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ABSTRACTS

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O1. Anxiety reduction using odors and frontal lobe function

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The 1/*f* frequency fluctuation of alpha waves in frontal sites is a very sensitive indicator for emotional processing. In this experiment, we assessed anxiety reduction by odor stimuli, i.e. lavender or tuberose, and examined whether frequency fluctuation coefficients of alpha waves were an indicator for anxiety reduction. Before the experiment, 14 high trait anxiety subjects and 14 low anxiety subjects were placed into two groups. The experiment was performed in two sessions, i.e. a non-odor and an odor session. In the second session, lavender or tuberose was presented to half of the subjects in each of the high and low groups for 5 min. State anxiety and psychological states (awareness, mood, relaxation) were estimated before and after EEC measuring. On the other hand, the time sequence of individual alpha wave cycles in the frontal locations (Fp1, Fp2) was determined using the filtering and zero-crossing method in two sessions, and coefficients of cycle fluctuations were calculated by a frequency analysis (FFT) and linear regression method. As a result, the state anxiety decreased when the subject felt in a good mood. Tuberose had a better effect for anxiety reduction as compared with lavender. The anterior frequency fluctuation asymmetry also correlated with the anxiety level. The left frontal site was more 1/*f*-like than the right side at the lower anxiety level. The state anxiety level was estimated using multiple regression analysis (state anxiety scores: y; and right and left slope coefficients: $X1$, $X2$). The estimation formula, i.e. $y = -36.2*X1 - 28.1*X2$, explained the anxiety level significantly ($n = 56$, $R^2 = 0.85$, $P < 0.001$), and this suggests that the slope coefficient of frequency fluctuation of alpha waves is as a good indicator to estimate the state anxiety.

O2. Effects of the odor of essential oils on brain function reflected in the results of the new EEG analysis program LORETA

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In order to investigate the effects of the odor of essential oils (lemon oil and lavender oil) on human brain function, the P300 component of event-related potentials was recorded in nine healthy adults (aged 21–25 years, all right-handed) while they were working on a visual oddball task. The P300 data were analyzed with the new EEG analysis program called LORETA (Low Resolution Electromagnetic Tomography). LORETA determines the current density within the brain volume from extracranial measurements of the electric potential and yields a 'blurred' image of the three-dimensional distribution of neuronal activity. Compared with the two-dimensional mapping of the scalp potentials, the results of analysis with LORETA revealed clearer differences in the effects of the odor of the two essential oils employed in the study. Differences in the results between the conditions with and without the odor and between the lemon oil and lavender oil conditions were especially observed in the distribution of current density in the anterior part and the deep areas of the brain.

O3. Evaluation of odor quality for five odorants of T & T olfactometer using concrete adjectives

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In a previous paper, odor quality was evaluated for five odorants of T & T olfactometer by a semantic differential method using 28 concrete adjectives. In the experiment, three subjects were used. Applying the principal component analysis to the ratings, the first to the third components were extracted as 'rotten smell', 'burnt smell' and 'floral odor'.

In the present paper, 32 subjects evaluated odor quality of the five odorants using the same adjectives to confirm the results of the previous paper. The subjects performed two replications at two concentration levels in the experiments. Mean values of ratings were calculated for 27 and 32 subjects in the first and second replications respectively. In the first replication, ratings of five subjects were excluded due to a high threshold value or large variation of threshold. Principal component analysis was applied to the mean values of the ratings. The first principal component was 'bad smell' (rotten smell)–'good odor' (including floral, fruity and sweet odors), and the second principal component 'burnt smell'. The results obtained were similar to and confirm the results of the previous study.

O4. Connections of the taste and tongue thermal regions in the parabrachial nucleus to the thalamic taste relay in rats

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The thalamic taste relay, the parvicellular part of the posteromedial ventral thalamic nucleus (VPMpc), bilaterally receives taste inputs from the taste relay of the parabrachial nucleus (PB) in rats. However, the VPMpc was claimed to have inputs mainly from the contralateral external medial subnucleus of the PB. We checked physiologically the properties of neurons in the external lateral subnucleus and also examined anatomically whether the two portions of the PB differentially connect to the VPMpc. Electrophysiological mapping studies showed that neurons in the external lateral subnucleus responded to the thermal stimulation of the tongue. When tracer was injected into the PB taste relay, anterogradely labeled terminal fields were found in the bilateral VPMpc with ipsilateral dominance; the labels were denser in ventromedial portions of the nucleus. Following injections into the external medial subnucleus, labeled terminal fields were seen in the VPMpc with contralateral dominance; the labels were denser in the dorsolateral portions of the nucleus. The present findings indicate that the taste and tongue thermal regions in the PB both project to the VPMpc and further that the VPMpc is divided into two portions, dorsolateral and ventromedial, on the basis of sensory functions and connections.

O5. Merkel-like basal cells in the taste organs of the frog, *Rana nigromaculata*

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Merkel-like basal cells in the taste organs of the frog were examined by fluorescence histochemistry, immunohistochemistry and electron microscopy. There were ~16–20 basal cells arranged in radial fashion at the base of each taste organ. These cells were strongly immunopositive for serotonin antiserum. They were characterized by the presence of numerous dense-cored granules in the cytoplasm, ranging from 80 to 120 nm in diameter, and of microvilli protruding from the cell surface. For 4 months after sensory denervation, all cell types of the taste organ were well preserved and maintained their fine structure. Even at 4 months after denervation, the basal cells exhibited strong immunoreaction with serotonin antiserum. To study the role of serotonin of the basal cells in taste organ function, a deficiency of serotonin was induced by administration of *p*-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, and of *p*-chloroamphetamine (PCA), a depletor of serotonin. After administration of these agents to normal and denervated frogs for 2 weeks, a marked decrease or complete absence of immunoreactivity for serotonin was observed in the basal cells. Ultrastructurally, degenerative changes were observed in both types of frogs; numerous lysosome-like myelin bodies were found in all cell types of the taste organs. The number of dense-cored granules in the basal cells was also greatly decreased by treatment with these drugs. Serotonin in Merkel-like basal cells appears to have a trophic role in the maintenance of morphological integrity of frog taste organ cells.

O6. Scanning electron microscopic findings on the taste pore of fungiform papillae of rats following disorder of the chorda tympani nerve

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In this study, the changes of the taste pore of fungiform papillae of rats following disorder of the chorda tympani nerve were studied by scanning electron microscopy. Twenty-three rats were used for this study and their chorda tympani nerves were disordered in their middle ear cavity by 10% formalin. The tongues were removed 3, 6 and 10 days, 2, 3 and 4 weeks, and 2 and 3 months after the treatment. Morphological changes of the taste pore have started by day 3, and obstruction of the taste pore was observed in 20% of the fungiform papillae. By day 14, almost all of the fungiform papillae showed disappearance of their taste pores. By week 4, regeneration of the taste pore was found and 60% of the fungiform papillae exhibited taste pores. The morphology of the taste pore seemed to be normal at months 2 and 3. Some different types of morphological changes were obtained from this study, and these findings could be useful for evaluation of the changes of the taste pore in some animal models for taste disorder. The time course of the effect on the taste bud of the chorda tympani nerve was suspected to be almost the same as that of the glossopharyngeal nerve which has been reported on foliate papillae.

O7. Effects of amiloride on the spinal nerve responses in the desert toad

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Toads obtain water by osmotic absorption across their skin. Behavioral and anatomical studies have suggested that ventral skin of the desert toad has a chemosensory function to avoid hyperosmotic salt solutions. This function was studied by extracellular recording of the spinal nerves (5th and 6th) innervating the ventral skin of the Colorado river toad (*Bufo alvarius*). The spinal nerves responded to NaCl, KCl and citric acid solution applied to the ventral skin with a much longer latency than to mechanical stimulation. However, the latency was reduced in stimuli of higher concentrations. When the ventral skin was exposed to 10 μM amiloride for 5 min, the neural response to NaCl was completely suppressed at 200 mM and significantly reduced to 72% at 300 mM. The effect of amiloride, together with the long latency of the response, suggests that neural excitation was induced by Na^+ flowing across the skin through amiloride-blockable channels. The ionic specificity of these channels seemed to be not very high, since the responses to KCl (at 200 and 300 mM) were also reduced by amiloride exposure. The results explain that avoidance behavior to NaCl solution (250 mM) in the desert toad is blocked by amiloride (10 μM) added to the solution.

O8. ADP receptor site in the sugar receptor cell of the fleshfly *Boettcherisca peregrina*

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In order to determine the structure–activity relationship of the adenine nucleotides in the labellar sugar receptor cell of the fleshfly, we tested the stimulatory effectiveness of ADP and other adenine nucleotides. The corresponding nucleotide receptor site was highly specific for ADP (the ADP site). While the insertion of a methylene group or an imido group between the β and γ phosphates of ATP did not affect the potency, the insertion of a methylene group between α and β phosphates resulted in almost complete loss of stimulatory activity. In these molecular characters, the ADP site resembled the nucleotide receptor found in cluicine mosquitoes.

We studied the effect of GTPγS, the activator of G protein, on the response of the sugar receptor cell. Although the responses to D7-glucose and L-Phe were significantly enhanced by introducing GTPgS into the sugar receptor cell, the response to ADP was not affected. This result suggested that the responses to D-glucose and L-Phe are mediated by the G protein-coupled transduction cascade, but that the response to ADP is mediated by some other transduction pathway.

O9. Differential screening and characterization of genes specifically expressed in taste sensory cells of *Drosophila*

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To identify molecules involved in the taste transduction of *Drosophila* we have developed a cloning strategy in which a cDNA library of enriched chemoreceptor-specific genes was constructed. Based on the morphology of the sensillum, the taste chemosensilla are considered to exist on the wing. We first examined whether the wing could be used as a starting material for the construction of a cDNA library. Using SEM, we observed a pore at the tip of the wing chemosensilla. The fluorescence dye, DAPI, was permeated from the pore of the wing sensilla and four nuclei of taste cells were identified. Electrophysiological recordings indicated that the wing chemosensilla respond to sugar. To construct a subtracted cDNA library of enriched taste-specific genes, the wings of two kinds of mutants were used. The *cut* mutant suffers a loss of mechanosensilla and the *poxn* mutant lacks chemosensilla. Using *cut* cDNAs from which *poxn* cDNAs were subtracted, a cDNA library was constructed. By combining the subtraction and differential screenings, we isolated 22 chemosensory cell-specific cDNA clones and mapped their genetic locations on polytene chromosomes. The expression pattern of isolated genes was determined by *in situ* hybridization to adult head sections and one clone was expressed in a labellum. Further studies on these clones may lead to the elucidation of the molecular mechanisms of taste transduction.

