

## ABSTRACTS

# The 34th Annual Meeting of the Japanese Association for the Study of Taste and Smell (JASTS XXXIV)

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### T1. Studies on the transduction mechanisms on bitter and umami taste perception in the isolated mouse taste cells

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Umami and bitter taste transduction mechanisms were studied by whole-cell patch-clamp and optical methods. Each three bitter compound elicited different types of responses under the voltage clamp. Naringine (the bitter compound from grapefruit) stimulation increased the IP<sub>3</sub> concentration, denatonium (chemical compound) response current was involved with both IP<sub>3</sub> and cyclic-nucleotide, and quinine (alkaloid) response was not coupled with G-protein transduction pathway. Monosodium glutamate and nucleotide are umami substances. Monosodium glutamate elicited three types of responses: two types of inward current response and an outward current response. Four kinds of glutamate receptor agonist—NMDA (NMDA-type ionotropic glutamate receptor agonist), AMPA (non-NMDA-type ionotropic glutamate receptor agonist), L-AP4 (metabotropic glutamate receptor type 4 agonist), ibotenic acid (metabotropic glutamate receptor group I and II, and NMDA type ionotropic glutamate receptor agonist) were used to classify the monosodium glutamate responses. Ibotenic acid—a strong umami substance—elicited large two types of inward current responses by patch-clamp recording. Umami is also characterized by the synergistic effect between monosodium glutamate and nucleotide. The mixture of monosodium glutamate and inosine monophosphate showed three types of response. Interestingly, two types of response were much larger than the response of glutamate only, but the outward response was the same amplitude.

### T2. Functional anatomy of the vomeronasal system

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My collaborators and myself have been characterizing the primary vomeronasal system (VNS) of rats by using lectin histochemical, neuroanatomical and immunohistochemical techniques. The sensory cells in the VNS, vomeronasal receptor neurons (VRNS), contain glycoconjugates (GCs) with terminal  $\alpha$ -galactose (Gal) and  $\beta$ -N-acetyl galactosamine (GalNAc). The density of these GCs is highest in the ‘mucomicrovillar complex’ of the vomeronasal sensory epithelium where perireceptor events take place. In contrast, olfactory receptor neurons do not contain terminal

$\alpha$ -Gal- and  $\beta$ -Gal-containing GCs. The terminal  $\alpha$ -Gal-containing GCs in the mucomicrovillar complex can be separated into cellular and mucus domains by using confocal laser scanning microscopy, indicating that dendritic terminals of VRNs contain abundant  $\alpha$ -Gal-containing GCs. Output neurons in the AOB, ‘mitral/tufted cells’ (MTCs) have a diverse dendritic branching pattern and most have a variable number of glomerular arbors per cell (up to six); we have tentatively classified the MTCs into simple, intermediate and complex. In complex MTCs, apical dendrites originating from the soma enter the glomerular layer to provide branches that form the glomerular arbors, and after that, some dendrites develop second arbor (s) (*en passant* and terminal arbors respectively). One of the major results of our immunohistochemical studies is that a subpopulation of MTCs contains immunoreactivity for somatostatin-28. We also observed the main olfactory bulb and found out that neither mitral nor tufted cells were somatostatin-28-immunoreactive. To extend the above findings, lectin cytochemical and gene histochemical studies on the VNS are underway in our laboratory.

### K1. Molecular biological study of the taste gene *Tre*, a candidate *Drosophila* sweet taste receptor

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Mutations of *Tre*, a *Drosophila* taste gene that is known to affect gustatory sensitivity to a disaccharide, trehalose, were induced by imprecise P-element excisions near the *Tre* locus. Among the available P insertions on 5A–5B regions, EP(X) 0964 induced *Tre* mutations in some P-excised progenies that showed a noticeable decrease in sensitivity to the sugar. Sequencing the genomic DNA in the mutants identified small deletions in the 5′ region of a G protein-coupled receptor gene *GPCR(5A)*. The *Drosophila* BLAST search of the 5′ sequence hit an EST and the cDNA clone was subsequently sequenced. The open reading frame of the gene codes a 392 amino residue in the wild-type allele. The mRNA was not detectable in the mutants where 5′ genomic region was disrupted by deletions. The deletions also uncover a small genomic region upstream of the *GPCR(5A)* but reverse transcriptase polymerase chain reaction analysis so far failed to show any detectable mRNA from the tissue preparations. We propose that *GPCR(5A)* is identical to the gustatory gene *Tre* and that the gene encodes a gustatory receptor for sweet taste in *Drosophila*.

### S1.1 Feeding-related hypothalamic neurons on the energy metabolic regulating system

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Body weight is a lot like the weather; everybody talks about it, but no one seems to be able to do much about it. However, in the past few years we have learned a great deal about the physiological mechanisms that help people keep their energy intake and expenditure in balance. The lateral hypothalamic area (LHA) and the ventromedial hypothalamic nucleus (VMH) have historically been implicated in ingestive behavior, energy balance and body mass regulation. The LHA is more closely associated with the initiation of eating, whereas the VMH mediates the cessation of eating. The parvocellular part of the paraventricular nucleus is also included in the suppressing mechanism. Recently, we have demonstrated the effects of several endogenous neuropeptides on the feeding-related hypothalamic neuronal activity, and the feeding behavior in the rats. In summary: the orexins, MCH (melanin-concentrating hormone), AgRP (agouti-related peptide) and NPY (neuropeptide Y) were involved in the hunger system; leptin, CRH (corticotropin-releasing hormone) and  $\alpha$ -MSH ( $\alpha$ -melanin-stimulating hormone) played a role in the feeding inhibitory system. These results are discussed in terms of hypothalamic neural circuits that are sensitive to endogenous food intake inducing and reducing substances.

### S1.2 Food choice in non-human primates: chemical substances and sense of tastes

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Nakagawa (1996) reviewed ecological studies on food choice in non-human primates, and concluded that these primates choose foods containing more protein and soluble carbohydrate, and less crude fiber and condensed tannin (digestion inhibitors). Such a food choice strategy enables them to maximize intakes of energy- essential nutrients and to minimize intakes of fiber and digestion inhibitors. This revealed the ultimate factor (i.e. adaptive meaning) but not the proximate factor (i.e. mechanisms) of food choice. On the other hand, physiologists have focused their attention on the mechanisms of food choice and revealed that tastes (e.g. sweetness, umami, bitterness, astringency) are the signals for chemical substances to be ingested (i.e. nutrients) or avoided (i.e. digestion inhibitors and toxins). Recently, interspecific differences in taste thresholds of primate species have been examined, and discussed from a standpoint of food habits. Such studies suggest not only the existence of mechanisms discriminating tasty substances but also the adaptive meaning of taste thresholds.

### S1.3 Nutritional condition and the taste preference in rats: the relationship between sour taste preference and organic acid metabolism in forced swimming rats

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It is generally considered that physiological condition affects taste preference in humans and animals. We have demonstrated that physical fatigue causes an increase in the preference for sour taste in rats. The present experiment was therefore undertaken to clarify the physiological mechanism of this effect. Six-week-old male Spague–Dawley rats were used for these tests. After acclimatization for 5 days, the rats were divided into two groups, control and forced swimming, before finally being killed. We quantitatively analyzed the activity of liver rate-limiting enzymes in the TCA cycle members (organic acids) and gluconeogenesis, i.e. citrate synthase, isocitrate dehydrogenase, 2-ketoglutarate dehydrogenase and phosphoenolpyruvate carboxykinase. Moreover, liver mitochondrial ATP productivity and ATP/ADP ratio were assessed by HPLC and oxygen electrode detection methods. It was clearly observed that the enzyme activities which participated in both the TCA cycle and gluconeogenesis were increased after forced swimming. We also observed that the decreased ATP production rate and mitochondrial ATP/ADP ratio in the liver due to forced swimming were both improved by ingestion of each organic acid (citric acid, malic acid and 2-ketoglutaric acid). These results suggest that the organic acid preference is possibly beneficial for the energy supply in physically fatigued rats.

### S1.4 Relationship between food properties and its preference in the human

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The relationship between food properties and its preference in humans was evaluated. Food was chewed in the oral cavity, mixed with saliva and swallowed. Food texture affects the preference. The following properties and preferences were considered: (i) food texture and chewing movement; (ii) food texture and oral stereognosis; (iii) eating pattern and food preference; (iv) taste and food preference; (v) food surface and food preference; (vi) physiological change in the body and food preference; (vii) physical response and food preference.

### S2.3 Calcium signaling cascade in taste transduction

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Two families of G protein-coupled receptors, T1Rs and T2Rs, have

been identified as taste receptors or their candidates which are expressed in subsets of taste bud cells. Previous physiological studies have indicated that taste transduction utilizes at least two signaling pathways: calcium and cyclic nucleotide signalings. Since IP<sub>3</sub>-dependent Ca<sup>2+</sup> release from intracellular stores is activated by many bitter tastants such as denatonium benzoate and by some non-sugar sweeteners such as saccharin and SC-45647, the calcium signaling cascade should consist of G protein-coupled receptors, G protein(s), phospholipase C (PLC), IP<sub>3</sub> receptor(s) and so on. In order to investigate the molecular mechanism of the calcium signaling pathway, we analyzed expression of its molecular components. We first identified PLC-β2 as a component downstream of G proteins G<sub>i2</sub> and G<sub>gust</sub>. Next, the type 3 isoform of inositol-1,4,5-triphosphate receptor, IP<sub>3</sub>R<sub>3</sub>, was identified and shown to express in the same cells as PLC-β2. In addition, we showed that taste bud cells expressing rT1R2 and those expressing rT2R9, a probable cycloheximide receptor, are included in PLC-β2- and IP<sub>3</sub>R<sub>3</sub>-positive cells. Our findings thus indicate that PLC-β2 and IP<sub>3</sub>R<sub>3</sub>, together with G<sub>i2</sub>, are involved as downstream components in the signalings of two different types of taste receptors, T1Rs and T2Rs, in taste bud cells.

## S2.4 Deficit in fear response and conditioned taste aversion in metabotropic glutamate receptor subtype 7 knockout mice

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Metabotropic glutamate receptors (mGluRs) consist of eight different subtypes and exert their effects on second messengers and ion channels via G proteins. The function of individual mGluR subtypes in the central nervous system, however, largely remains to be clarified. We examined the fear response of freezing following electric shock in wild-type and mGluR7<sup>-/-</sup> knockout littermates. Wild-type mice displayed freezing immediately following and 1 day after footshock. In comparison, mGluR7<sup>-/-</sup> knockout mice showed significantly reduced levels in both immediate postshock and delayed freezing responses. However, the knockout mice exhibited no abnormalities in pain sensitivity and locomotor activity. To further examine amygdala-dependent behavior, we performed conditioned taste aversion (CTA) experiments. In wild-type mice, the administration of saccharin followed by intraperitoneal injection of the LiCl caused strong CTA towards saccharin. In contrast, mGluR7<sup>-/-</sup> knockout mice failed to associate between the taste and the negative reinforcer in CTA experiments. Again, the knockout mice showed no abnormalities in taste preference. These results indicate that mGluR7 deficiency causes an impairment of two distinct amygdala-dependent behavioral paradigms. Immunohistochemical and immunoelectron-microscopic analyses showed that mGluR7 is highly expressed in amygdala and is preferentially localized at the presynaptic axon terminals of glutamatergic neurons. Together, these findings strongly suggest that mGluR7 is involved in neural processes subserving amygdala-dependent aversive responses.

## S3.1 Research trends on the olfactory transduction mechanism

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There has been an argument whether cAMP and IP<sub>3</sub> are alternative second messengers in vertebrate olfactory transduction. According to our EOG recordings, we supposed that the population of receptor cells for 'IP<sub>3</sub>-odorants' is so low that the increased cAMP could not be detected. By examining the correlation between the IP<sub>3</sub> gated and CNG conductances, we demonstrated that IP<sub>3</sub> channels might be from a part other than the olfactory cilia. It has already been demonstrated that all the odorants raise both cAMP and IP<sub>3</sub> in the cilia. Recent studies have shown that a given receptor has a relatively low specificity for odorants, and that output patterns formed among the 'overlapping' receptors would give a base for odor recognition. A single second messenger may be sufficient for such a recognition system. One of the characteristics of the olfactory cells is the response amplification by Ca<sup>2+</sup>-dependent Cl<sup>-</sup> channels. To confirm this, we have measured the intracellular Cl<sup>-</sup> concentration in rat olfactory cells, and found it to be ~80 mM. At this concentration, Cl<sup>-</sup> seems to boost the depolarization of the cell. However, it is also possible that Cl<sup>-</sup> suppresses the peak of the depolarization.

## S3.2. Multiple transduction pathways in olfaction independent of cAMP

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Odor responses in olfactory sensory neurons have been shown to be generated via a second messenger, cAMP. In previous studies, we have shown that the cAMP-independent pathway also contributes to the generation of odor responses. Using the whole-cell mode of the patch-clamp technique, we recorded inward currents in response to IP<sub>3</sub> in frog, clawed frog and turtle olfactory sensory neurons. Dialysis of IP<sub>3</sub> into the neurons induced inward currents with an increase in membrane conductance in a dose-dependent manner under the voltage-clamp conditions (holding potential -70 mV). To explore a novel second messenger in the cAMP-independent pathway, we investigated the possible role of cADP-ribose in olfactory transduction. The olfactory neurons responded to dialysis with cADP-ribose from a pipette with an inward current, an increase in membrane conductance and an increase in intracellular Ca<sup>2+</sup> concentration. The magnitudes of the inward current responses to cAMP-increasing odorants were greatly reduced by previous dialyses of a high concentration of cADP-ribose or 8-Br-cADP-ribose, an antagonist, suggesting that the cADP-ribose-dependent pathway greatly contributes to the generation of olfactory responses.

### S3.3 Optical analyses of signal propagation in guinea-pig piriform cortex slices

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In order to analyze spatiotemporal patterns of signal propagation, guinea-pig piriform cortex (PC) slices 400  $\mu\text{m}$  thick, stained with a voltage-sensitive dye (RH482), were subjected to optical recording. The most commonly observed pattern of propagation elicited by layer Ia stimulation was that excitation first propagated along layer Ia, then invaded layers II and III, and propagated along them. The other pattern often observed was that, while excitation propagated along layers II and III, it passed through the deeper part of layer III and finally reached the endopiriform nucleus. Signal propagation elicited by layer Ia stimulation was not affected by application of D-APV, but was completely suppressed by CNQX, suggesting that transmission between afferent fibers and pyramidal cells is mediated by non-NMDA glutamate (Glu) receptors. Effects of D-APV and CNQX on signal propagation elicited by layer Ib or II stimulation were similar to the above. A weak postsynaptic propagation component, however, was found to be resistant to both D-APV and CNQX and was suppressed by MCPG. These suggest that, although transmission between association fibers and pyramidal cells is mediated mainly by non-NMDA Glu receptors, participation of metabotropic Glu receptors is not negligible.

### O2. Effect of odor preference on the autonomic nervous system

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We aimed to investigate effects of odor preference on activities of the autonomic nervous system (ANS). Thus, for 6 min, 10 female subjects (age range 20–27 year) were asked to inhale three odors: favorite odor (FO), which comprised some essential oils using the compounding technique taking into account the large individual variation in odor preference; malodor (MO), the rotting odor of Welsh onion; and blank odor (BO). The effect of these odors was evaluated on eight ANS parameters: heart rate (HR); heart rate variability (LF component: 0.05–0.15 Hz, BF component: 0.23–0.43 Hz, LF/HF); systolic/diastolic blood pressure (SBP/DBP); spontaneous reflex of skin resistance (SR); and electromyogram of the metopic parietal area (EMG). It is recognized that these ANS parameters are affected by differing odor preferences. After inhalation of odors for 6 min, the MO gave significantly higher values for LF/HF, SBP and DBP analysis compared to those obtained with BO. This result suggests that MO caused stress in the ANS due to defense responses. In contrast, there is no significant difference between FO and BO after inhalation for 6 min. However, FO gave significantly lower values with SBP and EMG analysis compared with those obtained under BO by inhalation for the first 2 min. This result suggests that FO is not effective for relaxing, but accelerates relief from stress.

### O3. The effect that the existence of odor information has on the cyclical rhythm of $\alpha$ -waves

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An experiment was performed in which visual sense image information for the odor was either given or not given. The influence of this visual sense image was estimated from the change in the fluctuation rhythm of the  $\alpha$ -wave for right and left frontal regions of the subjects (32 females). The odors used were three type of perfume: peach, rose and sun (compound perfume). After attaching the sensor of a simple EEG rhythm measuring device to the right and left frontal areas of the head (Fp1 and Fp2), the subjects faced the apparatus. Three perfumes were presented by the bottle method, and initially no perfume information was given. The frontal brain wave was measured. After ~1 month, visual information for the perfume presented to the same subjects was given verbally, and the periodic rhythm of the  $\alpha$ -wave was measured and compared with the result of the first experiment. The rhythmicity of the  $\alpha$ -wave was increased when information was given, suggesting that the effect also increased.

