seawater. Since abalones are nocturnal, the experiments were conducted at night, from 1800 to 0800. We found that the abalones consumed all 4 types of bait. The 4 baits were consumed in the following order, from the largest amount consumed to the least: (1) powdered *wakame* seaweed, (2) sweet potato *shochu* waste stillage, (3) seawater, and (4) barley *shochu* waste stillage. The amount consumed was significantly greater for the baits comprising powdered *wakame* seaweed and sweet potato *shochu* waste stillage than for the control bait. The results of our experiments indicate that abalones are not averse to feeding on *shochu* waste stillage.

Evaluation of synergy among various flavor compounds containing in beer

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It is well-known that various beers contain many flavor compounds derived from barley malts, hops, yeast fermentation and other raw materials. Among these flavor compounds, branched- chain fatty acids (3-methylbutanoic acid, 2-methylpropanoic acid and 2-methylbutanoic acid) are mainly derived from yeast fermentation and hops. These branched-chain fatty acids are by-products from the metabolism of branched-chain amino acids (L-leucine, L-valine and L-isoleucine). On the other hand, the structures of acyl side chains of alpha acids and beta acids derived from hop correspond to those of these three branched-chain fatty acids. We analyzed test-brewing unhopped and hopped beer and confirmed that the concentrations of these branched-chain fatty acids in beer increased by both yeast fermentation and hopping. Recently, several researchers have reported that acetic acid and n-butanoic acid, below their threshold levels, could enhance the flavor intensity of several flavor compounds derived from coffee. Therefore, we assumed that branched-chain fatty acids might enhance other flavor compounds containing in beer. We focused on monoterpene alcohols (linalool, geraniol and citronellol) derived from hop as major flavor compounds contributing to hopped beer flavor, and tried to evaluate synergy among branched-chain fatty acids and these monoterpene alcohols. As a result, it was shown that the flavors of linalool and geraniol were enhanced by occurrence of these three branched-chain fatty acids at their threshold levels. In addition, the flavor impression of monoterpene alcohol mixture (linalool, geraniol and citronellol), having flowery and citrus flavor, was change under coexistence with these branched-chain fatty acids.

Effect of odor on neocortical responses to taste in fronto-temporal regions of the neocortex-Flavor creation using optical imaging

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During food intake, odor inputs influence taste perception through central mechanisms for the flavor integration. The fresh scent of lemon, for example, enhances the perceived intensity of both sourness and sweetness. Previously, we showed using multi-channel near-infrared spectroscopy (NIRS) that specific odorants modified the cortical responses to a tastant when the odor and the taste shared a common sensory quality. A sweet odor of ethylmaltol enhanced the cortical responses to a sweet taste of sucrose. In this research, we extended our previous study of the cortical responses to evaluate the effect of a lemon odor on the mixture of sour and sweet tastes. Using NIRS, we recorded neocortical responses to the mixture of sucrose and citric acid solutions with or without the lemon odor. First, we observed concentration-dependent increases in the amplitude of the responses to the mixture solutions. When the lemon flavoring was added to the taste solution, we observed a statistically significant increase in the amplitude of the responses to the taste solution as compared to that without the lemon odor. However, the addition of the lemon flavoring to water (without sugar and citric acid) caused no significant change in the amplitude of the cortical responses to water. These results indicate that the lemon flavoring enhances the cortical responses to the mixture of sweet and sour tastants, in a similar way as the increase in the concentration of the tastants enhances the responses. Thus optical imaging of neocortical responses provides a means to detect the central modification of responses to the mixture of multiple tastants by specific odors.

Added flavorings enhance neocortical taste responses in the fronto-temporal regions-Flavor creation using optical imaging

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Dried-bonito (Katsuobushi) broth, an important seasoning in Japanese cuisine, has long been used to reinforce the flavor of foods. Not only the taste but also the aroma components of the broth contribute well to the enhancement. The aroma components of dried bonito are important for the preference as well as the total characteristics of the flavor. We previously showed using multi-channel near-infrared spectroscopy (NIRS) that natural dried-bonito aroma enhanced the cortical responses to the mixture of amino acid and nucleotide solutions (odorless broth). In this research, we reconstituted a dried-bonito flavoring that comprised of odor-active compounds of dried bonito based on analysis of the aroma components from the natural dried-bonito extracts. To assess the effectiveness of the reconstructed flavoring, we compared the responses to the flavored broth with those to the odorless broth. Among the five subjects examined, four subjects (80%) showed a significant increase in the amplitude of the responses to the flavored broth as compared to that to the odorless broth. When smoky aroma, one of the important parts of dried-bonito aroma, was removed from the reconstructed flavoring, the reconstructed flavoring did not significantly enhance the cortical responses. These results indicate that the smoky-aroma components contribute to the enhancement of the cortical responses by the reconstructed flavoring. In addition, the results indicate that the reconstructed flavoring contains key aroma parts of dried bonito that enhance the neocortical taste responses in a similar way that the natural dried-bonito extracts aroma do. The optical imaging of the neocortical responses in the fronto-temporal regions thus provides a means to detect the influence of added aroma components on tastes.