O10. Effect of toki-syakuyaku-san on olfactory disorders

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In a previous study, the authors demonstrated that bifemeramin hydrochloride (BH) was effective on hyposmia of central origin. Toki-shakuyaku-san (TSS) was also reported to be effective on Alzheimer's disease similar to BH. For the next step, the efficacy of TSS on olfactory disorders was examined in 19 patients. Since all the patients showed no pathological findings in the olfactory cleft in the fiberscopic observation or in the radiological examination, their smell disorders were diagnosed as being of central origin. Fourteen of them were initially unresponsive to conventional treatment such as intranasal administration of betamethasone sodium phosphate. They were treated with 5.0 or 7.5 g of TSS a day for >2 months. The improvement of symptoms was noted subjectively in 57.9% and confirmed by the T&T olfactometer in 47.3%. The background of the patients, including age, duration of morbidity and severity of damage, did not affect the recovery. However, none of the three anosmic patients demonstrated any recovery following treatment. These results were comparable to these of BH. TSS therapy appeared to be useful for olfactory disorders of central origin.

O12. Spontaneous improvement of sensorineural olfactory disorders

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Sensorineural olfactory disorders caused by viral infection or head injury are often severe and refractory to treatment. However, some patients with this disorder exhibit spontaneous improvement prior to initiation of treatment. We examined the frequency and some clinical features of cases of spontaneous improvement of this type.

At the Olfaction Clinic of Osaka City University Hospital, we treated 249 patients with sensorineural olfactory disorder (177 patients with viral infection and 72 patients with head injury) between January 1992 and December 1997. The number of patients with any incidence of spontaneous improvement was 51 (28.8%) with viral infection and 8 (11.1%) with head injury.

Among those with viral infection, the rate of spontaneous improvement was higher in younger than in older patients. Patients with spontaneous improvement had better olfactory acuity on T&T Olfactometry and a faster response to treatment than those without spontaneous improvement. Among those with viral infection, there was no significant difference in the improvement rate after treatment between patients with spontaneous improvement and those without it, while among those with head injury, patients with spontaneous improvement had a better improvement rate than those without it.

Spontaneous improvement of sensorineural olfactory disorder may be due to the regeneration of olfactory receptor cells. Our

findings therefore suggest a difference in pathology between viral infection and head injury.

O13. Use of Kanpo for gustatory and oral disorders

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Since many cases of spontaneously arising abnormal taste, burning mouth syndrome, xerostomia and stomatitis are idiopathic and refractory, treatment of patients with these symptoms is quite difficult. We used Kanpo medications to treat 61 patients with these disorders at Osaka City University Hospital Gustation Clinic between January 1992 and February 1997. The prescriptions used were determined based on the literature and differed depending on the symptoms.

The most common prescriptions were (number of cases, effectiveness rate) Sai-boku-tou (19, 40.0%), Shou-saiko-tou (13, 44.4%), Hochu-ekki-tou (13, 23.1%), Ouren-gedoku-tou (11, 50.0%), Hachimi-jiou-gan (10, 37.5%), Bakumondou-tou (6, 20.0%) and Hange-shashin-tou (6, 66.7%). Some patients exhibited a good recovery following treatment with these prescriptions. The theoretical basis of traditional Chinese medicine is quite different from that of modern Western medicine. In the former, symptoms are directly related to the method of treatment used or Kanpo prescription given, while in the latter an understanding of the pathology or etiology of symptoms is required for treatment. Kanpo treatment is therefore expected to provide good therapeutic results for these gustatory and oral symptoms. However, there are certain difficulties and unsolved problems on the use of Kanpo: (i) the diagnostic procedure 'Sho' is not familiar to physicians practicing modern Western medicine. (ii) These gustatory and oral symptoms are usually refractory since they arise from the constitution and lifestyle of patients. (iii) Psychogenic factors play important roles in patients with these symptoms. (iv) The interactions of Kanpo medicine with other therapeutic drugs are unclear.

O14. The gustatory changes associated with the menstrual cycle in females

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We performed a gustatory examination by asking about palatability changes in correlation with the menstrual cycle in 30 healthy females (mean age: 29.1 ± 5.6 years) with a regular menstrual cycle (28.7 ± 2.2 days). The investigation was done by conducting electro-gustometry (EGM) and by the filter paper disk technique during the follicular and luteal phases. The responses to our questionnaire revealed that appetite became excellent before menstruation in 14 cases (46.7%). The results of the EGM showed that the threshold in the domain of the chorda tympani nerve was 0.313.4 dB in the follicular phase and –0.912.8 dB in the luteal phase. Although the the electro-gustatory threshold in the luteal phase showed a statistically significant decrease ($P < 0.05$), the difference was so weak that the change could not be subjectively discriminated. The threshold in the domain of the glossopharyngeal nerve was 2.6 ± 4.0 dB in the follicular phase and 1.7 ± 3.9 dB in the luteal phase, and there was no significant difference between the two values. The results of the filter paper disk technique showed no significant difference in taste between the follicular phase and the luteal phase.

P1. Postnatal development of taste buds in circumvallate and foliate papillae in the rat

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Development of the foliate (FL) and circumvallate papillae (CV) and their taste buds at different postnatal ages was examined histologically in the rat. After paraffin embedding, complete serial sections of 10 μm thickness were made and stained by hematoxylin & eosin. Digitized images for each section were examined carefully. To examine the development of these papillae, we measured the number and depths of the grooves and distances between grooves in the FL and the width of the CV papilla at the head and depth of the grooves. Both the height × width (i.e. volume) and height/width (i.e. shape) of each taste bud were calculated. The results indicated that these FL papillae were almost mature at 3–4 weeks of age, whereas the CV continued to grow until week 9. For both papillae, taste bud distribution was limited to two-thirds of the papillary wall from the bottom of the groove. The height × width rapidly increased until 4 weeks of age. In contrast, the ratio of height/width in the CV was ~1.8 at 1–9 weeks of age, whereas that in the FL was 2.37 at 1 week and decreased to 1.57 at 4 weeks. These results suggest that the process of maturation of the taste bud varies in different areas of the oral cavity.

P2. Postnatal development and function of taste buds on the soft palate in the rat

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Taste bud distribution at different postnatal age on the soft palate and within three types of tongue papillae (fungiform, foliate and circumvallate) were examined histologically in the rat. After paraffin embedding, serial sections of 10 μm thickness were made and stained by hematoxylin & eosin. Digitized images for each section were examined carefully. The existence of a taste pore was used to identify mature taste buds. At birth, the mean number of taste buds on the soft palate (SP) was 126.7, 67.7 (52.9%) contained those with a taste pore. The mean number of taste buds within fungiform papillae (FP) at birth was 110.3, whereas only 13.7 (12.2%) contained a taste pore. The number of mature taste buds increased rapidly, with 90% of the SP and 80% of the CT taste buds containing taste pores after the first week. In contrast, no taste buds with pores were observed at birth within foliate and circumvallate papillae. The increase in the number of mature taste buds occurred later in these two latter types of papillae, with 70–80% of the taste buds contained pores at 2–3 weeks. These results suggest that at an early stage of development, taste buds

within the soft palate play an important role in the detection of nutrients in the neonatal rat.

P3. Mash1 is expressed in rat taste bud

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Mash1, a mammalian homologue of *Drosophila achaete-scute* proneural gene complex, plays an essential role in differentiation of subsets of penpheral neurons. Using RT-PCR and *in situ* hybridization, we investigated whether Mash1 gene expression occurs in rat taste buds. Further, we examined the dynamics of Mash1 expression in the process of degeneration and regeneration in rat denervated taste buds. The results indicate that Mash1 is expressed in cells of the taste bud cell lineage. In denervation experiments, Mash1-expressing cells constitute an early stage of progenitor (transit amplifying) cells in rat taste buds, but are not self-renewing stem cells. We conclude that the expression of Mash1 in rat taste buds is required for neural induction.

P4. Localization of gustducin and Gα14 in taste cells of the rat

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Gustducin and Gα14 are guanine nucleotide binding proteins (G proteins) that are believed to be involved in bitter and sweet taste transduction. Gustducin has a similar structure to transducin and is considered to stimulate phosphodiesterase. Gα14 belongs to the Gq subfamily and is considered to stimulate phopholipase C. Gustducin was present in type II cells, while Gα14 was not only in type II cells but also in taste bud cells containing large, dense-cored vesicles. In order to ascertain the ultrastructural localization of these G proteins within taste cells, we utilized post-embedding colloidal gold immunocytochemistry. Rats were perfusion-fixed with a mixture of 0.1% glutaraldehyde and 4% paraformaldehyde. Circumvallate papillae were dissected and immersed in 1% tannic acid and then in 1% uranyl acetate solution. After dehydration with graded series of ethanol, the specimens were embedded in epoxy resin. Ultrathin sections were reacted with rabbit anti-α gustducin or anti-Gα14 primary antibody, followed by reaction with 12 nm colloidal gold conjugated with goat anti-rabbit secondary antibody. The specimens were observed in a JEOL 200cx transmission electron microscope. The gustducin immunoreactivity was observed diffusely in the type II cells. The Gα14 immunoreactivity was observed in type II cells and in cells containing large, dense-cored vesicles. The microvilli of these immunopositive cells also exhibited specific immunoreactivity to both antisera.

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P5. Distribution of taste buds in *Brachiodanio reno*

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Zebrafish, for which basic information on the general development and genome is well-known, should be suitable for studies of taste transduction. Thus, as a first step, we tried morphologically to reveal the distribution of taste buds with scanning electron and light microscopes. Taste buds distribute not only in the oral cavity but also on the external skin surface, particluarly densely on the lips and the two pairs of barbels. The outer shape of the taste buds of the oral cavity are different to those of the outer skin surface. The former protrude from the epithelia, which may be caused by the thin epithelial layer. The height and maximum diameter of the taste buds are the same in both areas, and are rather smaller than those known in other fish. The reason for this smallness might be attributed to the body length of this species.

P6. ATP pyrophosphatase activity in the rabbit taste bud

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Previously, we demonstrated cytochemically an enzymatic activity which hydrolyzed an ATP analog, 5′-adenylyl-imidodiphosphate, in the microvilli of taste bud cells in the rabbit foliate papillae; we reported that this activity seemed to be that of ATP pyrophosphatase, which hydrolyzes ATP into AMP and PPi.

In the present study, we further examined the characteristics of this enzymatic activity using basically the same materials and method (enzyme cytochemistry) as before. The enzymatic activity was localized in the microvilli and the neck region of taste bud cells. The cytochemical reaction product was observed mostly on the plasma membrane. The activity was inhibited by 10 mM dithiothreitol and enhanced by 5 mM $CaCl_2$, suggesting that it was a Ca-dependent ATP pyrophosphatase activity. 20 mM NaN_3, an apyrase inhibitor, showed no inhibitory effect. The enzyme also hydrolyzed another ATP analog, β,γ-methylene adenosine 5′-triphosphate, but to a much lesser extent compared with the 5′-adenylylimidodiphosphate hydrolysis.