### O5. Electroencephalogram in the intravenous olfaction test

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An objective olfaction test is not generally used in the clinical field. We tried to record the electroencephalogram (EEG) during smelling. When the thiamine propyl disulfide (Alinamin®, Takeda Pharmaceutical Company, Osaka, Japan) is injected into a vein, the subject experiences the odor of garlic. The latency and duration of the garlic smell are then measured. This test is called the intravenous olfaction test and is widely used in Japan. We recorded the EEG during the venous olfaction test and notes changes in the EEG when the garlic smell occurred. The subjects, who were healthy and had no olfactory dysfunction, gave their informed consent for participation in the study. Two electrodes were located bilaterally on the front of the head and a parietal electrode was saved as ground. EEGs were amplified, recorded on a digital audio tape recorder, and analyzed using a personal computer. When the garlic smell caused by intravenous olfaction occurred, the amplitude of spontaneous EEG increased. In the frequency spectral view, the increase is clearly noticeable in the 30–200 Hz region, but was not observed outside this band. Injection of physiological saline did not increase the EEG. Measuring EEGs when intravenous olfaction occurs is thought to be applicable for the objective olfaction test.



## 06. Involvement of ionotropic glutamate receptors in the olfactory bulb in early olfactory learning

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Two subtypes of ionotropic glutamate receptors exist in the olfactory bulb. These are involved in excitatory transmission from olfactory neurons to mitral cells, and from mitral to granule cells. After training with an odor paired with foot shock, rat pups show aversion to the odor. Since pharmacological manipulations of synaptic transmission in the olfactory bulb affect this olfactory aversive learning, intrabulbar plasticity is considered to underlie this olfactory learning. Therefore we examined whether infusion of ionotropic glutamate receptor antagonists could influence olfactory aversive learning in young rats. Infusion of the NMDA receptor antagonist D-AP5 facilitated olfactory learning not only for the odor exposed during infusion but also for an unfamiliar odor that was not exposed during infusion in a dose-dependent manner. The facilitatory effect of NMDA receptor blockade on olfactory aversive learning is probably due to a reduction of feedback inhibition of granule to mitral cells. Infusion of the non-NMDA receptor antagonist CNQX prevents olfactory learning, an effect that is probably brought about by blockade of excitatory transmission from olfactory neurons to mitral cells. These results suggest that NMDA receptors play an inhibitory role in the establishment of aversive learning in young rats.

## 07. Putative salt receptor cells in the epidermal cell layers of desert toads, *Bufo alvarius*

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Toads normally obtain water by absorption across their skin from osmotically dilute sources. We previously showed that desert toads (*Bufo alvarius*) have chemosensory function in the ventral skin to detect hypertonic salt solutions in a hydration source. The function is mediated by neural excitation of the spinal nerves that innervate putative chemosensory cells in the ventral skin. In the present study fluorescent dye translocated through the spinal nerves to those receptor cells in the epidermis was photoconverted in the presence of 3,3'-diaminobenzidine tetrahydrochloride (DAB) for electron-microscopic observation of the cells and associated nerve terminals. Most of photoconverted cells were located in the deepest layer of the epidermis, i.e. the germinativum cell layers. No labeled cell was seen in the outermost layer of living cells, i.e. the outer granulosum layer. In anuran amphibians generally, including desert toads, flask cells and Merkel cells, both with characteristic cytological features, are occasionally seen in the epidermis. However, cytological features of the photoconverted cells were reminiscent of neither flask cells nor Merkel cells, but were similar to those of surrounding epithelial cells in each layer of the epidermis. The photoconverted cells had a close association with nerve fibers. We hypothesize a sensory function for these cells.

## 08. Electrophysiological studies on the somatotopic organization of the facial lobe in the goatfish

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Previous studies have revealed that goatfish exhibit an unusual elaboration of the facial lobe (FL), which consists of dorsal and ventral divisions. The dorsal FL appears as a cauliflower-like protrusion from the dorsal surface of the medulla to form lobules. These lobules appear coarsely laminated with a superficial molecular layer, an intermediate layer of densely packed medium neurons and a deeper layer of elongated, larger neurons.

In order to understand the nature of the lobules of the dorsal FL, we utilized microelectrodes to map the mechano-receptive fields of neurons situated in various areas of the FL. The electrode was driven vertically throughout the FL in a systematic grid of points projected onto the dorsal surface of the lobe. The surface of the barbel was mechanically stimulated with the glass capillary (tip diameter 20  $\mu\text{m}$ ), and neural activity was recorded as integrated responses. Because of the convoluted nature of the dorsal FL, in any single dorsoventral electrode penetration, the electrode tip was likely to pass from one lobule into another. In any penetration, the maximum response was obtained when the electrode was positioned at the cell layers.

Penetrations through the rostromedial portion of the FL yielded receptive fields near the base of the barbel, whereas penetrations along the lateral edge of the rostral part of the lobe revealed fields near the distal tip of the barbel. There was not, however, smooth continuity of receptive fields between these areas. For example, moving the electrode  $\sim 500 \mu\text{m}$  laterally at the middle levels showed receptive fields moving from the base of the barbel to midway along its length without any intermediate representation. Such intermediately situated receptive fields were, however, evident at more caudal levels of the lobe. When all recordings are taken into account, despite apparent discontinuities at any particular antero-posterior level, a continuous, albeit convoluted, representation of the barbel could be discerned in the dorsal FL.

## 09. Properties of taste solution preference in geriatric disease model rats

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This study was conducted to clarify the relation between genetic background and the food factors that cause the geriatric diseases non-insulin-dependent diabetes mellitus (NIDDM) and spontaneous hypertension. Three rat strains—GK as the NIDDM model, SHR for spontaneous hypertension and Wistar as control—were used in the experiment. In each strain, six males aged 3 weeks, were housed individually in plastic cages. Preference tests were performed in a free choice situation with two bottles among water, 5% glucose, 5% sucrose and 0.9% saline. Daily intake of the solution/100 g of body weight was measured in the groups. All rats preferred a glucose solution more than water from the infant stage. There was no difference in intake volume between water and saline in Wistar rats. However, infant GK rats took a greater volume of

saline than water. In comparison with Wistar and GK, SHR rats showed a remarkable taste preference for both sweetness and saltiness. The above results suggest that the genetic factors of geriatric diseases are closely related to gustatory senses.

### O10. Evaluation of astringency using a multichannel taste sensor

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This paper reports evaluation of astringency using a multichannel taste sensor. We had previously developed this multichannel taste sensor, which is composed of lipid/polymer membranes. This sensor has 'global selectivity', which implies an intelligent sensitivity to chemical substances that enables it to classify five basic taste qualities: sourness, saltiness, sweetness, bitterness and umami. This taste sensor has been applied to a variety of foods/drinks such as beer, coffee, tea, mineral water, sake, fruits and vegetables. Its discrimination ability, durability and sensitivity are superior to those of humans.

We had been successful in terms of high selectivity and sensitivity to adsorptive taste substances such as astringent ones. The positively charged membranes show high selectivity and sensitivity to anionic astringent substances. The negatively charged membranes inversely respond to cationic astringent ones. By using the membranes and multiple regression analysis we tried to develop a method to evaluate astringency. Also we can detect synergism and suppression effects of astringency in tannic acid, an important astringent substance. Furthermore we found high correlations between the results of human sensory tests and these results. Therefore, it is concluded that this method is useful for the evaluation of astringency. Quantification of taste is largely improved by this method.

### O11. Electrophysiological study on bitter taste transduction mechanisms in mouse taste cells

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Multiple transduction mechanisms have been proposed for the perception of bitter taste. To investigate the detail of bitter taste transduction mechanisms, we recorded the electrophysiological responses to bitter stimuli in isolated mouse taste cells.

Taste cells were isolated from C57BL/6J mice (8-week-old, female). Denatonium- or quinine-induced responses were recorded under the voltage-clamp mode of whole-cell patch-clamp technique. Holding potential was -80 mV.

Denatonium (1 mM) induced outward current response with normal intracellular solution. IBMX (1 mM) in the intracellular solution had no effect on the outward current response. This is consistent with the results when cGMP was present in the intracellular solution.

Quinine (10mM) induced inward current response with normal intracellular solution. With 1 mM IBMX in the intracellular solution, quinine still induced the inward current response, but the amplitude of the response was larger than that observed with normal intracellular solution. This is consistent with the results when cGMP was added to the intracellular solution. Quinine induced the inward current response when 1 mM GDP- $\beta$ -S was

added to the intracellular solution. In Ca<sup>2+</sup>- or Na<sup>+</sup>-free extracellular solution, quinine induced the inward current response, but no response was observed when Ca<sup>2+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> were removed from the extracellular solution. These results suggest that quinine induces the influx of extracellular cation independent of the binding to taste receptors.

### P1. Expression of insulin-like growth factor and its receptor in the rat olfactory system

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It is known that the members of the insulin-like growth factor (IGF) family influence neuronal generation, survival and differentiation in the olfactory system. IGF-1 and its receptor, IGF-1R, are known to be expressed at high levels in the olfactory epithelium (OE); however, the cell types expressing these molecules have not been identified. In the present *in situ* hybridization study, we have shown that IGF-1R mRNA was expressed in the cells of middle to basal regions of the rat OE and vomeronasal organ (VNO) at embryonic day 15. At this stage, an antibody specific for the  $\alpha$ -subunit of the IGF-1R labeled a subset of immature olfactory cells. An antibody specific for IGF-1 labeled the supranuclear cytoplasm of supporting cells. Moreover, in the dorsal fossa of the posterior nasal cavity, IGF-1 and IGF-1R seemed to be involved in the process of olfactory cell death, followed by formation of the specialized epithelium consisting of supporting cells and horizontal basal cells during postnatal development. At postnatal day 1, the distribution of IGF-1R-positive immature olfactory cells in the dorsal fossa was similar to that in the OE in other regions. At P3, IGF-1R-labeled cells decreased in number and disappeared by postnatal day 14. In contrast, the number of IGF-1R-labeled cells in OE in other regions increased with age. The IGF-1 immunoreactivity in the supporting cells did not change during development in either the dorsal fossa or the OE. This suggests that IGF-receptor-depleted immature olfactory cells do not differentiate into mature olfactory cells, which results in the death of olfactory cells.

### P3. Chloride concentration in rat olfactory receptor cells measured with a fluorescent probe

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Inward directed, Ca<sup>2+</sup>-dependent chloride currents are thought to prolong and boost the transient receptor currents generated in olfactory cilia. Chloride inward current requires a sufficiently high intracellular Cl<sup>-</sup> concentration ([Cl<sup>-</sup>]<sub>i</sub>). In previous measurements using a fluorescent Cl<sup>-</sup> probe, MQAE, [Cl<sup>-</sup>]<sub>i</sub> of newt olfactory cells was estimated to be only 40 mM. This low value led us to re-examine the [Cl<sup>-</sup>]<sub>i</sub> by an improved procedure, using MQAE and *in situ* calibration. When isolated olfactory neurons of a rat were bathed in Tyrode's solution (150 mM Cl<sup>-</sup>) at room temperature, the [Cl<sup>-</sup>]<sub>i</sub> was ~82 mM. The corresponding Cl<sup>-</sup> equilibrium potential ( $E_{Cl}$ ) was ~-15 mV. Therefore, at the resting potential (~-90 to ~-50 mV), Cl<sup>-</sup> currents are predicted to be inward and able to

contribute to the depolarization induced by odorants. Yet, if the cell depolarized beyond  $-15$  mV,  $\text{Cl}^-$  currents would be outward and facilitate repolarization during excitation. The chloride content in the cilia may be expected to equilibrate with that in the other part of the cell in the resting state. The measured  $[\text{Cl}^-]_i$  may provide a starting point for the decrease in ciliary  $[\text{Cl}^-]_i$  predicted to occur during the transduction.

#### P4. Dopamine modulates the voltage-dependent ion channels in rat olfactory neurons

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Rat olfactory epithelium contains noradrenaline and dopamine derived from the sympathetic nerves, but  $\text{D}_2$  dopamine receptors only are expressed in the neurons. In the present experiments, we investigated the modulating effects of dopamine on the voltage-dependent ion channels of isolated rat olfactory neurons. The ionic currents were measured using the perforated whole-cell patch-clamp technique using amphotericin B. Rat olfactory neurons displayed a sustained inward current in  $10$  mM  $\text{Ba}^{2+}$  solution in response to depolarizing voltage steps from a holding potential of  $-91$  mV. The inward current was strongly inhibited by nifedipine ( $10$   $\mu\text{M}$ ), suggesting the expression of L-type  $\text{Ca}^{2+}$  channels. The magnitude of the  $\text{Ba}^{2+}$  current elicited by the ramp-voltage was decreased to 28% of the controls in response to dopamine ( $100$   $\mu\text{M}$ ) ( $n = 5$ ). An agonist of  $\text{D}_2$  dopamine receptor, quinpirole ( $10$   $\mu\text{M}$ ), similarly inhibited the  $\text{Ba}^{2+}$  current. Dopamine also inhibited the hyperpolarization-activated inward rectifying current ( $I_h$ ), but enhanced the voltage-dependent  $\text{Na}^+$  current. The results suggest that the excitability of rat olfactory neurons can be peripherally modulated by a neurotransmitter.

#### P5. Functional morphological analysis of olfactory receptor cells in the human fetus

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Although it has been suggested that the human fetus contains a functional olfactory system, little is known about the functional morphology of the fetal olfactory epithelium (OE). The aim of the present study was to observe the OE of 5-month-old fetuses—the age at which the OE is connected to the brain center.

Tissue blocks including the nasal region were dissected out from formalin-fixed fetuses ( $n = 6$ ), re-fixed in Zamboni's fixative overnight, and cut with a cryostat frontally at  $14$   $\mu\text{m}$  thickness. Using polyclonal antibodies to protein gene product 9.5 (PGP), the OE was examined by peroxidase-labeled streptavidin–biotin, immunofluorescence (Cy5.1-labeled second antibodies were used) and confocal laser scanning microscope (CLSM) techniques.

The OE of the 5-month-old fetuses contains PGP-immunoreactive cells. Thick nerve fibers in the lamina propria of the OE also exhibited intense immunoreactivity for PGP. Many cells in the OE had single dendrites that were oriented towards the epithelial surface. The CLSM technique clearly demonstrated the shapes of dendrites, olfactory knobs and axons. Furthermore, cells similar to

'microvillar cells' were scattered throughout the superficial region of the OE. Our present results suggest that the 5-month-old fetus contains functional OE.

#### P6. Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to pheromone candidates and urine in the rainbow trout

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The urine of ovulated female rainbow trout contains F-type prostaglandins (PGFS) and  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP), which have been thought to be pheromones for this species (Scott *et al.*, 1994; Yambe, unpublished). To address the issue of whether these pheromone candidates are effective olfactants for rainbow trout, and how these are detected by different types of olfactory receptor neurons (ORNs) in their olfactory organ, we studied the response characteristics of ciliated and microvillous ORNs of the rainbow trout, *Oncorhynchus mykiss*, to an amino acid mixture (L-Glu, L-Arg, L-Ala and L-Nva), single amino acids (Gly, L-Thr and L-Met), pheromone candidates (PGF<sub>2 $\alpha$</sub> , 15KPGF<sub>2 $\alpha$</sub> , DHP, DHP-acetate and DHP-sulfate) and urine samples collected from immature and ovulated female fish, using whole-cell voltage-clamp techniques. Inward current responses of ciliated ORNs were recorded from 39 of 160 ORNs. Eighteen ciliated ORNs responded specifically to the amino acid mixture or one of single amino acids, 13 responded to both the amino acid mixture and urine samples, and 8 responded specifically to one or both urine samples. Nineteen of 62 microvillous ORNs responded only to the amino acid mixture or one of single amino acids. None of the ciliated and microvillous ORNs responded to PGFs and DHPs. The response profiles of ciliated and microvillous ORNs did not change significantly by methyltestosterone treatment of immature fish. The results suggest that PGFs and DHPs may not be pheromones for the rainbow trout, and that ciliated ORNs may be generalists that respond to various odorants, whereas microvillous ORNs may be specialists that are specific to amino acid odorants.

#### P7. Study on perireceptor events in the blowfly antennae with a fluorescent olfactory substance

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Among olfactory perireceptor events, odorant-binding by carrier proteins has been well investigated in insect antennae. Many kinds of pheromone-binding (PBSs) and general odorant-binding proteins (GOBPs) has been identified as odorant carriers and their specific functions studied. Quantitative experiments on the binding specificity and affinity using radiolabelled ligands have also been reported, though not so extensively.