Evaluation of olfactory transport dysfunction by SPECT-MRI and nasal thallium-201 administration in patients with posttraumatic olfactory loss

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Injuries to the olfactory bulb and tract have been visualized with MRI in patients with posttraumatic loss of olfactory function. However, MRI does not sufficiently demonstrate olfactory transport. The aim of this study was to assess olfactory transport to the brain by using SPECT-MRI imaging with nasal administration of thallium-201 (^{201}Tl) in patients with posttraumatic olfactory loss.

Five patients with posttraumatic olfactory loss were enrolled in this study after providing informed consent (two males and three females; 31–64 years old). ^{201}TlCl was administered nasally into the right olfactory cleft. Uptake of ^{201}Tl was detected 24 h later by using a single photon emission computed tomography (SPECT)-X-ray computed tomography hybrid system. An MRI image was obtained for each patient, and merged with the SPECT image.

^{201}Tl olfactory transport to the olfactory bulb area was decreased in patients with posttraumatic olfactory loss. No patients experienced any adverse effects associated with the treatment.

The reduced olfactory transport in patients with posttraumatic olfactory loss support the hypothesis that olfactory fibers are disconnected between the olfactory bulb and olfactory epithelium in the nasal cavity of patients with posttraumatic olfactory loss. ^{201}Tl may be used in future studies to validate newer techniques, such as fMRI, and assess olfactory function without requiring patients to voluntarily respond to odors.

Numerical Conversion of Smell from Frozen Food and its Variation with Temperature

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Smell provides important information about the quality of food. Recent researches showed that electric nose devices had abilities for measuring the food smell and that the data suggested the freshness or pathogenic microorganisms. However, there are few reports about the smell of frozen foods and their variation with temperature. Here, we report the numerical conversion of smell from frozen

foods (*Broccoli*, Hamburger steak with demiglace sauce, and grated *Wasabi*), using FF-2A electric nose device (Shimadzu Corporation, Japan).

The samples were packed with N_2 in PET bag and incubated at -80, -30, 4, and 25 °C. The headspace gasses were syringed. The electric nose detected the smells of all the samples even at -80 °C and the total odour index was over 20. The index of broccoli was 21.9 – 34.6 at the indicated temperature; hamburger steak: 31.9 – 36.9; grated wasabi: 33.7 – 37. The total odour indexes increased with temperature, and the odour index in 9 standard gasses (hydrogen sulphide, organic acid, sulphur, aldehyde, ester, ammonia, aromatic group, carbon hydrate, and amine) had increasing tendency with temperature. In the groups of the standard smell, the odour index of the samples in sulphur group was higher than other groups.

Mere exposure effect of odor unconsciously contacted

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The mere exposure effect (MEE) is a phenomenon that the more frequently people contact with a stimulus -whether it is a word or a form as well as an odor-, the more they come to like it. In this study, participants were exposed by an odor stimulus which they were not aware the presentation of it. They were asked to work on a ten-minutes-spelling exercise during 20 days by using particular mechanical pencil lead in which microcapsulated odor was kneaded. Thereby they were able to contact with the odor stimulus repeatedly being out of their awareness. The pleasantness of the odor was rated and compared both within (Exp.1) and between participants (Exp.2). In Exp.1, participants rated the odor pleasantness before and after contact with the odor stimulus. As a result, no significant difference of likes of the odor was observed between before and after contact with it. Therefore the contact group and the noncontact group (by using normal pencil lead) were set up in Exp. 2, because it is possible that the pleasantness evaluation of the odor before contact might affect on the pleasantness of the odor after contact. It was examined whether the contact group rated the odor more pleasant than the noncontact group. As results, even the noncontact group liked the odor as well as the contact group, namely MEE did not occur in Exp.2 either. In consequence, occurrence of MEE for an odor stimulus may need to increase the familiarity following intentional contact with the odor.