P7. Intrinsic membrane characteristics and responsibilities to GABA of neurons in the lateral and medial parts of the parabrachial nucleus

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Whole-cell current-clamp recordings were made from neurons in the parabrachial nucleus (PBN) in an *in vitro* brain slice preparation in rats. Neurons were divided into three groups based on the recording site: neurons in the dorsolateral part of the PBN (DL-neurons), neurons in the dorsomedial part of the PBN (DM-neurons) and neurons in the ventromedial part of the PBN (VM-neurons). The mean input resistances of the DL-neurons were significantly smaller than those of the other two groups. The

mean action potential durations of the VM-neurons were significantly longer than those of the other two groups. The discharge frequencies of the DL-neurons induced by depolarizing currrent pulses were significantly lower than those of the other two groups. These results demonstrate that neurons in different locations in the PBN have different membrane and repetitive discharge properties. Superfusion of GABA resulted in a concentration-dependent reduction in input resistance in 67.5% of the neurons in the PBN. The effect of GABA was partly or completely blocked by the $GABA_A$ antagonist bicuculline in all neurons tested. Superfusion of $GABA_A$ agonist muscimol induced a decrease in the input resistance of all the neurons tested. It is considered that GABA works as a neurotransmitter in both the gustatory and the visceral parts of the PBN, at least, by way of $GABA_A$ receptors.

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P8. Taste rejection behavior and salivation after parabrachial lesions in rats

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The taste rejection behavior induced by oral injection of quinine-HCl is always accompanied by vigorous salivation. In the present study, we examined the role of the parabrachial nucleus (PB) in salivation by immunohistochemical and behavioral studies in rats. The following results were obtained: (i) immunohistochemical study revealed that oral injection of quinine induced the c-fos-positive neurons mainly in the so-called PB taste area (the medial part of the PB), the ventral part of the PB and the lateral part of the PB; (ii) when an electrolytic lesion was made in each PB area, the salivation during the taste rejection behavior almost disappeared by destruction of either the medial or the ventral part of the PB; (iii) destruction of the medial and ventral parts of the PB reduced the number of gapings (one of the taste rejection behaviors) by ~80 and 50% respectively; and (iv) destruction of the lateral part of the PB did not change the salivation, but reduced the number of gapings by ~40%. These results suggest that the unpalatable taste information related to salivation reached to the salivary center via the so-called PB taste area and ventral part of the PB.

P9. Licking behavior in parabrachial-lesioned rats

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The role of taste areas of the parabrachial nucleus (PBN) in the regulation of licking behavior was evaluated in laryngeal-deafferented rats. The lateral division of the PBN responsive mainly to sodium chloride (NaCl) and hydrochloric acid (HCl) was bilaterally lesioned in one group (PBN-l), while it was kept intact in the other group (PBN-i). Five bottles filled with distilled water (DW), 0.5 M sucrose (Suc), 0.5 M NaCl, 0.03 M HCl and 0.01 M quinine hydrochloride (QHCl) were used for testing. The number of licks and interlick intervals were measured during each test period of 1 min. The PBN-l as well as the PBN-i rats licked DW and Suc in a successive and regular pattern with high frequency. These two groups licked NaCl, HCl and QHCl in an intermittent and irregular pattern. The PBN-l tended to lick NaCl and QHCl more frequently than the PBN-i. The average interlick interval of the PBN-l for NaCl was shorter than that of the PBN-i. These results suggest that the lateral division of PBN plays a role in regulation of licking behavior guided by taste signals, especially of NaCl, from the oral cavity and pharynx.

P10. Cytoarchitecture and projections of the facial lobe in a teleost, *Plotosus lineatus*

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The primary taste center of the facial taste system, the facial lobe (FL), of *Plotosus* has a distinct somatotopical map. The entire body surface of the catfish is represented in the FL, and the barbels and trunk-tail are sharply defined in the lobule structures extending caudorostrally in the FL. Both small and large neurons are present in the lobules. The FL sends fibers to the superior (nGS) and inferior (nOl) secondary gustatory nuclei as well as other regions in the brainstem. The present study was undertaken to reveal the cell type of the projecting neurons to the two nuclei, their distribution in the lobule and the patterns of the projections from each lobule to the nGS.

To locate ascending and descending second neurons in the lobule, DiI was applied to the ascending secondary tract or descending secondary tract in the fixed brains. To examine the projections from the FL to nGS, DiI was also applied selectively to a single lobule.

Application of DiI to the ascending secondary gustatory tract labeled the large neurons. They were scattered throughout the lobules. Large neurons were also labeled after application of DiI to the descending secondary gustatory tract. They were distributed in the peripheral portion of the lobule rather than its center. These large neurons are much smaller in number than the ascending neurons. The application of DiI to a barbel or trunk-tail lobule labeled the entire nGS bilaterally, suggesting the convergent representation within the nGS of *Plotosus* from the highly specific somatotopic maps in the FL.

P11. 6-Hydroxydopamine-induced lesions of the midbrain ventral tegmental area reduce the consumption of palatable solutions

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In previous studies, we have found that electrolytic lesions of the midbrain ventral tegmental area (VTA) disrupt the normal intake of palatable solutions. To further clarify the possible involvement of the mesolimbic dopamine system in palatability, we examined the effects of 6-hydroxydopamine-induced lesions of the VTA on taste-guided behaviors in rats. (i) Twenty-four-hour two-bottle preference tests revealed that lesioned animals drank significantly more sucrose (0.1 M) than controls. The consumption of other solutions (NaCl, HCl and quinine hydrochloride) was not different between lesioned and control animals. (ii) When body sodium was depleted by injections of a natriuretic drug, furosemide, lesioned

animals consumed significantly less NaCl than controls. (iii) Injection of midazolam, a benzodiazepine agonist, or morphine 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 lesioned animals. Neither midazolam nor morphine modified intake of an unpalatable quinine solution in both lesioned and control animals. These results suggest that dopaminergic neurons in the VTA are important for hedonically positive responses to palatable taste stimuli.

P12. Disruptions of acquisition and retention of conditioned taste aversion by infusions of glutamate receptor antagonists into the rat amygdala

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To elucidate the possible involvement of the amygdala (AMY) in conditioned taste aversions (CTAs), the effects of reversible blockades of glutaminergic transmission by selective glutamate receptor antagonists on CTA formations were investigated. Soon after Wistar rats ingested 0.005 M Na-saccharin (conditioned stimulus, CS), they received bilateral intra-amygdala infusions of glutamate receptor antagonists: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) as an AMPA receptor antagonist, D-2-amino-5-phosphonovalerate (D7-APV) as an NMDA receptor antagonist and (+)-a-methyl-4-carboxyphenylglycine (MCPG) as a metabotropic glutamate receptor (mGluR) antagonist. With a 30 min interval, they received an i.p. injection of 0.15 M LiCl (unconditioned stimulus, US). The injections of CNQX, D-APV or MCPG produced significant impairments in the acquisition of CTA. In contrast, infusions of CNQX into the vicinity of the AMY showed no effect. To study their effects on the retentions of CTAs, each antagonist was infused into the AMY 20 min before the first retention tests. Retrievals of CTAs were disrupted by the infusions of CNQX, not by those of D-APV or MCPG. These results suggest that NMDA receptors- or mGluRs-dependent neuronal plasticity in the AMY are the underlying mechanism in the association of the CS with the US. It is also indicated that the neural pathways responsible for retrievals of CTAs are mediated by the amygdaloid AMPA receptors but not by its NMDA receptors nor by mGluRs.

P13. Effect of pungent stimulation within the oral cavity or intragastric administration of capsaicin on NaCl and KCl solution preference in SD rats

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We have previously shown that a capsaicin-supplemented diet and intragastric administration of capsaicin cause a decrease of salt preference in SD rats. However, it is not known whether the pungency of capsaicin is itself sufficient to produce an NaCl-preference-lowering effect of capsaicin. The present study was undertaken to clarify the effect of pungent stimulation by applying capsaicin to the oral cavity on NaCl or KCl preference in SD rats.

Fifteen male rats were divided into three groups (five rats each). The control group was administered the solvent (6% HCO-60 aqueous solution) intragastrically. In the capsaicin-stimulated group, the swab with 140 ppm of capsaicin was held in the mouth for 10 s after the solvent administration, and in the capsaicin-administered group, 3000 ppm of capsaicin in HCO-60 solution was administered intragastrically. These daily treatments were done for 10 days. The four bottle preference tests were performed during the 10 days [water, and 86, 154 and 239 mM solutions of NaCl (Exp.1) or KCl (Exp. 2)].

The NaCl preference was decreased in both of the capsaicin-treated groups (pungent stimulated and intragastric administered) compared with the solvent control group, whereas the KCl preference was increased in the pungent stimulated group only. The present experiment revealed for the first time that oral pungency (for 10 s stimulation a day) itself has a reducing effect of NaCl preference and an increasing effect of KCl preference, though further detailed studies should be done to clarify this mechanism.

P14. Synergistic umami responses in cortical taste neurons in rats

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Synergistic responses of sucrose-sensitive neurons to a mixture of two representative umami-substances, Na-glutamate (MSG) and 5'-guanyl monophosphate (5'-GMP), have been reported in the chorda tympani and brainstem nuclei of rats. However, cortical responses to the mixture have not been examined yet. In this study, we examined whether the responses of cortical taste neurons were synergistic compared with those to 0.3% MSG in rats fed with usual laboratory chow in which nutrients are not well prepared. We recorded single cortical taste neurons in urethane-anesthetized adult SD rats. In several neurons, MSG was more effective than the four basic tastes (0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl and 0.02 M quinine-HCl). No synergistic response was seen in any best-stimulus category of cortical neurons. Cluster analysis showed that neurons predominantly sensitive to umami substances did not belong to the same group as sucrose-best neurons. It is evident that the synergistic umami responses were not transmitted from the brainstem to the cortex in these rats. This may be attributed either to the nutritional state of the animal, as suggested by behavioral studies, or to neural interactions of taste information in the thalamus or cortex. It remains to be studied whether good nutritional states might disclose synergistic umami responses in sucrose-best cortical neurons. This study is now in progress in our laboratory.

P15. Effects of cold exposure on taste responses in rats

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The effects of cold (4°C) ambient temperature on the taste responses of the chorda tympani nerve to various taste stimuli were examined in rats. The chorda tympani nerve response to

NaCl was enhanced when the animals were exposed to cold for 7 days. In contrast, the nerve responses to sucrose, HCl and quinine-HCl in cold-exposed rats were comparable with those in rats maintained at room temperature (24°C). The enhancement of the chorda tympani nerve response to high concentrations of NaCl (>0.01 M) became remarkable depending on the duration of the cold exposure. The results of the behavioral study with two-bottle preference test showed that the rats exposed to a cold environment refused to drink NaCl solutions at 0.05 and 0.1 M, although the control rats preferred those solutions. The preference ratio at 0.5 M NaCl was identical in the control and cold-exposed rats. These results suggest that the ambient temperature influences taste cell function, and the enhanced NaCl response of the chorda tympani nerve may relate with avoidance of NaCl intake in the cold environment.