In order to trace the odorant being carried to the receptor molecule, we first attempted to visualize the odorant-binding of a water-soluble protein in the blowfly antennae by using the fluorescent odorant, umbelliferone.

We isolated and homogenized the blowfly antennae, and the



homogenate was ultracentrifuged to obtain a water-soluble fraction as the sample for native polyacrylamide gel electrophoresis. Electrophoresis was performed in the absence (control) and the presence of umbelliferone (test). The gel was then stained with CBB (Coomassie Brilliant Blue) to determine coexistence of the fluorescence of umbelliferone and a staining of protein. From this, we could directly visualize a blowfly OBP carrying umbelliferone during migrating on the gel. This suggested that this OBP carries the odorants to the receptor molecules as PBPs or OBPs of other insects. In addition, it was apparent that this antennae protein was identical in terms of its molecular weight to the CRLBP, an OBP that has been reported in the blowfly taste organ.

### **P8. Experiential olfactory effects of coumarin and limonene on the proboscis extension reflex to sucrose in the blowfly, *Phormia regina***

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The blowfly, *Phormia regina*, has antennae and maxillary palps as its main and associative olfactory organs respectively. However, the functional difference between antennae and maxillary palps has not been clear. We therefore removed either the maxillary palps or antennae of flies after emergence and fed them for 5 days with sucrose flavored with coumarin or limonene. To investigate associative memories between taste of sucrose and these odors, we examined their appetite for sucrose by the proboscis extension reflex test.

Intact flies fed with sucrose flavored with either coumarin or limonene had reduced appetite for sucrose in the absence of any odors, but when fed with sucrose flavored by coumarin, recovered their appetite for sucrose flavored with coumarin. The odor of coumarin, which did not affect appetite for sucrose in intact naive flies, subsequently became the inseparable odor for sucrose after associative learning. On the other hand, limonene was innately toxic for flies, so that the associative memory of limonene induced appetite reduction for sucrose.

In operated flies, only when the maxillary palps were intact was the associative memory of coumarin odor recalled by the taste of sucrose as an inseparable odor for sucrose, and only when the antennae were intact was the memory of limonene odor recalled by the taste of sucrose as an aversive odor for sucrose. Thus, the maxillary palps and the antennae of the flies may share olfactory inputs to form memories involved in food searching or avoidance.

### **P9. Morphological studies on the human vomeronasal organ**

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The vomeronasal organ (VNO) is a vertebrate chemosensory apparatus that detects pheromones. Although the human VNO has received increasing attention in recent years, there have been few reports regarding its occurrence and structure. To ascertain the frequency of the human VNO in Japanese people, 920 living subjects and 25 cadavers were examined macro- and microscopically. VNOs were observed in 51.3% of the living subjects and 91.3% of

the cadavers. The frequency of occurrence increased with age with no preferential differences between males and females. In 33.3% of the cadavers, the VNOs were found microscopically in the subepithelial lamina propria. Immunohistochemistry for a neural marker, protein gene product 9.5 (PGP9.5), revealed the presence of bipolar PGP9.5-immunoreactive cells in the VNO epithelium. Interestingly, PGP9.5-immunoreactive nerve fibers of the varicose type were often associated with PGP9.5-immunoreactive cells within the epithelium.

### **P10. Change in odor sensitivity of male mice vomeronasal receptors according to sexual experience**

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The urinary marking behavior of mice is affected by various conditions. In particular, the number of counter urine marks of males to female urinary marks is increased in the evening or after sexual experience. It is therefore to be expected that the sensitivity of the vomeronasal receptor to urinary sex pheromones will also vary according to day/night, or sexual experience. To demonstrate this possibility, the responses of the mouse vomeronasal receptors to urine odors were recorded and compared from day- to night-time, or before and after copulation. The sensitivity of receptors was assessed by histochemical techniques, which label the activity of succinic acid dehydrogenase using nitro blue tetrazolium (NBT), and by electrophysiological methods, which recorded electro-olfactograms (EOGs). As a result of EOG recording, it was shown that the sensitivity of male vomeronasal receptors showed a nocturnal increase whether they were sexually experienced or naïve, and that the sexual experience also resulted in an increase in pheromone sensitivity. These changes in odor sensitivity of vomeronasal receptor cells correspond to urine marking activity. The result of NBT labeling also showed the same tendency, but was partially statistically significant. Thus it was shown that the sensitivity of peripheral receptors varied with these changing conditions.

### **P11. $G_{i2\alpha}$ and $G_{o\alpha}$ expression in the vomeronasal system of the shiba goat**

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Previous studies have shown that  $G_{i2\alpha}$ - and  $G_{o\alpha}$ -immunoreactive vomeronasal receptor cells have segregated projection to the accessory olfactory bulb (AOB) in opossum, mouse and rat. However, these segregated projections have not been studied extensively in other mammals. In the present study, immunocytochemical localization of the  $G_{i2\alpha}$ - and  $G_{o\alpha}$ -receptor cells and their projection were investigated in the shiba goat, artiodactyla.  $G_{i2\alpha}$  and  $G_{o\alpha}$  immunoreactivity was detected in the vomeronasal sensory epithelium, and an especially strong reaction was observed at the



luminal surface of the sensory epithelium.  $G_{i2\alpha}$ - and  $G_{o\alpha}$ -immunoreactive axons were seen in the lamina propria. In the AOB,  $G_{i2\alpha}$  immunoreactivity was observed in the vomeronasal nerve layer and the glomerular layer, whereas  $G_{o\alpha}$  immunoreactivity was rarely recognized in these two layers. These results showed that  $G_{i2\alpha}$ -immunoreactive cells project the axons to the AOB, but majority of the  $G_{o\alpha}$ -immunoreactive cells probably do not project axons to the AOB. One possibility is that these  $G_{o\alpha}$ -immunoreactive cells project the axons to the other part of the olfactory bulb, although there is as yet no direct evidence for this. These results suggest that the projection patterns and pheromone receptor systems in artiodactyla are different from those of marsupial and rodent.

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### **P12. Electron-microscopic study of $G_{i2\alpha}$ and $G_{o\alpha}$ on the surface of the rat vomeronasal epithelium**

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There have been no detailed studies examining G protein expression patterns at the vomeronasal epithelium surface even though it is considered to be the region of pheromone-receptor interaction. In the present study, we investigated in detail the distribution of G protein subtypes  $G_{i2\alpha}$  and  $G_{o\alpha}$  at the surface of the vomeronasal epithelium using immunocytochemical double-labeling methods and electron microscopy. Results show that  $G_{i2\alpha}$  and  $G_{o\alpha}$  immunoreactivities can be observed at the epithelial surface and that the cells are evenly distributed. Electron-microscopic observation revealed that immunoreactivities to both antibodies were observed on the knob-like structures and microvilli of vomeronasal receptor cells. This expression pattern is similar to that reported for putative pheromone receptors. These data confirm that there are two distinct classes of vomeronasal receptor cells expressed at the surface of the epithelium. These two classes of receptors correspond to the same  $G_{i2\alpha}$  and  $G_{o\alpha}$  positive cells distributed in cell body layers of the epithelium and in the axon terminals in the accessory olfactory bulb.

### **P13. Projection of the vomeronasal neurons to the main olfactory bulb in the rat**

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It is known that olfactory receptor neurons project to the main olfactory bulb (MOB), while the vomeronasal (VN) receptor neurons project to the accessory olfactory bulb (AOB). In the present study, a novel projection pattern of vomeronasal neurons was observed using a horseradish peroxidase-wheatgerm agglutinin (HRP-WGA) neurotracer technique. The VN receptor neurons of an adult rat were labeled with HRP-WGA. The labeled axons were traced. The HRP-labeled bundles were observed on the medial wall of the olfactory bulb. The thick HRP-labeled bundles passed through the surface of the medial wall from the rostro-

ventral part to the dorsal surface of the caudal olfactory bulb in which the AOB is located. The bundles penetrated into the AOB. Aside from these thick bundles, thin HRP-labeled bundles were observed. The thin bundles passed through the ventral part on the surface of the medial wall from the rostral to the caudal area. In the most caudal portion of the MOB, the labeled axons terminated in the glomeruli. The terminals of the labeled axons made synaptic contacts with the dendrites in the glomeruli. HRP-labeled bundles were detected in the MOB glomeruli of 6 out of 20 rats in which HRP-labeled bundles were detected in the AOB.

### **P14. Time-dependent changes in expression of BrdU-positive cells in the main and accessory olfactory bulb**

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Sexually experienced male rats have been shown to prefer estrous to diestrous female urine, while sexually inexperienced males do not exhibit these preferences. In a previous study, we compared the expression of Fos-immunoreactive (ir) cells in the accessory olfactory bulb of sexually experienced and sexually inexperienced male rats following exposure to estrous urine. In the localized region (lateral and rostral regions) of the periglomerular cell layer, many more Fos-ir cells were expressed in the sexually experienced rats than in the inexperienced rats. A discrete region of the anterior part of the neonatal subventricular zone contains neuronal progenitor cells, which migrate into the granule cell and glomerular layers of the olfactory bulb and differentiate into granule and periglomerular cells. To explore the role of newly generated cells in the memory formation for sexual experiences in the accessory olfactory bulb, we quantified BrdU-positive cells in the accessory olfactory bulb and main olfactory bulb.

### **P15. Appearance of Fos-immunoreactive cells in the accessory olfactory bulb of the female rat after exposure to a combination of two male urinary components**

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Pheromones affect the sexual behaviors of conspecific male and female animals via the vomeronasal organ. Exposure of the rat vomeronasal organs to urine induces the expression of Fos-immunoreactive (ir) cells, which is correlated with cellular activity, in the accessory olfactory bulb (AOB). We have previously shown that at least two urinary peptides (one papain-sensitive and the other papain-insensitive) with the ability to stimulate the vomeronasal organ of female rats are contained in the urine of male Wistar rats. These results indicate that there must be multiple types of pheromones in male urine that influence female endocrine conditions. The molecular properties of pheromones contained in rat urine are still unclear. In the present study, in order to characterize active components that stimulate the rat vomeronasal organ, a male urine preparation was subjected to dialysis, and its effects on the induction of Fos-ir cells in the AOB were examined. Exposure to either the dialyzed urine preparation (<500 Da) or the

remaining substances (>500 Da) did not induce expression of Fos-ir cells in the mitral/tufted cell layer of the AOB, whereas exposure to a mixture of these preparation did induce expression. These results suggest that a combination of low and high molecular weight substances is necessary for the increases in Fos immunoreactivity in the AOB.

### **P17. Developmental observation of the vomeronasal nerve projections in the accessory olfactory bulb of the musk shrew**

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In several mammals (rat, mouse, guinea-pig, opossum and rabbit), vomeronasal (VN) neurons have been known to express two  $\alpha$ -subtypes of G protein, namely  $G_{i2}$  and  $G_o$ , which project to the rostral and caudal regions respectively of the accessory olfactory bulb (AOB) (segregated type). Recently, we found that only  $G_{i2}$ -expressing VN nerves terminate in the AOB of goat, horse, dog and marmoset (uniform type). To study phylogenetically the distribution of the VN nerves in the AOB, we examined the musk shrew, which is a primitive mammal, by immunohistochemical staining with antibodies against  $G_{i2}$  and  $G_o$ . Only  $G_{i2}$  immunoreactivity was found in the VN nerve and the glomerular layer throughout the AOB. This result shows that the VN system in musk shrew was of uniform type. Moreover, we observed ontogenetically the embryonic AOB of musk shrew. We could recognize  $G_{i2}$ -expressing VN terminals in the embryonic AOB, but not  $G_o$ -expressing VN terminals. The embryonic AOB of the musk shrew was of uniform type while the embryonic AOB of rat was of segregated type with respect to the VN nerve projection pattern. These results suggest that uniform-type VN systems are developmentally different from segregated-type VN systems. It is necessary to analyze the significance of the two types of VN system to pheromonal communication.

### **P18. Effects of the glutamate receptor agonist and antagonists on reciprocal synaptic currents measured from mitral cells in the mouse accessory olfactory bulb in slice preparations**

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To investigate the properties of synaptic transmission, evoked synaptic currents were measured from mitral cells in slice preparations with the patch-clamp technique in nystatin-perforated whole-cell configurations.

Accessory olfactory bulb (AOB) slices were prepared from 23- to 34-day-old BALB/c mice. To evoke dendrodendritic inhibition, a depolarizing voltage step from  $-70$  to  $0$  mV ( $5\sim 20$  ms) was applied to a mitral cell. Under control conditions, the voltage step evoked inhibitory postsynaptic currents (IPSCs), which were greatly enhanced after the reduction of extracellular  $Mg^{2+}$ . The IPSCs persisted in the presence of tetrodotoxin and were reversibly

blocked by addition of a GABA<sub>A</sub> receptor antagonist, bicuculline ( $10\ \mu M$ ), demonstrating that the IPSC in mitral cells can be elicited through purely dendritic interactions and is mediated by GABA<sub>A</sub> receptors. In  $Mg^{2+}$ -free solution, the NMDA receptor antagonist D,L-APV, as well as an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, significantly reduced dendrodendritic inhibition. On the other hand, the non-NMDA receptor antagonist CNQX moderately blocked the IPSCs. The present results suggest that NMDA and mGluR2 receptors play an important role in reciprocal transmission between mitral cells and granule cells in the mouse AOB.

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### **P19. Contribution of reciprocal synapses between mitral and granule cells to oscillatory excitation in the guinea-pig accessory olfactory bulb slice**

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By using an optical recording technique, we recently found that a characteristic oscillatory excitation was elicited in the external plexiform and mitral cell layers (EPL/MCL) by electrical stimulation of the vomeronasal nerve layer (VNL) of guinea-pig accessory olfactory bulb (AOB) slices. To examine the mechanism underlying the oscillatory responses, whole-cell voltage-clamp techniques were applied to mitral and granule cells. In mitral cells, stimulation of the VNL evoked two types of postsynaptic currents (PSCs): a long-lasting excitatory (E) PSC which reversed at a holding potential of  $\sim 0$  mV and periodic inhibitory (I) PSCs which were superimposed on the EPSC and reversed at  $\sim -50$  mV ( $E_{Cl} = -57$  mV). The frequency of the periodic IPSCs correlated well with those of the oscillation observed in the optical recordings. Furthermore, periodic EPSCs were blocked by the puff application of bicuculline to the EPL/MCL where mitral cells make reciprocal dendrodendritic synapses with granule cells. In granule cells, VNL stimulation evoked periodic EPSCs, the frequency of which corresponds to those of IPSPs recorded in mitral cells. These data indicate that periodic EPSCs of granule cells would evoke periodic IPSCs in mitral cells through reciprocal synapses, suggesting that this feedback inhibition may be involved in the generation of oscillatory activities in the AOB.

### **P22. Cerebral characteristics of lateralities for the MEG response of smells**

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The olfactory oddball paradigm was tested using contrasting sweet and repugnant smells, and the results compared with our previous reports for P300m data. Although the latency of geraniol tended to increase by only several tens of milliseconds in comparison with that of isoamyl acetate, there was no rare stimulation on the dominant difference in both right and left nasal cavity stimulation.

In left nasal cavity stimulation, in comparison with the latency of isoamyl acetate, geraniol and isovaleric acid, the difference which was predominant for all smells in the right and left interhemisphere could not be seen. However, the relationship between the sweet/repugnant odors was compared, there was a shortening of latency of 79 ms in the left hemisphere and 62 ms in the right hemisphere with isovaleric acid. On the other hand, there was a dominant difference in the right nasal cavity stimulation in the right hemisphere. Although the difference showed an increased tendency to shorten the latency for isoamyl acetate (88 ms) and geraniol (93 ms), the isovaleric acid gave the greatest shortening in latency (118 ms).

### P23. Human brain areas activated by odorants

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We aimed to trace the activated cortical areas of human brain by olfactory stimulation using a 64-channel whole head SQUID. We recorded magnetic fields (MFs) during stimulation of low concentrations of phenylethyl alcohol (rose-like odor) using a Kobal's olfactometer, and simultaneously recorded OEP (Cz). The duration of a recording lasted for 2000 ms and a pretrigger time of 400 ms was adopted in each trial. The duration of the stimulus was 200 ms and the interstimulus interval was 40 s. Thirty stimuli were presented during one session. Five healthy volunteers participated in this experiment several times. Clear magnetic responses started at ~300 ms after stimulus onset and peaked ~600–700 ms. We estimated equivalent current dipoles (ECDs) by using a spheres model. We localized ECDs around insular cortex which was generally activated at around the latencies of the N1 and P1 components of the OEP responses and STS at the P2 bilaterally. The activation in insular cortex seemed to move from anterior to posterior areas. These results were nearly consistent with those reported by Kettenmann *et al.* We estimated these ECDs by using a realistic model and found almost identical results.

### P24. The correlation between brain activity measured with functional MRI and pleasantness for the odors in humans.

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To clarify the brain mechanisms used to process pleasant/unpleasant odors in humans, we investigated human brain activity during smelling with functional magnetic resonance imaging (fMRI). This study consisted of two experiments: the psychophysical experiment and the fMRI experiment. In the psychophysical experiment, the participants were asked to smell three kinds of odor (tri-ethylamine: TEA, roseP and citral). The participants were also asked to evaluate the intensity and hedonics of the each olfactory stimulus. The intensity of the stimuli was evaluated on a scale of 0 (no odors) to 5 (extremely strong) with the decimal. The hedonics of the stimuli was evaluated on a scale of -3 (extremely

unpleasant) to +3 (extremely pleasant) with the decimal. The participants evaluated the strength of the odor for all three stimuli equally. But the hedonic ratings for the odor were significantly different: the participants' evaluation for the pleasantness of the TEA was significantly less than those of the roseP and citral. In the fMRI procedure, the participants were also asked to smell and evaluate the intensity and the hedonics for the three odors during acquisition of echo-planar imaging of the brain. A significant strong correlation was found between the size of the voxels that were activated by the odor and the hedonic rating.

### P25. Assessment of human olfactory cortex by near-infrared spectroscopy

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Non-invasive blood flow measuring of the human cortex is a useful method for investigating brain function. We measured the blood flow in orbitofrontal gyri of the frontal lobe under odor stimulation by using near-infrared spectroscopy (NIRS). Six healthy subjects closed their eyes and wore noise-excluding headset devices, and NIRS probes were positioned at the point of bilateral upper orbitas. When the odorant (mainly T&T olfactometer A5) was administered in front of their noses, bilateral increases of oxy-hemoglobin in orbito-frontal gyri of the frontal lobes were observed in four subjects. These observations indicate that the bilateral orbito-frontal gyri of the frontal lobes are activated by olfactory stimulation.

### P26. Clinical application of the electroencephalogram induced by intravenous olfaction

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Measurement of olfactory ability is the first step in the diagnosis of olfactory disturbance. This measurement is usually accomplished using T&T olfactometry and/or the intravenous olfaction test, using the garlic-smelling VBI solution called Alinamin® (Takeda, Osaka, Japan) in Japan. However, these are psychophysiological tests and are not applicable for some patients, such as those faking illness. We discovered gamma band oscillation of EEG ( $\gamma$ -EEG) on the frontal scalp when the garlic smell of the intravenous olfaction test was recognized. Therefore, to investigate the possibility of measuring  $\gamma$ -EEG caused by intravenous olfaction as a clinical test, 31 patients who complained of olfactory disturbance and consulted our hospital were studied. Eighty-four percent of these patients recognized the garlic smell caused by intravenous olfaction. The relationships of latencies between psychophysiological and  $\gamma$ -EEG was significant but the relationships of duration between psychophysiological and  $\gamma$ -EEG was not significant.

The  $\gamma$ -EEGs were observed in three patients who had undergone neurosurgery, Alzheimer's disease and unknown cause though they



could not be recognized psychophysically. Because  $\gamma$ -EEG is suspected to originate in the olfactory bulbs, olfactory dysfunction of these patients may occur in the upper streams of the olfactory bulbs.

Measuring  $\gamma$ -EEG during the intravenous olfaction test is applicable not only for electrophysiological olfactometry but also to differentially diagnose the level of dysfunction of olfaction.

### **P27. Physiological responses due to odorant stimulation during slow and fast vehicular driving in a graphic simulator**

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The present experiments investigated the effect of odorant stimulus on boredom and tension. After inducing boredom and tension, respectively, using low-speed (40 km/h) and high-speed (160 km/h) driving in a graphic simulator, 100% jasmine abs. and lavender oil fragrance (KIMEX Co. Ltd) were presented to elucidate the effect of odorant stimulation. Autonomic responses of electrocardiograph, skin temperature and photoplethysmogram were measured for 10 healthy subjects. The sickness caused by simulator itself was also measured before and after the experiment. When the odorant stimuli were presented to bored subjects, the autonomic nervous system in low-speed driving was activated and the effect of alertness was produced. But there was no significant difference between the effect of the two odors. This result shows that the boredom or drowsiness that occurred due to the low-speed driving could be reduced by using odorant stimulus. It was also observed that in the high-speed driving condition, tension was reduced significantly when the odorant stimuli were used. This result shows that the tension that occurred due to high-speed driving could be reduced by using odorant stimuli. The present experiments show that the odorant stimulus can reduce the boredom and tension during low-speed and high-speed driving, respectively.

### **P28. EEG changes during the intravenous olfactory stimulation in humans**

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The intravenous olfaction test with thiamin propyl disulfide (Alinamin®) is a simple procedure and widely used in Japan. An olfactory stimulus is provided by the intravenous injection of Alinamin (2 ml) for 20 s. The subject smells n-propyl mercaptan (a product of the decomposition of Alinamin discharged from the blood into alveoli) in expired air after intravenous injection.

In this study we recorded EEGs during three epochs: (i) eyes-closed rest (pre-stimulus), (ii) olfactory sensation after Alinamin injection and (iii) disappearance of sensation. In each of these

epochs, we calculated the EEG power and compared the band components of each electrode position. This preliminary study in normal subjects was designed (a) to evaluate, using frequency analysis, EEG changes during olfactory sensation after Alinamin injection, and (b) to identify the most significant changes in EEG power according to frequency bands and electrode locations.

During intravenous olfactory stimulation, alpha2 and beta2 waves were activated over the frontal and temporal area. After the disappearance of olfactory sensation, these waves were reduced over the same area.

### **P30. Wine aroma on relaxation using quantitative EEG analysis**

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Aroma often affects brain function in humans. In general, the aroma of wine amuses us. Brain function was analyzed using quantitative electroencephalography (QEEG) analysis from normal female subjects to examine the psychophysiological effects of wine aroma. Furthermore, we analyzed the relationship between brain activity and subjective mood state.

According to two-factor ANOVA (Sample  $\times$  Electrode), a significant main effect of Sample was found in several EEG alpha-frequency bands. Follow-up ANOVA suggested that Chardonnay, Concord and/or Sauvignon Blanc significantly increased power spectrum for 910 and 12–14 Hz bands compared to ethanol solution ( $P < 0.05$ ). Total power activity for mainly higher alpha-frequency band (12–14 Hz) was increased by wine aroma. Subjective scores for relaxation and mood state indicated that the majority of subjects felt better with wine aroma compared with ethanol or distilled water solution exposure. These results suggested that the aroma components of wine samples affect the brain mechanism for relaxation. Further analysis of the data on relationship between EEG activity and subjective scores suggested that these relaxation effects were different for each wine.

### **P31. Somatosensory evoked potentials and their changes with lavender odor**

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We examined scalp topography of somatosensory evoked potentials (SEP) and their change with lavender odor. The subjects were five healthy students, aged 18–25 years. Electroencephalograms (EEGs) were recorded from 19 electrodes placed on the scalp according to the international 10–20 recording system. Electrical stimulation was applied to the median nerve (0.3 ms duration, 1 Hz) of the left hand. SEPs were averaged 250 times following electrical stimulation of the median nerve. Simultaneously, electro-oculography was performed to monitor the combination of eye-blinking potential with EEG. The increased amplitude of the N20 component of the SEP was located at the C4 and P4 areas on the contralateral side of the cortex. The amplitude of the N20 component of the SEP was classified into two categories: the

increased type (Type I) and decreased type (Type II) with lavender odor. On the other hand, the increased amplitude of the N27 component was located at the frontal area of the cortex. The amplitude of the N27 component was increased with lavender odor. There were four Type I and one Type II SEP cases.

These results suggest that the patterns of occurrence of SEPs in the somatosensory area have two different types of neuronal circuit for lavender odor.

### P32. Causal relationship study of human sense by odor stimuli

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The aim of this report is to clarify the origin of individual differences in sensory evaluation of odor stimuli. Nine subjects in their twenties smelled 15 kinds of odorants and graded them on a seven-point scale in terms of 20 adjective pairs. Their reactions were quantified using factor analysis. Three types of impressions of odor stimuli were observed: preference sensation, arousal sensation and continuance sensation. The preference sensation was related to the continuance sensation. The relationships between taste and continuance sensation were the opposite. The individual differences for odor stimuli were classified into three groups by causal relationship study, analysis of covariance and graphical modeling. The first group demonstrated that the arousal sensation and average model were mutually influenced. The impressions of the second group were an averaged sensation of the odors. The third group had preference sensation which was not influenced by arousal sensation but was determined by continuance sensation.

### P33. The objective valuation method of mood by the fingertip temperature recovery rate measurement

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The fingertip temperature recovery rate (FTRR) is considered to reflect the balance between the sympathetic and parasympathetic nervous systems of the human autonomic nervous system. We studied the possibility of FTRR measurement as an objective valuation method of mood in common situations. In our FTRR measurement, the fingertip was cooled by a Peltier device for 30 s, and the temperature recovery rate was calculated by measuring the initial, lowest and final (3 min after cessation of cooling) temperatures. We selected smoking by normal smokers as the inducement of mood change. FTRR was measured before and after smoking, and the relaxation value of subjective mood was rated using a questionnaire at the same time. The results showed the tendency of FTRR to shift towards 100% (equivalent to the initial temperature before cooling), and the relaxation value increased as a result of smoking. When data for the before-smoking FTRR were divided into two groups, lower and higher than 100%, FTRR considerably correlated with the relaxation

value. Therefore, we believe that FTRR can provide a useful objective evaluation of mood, especially the state of relaxation.

### P34. Evaluation of odor quality for 10 odorants from the T&T olfactometer using concrete terms for odor description

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In a previous paper, odor quality was evaluated for 10 odorants from the T&T olfactometer (A~J) by semantic differential method using 28 concrete adjectives. Applying principal component analysis to the ratings for one subject, the first component was extracted as 'bad smell' (including rotten smell)–'good odor' (including floral, fruity and sweet odors), and the meanings of higher-order components were not clear. It seemed to be caused by an insufficient number of evaluation items.

In the present paper, 54 concrete odor terms as well as the previous 28 were used for odor description as proposed by Shimoda and Osajima *et al.*, and two subjects evaluated odor qualities for 10 odorants.

The following conclusions were obtained from the evaluation experiments. (i) Using 54 concrete odor terms, odor qualities of the six odorants (A, C, E, F, H, J) are expressed with suitable adjectives. However, expressions are insufficient for the remaining four odors. (ii) Twenty-nine of 54 odor items have an evaluation value of zero. The number of the items should be increased for accurate odor discrimination.

### P35. Toward selection of standard odor molecules. II: Calculation of molecular frequency and examination of odor evaluation method

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The primary goal of this experiment was to select standard odoriferous molecules for olfactory experiments or for odor evaluation, based on molecular properties. Rigid odoriferous molecules were selected and their infra-red wavenumbers and intensities were calculated using Gaussian98 with B3LYP, 6-31G(d), since the importance of the frequency of odoriferous molecules has been suggested elsewhere (Turin, 1996, *Chem Senses*, 21: 773–791). Molecular frequency data were converted into a bar graph and compared. At the same time, the odor similarity of the pairs of the six rigid molecules in the gas phase were evaluated by six males using a 10 cm scale (10 cm corresponds to 100% similarity). This revealed that the odor similarity of a pair of identical compounds was evaluated as less than 100%, and the similarity score depended on a pair of compounds. Some pairs were evaluated as ~10% similarity and the standard deviation (SD) was ~15, but other pairs were evaluated as ~50% and the SD was as high as 30 or

40. The similarity scores were compared with the calculated frequency data. From this comparison, the relationship between wavenumbers and odor qualities was recognized, in addition to other molecular features like size for the selection of odoriferous molecules.

### P36. Electroencephalogram change caused by the aroma of coffee and whisky: fluctuation of the $\alpha$ -wave with time

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Changes in the electroencephalogram (EEG) caused by the aroma of coffee and whisky were examined. Three subjects who did not drink coffee or whisky and disliked the aromas were chosen. Their EEG was divided into four stages: stage 1 (0–3 min), stage 2 (6–9 min), stage 3 (12–15 min) and stage 4 (18–21 min). The power and average spectrum of each stage were measured.

The following results were obtained. (i) The tension caused by a special situation (EEG measurement) can be reduced by the disagreeable aroma (coffee). (ii) The tension can be maintained by the disagreeable aroma (whisky). (iii) Brain activity can be kept regular by the disagreeable aroma (coffee, whisky). The time taken for an influence to have an effect differs from person to person.

### P37. Chemical structure–odor relationships of ‘green odor’ in fruits

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C<sub>9</sub>-aliphatic compounds such as violet leaf aldehyde [2(*E*),6(*Z*)-nonadienal] and cucumber alcohol [2(*E*),6(*Z*)-nonadienol] have been reported as important volatile components that impart a ‘green odor’ in fruits and vegetables.

The sensory odor characteristics of 32 C<sub>9</sub>-aliphatic compounds (n-nonanol, 13 nonenols, 10 nonadienols, 1 nonadienal and 7 nonynols) were evaluated by score sheets with a four-point scale using eight sensory descriptive terms [grassy–leafy green (GLG), vegetable green (VLG), fruity (FRT), sweet (SWT), fresh (FRS), spicy (SPC), oily–fatty (OLF), herbal (HRB)] and the sensory evaluation data were statistically analyzed using principal component analysis. It was found that the compounds, which had a *Z* double bond at the C-6 site, possessed some unique and important aspects of green odor in fruits and vegetables. We had previously reported that a *Z* double bond at the C-3 site of C<sub>6</sub>-compounds was an important factor for green odor in green leaves. From these two observations it could be indicated that the *Z* form at the  $\omega$ -3 position (C-6 site of C<sub>9</sub>-compounds, C-3 site of C<sub>6</sub>-compounds) is important in the positional and geometrical structure of monoen system for the characteristic green odor. These tendencies may suggest that humans perceive odor caused by each chemical structure via specific receptors.

### P38. A survey concerning essential oils that aromatherapists consider effective and evaluation of their effect in aromatherapy

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We confirmed that lavender, marjoram and rosemary are the most frequently used essential oils for stiff neck and shoulder, and neroli, lavender and rose otto for insomnia. These findings were based on replies from 44 aromatherapists to questionnaires concerning the effect of essential oils. We then made two types of test blends, each consisting of three of the essential oils combined in jojoba oil at a concentration of 1%. We labeled them the ‘stiff neck blend’ and the ‘insomnia blend’; we then tried to evaluate their effectiveness in acute aromatherapy. The participating aromatherapists were informed what types of essential oils were in each blend and their concentration, but not of any relationship between the blends and stiff neck or insomnia. These blends were used only for clients who had given their consent. The evaluation of the effect of each blend was based on questionnaires answered by the clients. The results indicated that both the ‘stiff neck blend’ and the ‘insomnia blend’ showed significant effectiveness. Moreover, their effect just after treatment and the next morning was found to be different.

### P39. Expression of seaweed-like odor in the marine alga *Ulva pertusa*

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The characteristic odor components of marine green alga *Ulva pertusa* such as (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrienal, (8*Z*,11*Z*)-8,11-heptadecadienal, (8*Z*)-8-heptadecenal and pentadecanal were synthesized. The variation of the odor of these aldehydes showed that the position of the double bonds obviously exerts the most important influence. (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrienal was important for the typical seaweed odor. On the other hand, since the odor intensities of the alcohols were weaker than those of aldehydes, the odor profile seemed to be attributed to the functional groups.

### P40. The association between the quality of taste and odor in the rat

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Flavor perception is formed from the association between gustation and olfaction. The mechanisms of the development of flavor perception have not yet been fully understood. Sakai and Yamamoto (in press) found that the rats could associate odor and the hedonic aspects of taste: the rats preferred the odor that had been paired with saccharin to the odor that had been paired with quinine. However, some research on human psychophysics (e.g.



taste enhancement by the odor) suggested that flavor perception is formed from an association between the qualitative information about taste and the odor. Thus, we aimed to study whether the rats can also acquire the association between the qualitative aspect of taste and odor. First, the rats were presented with pairings of the odor and 0.3 M NaCl solution. The rats were then given furocemide to cause sodium deficiency. The rats fed sodium solution ingested the water flavored with the odor that had been paired with NaCl. On the other hand, the rats fed a natural sodium balance avoided ingesting the water flavored with the odor that had been paired with NaCl. Thus, we concluded that the rats could acquire associative learning between the qualitative aspect of taste and odor.