P16. Response characteristics of laryngeal taste nerves in the rat and rabbit

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To elucidate the mechanism of taste reception of alcohol, particularly in the larynx, we examined the reponsiveness of the superior laryngeal nerve (SLN) to several kinds of alcohols. Afferent neural activity was recorded from the SLN of urethane-anesthetized rats and rabbits. Test solutions of alcohol were ethanol, ethylene glycol, 1-propanol, propylene glycol, glycerine and 1-butanol. These solutions were applied to the laryngeal surface of the epiglottis. After application of test solutions for a period of 20–30 s, the surface of the epiglottis was rinsed with the solution of 0.15 M NaCl. The solution of 1 M ethanol elicited a remarkable response; however, ethylene glycol (1 M) and propylene glycol (1 M) did not produce conspicuous responses. Glycerine (1 M) and butanol (0.7 M) strongly depressed the spontaneous activity of the nerve. These results suggest that the magnitude of response to alcohol in the SLN is inversely related to the number of hydroxyl groups and the length of carbon chain of alcohol. These characteristics of taste receptors in the larynx are profoundly different from the response properties of the tongue receptors, because recently it has been reported that the tongue receptors respond only to alcohols which have two or three hydroxyl groups, and that the magnitude of response does not depend on the length of the carbon chain of the alcohol.

P17. Analysis of single glossopharyngeal taste fiber responses to various taste stimuli in the aquatic toad, *Xenopus laevis*

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We recorded the responses of 51 taste fibers in the glossopharyngeal nerve of the aquatic toad, *Xenopus laevis*, during taste stimulation with 20 stimuli. Responses were quantified as the number of impulses evoked at the first 5 s after stimulation. The average of spontaneous activity of 20 amino acid-responsive fibers most sensitive to L-proline was <1 spike/5 s (0.00 ± 0.00 SD), and the average response of L-proline was 83.5 ± 30.35 spikes/5 s.

Hierarchical cluster analysis of 51 fibers for the four different stimuli (L-Pro, HCl, Q-HCl, $CaCl_2$ and galactose) identified one large and two small groups of units. The largest group was dominated by 34 fibers stimulated best by L-Pro and HCl. One small group comprising nine fibers was stimulated considerably by Q-HCl and HCl. The other small group, comprising eight fibers, was stimulated by $CaCl_2$ alone.

The clusters identified from 35 fibers for amino acids included a large group of 34 fibers and one separate fiber. The large group was divided into four subgroups; L-tryptophan/L-tyrosine, L-valine, L-tyrosine/L-valine, and L-tyrosine subgroups. The most effective stimulus to the remaining single neuron was L-arginine.

Hierarchical cluster analysis for the 18 basic taste stimuli identified four major clusters, amino acids-acid (n = 10), alkaloid (n = 3), salt (n = 4) and $CaCl_2$ (n = i) that was completely independent of other stimuli.

P18. Amiloride does not affect the taste responses of the frog glossopharyngeal nerve and submandibular branch of the facial nerve to NaCl

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Amiloride-blockable sodium ion channels have been found in isolated frog taste cells. Several researchers have reported that the summated response of the frog glossopharyngeal nerve to NaCl was reduced by amiloride. Since the sensitivity of the frog glossopharyngeal nerve to chemical stimuli tends to wane with repeated applications of stimulating solution to the tongue, the reduction of the response to NaCl in the frog glossopharyngeal nerve by amiloride might be due not to amiloride but to fatiguing of receptors. Thus, we carefully examined whether amiloride affected the response of the frog glossopharyngeal nerve and submandibular branch of the facial nerve to NaCl. The frogs were anesthetized with urethane. We recorded the response to NaCl from the whole nerve, and also recorded unitary discharges from single fibers. Amiloride at 0.5 mM did not affect the response to NaCl at concentrations of 0.1–0.5 M. In another experiment, a solution of 0.5 M NaCl was applied to the tongue for 20 s, followed by a mixture of 0.5 M NaCl + 0.5 mM amiloride. The tonic response to prolonged stimulation with NaCl was not affected by addition of amiloride. We concluded that amiloride-blockable sodium ion channels in the apical membrane of taste cells are not involved in salt taste reception in the frog.

P19. Responses of the superior laryngeal nerve to water and NaCl stimulation of the larynx in STR/N mice

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Responsiveness of the superior laygneal (SL) nerve in polydipsic inbred mice (STR/N mice) to water and NaCl applied to the pharyngolaryngeal area were investigated. The taste stimuli used were seven concentrations of NaCl (0.01–1.0 M) and deionized

water. Saline (0.15 M NaCl) was used as a rinse between stimulations. Both integrated and rate (spikes/s) responses were obtained by whole nerve recording. The spontaneous rates in STR/N mice (314.2 ± 43.0 spikes/s, $n = 9$) were significantly lower (two-tailed t-test, $P < 0.05$) compared with those in ICR mice (control) (539.8 ± 79.6 spikes/s, $n = 10$). The responses to water and the concentration–response functions for NaCl between STR/N and ICR mice showed no significant difference when the responses were measured as deviations above or below the spontaneous rate. However, when the responses were derived by subtracting the responses to 0.15 M NaCl, which is considered as a tactile response to mechanical delivery of the solution, the concentration–response functions for NaCl were significantly different between STR/N and ICR mice [$F(1,17) = 4.813$, $P = 0.042$]. This difference was also shown using the data obtained from the integrated response recording. There is the possibility that the characteristic neural activities in the SL for STR/N mice may have some relation to the polydipsia in this strain.

P20. Responses of the glossopharyngeal nerve to binary taste mixtures in the clawed toad, *Xenopus laevis*

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An interaction of binary taste mixtures with oral chemoreceptors was examined recording the neural activity of the glossopharyngeal nerve of the clawed toad, *Xenopus laevis*. The neural activity decreased with an increase in HCl concentration when L-proline was added to 1–5 mM HCl in L-proline–HCl mixtures. At each concentration of HCl the response to7 L-proline–HCl mixtures was smaller than the response to L-proline alone. This inhibition of the response to L-proline occurred regardless of the L-proline concentration used.

Sirnilar inhibitions of the binary mixtures were also observed in Q-HCl–HCl mixtures including 0.5–5 mM HCl or in L-proline–Q-HCl mixtures including 1 nM–0.1 mM Q-HCl.

The neural activity elicited by 5′-guanylate (5′-GMP)-L-tyrosine mixtures was greater than the activity elicited by the component stimulus presented alone. However, the inhibition of the response to 5′-GMP was observed when 5′-GMP was added to 1 nM–0.Ot mM L-arginine in 5′-GMP7-L-arginine mixtures. The results demonstrate that gustatory mixture interaction in binary mixtures may occur at the level of the taste receptors or peripheral nerve.

P21. Effects of pH on chorda tympani nerve and behavioral responses in rats

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While sour taste is essentially dependent on H^+, it has been suggested that the accompanying anion also influences taste nerve responses. In the present study, we examined the effects of pH on chorda tympani nerve response and behavioral response in rats. When the pH of the acid solutions was adjusted to pH 3, chorda tympani nerve responses varied considerably, confirming the modulatory effects of anion for sensing acid. The order of magnitude of the nerve responses was acetic acid >> ascorbic acid ≥ citric acid = lactic acid > HCl, and the response to acetic acid was ~10 times larger than that to HCl. The behavioral response to each acid solution, which was assessed by a two-bottle preference test, was fundamentally coincident with the chorda tympani nerve response in its threshold concentration and magnitude of response. These results indicate that anion in acid solution contributes not only to the taste nerve responses but also to behavioral refusal of acids. It is also suggested that the chorda tympani nerve plays a pivotal role in the refusal of acids by rats. It should be noted, however, that this suggestion does not exclude the possible contribution of other nerves, including the glossopharyngeal nerve and the trigeminal nerve. Further study is needed to evaluate the relative importance of these nerves in acid sensing.

P22. Ibuprofen can modify responses of rat gustatory nerve fibers to NaCl

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Single- and multi-fiber preparations of the rat chorda tympani (CT) nerve were used to study the effects of ibuprofen, a non-steroidal anti-inflammatory drug, to salt-taste transduction. 10–100 mM ibuprofen was suspended in borate–Na_2CO_3 buffer (pH 7.5) or dissolved in the same buffer at pH 10.0 (ibuprofen–sodium salt). The former suspension produced a tingling sensation on the tongue and a strong stinging sensation on the throat with some time lag after its application, but no irritation was shown by the latter solution. The response of all multi-fiber preparations of chorda tympani nerve to 100 mM NaCl were suppressed by ibuprofen suspension (pH 7.5). The suppressive effect appeared 10–20 s after the simultaneous application of 10–100 mM ibuprofen–100 mM NaCl, and it was significantly stronger at 100 mM ibuprofen than at 10 mM. On the other hand, ibuprofen–sodium salt solution (pH 10.0) had little or no effect on CT nerve response to NaCl. From these results, it is suggested that there is a interrelationship between the oral-irritation and the taste-suppressing effect evoked by ibuprofen.

P23. Temperature dependency of chorda tympani responses and synergistic effects of umami taste in the rat

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It is known that strong synergism occurs between umami substances, such as monopotassium glutamate (MPG) and inosine 5′-monophosphate (IMP), in several animals. In the present study, to study temperature dependency of chorda tympani responses to umami and their mixture solutions, the neural responses were recorded in Wister rats. 1×10^{-3}–1.0 M MPG, 1×10^{-4}–1.0 M IMP and their mixture solutions at 5, 25 and 45°C were applied to the tongue after its adaptation for 1 min at all concentrations. The order of magnitude of responses were 45°C > 25°C > 5°C. On the other hand, the order of synergistic mixture effects expressed by

potentiation ratios (= magnitude of the response to the mixture solution/sum of the magnitude of the response to individual components in the mixture) were 5°C > 25°C > 45°C. These results suggest that synergistic mixture effects between umami substances are affected by the temperature of their solutions.

P24. Electrophysiological responses to denatonium stimulation in isolated mouse taste cells

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It has been considered that perception of the bitter substance denatonium involves two different transduction pathways in taste cells. One is an activation of phospholipase C increasing the concentration of intracellular IP_3, the other is activation of gustducin and/or transducin decreasing the concentration of intracellular cyclic nucleotides. We investigated the electrophysiological responses of denatonium in mouse isolated taste cells by the whole-cell patch clamp technique, and examined whether those two different pathways function independently or not.

Taste cells were isolated from 8-week-old female mice (C57BL/6J) by enzyme treatment. 1 mM denatonium dissolved in Ringer solution was applied to the taste cells by pressure ejection from capillary glass.

In the voltage-clamp mode (holding potential –80 mV) using a normal pipette solution, denatonium induced outward current responses, and these responses were detected repeatedly. Denatonium activated or inactivated a voltage-dependent potassium current in the voltage-step from –80 mV. 8-Br-cGMP and the phospholipase C inhibitor U73122 did not affect the responsive outward currents when they were applied separately. However, when both 8-Br-cGMP and U73122 were applied, the outward current was observed only in the period just after the establishment of the whole cell configuration, but not in the repeated stimulation. These results suggest that two different mechanisms of denatonium transduction function cooperatively in mouse taste cells.