#### P41. Verbal label changes odor perception

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We experimentally gave participants odors with true or false labels to investigate labeling effect on odor perception. Sixty students smelled 10 everyday odors and judged their perceived intensity, pleasantness and quality (fitness to 18 descriptors). The participants were divided into two groups: odors without labels and odors with labels. Labels were either true or false. Misidentified labels were used as false labels. One week later the judgments were repeated again.

Odors tended to be perceived stronger with the labels than without the labels. Pleasantness and quality depended on meanings of labels. For instance, raisin odor was judged more pleasant with raisin label than with sweaty-shirt label. Quality judgments of raisin odor with sweaty-shirt label were very similar to judgments of sweaty-shirt label itself. Judgments were relatively stable between sessions when odors were presented with labels.

These results indicate that an odor is encoded into an olfactory code then compared with inner olfactory representations. In the case of an odor with a label, it is encoded into both olfactory and verbal codes. A semantic image activated by the verbal label could associate with proper olfactory representation. It is suggested that odor perception is influenced by concrete meanings of labels.

#### P42. Olfactory mucosa and olfactory disturbance

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The olfactory mucosa at the olfactory cleft of patients with olfactory disturbance was examined using a rigid endoscope. In cases of rhinosinusitis, 81 of 88 (92.0%) patients had sick clefts and most of them had the impediment at the olfactory mucosa and/or at the intra-nasal cavity. On the other hand, in cases of olfactory disturbance after common cold, 53 of 80 (66.3%) patients had normal olfactory clefts, and in cases of anosmia following head trauma, 19 of 25 (76.0%) patients had normal clefts. In cases of unknown etiology, 35 of 47 (74.4%) patients had normal clefts. It could be said that the patients with normal olfactory clefts had the impediment at the olfactory nerve and/or the olfactory bulb. There was no relation between the degree of the olfactory disturbance

evaluated by T&T olfactometry and intravenous olfaction test (Alinamin® test) and the findings from the olfactory clefts. In cases after common cold, 27 of 80 (33.7%) patients had edematous olfactory clefts. This meant that several cases of common cold still had inflammatory changes to the olfactory mucosa though the majority of the patients had normal clefts. These findings regarding olfactory mucosa are important as they enable clinicians to determine the site of the dysfunction in the olfactory tract.

#### P43. Taste and smell disturbance associated with interferon treatment

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Interferon treatment is associated with various adverse reactions, including taste and smell disturbance—the focus of this study.

Before and after interferon treatment, interview, hematological analysis, taste and smell examinations were performed on 28 hepatitis C patients who received interferon in the Third Department of Internal Medicine at our hospital.

(i) Eight patients had taste disturbance, including one with symptoms. (ii) There was no relationship between taste and smell disturbance and age, sex, effect of interferon treatment or interferon type. (iii) Serum zinc level decreased in three of eight taste disturbance patients, while it was not reduced in any smell disturbance patients. Most patients with taste and smell disturbance did not have symptoms. Fortunately, there was no serious adverse reaction such as loss of taste and smell function. In addition, all patients recovered within several weeks after the treatment. However, since examinations may reveal taste and smell disturbance even on patients without symptoms, some serious adverse reactions will occur. These results suggest that pharmacological treatment should be done even on patients without symptoms.

#### P44. The clinical evaluation of patients with smell and taste disorder

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In our experience, we sometimes find patients who complain of a smell and taste disorder at the same time. The 35 patients with smell and taste disorder who were treated in our clinic in the period October 1997–September 2000 are reported in this study. Twenty-one patients who presented abnormal in the smell and taste test are called ‘group A’, and 14 patients who presented abnormal in the smell test but normal in the taste test are ‘Group B’. Fifteen cases in group A (71.4%) and the 10 cases in group B (71.4%) were caused by common cold. Of the 12 cases from group A that were able to retest after the treatment, 5 cases (41.7%) showed an improvement in smell and taste tests. In 10 cases of group B that

could be retested after treatment, 5 cases (50%) showed an improvement in the smell test only. On the other hand, regarding the subjective symptoms, there were improvements in nearly 70% of the patients in both groups.

#### **P45. A putative neurotransmitter for taste reception in the frog taste organ**

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Pharmacological and histochemical reports have suggested nor-epinephrine (NE) or serotonin as a candidate for the neurotransmitter between taste cells and the glossopharyngeal nerve in frogs. However, precise identification of the transmitter has not yet been achieved.

The present study was undertaken to pharmacologically identify the transmitter, using an isolated lower jaw and tongue of the frog *Rana catesbeiana*. Intravascularly administered NE increased the spontaneous neural activities in the glossopharyngeal nerve. When a few neural units of impulses were recorded from a branch of the glossopharyngeal nerve, similar amplitudes of impulses were induced by  $\text{Ca}^{2+}$  applied on the tongue surface as a taste stimulus and by NE applied to the taste organ intravascularly. An  $\alpha$ -adren-ergic blocker (phentoleamine) reversibly suppressed taste responses to  $\text{Ca}^{2+}$  and quinine. The taste responses were enhanced when NE was applied to taste organs in frogs, in which a monoamine depleting agent (reserpine or guanethidine) had been administered intraperitoneally. In normal frogs, this enhancement was not observed. These results support the argument that NE works as a neurotransmitter in taste reception.

#### **P46. Electrophysiological properties of taste cells in frog taste organs following denervation**

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The taste buds in mammals disappear shortly after denervation, indicating that the morphological integrity of the taste buds is dependent on an intact nerve supply. The taste organs in frogs, however, maintain their morphology after a long period of denervation. In the present study, we investigated whether the electrophysiological properties of taste cells in frog taste organs alter following denervation. Bullfrogs, *Rana catesbeiana*, were used in this study. Under 0.2% ethyl *p*-aminobenzoate anesthesia, the glossopharyngeal nerves and the hypoglossal nerves were cut bilaterally. The tongues were removed at ~1 month after denervation. Isolated rod cells and wing cells were obtained from papain-treated taste organs. The shape of the isolated cells in denervated frogs was similar to those obtained in normal frogs. Whole-cell patch-clamp recordings were made from individual cells. Voltage-dependent transient inward ( $\text{Na}^+$ ) current and outward ( $\text{K}^+$ ) current were observed in both rod cells and wing cells isolated from denervated frogs. Electrophysiological properties of rod cells and wing cells in denervated frogs were similar to those in normal frogs. The present findings suggest that electro-physiological properties of frog taste cells are largely independent of sensory innervation.

#### **P47. Differential effects of serotonin on voltage-gated potassium current ( $I_K$ ) in different subsets of bullfrog taste receptor cells**

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Merkel-like basal cells in frog taste disk contain serotonin (5-HT). We have investigated the effect of 5-HT on the membrane properties of isolated bullfrog taste receptor cells (TRCs) using the patch-clamp technique. External application of 1  $\mu\text{M}$  5-HT reversibly suppressed the peak voltage-gated sodium current ( $I_{\text{Na}}$ ) by 26% and inhibited the steady-state voltage-gated potassium current ( $I_K$ ) by 46% at +40 mV in 45% of the TRCs sampled. Suppression of  $I_{\text{Na}}$  was completely recovered by washing the drug with normal Ringer's solution in 3–5 min. 8cpt-cAMP (300  $\mu\text{M}$ ) suppressed the peak  $I_{\text{Na}}$  by 26% and internal dialysis with 50  $\mu\text{M}$  protein kinase-A inhibitor potentiated the peak  $I_{\text{Na}}$  by 114%. In 20% of the TRCs sampled, 5-HT enhanced the steady-state  $I_K$  by 163%, but did not affect the  $I_{\text{Na}}$ . Effects were larger on steady-state  $I_K$  than peak  $I_K$ . In the residual 35% of TRCs sampled, 5-HT affected neither  $I_{\text{Na}}$  nor  $I_K$ . These results suggest that  $I_{\text{Na}}$  and  $I_K$  are differentially modulated by 5-HT in different subsets of bullfrog TRCs, resulting in modulation of taste responses.

#### **P48. Efferent innervation of frog taste cells**

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The properties of postsynaptic potentials were examined in bullfrog taste cells while the glossopharyngeal nerve was stimulated. Postsynaptic potentials were recorded from the taste cells of the fungiform papillae with 3 M KCl-filled glass microelectrodes. Before starting the experiment, atropine was injected to remove the large slow potentials generated between lingual surface fluid and lingual gland saliva secreted by stimulation of parasympathetic nerve fibers. Slow depolarizing and hyperpolarizing postsynaptic potentials were observed. The former were regarded as slow excitatory postsynaptic potential (slow EPSP) and the latter as slow inhibitory postsynaptic potential (slow IPSP). The mean duration of slow EPSP and slow IPSP was  $15.8 \pm 0.8$  s ( $n = 27$ ) and  $14.9 \pm 0.8$  s ( $n = 54$ ). The mean amplitude of slow EPSP and slow IPSP was  $2.0 \pm 0.8$  mV ( $n = 57$ ) and  $-2.1 \pm 0.2$  mV ( $n = 153$ ). After intravenous injection of  $\alpha$  and  $\beta$  receptor blockers, slow EPSP and slow IPSP were still induced. It is concluded that slow EPSP and slow IPSP in taste cells are induced by non-cholinergic and non-adrenergic efferent fibers.

#### **P49. Modification of rat chorda tympani responses by cardiovascular medications**

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Cardiovascular medications, which include calcium channel blockers (diltiazem hydrochloride), adenylyl cyclase-enhancers (ticlopidine hydrochloride) and anti-arrhythmic drugs (mexiletine hydrochloride), are known to cause abnormal taste sensations, such as dysgeusia,

metallic taste or hypersensitivity to bitter stimuli. We investigated whether or not these drugs taken orally (25–300 mg/kg/day for 3 days), modify rat chorda tympani nerve responses to the four basic taste stimuli. All the drugs potentiated the nerve responses to a low concentration of bitter stimulus ( $3 \times 10^{-4}$  M quinine–hydrochloride, Q–HCl) so as to lower the threshold, but suppressed the nerve responses to a high concentration of sweet stimulus (1.0 M sucrose, Suc). Furthermore, ticlopidine greatly potentiated the phasic responses to a low concentration of salty stimulus (0.03 M NaCl) so as to lower the threshold, but mexiletine potentiated the tonic nerve responses to higher concentrations of salty stimuli (0.3 and 1.0 M NaCl). Interestingly, all three drugs did not affect the responses to sour stimuli (0.001 to 0.03 M HCl) significantly. All these results were different from those obtained from human taste tests after direct application of these drugs to the tongue surface. Therefore, these results suggest that the cardiovascular medications directly modulate the gustatory transduction mechanisms.

### P50. Cloning of nucleotide receptor genes expressed in taste tissue

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Umami taste is strongly potentiated by the coexistence of monosodium glutamate (MSG) and 5'-ribonucleotides such as 5'-GMP and 5'-IMP. Therefore, it is likely that 5'-ribonucleotide-specific receptors exist in taste receptor cells. In this study, we attempted to clone nucleotide receptor genes expressed in rat and mouse taste tissues. We performed polymerase chain reaction with cDNA prepared from the taste tissues and degenerate primer pairs corresponding to the conserved regions of known purinoceptors (P2X and P2Y receptor families) and the adenosine receptor family. Amplified products were cloned into plasmid vector and sequenced. A database search revealed that several products obtained were highly similar to known nucleotide receptors such as P2Y2 and adenosine A2a receptors (>95% homology). It is not known whether the cloned receptor genes are identical to the known receptor genes, because the clones were partially sequenced. However, it is possible that these nucleotide receptors function as 5'-ribonucleotide receptors in the taste tissue. Further study is needed to clone the full-length receptor genes.

### P51. Expression of receptors (Ob-Rb) and sweet-suppressive effects of leptin in the mouse taste buds

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We have previously demonstrated that genetically diabetic (*db/db*) mice, which have a defect in leptin receptors (Ob-Rb), show greater sensitivities of the chorda tympani nerve (CT) to various sweeteners,

and an administration of leptin into lean mice suppresses sweet responses of the CT. In this study, we examined the expression of Ob-Rb mRNA, which is the signal-transducing long form of the Ob-R as leptin receptor by reverse transcriptase polymerase chain reaction (RT-PCR) in the mouse tongue. We also investigated possible leptin effects on the responses of the glossopharyngeal nerve (IXth) to taste substances. The results showed that Ob-Rb mRNA expresses in both fungiform and circumvallate papillae innervated by the CT and IXth respectively. No RT-PCR product was observed in the tongue epithelium without taste buds. The selective suppression of sweet responses by leptin was observed in the IXth as well as the CT. The *db/db* mouse with impaired leptin receptors showed no such leptin suppression. Taken together, these observations suggest that the taste organ is a new peripheral target for leptin, and that leptin may suppress responses of both CT and IXth nerves to sweet substances in taste buds all over the tongue. Defects in this leptin suppression system in *db/db* mice may lead to their enhanced neural sensitivities to sweet stimuli.

### P52. Taste responses of mouse taste bud cells and their distribution in the taste bud

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Although single taste buds in mouse fungiform papillae contain ~50 cells (TBCs), only a few TBCs have synaptic contacts with taste nerves. We investigated whether the non-innervated TBCs were chemosensitive with a calcium indicator, calcium green-1 under an *in situ* optical recording condition. The application of 0.2 M NaCl on receptor membranes increased cytosolic calcium concentration ( $[Ca^{2+}]_i$ ) in 27–33% of TBCs. These results were consistent with the results of our voltage-clamp experiments on the number and distribution of functional HVA- $Ca^{2+}$  channels in single taste buds. The application of 1 mM denatonium on receptor membranes increased  $[Ca^{2+}]_i$  in 17–79% of TBCs, indicating that the number and distribution of TBCs responding to denatonium in single taste buds was different from those eliciting HVA- $Ca^{2+}$  channel currents. Arrangements of TBCs which responded to NaCl and denatonium were also different each other in a single taste bud. Although the number of chemosensitive TBCs found in the present study was underestimated, it was much larger than three. Therefore, we concluded that non-innervated TBCs responded to taste substances. We consider that non-innervated chemosensitive TBCs may modify the output of innervated TBCs with electrical synapses and/or chemical synapses forming cell-networks.

### P53. Electrophysiological recordings of glutamate receptor agonists and their synergism with 5'-nucleotide in mouse taste cells

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Monosodium glutamate (MSG) elicits a unique taste in humans, called 'umami'. Recent studies suggest that several mechanisms are involved in the transduction of umami taste. We report here



responses of mouse taste cells to five kinds of stimulating solutions: the glutamate receptor agonist MSG (10 mM), L-AP4 (1 mM), IMP (0.5 mM), a mixture of MSG (10 mM) and IMP (0.5 mM), and a mixture of L-AP4 (1 mM) and IMP (0.5 mM) under whole-cell voltage-clamp (-80 mV).

The mixture of MSG and IMP induced three different types of responses (transient inward, sustained inward and outward currents), similar to MSG alone. The amplitudes of both the transient and sustained inward currents were larger than observed with MSG alone. L-AP4 induced only outward currents in taste cells, while the mixture of L-AP4 and IMP induced both sustained inward currents and outward currents. But application of IMP showed no response. The addition of IMP to MSG or L-AP4 changed the responses by either MSG alone or L-AP4 alone. This study showed the synergistic responses of umami electrophysiologically for the first time. The electrophysiological data of umami synergism has not previously been reported. The results shown here may thus be significant in providing the evidence for umami synergism. Further studies will be needed to examine the transduction mechanisms involved in umami and umami synergy.

### **P55. Modulation of the pattern of rhythmical jaw movements by taste stimulation**

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Taste stimulation to the mouth induces various oral movements. Sucrose or salt solution induces rhythmical jaw movements (RJMs) or tongue protrusion as ingestive behavior. Bitter taste induces a gaping or tongue retraction as aversive behavior. There is no report that describes the precise pattern of jaw movements induced by taste stimulation. The purpose of this experiment is to clarify the pattern of taste-induced RJMs. When water was injected into the mouth, an RJM was induced. In this type of RJM, the lower jaw swung alternately to the right and left in each open-close cycle. This alternate lateral jaw movement was prominent when the oral tissue was stimulated mechanically. The RJMs induced by water were opening-dominant movements, although mechanically induced RJMs were closing-dominant. Sucrose or salt solution induced a similar pattern of RJMs to water-induced RJMs. When acetic acid was injected, the amplitude of the alternate lateral jaw movement was significantly larger than that in the water-induced RJMs. In the case of quinine hydrochloride injection, the pattern of induced RJMs was characterized by small lateral, large open-close and large antero-posterior movements. Tastes that are reported to induce ingestive behavior, such as sweet or salty, do not alter the pattern of RJMs; however, the tastes that induce aversive behavior make the pattern of RJMs different from water-induced RJMs.

### **P56. Recovery of amiloride sensitivity after denervation of the mouse chorda tympani nerve**

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Synaptic reformation between taste cells and axons after denervation

of the chorda tympani (CT) nerve was studied by examining recovery of behavioral discrimination between sodium and potassium salts and taste responses of regenerated CT to salts and other taste stimuli in mice. About 2 weeks after denervation of the CT, behavioral discrimination between NaCl and KCl disappeared, and no significant responses to taste stimuli were observed in the CT. At ~3 weeks after denervation, responses to salts recovered in both behavioral and neural measurements. However, Na responses of single CT fibers were not inhibited by amiloride and the salts did not exhibit any discrimination. At ~4 weeks after denervation, mice discriminated between NaCl and KCl, and showed amiloride inhibition of NaCl responses of the CT. After more than a month, mice showed complete recovery in amiloride sensitivity (AS) in neural responses and behavioral discrimination between the salts. These results suggest that ~4 weeks after denervation some of taste cells possessing AS reappeared and these AS taste cells were specifically reinnervated by N-type fibers. AS-N-type fibers play a crucial role on behavioral discrimination between sodium and potassium salts.

### **P57. Comparison in physicochemical properties of salivary gurmarin-binding proteins between C57BL and BALB mice**

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Gurmarin has been known to suppress sweet responses of the chorda tympani in rats and some strains of mice. We have suggested that in submandibular saliva of C57BL mice fed a *Gymnema* diet, gurmarin-binding proteins attenuate the effects of gurmarin. The aim of this study is to separate gurmarin-binding proteins from submandibular saliva of C57BL and BALB mice fed a *Gymnema* diet and to investigate physicochemical properties of these proteins. Fractionation of gurmarin-binding proteins was performed by affinity chromatography on gurmarin-coupled Sepharose columns. SDS-electrophoresis of the resulting fraction revealed six major bands (60–70 kDa) and two inconspicuous (14–20 kDa). Of these bands, two corresponded to albumin bands. There was no difference in banding pattern between the two strains. When gurmarin was applied to the tongue with albumin inhibition of gurmarin, as caused by saliva, was observed in behavioral studies using the conditioned taste aversion paradigm. These results suggest that there are at least six binding proteins, not including albumin, that probably inhibit gurmarin in saliva of C57BL and BALB mice fed a *Gymnema* diet.

### **P58. Mechanisms of synergistic responses elicited by mixtures of metabotropic glutamate receptor agonist and sweet substances**

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We have recently found in rats that mixtures of a metabotropic

glutamate receptor agonist (L-2-amino-4-phosphonobutyric acid; L-AP4) and sweet substances elicit strong synergistic effects, but these synergistic responses are not suppressed by sweet suppressants such as gurmarin and pronase E. In the present study, to investigate the characteristics of these synergistic responses, whole nerve and single fiber responses of the chorda tympani nerve to binary mixtures of L-AP4 and sweet substances were measured in Wistar rats. Results were as follows: (i) the synergistic responses elicited by the mixtures of L-AP4 and one of 0.1 M sweet substances such as sucrose, glucose, fructose and maltose were not suppressed by either metabotropic or ionotropic glutamate receptor antagonists; (ii) the fibers that responded well to the binary mixtures of L-AP4 and sweet substances also responded well to NaCl and HCl but very weakly to sucrose. On the other hand, the fibers that responded well to the binary mixture of monopotassium glutamate and 5'-inositol monophosphate were not responsive to NaCl or HCl but responded well to sucrose. These results suggest that there are different transduction mechanisms for umami substances in the taste cells of rats.

### **P59. Behavioral taste similarities and differences among monosodium L-glutamate, L(+)-2-amino-4-phosphonobutyrate and N-methyl-D-aspartate in C57BL mice**

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Monosodium L-glutamate (MSG) elicits umami taste in humans and probably in some species of animals. Our previous electrophysiological and biochemical studies suggest that metabotropic and ionotropic glutamate receptors may be involved in receptor mechanisms for umami taste in mice. In the present study, therefore, we compared behavioral similarities and differences among MSG, L(+)-2-amino-4-phosphonobutyrate (L-AP4) and N-methyl-D-aspartate (NMDA) in C57BL mice using conditioned taste aversion procedures. Water-deprived mice were conditioned to avoid 30 mM MSG, 1 or 10 mM L-AP4, 50 mM NMDA, or 0.1 M sucrose by using LiCl. After conditioning, lick rates in response to various taste stimuli were counted for 10 s. Mice conditioned to avoid either MSG or 10 mM L-AP4 appeared to avoid MSG, 5'-IMP and mixtures of MSG and 5'-IMP, and L-AP4, but not NMDA. Aversive conditioning to NMDA was generalized to NaCl, but not to the umami substances and L-AP4. However, aversive conditioning to L-AP4 at 1 mM was generalized to NMDA and the umami substances. Lick rates for L-AP4 increased by mixing with (RS)- $\alpha$ -cyclopropyl-4-phosphonophenylglycine when animals were conditioned to avoid MSG and L-AP4. Lick rates for NMDA also either increased or decreased by mixing with D(-)-2-amino-5-phosphonopentanoic acid or glycine when animals were conditioned to avoid 10 mM L-AP4 and NMDA. Gurmarin, a sweet-inhibiting peptide, was ineffective in avoidance of L-AP4, but not sucrose. The results suggest the possibility that mice may taste MSG similar to L-AP4 but different from NMDA, although both taste-mGluR4 and NMDA receptors may be involved in perception of umami taste in mice.

### **P60. The influence of lesion of the rat taste ascending tract upon novel protein synthesis induced by capsaicin-containing food**

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It has been reported that capsaicin (CAP) can induce a novel protein in rat submandibular saliva. CAP could produce not only the sensation of spices but also mucosal inflammatory responses, and its information was transmitted through the glossopharyngeal nerve. In this study, we investigated possible influences of lesion of the medial part of the parabrachial nucleus (PBmed) upon protein synthesis, since the PBmed is the second relay station in the taste ascending pathway.

Male Wistar rats, weighting 300–400 g were used for the experiment under anesthesia. Rats were fed with standard food. After lesion, the rats were fed with food containing 0.005% CAP. We collected saliva and the aliquot was applied to SDS-polyacrylamide gel electrophoresis. Separation gels were of 15% acrylamide and were treated with silver stain reagents.

The PBmed is situated just under the superior cerebellar peduncle (scp). Therefore, rats showed temporary paralysis after the lesion operation. Three to five days later, rats had recovered from that state. From the electrophoresis, we found the band of the novel protein in the saliva of the CAP-fed rats, and this corresponded to that of cystatin S-like protein. Bilateral lesion of PBmed caused the band to disappear, whereas unilateral lesion of PBmed did not.

In this study, we tried to investigate whether input of sensory information into the upper central nervous system (CNS) is necessary for the production of the new protein or whether the same information works via the brain stem reflex arc. This result suggests that the sensory information must be sent to the CNS and processed there. Moreover, it suggests that of all the sensory information induced by CAP, the taste sensation was mainly related to the phenomenon. However, the precise pathway of brain stem reflection is still unclear. We cannot deny the hypothesis that the reflex arc does not include PBmed. Further studies are necessary to investigate the precise route and its function for the production of the protein.

### **P61. Study of evoked potential due to taste stimulation of solutions with the same acidity**

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It has been reported that solutions with the same acidity are not necessarily recognized to have the same degree of the acidity. To elucidate the mechanism of this effect, solutions of lactic acid (0.14 M), formic acid (0.27 M) and acetic acid (1.27 M), each of which is pH 2.75, were used, and the potentials these solutions evoked were measured. The mean latencies of the lactic, formic and acetic acids were 158.0, 172.5 and 179.5 ms respectively. The mean

amplitudes between the peaks of evoked potentials, N2 and P2, for the acids are 18.6, 20.9 and 25.1  $\mu\text{V}$  respectively. The order of the subjective evaluation for the strength of the acidity shows the same order as the latency and the amplitude. In other words, the latency and amplitude of the acids are well reflected in the subjective evaluation. The mechanism behind the different tastes of acid solutions with the same acidity was investigated. The mechanism by which the response of the  $\text{H}^+$  ion channel to  $\text{H}^+$  ion from the acid solution depolarizes the taste cell does not take place, since the solutions have the same pH. We propose that the different tastes are caused by the different degrees of adsorption to the taste cell by the anion molecules contained in the acids.

## P62. Cognitive mechanisms of umami ingestion by the hypothalamus

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Monosodium L-glutamate (MSG) elicits a unique taste termed 'umami'. Several lines of data suggest that umami preference may be regulated by the hypothalamus. In the present study, we investigated the relationship between MSG ingestion and nor-epinephrine (NE) release in the lateral hypothalamic area (LHA). We also investigated, using 4.7 T/400 magnetic resonance imaging, the changes in brain blood flow in response to intraduodenal infusion of MSG in awake rats. In rats fed normal protein diet, no significant responses of NE release to the drinking of distilled water, 0.06 M MSG, 0.06 M NaCl and 0.6 M glucose solution were found. In rats fed non-protein diet, however, a specific decline in NE release was detected during MSG drinking, suggesting that the activity of NE neurons in the LHA may serve as a neurochemical substrate that links MSG preference and protein intake. The brain blood flow signal was increased by infusion of 0.15 M MSG solution, but decreased by infusion of 0.3 M glucose solution. Infusions of 0.15 M NaCl or water were ineffective. Therefore, the changes in cerebral blood flow induced by MSG and glucose infusions were neither nonspecific volume nor osmotic effects. Specific chemosensory sensors in the alimentary tract may play an important role in recognizing the digesting component of nutrients.

## P63. Analyses of human brain magnetic fields evoked by several taste stimuli

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We analyzed a brain function for gustation in humans using a 122-channel whole-head neuromagnetometer, which measures time and space to a high degree of accuracy. These imaging techniques play an important role in understanding the gustatory mechanism in human brain by detecting activated brain regions.

In a previous study, we attempted to use a single taste stimulus for each session, and compare the responses for different tastants between sessions. Measuring the responses for two or more taste

stimuli given during the same session caused technical problems because the total time for each session was prolonged by a factor of two or more. For this reason, we examined how to shorten inter-stimulus intervals (ISI) in our paradigm. We found that the ISI for taste stimulant needed to be  $>15$  s; if the ISI was  $<15$  s, the responses were not clear enough to detect the activation area.

Second, we tried to use two taste stimuli during the same session with a 15 s interval. When the two stimuli were given alternately, the responses of evoked magnetic fields were clearly distinguished for each tastant. However, when two stimuli were given randomly, the responses for the two tastants could not be distinguished. These results suggest that gustatory primary responses in brain are easily modified by their leading stimuli. Taste recognition needs another priming sensation.

## P64. The role of the ventral pallidum in the consumption of palatable taste solutions in rats

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The ventral pallidum (VP) has recently been shown to play an important role in reward. To clarify whether the VP is involved in the intake of hedonically positive taste fluids, we examined the effects of ibotenic acid lesions of the VP. (i) Two-bottle preference tests revealed that VP-lesioned (VPx) animals drank significantly less sucrose (0.06, 0.1 and 0.3 M) than controls. In addition, VPx animals consumed less NaCl (0.1 M) and saccharin (0.0025, 0.005 and 0.025 M) than controls. The consumption of sucrose, NaCl and saccharin at other concentrations was not different between VPx and control animals. (ii) Injection of midazolam (3 mg/kg, i.p.), a benzodiazepine agonist, significantly increased the consumption of 0.1 M sucrose solutions in control animals. The same injections, however, failed to increase intake of 0.1 M sucrose in VPx animals. Midazolam did not modify intake of an unpalatable quinine (0.0003 M) solution in both VPx and control animals. These results obtained in VPx animals are similar to those with lesions of the ventral tegmental area (VTA) or pedunculopontine tegmental nucleus (PPTG) reported previously. The enhanced intake of highly palatable solutions is likely to be mediated by a neural circuit involving the VTA, PPTG and VP.

## P65. Corticofugal influence on taste responses in the parabrachial nucleus of the rat

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The parabrachial nucleus (PBN) plays an important role in taste information processing in the brain. Although several studies indicate that there is a reciprocal connection between the cerebral cortex and PBN, it is still unclear how cortical outputs modify the PBN taste responses. In this study, we examined the effects of electrical stimulation to the orbitofrontal cortex (OBF) and gustatory cortex (GC) on neuronal responses of the PBN. After observing spontaneous activity for 30 s, we successively infused water, a taste stimulus and water for 10 s each. Taste stimuli presented were



0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, and 0.02 M quinine HCl. A 5 s train of 5 Hz pulses was applied to the OBF and GC during the period of taste stimulation or baseline. Among 15 units recorded, the baseline firing rate was suppressed by the electrical stimulation of the OBF and/or GC. In some units, taste responses to NaCl increased during the electrical stimulation of the OBF or GC. Thus, the effects of electrical stimulations on spontaneous activity and taste responses were not always similar. Although the number of recorded units was small, these results suggest that cortical–parabrachial connections have some effects on neural encoding of taste in the PBN.

### **P66. POMC gene expression in the hypothalamus is induced by sweet taste stimulation**

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To determine whether or not the transcription of the *Proopiomelanocortin (POMC)* gene is regulated by sweet and palatable taste stimulation in the hypothalamus, we analyzed RNA expression in the hypothalamus of Wistar rats that had ingested 5 mM saccharin solution or distilled water (DW). The animals were deprived of water for 20 h and chow for 19 h, and were trained to ingest saccharin solution or DW from a nozzle. On the test day, rats were decapitated after ingestion of either one of the liquids. The hypothalamus was taken, frozen and stored at  $-80^{\circ}\text{C}$  until RNA extraction. The relative amount of *POMC* mRNA was quantified with Northern blot analysis, using a digoxigenin-labeled probe. *POMC* mRNA was significantly increased at 90 and 150 min after saccharin ingestion. To assess whether the *POMC* mRNA increase is due to sensory or rewarding information or both, we converted the sweet palatable taste of saccharin to the sweet aversive one by means of the conditioned taste aversion (CTA) paradigm. *POMC* mRNA expression was not induced in CTA rats and the level was equal to that exhibited by rats that ingested DW. These results suggest that *POMC* gene expression in the hypothalamus is correlated with palatability of sweet taste stimulation.

### **P67. Contextual effects on the formation of conditioned taste aversions in rats**

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In the ingestive behavior after the establishment of conditioned taste aversion (CTA), animals often show cautious or fearful reactions toward ingestion itself. To try to separate the fearful learning from the taste aversion learning, two groups of rats were trained to avoid drinking 0.005 M saccharin by pairing its ingestion with 0.15 M LiCl (2 ml/100 g body wt, i.p.). One group (Novel rats) was conditioned in a novel test box, and the other group (Familiar rats) was in the familiar home cage. Both groups were tested in the test box. Novel rats avoided (1) entering the test box, (2) drinking any liquids, (3) drinking taste solutions but not water, and (4) drinking only the saccharin conditioned stimulus (CS). These behaviors were expressed in this sequence on successive days. The selective aversion to saccharin was attained ~10 days after the conditioning day. Behaviors 1–3 may imply fearful reactions. On

the other hand, Familiar rats avoided drinking only the CS, indicating a typical CTA. The present results suggest that the acquisition of CTA without fearful reactions can be attained when the conditioning procedure is performed in a different place to the test place.

### **P68. Study on bioactive substances in the rat brain after stimulation by a bitter taste**

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We examined substances after stimulation of a bitter taste in the rat brain. Cerebrospinal fluid (CSF) from rat after intraoral stimulation of quinine–HCl (quinine CSF) suppressed the intake of 5% sucrose when it was injected into the fourth ventricle in mice. We supposed this suppression to be induced by substances in the quinine CSF. We then analyzed a candidate for the active substance in the quinine CSF using the hydra behavioral test, which utilizes a tentacle ball formation (TBF) of hydra. The effect of the quinine CSF on TBF was different from that of CSF from control rats (control CSF). From an analysis of this pattern of TBF, we hypothesized that diazepam-binding inhibitor (DBI) is responsible for this effect. The effect of the mixture of DBI and control CSF on TBF was similar to that of quinine CSF. Furthermore, we examined the effect of the injection of DBI peptide fragment into the brain on the intake of 5% sucrose in mice and also observed suppression of the intake. These results suggest that a DBI-like peptide increases in CSF after stimulation of the aversive quinine taste.