P25. Intracellular calcium concentration changes of mouse taste cells to monosodium glutamate analogues recorded by optical imaging

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Monosodium glutamate (MSG) elicits a unique taste called umami. In this study, fluorescent dyes and digital imaging techniques were used to record the responses of vallate and foliate taste cells from C57BL/6J mice to MSG (10 mM), the metabotropic receptor agonist L-AP4 (1 mM) and the ionotropic receptor agonist, NMDA (1 mM). Cells in isolated taste buds were stained with the Ca^{2+}-sensitive dye, fura-2. Of the 296 cells examined, ~65% responded to glutamate of the other glutamate receptor agonists. Increases in $[Ca]_i$ were observed in 23% of the cells (46/202) stimulated with MSG, 38% of the cells (74/194) stimulated with L-AP4 and 14% of the cells (7/50) stimulated with NMDA. We also examined the effect of the K channel blocker, tetraethyl ammonium (TEA), on taste cells responses. TEA markedly reduced the number of taste cells showing increases in [Ca] in response to MSG (5168 cells) and L-AP4 (4/68 cells). These results are consistent with previous patch-clamp data, suggesting that MSG activates several different mechanisms in taste cells, including processes mediated by ionotropic and metabotropic types of receptors.

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P26. Possible transduction pathways involved in umami perception in mouse taste cells

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To elucidate the taste receptor mechanisms for umami, we examined the responses of isolated C57BL/6J mouse taste cells to monosodium glutamate (MSG) or a potent agonist of metabotropic glutamate receptor 4, L-AP4 (2-amino-4-phosphonobutyrate), by using the whole-cell patch-clamp method. Under voltage clamp (holding potential –80 mV) with a KCl-containing pipette, MSG (10 mM) elicited both inward currents with an increase in membrane conductance and outward currents with a decrease in membrane conductance. L-AP4 (1 mM) elicited only outward currents with a decrease in membrane conductance. With an NMDG-containing pipette in an NMDG–TEA bath solution, MSG elicited a transient inward current response and an outward current response, and L-AP4 elicited only the outward current response. And with a K-gluconate- containing pipette in an NMDG–gluconate bath solution, MSG elicited two kinds of inward current responses (sustained, transient inward) while L-AP4 elicited only the outward current response. These results suggest that MSG perception involves at least three types of mechanisms: one is mediated by the metabotropic glutamate receptor 4 that decreases the membrane conductance and the others are mediated by other receptor types that increase the membrane conductance.

P27. Mechanism for the specific recognition of amino acid ligands by the chemoreceptors of *Escherichia coli*

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Escherichia coli has four closely related transmembrane receptors responsible for chemotaxis to a variety of chemicals. Among them, Tar and Tsr specifically detect aspartate and serine, respectively, and so are ideal for studying the mechanism underlying subtle molecular recognition. Crystallographic studies on an extracellular fragment of Tar have identified several aspartate-binding residues. Most of them are conserved in Tsr but two are not conserved. We exchanged these two non-conserved residues between Tar and Tsr individually or in combination, but none of the resulting mutant Tar or Tsr receptors showed altered ligand specificity, suggesting that some other residues may determine

ligand specificity without directly interacting with the ligand. A chimeric receptor Tsasr-61–90, in which residues 61–90 of Tsr were replaced by the corresponding residues of Tar, sensed aspartate but did not sense serine at all. Random exchange of residues 61–90 of Tsr with the corresponding residues of Tar identified six residues important, but not always required, for discrimination of aspartate from serine. These residues seem to participate in interaction between α-helices from different subunits of the Tar homodimer, which may be critical for the three-dimensional arrangement of the ligand-binding residues.

P28. Distributions of voltage-gated channels in mouse taste buds

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We investigated the distributions of voltage-gated channels in single fungiform taste buds by recording voltage-clamp currents from non-isolated taste bud cells under in-situ tight-seal recording conditions. Each taste bud was classified into four concentric circles with the same width, Divisions I, II, III and IV, from the center to the periphery of taste buds. The mean of pooled TTX-sensitive inward currents was, in decreasing order, D-II > D-III > D-I > D-IV. The difference between D-II and D-IV was significant (one-way ANOVA, Scheffé's multiple comparison, $P < 0.01$). The same order was found in the mean of pooled TEA-sensitive outward currents. The difference between D-II and D-IV was also significant ($P < 0.05$). There was no significant difference in the mean of inward rectifier currents. These circular distributions of voltage-gated Na and K currents indicate that the number or characteristics of voltage-gated Na and K channels are different among concentric circles. Since taste bud cells of mammals are short-lived (~10 days) and are suggested to be produced by multiple stem cells, taste buds consist of cells of different ages and lineage. The present results suggest that there is a mechanism for the controlled distributions of the varieties of taste bud cells.

P29. Recovery of bullfrog taste responses by NH_3- and CO_2-induced suppression

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We investigated the suppressive effect of CO_2 and NH_3 on the taste nerve responses of bullfrogs to 0.1 mM quinine. $NaHCO_3$ and NH_4Cl were used to yield CO_2 and NH_3 respectively. The responses were decreased as a function of CO_2 and NH_3 concentration calculated with the Henderson–Hasselbalch equation. These results indicate that CO_2 and NH_3 are responsible for the suppression, whereas other forms, such as HCO_3^- or NH_4^+, are ineffective. Since CO_2-induced acidosis and NH_3-induced alkalosis of taste cells or taste nerve endings appear to be responsible for the suppressive effect, we investigated the antagonism between these suppressive effects. The irrigation of the lingual artery with NH_3 reversibly suppressed the quinine responses. The suppressed responses were recovered with the surface application of CO_2 for 4 s prior to 0.1 mM quinine. The recovering effect was decreased with the longer application of CO_2. It is likely that the diffusion of CO_2 neutralizes the intracellular solutions of taste cells or taste nerve endings to recover the responses, and the longer application acidifies inside taste cells to suppress the quinine responses. The time-dependent recovery suggests that the neutralization of taste cells at a point or layer is critical in taste transduction.

P30. Changes in cAMP and IP_3 mass levels of the fungiform papilla in response to umami substances in mice

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Transduction mechanisms for umami have recently been proposed to involve metabotropic glutamate receptor 4 and a decrease in cyclic adenosine monophosphate (cAMP) levels in the circumvallate and foliate papilla in rats. We tested this hypothesis using taste buds of the fungiform papilla in C57BL mice *in vivo*. Within ~10 s after the onset of taste stimulation, each fungiform papilla was removed with fine forceps to pool 60 fungiform papillae in each sample. Mass levels of both cAMP and inositol 1,4,5-triphosphate (IP_3) in each tissue pool were measured by radiobinding assay kits. The levels of cAMP in the fungiform papilla significantly increased with 0.5 M sucrose, 0.1 M MSG and 0.5 mM IMP; and those of IP_3 in the taste tissue increased with 0.03 and 0.1 M MSG and 0.5 mM IMP. Sodium chloride at 0.1 M elicited no significant changes in cAMP and IP_3 levels of the fungiform papilla. Importantly, increases in cAMP and IP_3 levels of the fungiform papilla elicited by 0.03 M MSG + 0.5 mM IMP were larger than the sum of the increases to each component of the mixture. These results suggest that both cAMP and IP_3 may be involved in the transduction for umami in the fungiform papilla in mice.

P31. Comparison of a GTPγS-induced current and a cGMP-induced current in frog taste cells

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GTP-binding protein may be involved in the taste transduction mechanism. In frog taste cells, we compared the characteristics of the conductances activated by dialysis of GTPγS (0.5 mM) and 8-Br-cGMP (1 mM). In the conventional whole-cell clamp method with internal 104 mM Cl^-, GTPγS dialysis induced fast transient and slow sustained currents in 6/16 rod cells. Magnitudes of fast and slow components were –127 ± 26 pA and –238 ± 35 pA (mean ± SE, $n = 6$), respectively, at a membrane potential of –50 mV. When internal Cl was reduced from 104 to 10 mM, the slow component disappeared, but the fast one was still observed. Forked cells displayed the enhancement of a Ba^{2+}-sensitive outward current in 11/19 cells in response to GTPγS dialysis. Intracellular dialysis with 8-Br-cGMP in 104 mM Cl^- induced an inward current of–140 ± 20 pA ($n = 21$) at a membrane potential of –50 mV in rod cells. The cGMP-induced current was observed in 47% of rod cells, but not in forked cells. It was inhibited by

reducing intracellular Cl^- from 104 to 10 mM, but not by eliminating external Na^+. The slow component of GTPγS-induced current may be mediated by guanylate cyclase, but the fast component is mediated by an unknown mechanism. The membrane property of rod cells in response to GTPγS and cGMP was different from that of forked cells, suggesting that two types of taste cells play differential roles in taste transduction.

P32. Sour taste transduction mechanism of mouse non-dissociated taste cells

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We examined acid-induced responses in non-dissociated taste cells of mouse fungiform papillae using the whole-cell clamp technique and a localized taste stimulation method. When taste cells were adapted to Na^+-free Tyrode solution, 36% of cells responded to both 0.5 M NaCl and 25 mM citric acid with depolarizations under current clamp or with inward currents under voltage clamp. In five taste cells which responded to both salt and acid stimuli, 5 μM amiloride suppressed 80% of salt-induced responses but no acid-induced responses. In addition, the I–V relationships of acid-induced current responses showed an outward-rectifying property, while the I–V relationships of most salt-induced responses showed an inward-rectifying property. The results suggest that the mechanism for the sour taste transduction is different from that for the salty taste transduction.

On the other hand, we found that a Cl^- channel blocker, NPPB, greatly suppressed acid-induced responses and the E_r of NPPB-sensitive current of acid-induced responses was close to the E_{Cl}, indicating contribution of Cl channels to sour taste transduction. However, the E_r of the total current responses induced by acid was different from those of the NPPB-sensitive current or the E_{Cl}, suggesting that multiple mechanisms are involved in the sour taste transduction.

P33. A sugar taste receptor hidden in the proboscis of the swallowtail butterfly

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We have been studying the perception of sugars in the *Papilio* butterflies. From experiments on feeding behavior (drinking droplets of sugar solutions through the proboscis), feeding thresholds were found to be sucrose < fructose < glucose. It was also supposed that starch antagonistically binds to the sucrose receptor, because starch itself did not induce the feeding behavior but increased the feeding threshold for sucrose when mixed with sucrose. Hence, a sugar receptor protein was searched for by affinity electrophoresis using starch as the affinity ligand. A putative sugar receptor protein, whose migration was retarded by starch, was detected in the proboscis extract. Moreover, we discovered several types of uniporous sensilla lining both sides of the inside wall of the proboscis. Some of them showed a less adopting electrophysiological response to sugars. In *Papilio* butterflies, then, the sugar taste is perceived by the sugar sensitive cells in these sensilla hidden in the proboscis.

P34. The molecular mechanism of the taste enhancement effect of umami substances in the fly

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Umami is regarded as the fifth basic taste, having an independent quality of sweet, salty, sour and bitter. Umami is not a very impressive taste by itself but is conspicuous in synergy with tastes of ingredients of foods. Monosodium glutamate (MSG), a representative umami substance, shows a remarkable taste-enhancing effect. This is a highlight of taste synergism in humans, and the mechanism of action has been discussed. In the blowfly, MSG enhances the sugar taste via a particular receptor protein. We proved that the affinity of the sugar-binding region of a putative sugar receptor protein in the fly was increased by MSG. This mechanism consistently explained the behavioral aspect on the taste-enhancing effects of MSG. The taste-enhancing mechanism of umami substances should be different in detail between the human and the fly, but it could be considered generally that a taste-enhancing substance interacts with a particular taste receptor protein to improve its tastant-binding ability.