### **P69. Efferent and afferent connections of the insular cortex in rats: an electrophysiological study**

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The anterior and posterior portions of the insular cortex are considered to be a cortical taste area and a visceral sensory area respectively. We have reported that the neurons in the posterior insular cortex (posterior to the region where the chorda tympani projects) respond to gustatory, visceral and nociceptive stimuli. Although many studies concerning neural connections in the insular cortex have been reported, few electrophysiological studies have been undertaken. In the present study, we recorded the extracellular neural responses from the posterior insular cortex following electrical stimulation of various brain sites, 1–3 mm posterior to the bregma, using a stainless-steel electrode implanted in the dorsal surface of the brain. In the insular cortex, 43 cases responded with action potential and/or compound action potential to electrical stimulation of the various brain sites. Of the 43 stimulation sites, 16 were found in the internal capsule, 13 in the thalamic reticular nucleus, 4 in the amygdaloid complex, 4 in caudate putamen, 3 in the ipsilateral posterior-most portion of the insular cortex, 2 in the mediodorsal thalamic nucleus and 1 in the ventral posterolateral thalamic nucleus. The average latency was

5.8 ms ( $n = 43$ ), ranging from 0.8 to 42.3 ms. In the present study, 15 cases were examined the types of connection: 7 showed an antidromic response, mainly from the internal capsule (6/7) and 8 showed an orthodromic response, mainly from the thalamic reticular nucleus (5/8). These data suggest that input from the thalamic reticular nucleus and output via the internal capsule may play an important role in information processing in the posterior insular cortex.

### **P70. Connections between the insular cortex and the taste relay zone of the solitary tract nucleus in rats**

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The present study examined distribution patterns of neurons in the insular cortex (IC) projecting to the taste relay zone of the solitary tract nucleus (NTS) with a tract tracing method using wheat germ agglutinin-conjugated horseradish peroxidase. The tracer was injected into the region in the NTS that showed neural activities in response to taste stimulation of the anterior tongue. Labeled cell bodies were seen in layer V of almost the entire extent of the rostrocaudal axis of the IC with contralateral dominance. Their density was highest in the cortical taste area and second highest around the bregma level, showing a bimodal distribution of the cells. Cytoarchitecturally, they were located both in the granular and dysgranular ICs and also in the posterior IC. Tracer was also injected into the portion of the NTS that responded to tactile stimulation of the anterior tongue. The distribution of labeled cell bodies in the IC was similar to those in the case of the injection into the taste zone, except for (i) their distribution was unimodal with the densest portion in the cortical taste area and (ii) more dorsal distributions in the IC. The results indicate that the taste relay zone of the NTS receives a descending control from the two zones in the IC.

### **P71. Evidence of functional column in cortical taste area in rats, obtained for mechanoreceptive neurons with receptive fields in the oral cavity**

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In the neocortex of mammals, neural information is reported to be processed within the so-called functional column. However, nothing has been reported about it in the cortical taste area (CTA). The present study was aimed to study the possible columnar organization in the CTA. Recording electrodes were inserted vertically into the CTA on the pseudo-horizontal plane in urethane-anesthetized rats, and mechanoreceptive neurons that had receptive fields (RFs) in the oral cavity were recorded at 50 or 100  $\mu\text{m}$  intervals. The response to brushing, stroking with a glass rod or pinching the tissue with a pair of non-serrated forceps was examined. Three major receptive types were noticed: one with RFs confined within the oral cavity, one with additional RFs on lips, and one with RFs on lips and external body surface, such as tails. Mechanoreceptive neurons with the same receptive features were

recorded over several consecutive recording points. The diameter of the column in the cortex, where neurons with the common receptive features, RFs and adequate stimulus were recorded, was estimated to be 50-100  $\mu\text{m}$ . The results indicate that mechanoreceptive information is processed within the columnar organization in the CTA.

### **P72. Cortical areas related to taste, measured by fMRI and MEG (2)**

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MEG has a good temporal resolution and can provide a good estimation of the location of neural activity because the magnetic field generated from the living brain is free from distortion by the skull. However, when there are more than two activated areas, the reliability of estimation of the equivalent current dipoles might decrease. fMRI, on the other hand, can reliably detect many activated regions simultaneously, but with very poor temporal resolution. In the present study, we tried to measure changes in the regional cerebral blood flow (rCBF) induced by gustatory stimulation, using the fMRI technique to find activated areas with 12 participants, and compared the results with the findings from MEG.

Changed rCBF was observed in the transition area between insula and Rolandic operculum, which was observed in short latency, in MEG experiment. Furthermore, the frontolateral operculum, postcentral gyrus, supramarginal gyrus, angular gyrus, middle frontal gyrus and superior temporal gyrus were observed in long latency in MEG.

### **P74. Structure of sweet liking**

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Sweet liking is related to many factors such as liking for strong sweet taste, eating sweets frequently, eating a large quantity of sweets, etc. In this study, we examined the relationships between sweet liking and such factors, particularly the strength of sweetness, frequency of eating and quantity eaten by means of a questionnaire survey and sensory evaluation using 354 female students from our universities. The questionnaire survey consisted of four statements: 'I like sweets very much', 'I like rather stronger sweets better than weaker ones', 'I want to have something sweet at least once a day' and 'I cannot eat a large amount of sweets at a time'; agree or disagree ratings were made using a 7-point category scale. In sensory evaluation, agar gel containing 16% of sugar was evaluated for its appropriateness of sweetness for each subject, using a 7-point category scale. The questionnaire survey showed

that subjects who like strong sweetness always like sweets, but those who like sweets do not always like strong sweetness, and subjects who dislike sweets never like strong sweetness, but those who do not like strong sweetness do not always dislike sweets. This means that the variance in the desired strength of sweetness increased as the degree of liking for sweets increased. Sweet liking was more closely related to frequency and amount. The results of sensory evaluation showed that those who evaluated the sweetness of the gel as not too strong liked more sweets, both in terms of frequency and amount, than those who evaluated it as too strong. However, the variance in the evaluation scores for the sweetness did not increase as the degree of liking for sweets increased.

### P75. Increased NaCl preference and other mineral imbalance in zinc-deficient rats

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It is well known that zinc deficiency causes abnormalities in general taste sensation. We have found out that feeding zinc-deficient or low-zinc diets causes an abrupt increase of NaCl preference even after 4 days of feeding. Therefore, the present study was undertaken to clarify the mechanism for the increased NaCl preference observed in zinc-deficient and low-zinc rats at an earlier stage of the feeding. Male 4-week-old Sprague–Dawley rats were divided into three groups (Zn-Def, Low-Zn and Pair-fed). Plasma sodium concentration and urinary sodium excretion were measured after 0, 4, 14, 21 and 28 days of feeding. Through the feeding period, there was no change of plasma sodium concentration in the zinc-deficient rats. At an earlier stage of the feeding period, there was no increase of urinary sodium excretion in the zinc-deficient rats. These results indicate that the dietary signal of zinc-deficiency may reflect NaCl preference, probably through the central nervous system.

### P76. The study of the taste of 'gyokuro', a specially produced green tea

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'Gyokuro' is a specially produced, high-grade Japanese green tea. It is rich in glutamic acid and theanine, which are the amino acids that elicit the umami taste. When a model solution of gyokuro that contains catechins, caffeine, theanine and glutamic acid is tasted, the strong feeling of astringency originating from the catechins masks the umami taste of these amino acids. We found that polysaccharides extracted from 'gyokuro' tea infusion reduced the precipitation between tannins and proteins. Polysaccharides were supposed to depress the astringency of 'gyokuro' infusion.

The concentrations of oxalic acid in 'gyokuro infusion' are ~1000 mg/l, and oxalate itself has an unpleasant aftertaste. We hypothesized that the taste of oxalic acid is the result of the precipitation of calcium oxalate. Citric acid, which is also a component of tea, inhibited the precipitation between oxalate and calcium, and may reduce the aftertaste of oxalate in 'gyokuro' infusion.

Our conclusion is that polysaccharides and citrate lessen the unpleasant taste and we enable us to taste the umami when drinking 'gyokuro' infusion.

### P77. Bitterness of a novel, sulfur-containing amino acid, pulcherrimine, from the green sea urchin, *Hemicentrotus pulcherrimus*

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To investigate the effects of the bitter taste of pulcherrimine, a novel, sulfur-containing amino acid isolated from the green sea urchin *Hemicentrotus pulcherrimus*, we carried out sensory tests. The relationship between the contents of pulcherrimine and its bitter taste was examined and discussed in relation to detection and recognition thresholds.

A significantly positive correlation was observed between pulcherrimine content and bitterness of the sea urchin gonads ( $r = 0.706$ ,  $n = 96$ ,  $P < 0.0001$ ). The detection and recognition thresholds determined by the single and triangle tests were 0.19 and 0.31 mM, and 0.17 and 0.34 mM, respectively. Furthermore, according to the triangle tests, the thresholds for pulcherrimine added to the extracts from green sea urchin gonads were estimated to be 0.25 and 0.52 mM (immature gonads), 0.22 and 0.46 mM (ovaries), and 0.27 and 0.46 mM (testes), respectively. These findings suggest that the compound confers a remarkable effect of bitter taste on extracts from both immature and mature green sea urchin gonads.

### P78. Taste-modifying effects of soluble starch in human

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The effect of soluble starch on taste intensity was investigated in 135 human subjects. The starch solution was made up with distilled water (DW) and was gelatinized by heating to 80°C. Taste substances (sucrose, NaCl, quinine–HCl and citric acid) were dissolved in either DW (standard) or starch solution (test solution) at different concentrations. The solutions were presented to naïve subjects, and each subject was requested to taste and compare the taste intensity between the standard and test solutions based on a scale ranging +3 (stronger) to –3 (weaker). A greater sweetness intensity occurred to 1 M sucrose dissolved in a soluble starch (0.125–4.0%) than for 1 M sucrose dissolved in DW, while sweet enhancement did not occur for the other six sweet substances including artificial sweeteners. Saltiness with NaCl (0.0–0.3 M) was not affected, but bitterness by quinine–HCl (0.00003–0.001 M) or sourness by citric acid (0.0003–0.01 M) tended to be inhibited by the soluble starch. These results suggest that the taste-enhancing effects of soluble starch on sweetness and the inhibiting effects on bitterness or sourness might depend not on the taste transduction mechanisms



but on the molecular structures of the soluble starch and taste substances.

### P79. Taste-modifying effects of citric acid on umami taste

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The effect of citric acid on umami taste intensity was investigated in human subjects and in rats. In humans ( $n = 42$ ), an umami substance [monosodium glutamate (MSG) at 0.005–0.08 M] was dissolved in either distilled water (DW) or sodium citrate solution (CitNa, 0.001–0.01 M). A greater umami intensity occurred in response to CitNa than to NaCl (0.002–0.02 M). A two-bottle preference test was performed in the rat. Rats were individually housed in a cage and the two different paired solutions were presented simultaneously. The position of the bottles was changed after 24 h presentation, and the consumption of each solution was measured for 48 h. Rats significantly preferred 0.05 M ( $P < 0.05$ ) or 0.08 M ( $P < 0.005$ ) MSG to DW and 0.003 M CitNa ( $P < 0.05$ ) to DW. The preference ratio for MSG + CitNa tended to be larger than that for MSG. Also, the rat tended to prefer MSG + IMP + CitNa to MSG + IMP. These results suggest that CitNa may have enhancing effects on umami taste in mammals.

### P80. Effects of taste and smell on preference for corn oil in mice

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Oily and fatty foods are strongly preferable stimuli for human and rodents. We previously observed that mice showed a preference for vegetable oils in the two-bottle choice test after only 10 min of feeding. Further, corn oil was suggested to have rewarding effects which were measured by the conditioned place preference (CPP) test in mice. These effects were observed when corn oil was voluntarily ingested in the conditioning box. These results suggested that the stimuli of the oil in the oral cavity were important for the preference. However, it still remained to be elucidated what kinds of stimuli contribute to the preference. In the present study, we investigated the contribution of olfaction to the preference for corn oil in the CPP test and the two-bottle choice test in mice. In the CPP test the rewarding effects were observed by voluntary intake of 100% corn oil in olfactory-blocked mice as well as normal mice. In the two-bottle choice test, the normal mice but not the olfactory-blocked mice significantly selected low concentrations (1 and 3%) of corn oil compared to vehicle (a 0.3% xanthan gum solution). These results suggested that the olfactory stimuli of corn oil are not needed for its rewarding effects although they may play a role as a signal of the presence of the oil in the lower concentrations.

### P81. Preference for 'dashi': importance of unknown umami factor

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We studied the effects of tastes of amino acids and nucleotides on preference for 'dashi' prepared from dried skipjack (*katsuobushi*) which has traditionally been used as a Japanese stock. In a two-bottle preference test, C57BL mice showed preference for a 'dashi' solution compared with water. However, there is no difference in intake of the 'dashi' and an amino acid–nucleotide solution containing the same composition of amino acids and nucleotides as the 'dashi' in this test. These results suggest that the tastes of amino acids and nucleotides are important factors in the preference for 'dashi' in C57BL mice. ICR mice preferentially selected the 'dashi' compared with water or the amino acid–nucleotide solution but showed no difference in intake of the amino acid–nucleotide solution and water in this test. Therefore the preference of the ICR mice for 'dashi' may be not derived only from the tastes of amino acids and nucleotides. In addition, the decreased preference for 'dashi' was observed in ICR mice with glossopharyngeal (GL) denervation. Therefore, the GL nerve plays an important role in preference for 'dashi' in ICR mice. These observations suggested that mice preferred 'dashi', and this preference was involved in not only the taste of amino acids or nucleotides but also other factors mediated by the GL nerve.

### P82. The influence of taste memory and suggestive taste behavior on the taste decision ability

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We surveyed if the memory of taste experience influenced taste decision ability, when people judged the concentration of a taste material that was experienced just before the judgment was made. We investigated whether taste decision ability was decided by given information in cases where some information about the taste was given just before the decision. We also surveyed if the effect of these memories and suggestive actions on taste decision ability was affected by age and gender.

For taste stimulus, we adopted the basic tastes of sweetness, saltiness, sourness and bitterness. The subjects were 20- to 40-year-old men and women (Y = young), 41- to 64-year-old men and women (M = middle aged), and men and women over 65 years old (O = old people). There were a total of six groups of 30 people each, including three male Y, M, O, and three female Y, M, O. We analyzed data using Kendall's  $W$  coefficient, which signifies taste consideration according to the group decision ability as a whole.

The results were as follows. In the control groups where both memory and suggestions were not given, the decision ability was high in order of the Y, M and O groups with no difference in gender. As to gender differences, the women's decision ability was higher than men's.

For the memory taste test, there was little fluctuation of  $W$  by memorizing taste: subjects did not memorize the taste experience just before the decision.

Regarding the suggestive taste test,  $W$  was reduced in all age groups for both genders. When false information about taste was

given just before the decision, it was found that the taste decision ability was influenced by that false information.

### **P83. Effect of physical exercise on taste preference in hot-wet and cold-dry environments**

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The effect of physical exercise on taste preference in hot-wet (30°C, 70% relative humidity) and cold-dry (10°C, 40% relative humidity) environments was studied in 16 healthy university students. After 30 min exercise using a bicycle ergometer at an intensity of 50%  $\dot{V}O_2$ max (maximal oxygen uptake), a rating scale test on taste hedonic tone was performed. The test solutions were sucrose, citric acid, NaCl, caffeine and monosodium glutamate (MSG). Preference scale values for sucrose, citric acid and NaCl increased after exercise in the hot-wet environment, while the values for caffeine and MSG were unchanged. Preference scale values for sucrose increased after exercise in the cold-dry environment, while the values for citric acid, NaCl, caffeine and MSG were unchanged. These results suggest that the effect of physical exercise on taste preference might depend on environmental condition.

### **P84. Influence of pregnancy on the taste preference in women**

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In order to evaluate the influence of pregnancy on the taste and preference for food, the taste threshold test and preference were examined by questionnaire in pregnant women.

The threshold for monosodium L-glutamate was lower late in pregnancy and just after birth than that during early pregnancy, while the threshold for sucrose, NaCl, L-tartaric acid and quinine-HCl did not change during pregnancy or just after birth. Regarding taste preference, the preference for sour and sweet tastes during pregnancy increased compared with before pregnancy. The preference for sweet, salty and sour food increased. These results suggest that physiological changes during pregnancy may change taste preference.

### **P85. Evaluation of zinc concentration in serum and TIMP-1 activity in saliva from patients with taste disorder**

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The purpose of this work was to evaluate the concentration of zinc in serum and TIMP-1 activity in saliva from the patients with taste disorder.

Mixed saliva materials were collected from 10 patients with taste disorder referred to the gustatory clinic, Niigata University Dental Hospital. The control samples (healthy subjects) were obtained from 10 volunteers with normal taste acuity. The samples were quickly frozen until assay and centrifuged at 12 000 r.p.m. for 5 min to remove suspended matter. Supernatants were used for ELISA for TIMP-1 enzyme activity. In addition, sera were obtained from

patients with taste disorder, and normal sera were obtained from individuals without any abnormality for use as a control.

The value for TIMP-1 activity of the control groups (age 32–78 years) was mean  $50.4 \pm 13.0$  (mean  $\pm$  SD), whereas activity of the taste disorder patients (age 36–79 years) was  $35.6 \pm 13.7$  ( $P < 0.05$ ; *t*-test). The serum zinc concentration in patients with taste disorder is slightly lower (~14% decrease) than the control group ( $P < 0.05$ ; *t*-test). But the low activity level of TIMP-1 in taste disorder patients was not related to the low zinc concentration level in serum.

We hypothesize that low TIMP-1 activity might depress cell proliferation activity and alter the function of intraoral organization.

### **P86. The influence food taking and taste stimuli on the clinical course of mandible osteoradionecrosis: a case report**

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A 58-year-old man received radiation therapy for carcinoma of the mouth floor (T2N0M0) and obtained a complete response. However, 2 years after radiation treatment, osteoradionecrosis of the mandible occurred, for which alcohol consumption and poor nutrition were contributory factors. The patient was treated with antibiotics at a dose of over 10 g/year, and was repeatedly hospitalized. After 4 years the patient began to take food according to JASTS recommendations (Japanese Association for the Study of Taste and Smell abstract; Chem. Senses, 24:217), which included intake of seasonal vegetables, and fish and umami. His condition improved to the point where it was controlled without antibiotics for a year. In addition, the patient's psychological condition was much good. Food taking and taste stimuli, including umami, can induce positive emotions, and can markedly improve the condition of oral cancer patients. Even in modern clinical medicine it is important to consider the usefulness of (evidence-based medicine and critical pass, particularly in the treatment of cancer patients.

### **P87. Taste disturbance caused by a strong alkaline reagent: a case report**

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A number of laboratory reagents are very dangerous. In particular, strong acids and alkalis have a serious effect on the human body, causing severe inflammation or ulceration to the mucous membrane in the oral cavity, esophagus and stomach. In the present study, we report a case of taste disturbance caused by a strong alkaline reagent, and examine the mechanism of bodily injury of this reagent.

A 20-year-old female laboratory worker mistakenly sucked up a strong alkaline reagent. She received rapid first aid to wash her oral cavity with tap water. She felt a taste disturbance on the tip of her tongue, and then visited our taste clinic. Medical examination showed inflammation and taste disturbance on the tip of her tongue, but her taste in the area controlled by the chorda tympani nerve was within the normal range. Treatment, using steroids, improved the taste disturbance within 2 weeks.

### P88. Higher recovery of taste function in younger people after middle ear surgery

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The purpose of this study was to investigate, using electro-gustometry (EGM), patients' symptoms and the functional recovery of taste after surgery. We analyzed 84 ears (35 ears with cholesteatoma, 25 with chronic otitis media and 24 with non-inflammatory diseases) from 76 patients who underwent middle ear surgery between January 1998 and December 1999. The subjects' taste functions were examined 2 days before and 2 weeks after surgery. Two weeks after surgery, numbness in the tongue and taste disturbance were more frequently found in patients in whom the chorda tympani nerve (CTN) had been preserved than in those with a sectioned CTN. In patients with intact CTN, the recovery rate of EGM threshold 2 weeks after surgery was 90.9% in the 4–20 year group, 55.6% in the 21–40 year group and 51.9% in the 41–60 year group. Younger age is an important factor for recovery of taste function after middle ear surgery, which is useful in explaining the complication to patients.

### P89. Theobromine induces new proteins in submandibular saliva of the rat

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Irritating dietary substances such as tannin, papain and capsaicin have been reported to alter the morphology of salivary glands and their secretions. We investigated the effects of dietary theobromine on rat submandibular saliva. Theobromine is the principal alkaloid of cacao beans, which contain 1.5–3% of the base. Several groups of animals were offered either control diets or diets containing theobromine for 7 days. Higher concentrations (1%) suppressed food consumption for 3 days, and body weight decreased until the day 4 of the feeding trial. After day 4, the animals began delayed recovery of the intake. New theobromine-induced protein bands (~15 kDa) were found in an SDS-PAGE gel. The electrophoretic properties of these proteins were similar to those isoproterenol-induced proteins. In addition, inconspicuous bands (~18 and ~40 kDa) were also observed.

### P90. Responses of the surface plasmon resonance (SPR) chemical sensor for vinegar

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A chemical sensor based on four-channel surface plasmon resonance (SPR) was studied for quality control of the fermentation process of vinegar. Each channel of the SPR sensor with distilled water as a reference liquid responds to a change in the quality of the fermenting vinegar. The SPR sensor with aqueous acid solution as the reference liquid also responds to different kinds of vinegar.

These results strongly suggest that the SPR chemical sensor is useful for quality control and for identification of the type of vinegar.

### P91. Development of a monitoring system for research on drinking behavior in rats

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We have designed a computer-controlled device that monitors drinking behavior in rats. This system simultaneously presents two bottles to rats. The problem with the traditional two-bottle technique is that rats may use the position of spouts as a cue to discriminate taste stimuli when the position of spouts has been fixed for a long period. This new system monitors the consumption of water or fluid at 1 min sampling interval for periods of up to 4 days. It also automatically changes the position of spouts at a regular interval (from 1 min to 2 hours), thereby excluding the possible influence of fixed spout position. The present experiment examined whether the position of spouts affects the memory of the location of a taste stimulus in rats. Rats were trained to drink sweet ( $5 \times 10^{-3}$  M saccharin) or bitter ( $10^{-3}$  M quinine) taste stimuli from two bottles. In one group, the position of the spouts was fixed (Fixed group), and in the another group, the position of the spouts changed (Rotation group) at 6 min intervals. While the Fixed group learned the position of the spouts, the Rotation group did not. These results suggest that this new monitoring system eliminates the above-mentioned problem of the two-bottle technique.

### P92. Chemical stimuli and behavioral responses of the blue swimming crab, *Portunus pelagicus*

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The blue swimming crab, *Portunus pelagicus* L., was stimulated in an aquarium with four amino acids, two saccharides, betaine, quinine-HCl and sugar-cane extract. The chemicals were tested cumulatively from  $10^{-13}$  to  $10^{-3}$  M, at intervals of every 15 min. The antennular flicking rate before and after stimulus application was statistically analyzed to determine the detection threshold. Glycine and L-alanine were detected at  $10^{-13}$  M; galactose, glucose and betaine at  $10^{-12}$  M; L-serine and L-glutamic acid at  $10^{-6}$  M; and sugar-cane extract at  $10^{-5}$  ml/l. The sequence of behaviors observed after an increase in the antennular flicking rate was the following; maxillar movement, dactyls digging into the substrate, chelae or dactyl probing, and searching behavior. This study showed that an increase in the chemical concentration induces different behavioral stages.

### P93. An electrophysiological study on the taste responses of *Oreochromis niloticus*

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The stimulatory effectiveness of six amino acids and betaine on the



taste system of the Nile tilapia, *Oreochromis niloticus*, was studied electrophysiologically. Responses were recorded from a branch of the facial nerve. The chemicals were tested at a concentration of 1 mM. All the tested chemicals evoked neural responses. The taste receptors of this species responded well to both the basic (L-arginine) and acidic (L-glutamic acid) amino acids. The order of the relative stimulatory effectiveness of tested chemicals was L-arginine > L-serine > L-glutamine > L-glutamic acid > L-alanine (std) > L-proline > betaine. The stimulatory effectiveness of these amino acids was similar to that found in a previously studied herbivorous fish, but L-proline was not quite as stimulatory in this species. The results of this study indicate that *O. niloticus* has adequate gustatory sensitivity to amino acids.

#### **P94. Studies on the motor control system of barbels in goatfishes**

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The goatfish has a large pair of barbels extending downward from its lower jaw. These barbels are fairly rigid and move rapidly to probe and stir the substrate material. This study was performed to reveal the nature of barbel movement and to identify the muscles and motor neurons that control this movement.

The application of an electrical pulse (6 V, 1 ms duration) to the peripheral cut stump of the barbel nerve caused one movement of the barbel. The barbel could move in response to each pulse in the range of 1–20 Hz and each movement decreased in magnitude for higher frequencies.

A frequency of >20 Hz caused the barbel to extend rigidly toward the anterior direction without any movement to each pulse. At least five kinds of muscles were found to contribute to the barbel movement. Two muscles function as flexor or extensor. The other muscles contract to modify simple movements caused by the flexor or extensor. Application of neural tracers to the central cut stump of barbel nerve labeled motor neurons in the facial motor nucleus as well as fibers in the sensory roots of trigeminal and facial nerves. A total of 295 motor neurons were labeled and these neurons send dendrites toward the spinal trigeminal nucleus and reticular formation under the facial lobe.

These results suggest that monosynaptic and multisynaptic reflex pathways are respectively responsible for the trigeminal and facial inputs received in the barbel.

#### **Glycyrrhizin induces sugar response in blowfly, *Phormia regina***

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Several studies have been performed to evaluate the taste profile of glycyrrhizin (GL) in vertebrates, but little is known about its effects in invertebrates. GL, a triterpenoid saponin found in the root of licorice (*Glycyrrhiza glabra*) is used as an artificial sweetener and a medicine for humans. GL was found to induce sugar response

in blowfly, *Phormia regina*. This has been confirmed by both electrophysiological and behavioral experiments. The optimum concentration of GL was 3.0 mM, which is much lower than that of sucrose. This strengthens the idea that GL is much more sensitive than sugar molecules. The glycosidic moiety of GL is composed of two molecules of glucuronic acid. We suspect that the whole GL molecule is involved in the induction of sugar response.

It is known that the sugar receptor cell possesses at least two types of receptor sites which are distinguished by selective inhibition with polysaccharides. GL-induced responses were inhibited by starch but not by levan, indicating that the starch-sensitive P site is involved in the GL action. Surprisingly, repeated GL-stimuli reduced adaptation but enhanced the electrophysiological responses.

Previously, we have shown that chromosaponin I (CSI), a  $\gamma$ -pyronyl-triterpenoid saponin isolated from pea and other leguminous plants, stimulates the sugar taste receptor cells of *P. regina*. We propose that GL, a structural analog of CSI, exerts dual functions to induce sugar response: first, by binding to the P site on the sugar receptor cells as a stimulant; second, by penetrating the cell membrane and interacting with a part of the sugar signal transduction pathway inside the sugar receptor cell.

#### **P96. Participation of learning and memory in the gustatory discrimination of *Drosophila melanogaster***

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Several mutants of *Drosophila melanogaster* have been isolated that show deficiency in olfactory associative learning. The first one of these was named *dunce*. No one, however, has examined the ability of learning that couples with taste cues. We investigated the discrimination ability of *dunce* between different concentration levels of sucrose solutions. Based on a learning index (LI) defined by the feeding behavior, we found significantly smaller LI values for *dunce*, compared with that of the wild-type flies. We conclude that learning and memory processes participate in the food selection behavior of *Drosophila*.

#### **P97. Taste-enhancing effect of glycine on the sweetness of glucose in the relationship between the ant, *Camponotus japonicus*, and the lycaenid butterfly, *Niphanda fusca***

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In the symbiotic relationship between the ant, *Camponotus japonicus*, and the larvae of lycaenid butterfly, *Niphanda fusca*, the ants shelter the larvae in their nest, and the larvae provide the ants with a sweet secretion. The secretion includes a significant amount of glycine (76% of total amino acids) as well as glucose (75% of total sugars) (Nomura *et al.*, 1992).

Focusing on glycine in the secretion, we investigated the feeding threshold of the ants to glucose in the absence or the presence of glycine. The feeding threshold of glucose was clearly decreased in a glycine concentration dependent manner.

We let the ants choose between plain glucose and glucose plus

glycine. As the result, they preferably drank glucose plus glycine, even if the concentration of glycine was too low to induce their feeding behavior by itself.

We also found in some chemosensilla on the labial pulps of the ant that glycine synergistically enhanced the electrophysiological response to glucose.

These results suggest that glycine makes the larval secretion attractive to the ants. One of the reasons the ants assiduously feed the larvae might be to obtain this glycine-flavored secretion.

### **P98. Sensitivity of taste receptor cers in the galea of the honeybee, *Apis cerana japonica***

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Honeybees have often been studied ecologically, but little work has been done on the electrophysiology of their taste. We investigated the honeybee's taste sensitivity using electrophysiological techniques. We used the Japanese honeybee (*Apis cerana japonica*), which is a subspecies of *Apis cerana* that lives in Japan. There are taste hairs on the honeybee's proboscis, or galea, and we succeeded in recording sugar, salt and water impulses from the taste hairs by the tip-recording method. From the results, we concluded that there are at least three taste receptors: the sugar, salt and water receptors. We concentrated on the sugar receptors. By constructing the dose-response curves of sugar receptor for sucrose, glucose and fructose respectively, we discovered that the honeybee's sugar receptors are more sensitive to glucose solution than the blowfly's.

Next, by applying glucose and fructose solutions mixed with starch (the P-site inhibitor of the blowfly) to the honeybee's taste hairs, glucose responses were significantly reduced, while fructose responses were unchanged. The impulses evoked by the application of solutions of nucleotide or amino acid to the hairs were derived from the sugar receptor, judging from the impulse height. On these findings, we speculated that honeybees must have multiple sites on their sugar receptor cells.

### **P99. The change of salt taste response by organic acids: study on the membrane of salt receptor cells of the blowfly**

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We electrophysiologically investigated the enhancement and depression of the salt taste responses to 100 mM NaCl or 100 mM KCl mixed with various amounts of acetic, succinic or citric acid, using the labellar salt receptor cells of the blowfly, *Phormia regina*. The organic acid concentrations that gave the maximum response varied: citric acid < succinic acid < acetic acid. The pH values for the 100 mM NaCl solutions mixed with each organic acid, which gave the maximum salt response, were different from one another. Therefore, the enhancement of salt taste response due to organic

acid could not be explained by pH-dependence. These findings are consistent with the results from our previous study. The application of 100 mM NaCl mixed with HCl to the chemosensillum also increased the salt taste response in a similar pH range to where the mixture with organic acids gave the maximum response. This suggests that protons from organic acids could amplify the response of salt receptor cells. However, the response to 100 mM NaCl tended to be decreased by organic acids at the pH where HCl most increased it. Organic anions or undissociated molecules from organic acids might be more effective in depressing salt taste response. TNBS and NBS treatment of the salt receptor cell membrane depressed the response to 500 mM NaCl. These results suggest that the amino groups and the tryptophan or tyrosyl residues might be related to the salt taste response, and that organic acids might interact with them on the membrane surface of salt receptor cells.

### **P100. Sequence analysis of the polymorphism of the candidate sweet taste receptor gene *GPCR(5A)* in *Drosophila***

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Genetic variation in sensitivity to the disaccharide trehalose is known among wild and laboratory strains of *Drosophila melanogaster*. The variation has been shown to be due to a single gene dimorphism in the taste gene *Tre* on the X chromosome. Since a P-mutagenesis experiment showed that disruption of a G protein-coupled receptor gene *GPCR(5A)* is associated with *Tre* mutations, we analyzed the gene to find out if there is any correlation between the dimorphism in trehalose sensitivity and changes in the genomic DNA and the amino acid sequences of *GPCR(5A)* in the wild populations. Among four wild strains—HG84, Shanghai, Tananarive and Singapore—HG84 had a wild-type allele *Tre*<sup>+</sup> and other three strains had the spontaneous mutant allele *Tre*<sup>01</sup> according to the two-choice sugar preference test. Throughout the open reading frame of the *GPCR(5A)* of the wild populations we identified a total of 30 polymorphic nucleotide substitutions and three insertions, of which one insertion and two substitutions lead to changes in the amino acid sequence. However, none of the polymorphic changes were found to be significantly correlated with the taste sensitivity to trehalose.

### **P101. The taste quality discrimination system of the fly consists of various mouthpart gustatory organs**

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The fly adjusts its intake of sugary and proteinaceous foods according to its nutritional state. Here we report that mouthpart gustatory organs (labellar taste hair, intersegmental papilla, labial chemosensory organ, cibarial chemosensory organ) are mainly responsible for discriminating between sucrose solution and brain-heart infusion (BHI) solution, and the olfactory organ and tarsus gustatory organ took little part in this discrimination. Behavioral experiments revealed that the labellar taste hairs play a

vital role in this gustatory discrimination because this discrimination did not occur when sensory input from the labellar taste hair was absent. On the other hand, electrophysiological experiments showed that both sucrose solution and BHI solution mainly stimulate the sugar receptor cell in the labellar taste hair, and the response characteristics of the sugar receptor cells in the four kinds

of the labellar taste hairs were similar. It seemed impossible to make this taste quality discrimination only by the sensory input from the labellar taste hair. We hypothesize that taste quality discrimination between sugary and proteinaceous food occurs as a result of integrating sensory inputs from the various mouthpart gustatory organs.