P35. Sugar-induced inward currents at the sensory dendrites of the fleshfly recorded with the patch-clamp technique

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We studied the transduction mechanisms in the taste cells of the fleshfly *Boettcherisca peregrina* with patch-clamp techniques.

When taste hairs were cut and incubated for 6–18 h in fly Ringer solution, it was possible to make a gigaohm-seal and rupture the patch membrane leading to the whole-cell clamp with a patch pipette onto the tip of the sensory dendrites.

When 0.1 M sucrose was applied to the sensory cilia for 0.6 s from a puff pipette under a cell attached mode at its tip, eight impulses were observed to come backward along the sensory process distally to the tip. Under a whole-cell voltage clamp configuration at –60 mV, inward currents of 10 pA were induced. As the inward current increased, the noise level increased. These increments were suppressed relatively slowly after stimuli. We never observed the induced currents at more positive holding potentials. To estimate single transduction channel behavior, we applied noise analysis for the inward current fluctuations. The noise currents had the fast kinetics of the transduction channels, and single channel current amplitudes were estimated to be ~0.1 pA.

P36. Effects of xanthine derivatives on the salt receptor cell of the fleshfly, *Boettcherisca peregrina*

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We studied the effects of xanthine derivatives on the salt receptor cell of the fleshfly. 1 M NaCl mixed with 10 mM caffeine or 10 mM theophylline activated the response of the salt receptor cell compared with the response to 1 M NaCl. In contrast, 1 M NaCl mixed with 5 mM 3-isobutyl-l-methylxanthine (IBMX), a phosphodiesterase (PDE) inhibitor, suppressed the response of the receptor cell. In addition, the effects of ATP were studied. ATP dissolved in 1 M NaCl had no effect on the response of the salt receptor cell at concentrations of 1 and 5 mM.

In order to explain the difference between the effects of theophylline and IBMX, both of which are known as PDE inhibitors, we propose a working hypothesis that (i) methylxanthine binding sites exist on the outside of salt receptor cells and when they are occupied with methylxanthine the salt response is are enhanced and (ii) IBMX can penetrate the cell membrane and inhibit PDE, which causes the inhibition of the response.

Purinoceptor may be responsible for the enhancement of the response of the salt reseptor cell by caffeine amd theophylline. To clarify the hypothesis, the effects of nucleotides on the salt receptor need to be resolved.

P37. Ion selectivity of the transduction current induced by sucrose stimulation to the cell of the fleshfly

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The transduction channel for sugar taste on the fleshfly, *Boettcherisca pergina*, has been inferred to be a non-selective cation channel with a reversal potential of ~0 mV from the finding that the driving force of this channel is supplied mainly by trans-epithelial voltage and Na^+ and K^+ have enhancing effects on the sugar response.

We studied the effect of replacing Na^+ with an large organic ion triethanolamine$^+$ (TA^+) in a sucrose solution for stimulation to test this hypothesis.

Comparing the relations between sucrose concentration, response frequency and variance of transduction current induced by sucrose in Na-citrate solution or TA-citrate solution, it was revealed that (i) the open probability of the transduction channel is lowered by adding TA^+; (ii) the apparent single channel current is lowered too; and (iii) the time constant of autocorrelation functions is increased by replacing Na^+ with TA^+.

The result (ii) means that TA^+, which has a large molecular radius, is not less permeable to this transduction channel than Na^+. The result (iii) suggests that TA^+ might block this channel. These two results support our non-selective cation channel hypothesis.

P38. Sweet and sweetness-inducing activities of strogin and curculin

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In a previous study we isolated five new oleanane-type triterpene glycosides from the leaves of *Staurogyne merguensis* Kuntze named strogins and determined their structures. In the present study, we examined properties of their sweet and sweetness- inducing activities and compared their properties with those of curculin. Strogins **1**, **2** and **4** elicited a sweet taste. The sweetness was diminished during holding the strogin solutions in the mouth but then application of water elicited a sweet taste. The maximum sweetness was induced by water at 4–10°C and the sweetness-inducing activity was decreased with increasing temperature of water. The sweetness-inducing activity lasted for 1–2 h. Application of γ-cyclodextrin to the mouth after the strogins were held in the mouth immediately eliminated the sweetness-inducing activity in response to water, which suggests that strogin is adsorbed on the taste receptor membranes and is eliminated by the inclusion action of γ-cyclodextrin. While the sweetness- inducing activity of curculin in response to water was suppressed by the presence of divalent cations such as Mg^{2+} or Ca^{2+}, that of strogin was not suppressed by the divalent cations. The mechanism of the sweetness-inducing activity was discussed in comparison with that of curculin.

P39. Nature of the active site of gurmarin as deduced from physicochemical interaction with β-cyclodextrin

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Our recent study in mice revealed that the recovery of sweet taste responses which had been suppressed by gurmarin was greatly facilitated by washing the tongue with β-cyclodextrin (CD). To investigate interaction of β-CD with gurmarin, the effects of various CDs were examined on the UV absorption spectrum of gurmarin and on the elution behavior in gel permeation chromatography (GPC). The addition of β-CD to gurmarin solution produced significant changes in the UV absorption spectra which were characteristic of those found in the inclusion complex formation of tyrosine and tryptophan with β-CD. An unexpected retention behavior of gurmarin on a GPC column due to hydrophobic interaction with the gel matrix was reverted to normal by the presence of β-CD in the elution buffer. These results strongly suggest that the unique domain of gurmarin, in which five aromatic amino acid residues are all directed outwardly and form a hydrophobic cluster, is a possible site of interaction with the sweet taste receptor and steric hindrance, and that the weakening of the hydrophobicity of the site caused by formation of the inclusion complexes of tyrosine(s) and/or tryptophan(s) with β-CD results in interference with gurmarin–receptor interaction.

P40. Intake of diets containing leaves of *Gymnema sylvestre* induces salivary gurmarin-binding protein in the rat

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The reactions of animals to a novel constituent of food were investigated by analyzing taste preference and salivary constituents in rats fed diets containing leaves of *Gymnema sylvestre* (GSL-diet). Male Wistar rats were fed diets containing the ground leaves in concentrations of 1, 3 and 10%. Changes in preference behavior, measured with a 48 h two-bottle preference test, followed a similar pattern in all the GSL-diet groups. The percent preference for 0.01 M sucrose of animals at 1–3 days after the beginning of feeding significantly decreased compared with those of the same individuals fed ordinary chow (control). Gurmarin inhibition of the sweet taste response possibly participates in this decrease in the preference. The preference started to return to control levels from 5 days after the beginning of feeding. Intake of GSL-diets had no effect on preference for 0.03 M NaCl, 0.03 M MSG and 0.01 mM quinine-HCl. Gurmarin-binding protein in rat saliva has been reported which inhibits the cross-reaction of gurmarin with antibody against the peptide. Submandibular saliva of rats fed GSL-diet strongly inhibited the cross-reaction compared with that of rats fed an ordinary chow. This suggests that intake of GSL-diet stimulated production of salivary gurmarin-binding protein. The return of the preference for sucrose to normal levels was attributable to the inactivation of gurmarin by this protein. The increased production of the protein could be a form of adaptation for obtaining energy in the presence of gurmarin.

P41. Genetic analysis on behavioral preferences for sweet and bitter substances in mice

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Behavioral preferences for sucrose, saccharin, D-phenylalanine, isohumulone and ethanol in C57BL and BALB mice and their F_2 hybrids were measured by use of a two-bottle test. C57BL mice showed higher preference scores for all five taste substances than BALB mice. F_2 mice showed wide varieties of preference scores for these substances and their mean scores were intermediate between those of the parent strains. Across-mouse correlations among five substances were calculated based on the data obtained from 34 F_2 mice. The results showed that there are significant correlations not only between preference scores for sweet substances (sucrose versus saccharin and D-phenylalanine) but also among those for sucrose versus several concentrations of isohumulone (0.02–0.2 and 20 mg/l) and ethanol (1–15%). These results suggest that although peripheral receptor sensitivities for some sweet and bitter substances are suggested to be controlled by different genes on different chromosomes (4 and 6), behavioral preferences for sweet and bitter compounds measured by a two-bottle test may not necessarily be independent with each other. In the strains of mice tested, higher behavioral preferences for sweet substances may account for lower behavioral aversions for bitter compounds.

P42. Influences of genetic backgrounds on sweet taste preferences in the diabetic db/db mouse

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Genetically diabetic C57BL/KsJ-db/db mice showed greater neural and behavioral responses to sugars and non-sugar sweeteners except D-phenylalanine (D-phe). In the present study, we made crosses between C57BL/KsJ-+/db heterozygotes at the db locus showing sweet taste responses to D-phe (dpa^+) and BALB mice lacking sweet responses to D-phe (dpa^+), and produced diabetic and lean mice with mixed genetic backgrounds of two inbred strains in their F_2 generations. Behavioral preferences for D-phe, sucrose and saccharin, measured by a two-bottle test, were compared among diabetic and lean control F_2 mice. Diabetic and control mice were divided into two subgroups (dpa and dpa) according to their preferences for D-phe, and in total four groups—dpa^+-db/db, dpa^--db/db dpa^+-+/? and dpa^--+/?. The results showed that preferences for sucrose and saccharin of dpa^--db/db mice were significantly higher than those of dpa^-+/? mice, whereas no such difference was observed between dpa^+-db/db and dpa^+-+/? mice. These results suggest that enhanced preferences for sweet substances of db/db mice are evident in dpa^- but not dpa^+ groups, and mixing of genetic backgrounds of BALB with C57 mice may reduce differences in sweet preferences between db/db and lean control mice.

P43. Comparison between young and elderly people in quality discrimination and identification of mixed odors and tastes

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The discrimination and identification of odors (a mixture of suprathreshold concentrations of β-phenylethyl alcohol and γ-undecalactone) and tastes (a mixture of suprathreshold concentrations of sucrose and tartaric acid) in young and elderly people were compared using the triangular test. The short-term memories of young and elderly people were also compared. The elderly had a significantly lower discrimination and identification of odors and tastes compared with the young. The percent correct in both the discrimination and the identification tests showed a correlation between olfaction and taste. The elderly had a significantly poorer short-term memory compared with the young. The percent correct in the short-term memory test showed a correlation with the percent correct in discrimination or identification test of odors or tastes. Therefore, the reults supported the hypothesis that the olfactory and gustatory senses of humans decrease with aging. It was thought that the decrease in discrimination and identification abilities for odors and tastes with

aging would be due to the decreases not only in the peripheral but also in the central nervous system.

P44. A study on the judgement ability of taste—how the sense of sight, smell and hearing have influence on the taste ability

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A purpose of this study was to define how the sense of sight, smell and hearing have an influence on the appreciation of taste. The method used involved covering the eyes for sight, stuffing the nose for smell and closing the ears for hearing ability. The basic taste substances we selected were sodium chloride as salty taste, sugar as sweet taste, vinegar as sour taste, monosodium glutamate as umami taste and coffee as bitter taste. These substances were used after appropriate dilution with water. The testing method we conducted was a 'sensory evaluation' survey of a mass of tasters. Their judgement abilities of taste were evaluated by use of Kendall's coefficient *W*. Our results are as follows: 'stuffing the nose' is an important factor, weakening the judgement ability of taste. On the contrary, 'covering the ears' is a less influential factor. The sense of hearing does not have so much influence on the judgement ability of taste. From another viewpoint, sweet taste does not seem to be affected by any of the factors. But umami is easily influenced by all factors, especially the sense of smell. From this study, we can recognize that sensitive factors have a considerable influence on the judgement ability of taste.

P45. Analysis of the concept of koku-flavor from the viewpoint of evaluation terms

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'Koku', a Japanese word, is a decisively important factor for palatability of foods. It is usually translated into English as 'body' or 'substance'. However, the scientific definition of koku as a flavor evaluation term has not yet been given. In order to characterize the term, a questionnaire survey was conducted for people working in sensory evaluation fields in food companies. From a preliminary survey, 17 words expressing koku and 30 items of foods considered to have plenty of koku were selected. Participants were asked to evaluate the degree of importance of koku for the palatability of each food and also the importance of each word to explain the meaning of koku in each food. Foods that koku was evaluated to be most important in were beef stew, followed by curry soup, Chinese noodle soup, milk, red wine, sake etc. Principal component analysis revealed that the meaning of koku has a distribution expressed by a continuum of words—mouthful, profound, complex, round, long after-effect, thick, heavy, viscous and greasy—which corresponded with light to heavy foods.

P46. The perception of umami and salt taste by Japanese and American subjects

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Freshly cooked chicken broth was prepared with several concentrations (0.056–0.32 M) of added NaCl and 0.01 M monosodium glutamate (MSG) to examine the perception of umami and salt taste in Japanese and American subjects. Both groups of subjects detected and preferred the sample with added MSG when the salt levels were low to moderate. The Japanese subjects described the taste of MSG in the chicken broth with the temporal and spatial attributes of umami taste, while the Americans tended to focus on the intensified chicken flavor as well as saltiness. The effects of adding 0.01 M NaCl to the chicken broth were not identical to adding 0.01 M MSG, but American subjects did prefer the soup sample with more salt at the two lowest salt levels. In the last study, the concentration of sodium was held constant in the broths and only the glutamate was varied by adding 0.01 M MSG to one sample and 0.01 M NaCl to the other. Both groups of subjects preferred the sample with added glutamate to the one without, demonstrating a role for glutamate alone in enhancing palatability. These studies, in sum, demonstrate that MSG increases palatability of salted soups and that both the sodium and the glutamate independently contribute to this enhancement. Morever, the glutamate enhancement of food flavors is not restricted to a single cultural group.

P47. Effect of physical exercise on the preference of various sweet taste solutions

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The effect of physical exercise on the preference of various sweet taste solutions was examined. After 30 min of exercise using a bicycle ergometer at intensity 50% VO_{2max} (maximal oxygen uptake), a taste preference test was performed in 44 healthy university students aged between 19 and 24 years. Test solutions were sucrose, glucose, stevioside, D-sorbitol, erythritol and saccharin.

Preference scale values of sucrose, glucose, stevioside, D-sorbitol and erythritol increased after exercise. The degree of change was especially large in high concentrations of the taste solutions. However, the preference scale values of saccharin were not changed between pre-exercise and post-exercise in all concentrations.

These results suggest that effect of preference of sweet taste by physical exercise varied between different sweet taste substances.

P48. Electro-physiological analysis of regional differences underlying gustatory sweating in women

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To determine autonomic nervous functions induced by taste stimuli, we recorded the sympathetic skin response (SSR) from

the skin surface of the fingertip, palm, dorsal hand, frontal region and upper lip as an indicator of gustatory sweating. SSR attributed to sudomotor activities. Six healthy women were employed and were tested by five kinds of tastes: sweet, salty, sour, bitter and spicy. When taste stimuli were applied to the surface of the tongue, electrical potentials as SSRs were obtained. The SSR was a monophasic deflection in almost all cases, and its changes were presented as amplitudes and durations. In a few cases, potentials showed bi- and multi-phasic deflection. From the size of the responses we evaluated the degrees of the responses in gustatory sweating. There were regional differences among recording sites; for example, the upper lip and frontal region showed greater responses than the fingertip. Responses at the fingertip were not always invoked by taste stimuli. In contrast, the most effective responses from the fingertip were elicited by mental arithmetic, association and sound. In the five kinds of taste, the most effective responses were observed with the bitter and sour, then the spicy and salty, with the weakest response from the sweet.

P49. The effect of taste stimuli on human eating behaviour from the viewpoint of movement of the body's centre of gravity

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The process of cognition of human taste is affected by the individual's condition and external stimuli. In the daily meal, it is different between the starting taste and the ending taste. Therefore the taste sensation seems to show resting and adaptation. After taste stimuli, the contact behaviour shows a difference between pre-cognition and non-cognition of the origin of stimuli.

The authors hypothesized that it was possible to observe the behaviour of eating with the movement of the body's centre of gravity. The movement of the body's centre of gravity was measured by a stabilometer (model IG06, NEC Medical Systems Co. Ltd, Tokyo). We examined the gustatory test twice: before and after the measurement of the body's centre of gravity. We used the whole mouth method for the gustatory test. Subjects were 11 adult males, divided into three groups; in two subjects the first results of the gustatory test were better (taste resting group), in six subjects the first results were the same as the second results (non-changing group) and in three subjects the first results were worse (taste adapted group).

Concerning the length from the left side to the right side, the taste resting group was the largest and the taste adapted group was the smallest under the condition of eye-open and also eye-closed. For the movement of vector analysis of the forward direction, under the eye-open condition the taste adapted group was the largest, and under the eye-closed condition the taste resting group was the largest.

P50. Chemosensory brain evoked potential induced by stimulation with four taste qualities: the influence of a sweet suppressing agent

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A measurement system using a laser beam device was employed to detect gustatory evoked potentials from the human scalp following stimulation with a taste solution. The evoked potentials for four taste qualities (sweet—sucrose, salty—sodium chloride, sour—tartaric acid and bitter—quinine-HCl) were measured before and after the treatment of a sweet suppressing agent (*Gymnema sylvestre* extract) to the tongue of a human. The solution was applied to the chorda tympani nerve located 20 mm from the apex of the tongue and 15 mm to the left side from the center line. The maximum potential level and its latency were evaluated. Artificial saliva was used as a control solution. The evoked potentials obtained were averaged over eight evoked potentials to detect the peak more clearly. The latencies for the taste stimuli were found to be two peaks at around 50 and 180 ms, which were called P1 and P2. The purpose of this study was to investigate the influence of a sweet suppressing agent on the peaks P1 and P2. It was found that the sweet suppressing agent had no influence on the potentials evoked by the salty, sour and bitter taste stimuli, but that the responses to sweet (sucrose) were abolished after treatment with the sweet suppressing agent. It was recognized that the peak P2 originated from the taste stimulus. The peak P1 was not influenced by the sweet suppression, so peak P1 was considered to be a response due to sensations other than the gustatory response, such as somatosense.

P51. Temporal process in taste reception studied by gustatory-evoked magnetic fields and reaction times.

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We previously located the primary gustatory area in the human cerebral cortex by using magnetoencephalography and a taste stimulator with a rapid-rise time. In the present study, we aimed to investigate the temporal process in taste reception based on the results of the gustatory-evoked magnetic fields (GEMs) and reaction times (RTs). We used 100 mM, 300 mM and 1 M NaCl and 3 mM saccharin. The duration of each stimulus was 400 ms, and the inter-stimulus interval was ~30 s. Four subjects participated in this experiment. The 64-channel whole-head SQUID system was used to measure GEMs. In each subject, GEMs and RTs to a given taste were measured separately by applying 40 trials of stimulation. Averaged GEMs were superimposed on the same sheet with all 64 channels to find the onset of GEMs. RTs became shorter with increased concentrations of NaCl, and RTs and GEMs onset latency were longer for sweet taste than salty taste. Sweet taste may take a longer time than salty taste at the receptor process, including the time for diffusion to

receptors. The value of ET minus the GEMs onset latency from RTs, presumably indicating the time for the higher brain process plus the motor process, tended to become longer for lower concentrations of NaCl.

P52. The location of human cortices related to taste stimulation

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In a previous study, we applied taste stimulation with an abrupt rise time to the tongue tip of human subjects and measured very subtle changes of magnetic fields (MFs) from the human brain with a whole-head SQUID system. From the short latency of the gustatory MFs and the anatomical location of the dipole estimated, we concluded that the primary gustatory area (area G) in humans was located at the transition between the operculum and insula. In this study, we covered two topics: one is the accurate location of area G and the other is the locations of subsequent activities after area G. With precise magnetoencephalography data analysis, area G was found to be located behind the central sulcus. The location of area G, therefore, is the transition part of the insula cortex and the parietal operculum. We identified that the superior temporal sulcus, hippocampus, frontal operculum and parietal lobe were sequentially activated after area G. Several cortical regions, such as the superior temporal gyrus and the hippocampus, were recognized as activated areas in a PET experiment but the seqeunce of activation was first clarified in the present study. The frontal operculum and the anterior part of the insula were observed to be activated after the activation of area G. These areas may function as a higher gustatory area like the PrCO and OFO of macaque monkeys.

P53. Electron microscopic observation of the vallate taste buds of rats with a taste disorder induced by tetracycline

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We investigated morphological changes of the vallate taste buds of rats with a taste disorder by using transmission electron microscopy. A taste disorder was induced in rats by intraperitoneal injection of tetracycline and the degree of that disorder was assesed by a two-bottle preference test. Clear cells in the taste buds decreased in number. Some clear cells showed degenerative processes: decreased cytoplasmic density and condensed nucleus were seen. Secondary lysosome-like dense bodies were various in number, shape and size in the cytoplasm of clear and dark cells. Dense bodies showed heterogeneous electron density. These results were different from morphological changes of vallate taste bud cells in rats with a taste disorder induced by a zinc-deficient diet. In addition, in the present study plasma levels of zinc and protein were lowered, but dermopathy and growth disturbance were not observed. Hence, the cause of a taste disorder induced by tetracycline is not zinc deficiency in serum, but tetracycline might directly affect sensory cells as well as clear cells in the taste buds in rats.

P54. A study on taste of the disabled with cerebrovascular diseases

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The sense of taste was evaluated in 104 patients who had cerebrovascular attack. The 104 subjects comprised 77 males and 27 females, aged 19–90 years, with an average age of 56 years. The kinds of acquired diseases were intracerebral hemorrhage (55 subjects), cerebral infarction (42) and subarachnoid hemorrhage (7), and the sequelae of these acquired diseases were right hemiparalysis (38), left hemiparalysis (38), ataxia (1) and 5 cases without any paralysis. As a control group, 68 healthy adults were examined in the same way. The test was held in this way: four basic taste solutions of sucrose, NaCl, citric acid and quinine sulfate were made up in three different concentrations—thin, medium and thick. From the thin concentrated solution to the thick one, a tiny amount of each solution was dropped on the examinee's tongue, and then tasted with whole oral cavity. The subject was then asked to identify the taste. The percentage of correct answers from the control group was 57% with the 'thin' concentration solution and 84% with the 'medium' one. There was no significant difference between males and females. Over age 60, the percentage of correct answers was low. On the other hand, among the disabled patients, the percentage of correct answers was 16% with the 'thin' concentration and 44% including the 'medium' one. The group of intracerebral hemorrhage could least distinguish the difference, especially the tastes of salt and sourness. The incidence of correct answers was slightly lower in the group with aphasia (38 cases) than in the group without it (66). Further investigation will focus on the relevance of the intake of drugs and on the analysis of MRI and CT scans.

P55. Establishment of functional synapses between rat olfactory epithelium and olfactory bulb under the culture conditions

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Olfactory neurons have an ability to regenerate throughout their lifetime even in adults. It is interesting to know how olfactory neurons newly innervate to the target neuron. In the present study, we co-cultured newborn rat olfactory epithelium explants and olfactory bulb slices to explore synaptic formation between olfactory neurons and neurons in the olfactory bulb by using the organotypic slice culture technique. At 28 days in culture, MAP2 (a maker of neuron) positive fibers were observed between the olfactory epithelium explant and the olfactory bulb slice. The formation of functional synapses between olfactory neurons and neurons in the olfactory bulb was examined electrophysiologically. Application of 50 μM forskolin to the olfactory epithelium explants evoked an inward current response in 5/6 olfactory bulb neurons (−13 ± 6 pA, $n = 5$), which were located at the region of innervating fibers from the olfactory epithelium explants under the whole-cell voltage-clamp conditions at −70 mV. It seems that

functional synapses were established between the olfactory neuron and the olfactory bulb neuron under the culture conditions.

P56. Immunohistochemical localization of proliferating cells, epidermal growth factors and epidermal growth factor receptors in mouse olfactory epithelium

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We examined the expression of proliferating cells, epidermal growth factors (EGF) and epidermal growth factor receptors (EGFR) in the olfactory epithelium of embryonal, neonatal, adult and aged mice using an immunohistochemical method. Many proliferating cells presented in the surface and basal layers of the olfactory epithelium in the embryonal and neonatal mice, but were found to decrease rapidly after birth. Only some proliferating cells presented in the basal layer in adult mice and only a few presented in the basal layer in aged mice. EGF were observed in the whole layer of the olfactory epithelium at all stages. EGFR were observed in the whole layer of the olfactory epithelium at the embryonal and neonatal stages, but were not identified in the olfactory epithelium at the adult or aged stages.

These results indicate that a decrease in the proliferating cells in the adult and aged olfactory epithelium is one factor underlying atrophy of the olfactory epithelium with aging, and is related to the decreasing sense of smell in the aged. We believe that both EGF and EGFR contribute to cell proliferation, growth and turnover of the olfactory epithelium, and that one causal factor of inhibition of cell proliferation is a decrease in EGFR.

P57. Basic fibroblast growth factor promotes olfactory cell proliferation *in vitro*

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We investigated the expressions of peptide growth factor receptors and the effects of basic fibroblast growth factor (bFGF) on olfactory cells *in vitro* using a primary co-culture system with brain astrocytes. RT-PCR of peptide growth factor receptors on the co-culture cells revealed the expression of fibroblast growth factor receptor (FGFR)2 and FGFR3, both of which could not be detected in the feeder cell layer of the astrocyte. FGFR1 and transforming growth factor β receptor (TGFβR) were detected both on the co-culture and on feeder cells. FGFR4, insulin-like growth factor 2 receptor and hepatocyte growth factor receptor could not be detected in either of the samples. bFGF, a prototypic FGF, was also found from the co-culture system and from feeder cells. FGFR2 was detected from day 2 and FGFR3 was detected from day 14 in culture. Colonization and neuronatin expression of olfactory cells were suppressed in the presence of neutralizing antibody of bFGF for 2 days and recovered after removing the antibody. The present study indicates that bFGF promotes olfactory cell proliferation *in vitro* and the effect might be expressed by way of FGFR2.

Intracellular architecture of rat olfactory epithelial cells

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The intracellular architecture of rat olfactory epithelial cells was studied with an O–D–O (osmium–DMSO–osmium) method. Many microtubules were observed in the apical side of the olfactory cell. These must contribute to the maintenance of the cell body. In the supporting cell, lysozome-like granules were seen. With this method, we could produce a three-dimensional image of the cytoorganelle of the cells.

P59. Regeneration of olfactory receptor cells after transection of the fila olfactoria

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It is well known that olfactory receptor cells reside in the epithelium of the nasal cavity and project axons to the olfactory bulb in mammals. In addition, newly generated ollactory receptor cells mature, live an average of 90 days and then die. In this study we observed the process of regeneration in rat olfactory receptor cells after transection of the fila olfactoria using *in situ* hybridization. The survival times were 3, 7, 14, 21, 30, 60 or 90 clays after transection.

We used olfactory marker protein (OMP), expressed in mature cells, growth associated protein (GAP43) and β-tubulin, expressed in immature cell oligonucleotide probes, for *in situ* hybridization. The expression of GAP43 and β-tubulin mRNAs increased in the lower part of the epithelium after 30 days and the expression of OMP mRNA increased in the middle of the epithelium after 60 days.

P60. The olfactory epithelium consisting of supporting cells and horizontal basal cells

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The olfactory epithelium of adult mice generally consists of mature and immature olfactory cells, precursors of olfactory cells (globose basal cells), supporting cells and horizontal basal cells. We examined the changes of olfactory epithelium of adult mice caused by colchicine treatment with electron microscopy and immunohistochemistry. A single intraperitoneal injection of colchicine (2–4 mg/kg body wt) induced apoptotic cell death in most of the olfactory epithelium regions in the septum and nasal turbinates within 1 day, followed by active proliferation of globose basal cells and the subsequent regeneration to normal olfactory epithelium within 1 week after colchicine treatment. However, in some epithelial regions in the third and fourth nasal turbinates, where olfactory cells and globose basal cells had died from the effect of colchicine, the regeneration did not occur even after 1 month, forming the epithelium of only supporting cells and

horizontal basal cells. During the next month, however, these regions became normal olfactory epithelium. This suggests that the globose basal cells in the surrounding normal olfactory epithelium might invade these regions to give rise to the olfactory cells. In the further study with control mice (1–10 months old), we found the epithelium consisted of only non-neuronal cells in the dorsal fossa of the third and fourth turbinates. The cell bodies of supporting cells in the regions formed two or three layers and were connected to horizontal basal cells by desmosomes. Bowman's glands were also observed. In newborn mice, however, normal olfactory epithelium occupied these regions, suggesting that migration or cell death of olfactory cells and globose basal cells occur during postnatal development to produce the epithelium of supporting cells and horizontal basal cells.

P61. The patch-clamp analysis of amino acid responses of isolated olfactory receptor neurons from the rainbow trout

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To elucidate functional roles of ciliated and microvillous receptor neurons in fish olfaction, their basic response properties to electrical and chemical stimulation were studied by means of patch-clamp techniques in the rainbow trout, *Oncorhynchus mykiss*. The two types of receptor neurons which were discernible by their knob structure were successfully isolated from the pre-dissected epithelial tissues of individual olfactory lamellae without using proteolytic enzymes. A mixture of four amino acids (L-glutamate, L-arginine, L-alanine and L-norvaline at 10 μM concentration) in a stimulating pipette (tip opening ~1.5 mm dia.) was applied to receptor neurons in normal Ringer's solution by a home-built pressure ejection system. Voltage-gated current responses of both types of neurons held at –60 mV to depolarizing and hyperpolarizing voltage steps were essentially similar in their properties. Spiking responses recorded in cell-attached mode to amino acids were more robust than in the neurons isolated by using proteolytic enzymes. Whole-cell responses of ciliated neurons held at –60 mV to amino acids, which were recorded with the nystatin-*N*-methyl-D-glucamine perforation method, were monophasic inward currents, of which the peak amplitude increased with an increase in amino acid concentration. The latencies were shorter (~20 ms) than the values reported previously in amphibians and rat, suggesting that olfactory transduction cascade in fish may differ from those in other species. Supported in part by JMESC 07044172.

P62. Tuning specificities to various odorants in mouse olfactory receptor cells

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We examined the responsivity to a panel of various odorants in individual olfactory receptor cells of mouse. The odorant panel included two homologous series of *n*-fatty acids and *n*-aliphatic alcohols, as well as the other different odorants: isoamyl acetate, citralva, isovaleric acid, pyrazine, pyridine, L-menthone, L-menthol, lyral, lilial, eugenol, geraniol, ethyl vanillin and L-carvone. Odor responses were recorded optically by measuring intracellular calcium increases with fura-2. Seventy-eight cells out of total 160 responsive cells responded to one or more of the *n*-aliphatic odorants, and their tuning specificities were dependent on both the carbon chain length and the functional group (carboxyl and hydroxyl). Fifteen of these cells also responded to other odorant(s), e.g. isoamyl acetate, isovaleric acid, citralva. Thirty-four cells responded to two or more odorants other than the *n*-aliphatic odorants. The number of cells responding preferentially to both eugenol (EU) and ethyl vanillin (EV) was 11. Eight of them were more sensitive to EV than EU, suggesting that the -CHO and $-OCH_2CH_3$ of EV may interact with the receptor site more strongly than the $-OCH_3$ and $-CH_2CHCH_2$ of EU. Although some cells responded to a few odorants which did not seem very similar, most olfactory cells responded to a few odorants with similar stereochemical structures. Our results suggest that the tuning specificity of the olfactory receptor cell is determined by the three-dimensional arrangement of intermolecular interactions.

P63. Odor responses of olfactory receptor neurons in the lateral diverticulum of *Xenopus laevis*

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The olfactory organ of *Xenopus laevis* was divided into two independent parts, the medial diverticulum (MD) and the lateral diverticulum (LD), by the valve structure. Olfactory neurons in the MD and LD have been considered to receive air-borne odorants and water-borne odorants respectively. Recently, it was reported that mammalian-like olfactory G-protein-coupled receptors (GCRs) exist in the MD while fish-like GCRs exist in the LD. In the present study, we measured current and voltage responses to electric and chemical stimulations in the olfactory receptor neurons in the LD epithelium slices using the whole-cell patch-clamp technique. Injection of depolarizing currents induced trains of action potentials in the receptor neurons, which were blocked by TTX. The application of an amino acid mixture induced inward current responses in 9/14 cells. The application of a cAMP-increasing volatile odorant mixture or an IP_3-increasing odorant mixture also induced inward current responses in 7/14 cells. The present results suggest that olfactory receptor neurons in the LD respond to both amino acids and volatile odorants.

P64. Bile acids in natural stream waters: an evaluation of bile acids as olfactory cues in salmonid homing

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In salmonid fishes, many studies have shown that olfactory systems play an important role in homing migration. It has been suggested that bile acids are olfactory cues for salmonids. To test this hypothesis, we determined the content of bile acids and main

cations in natural stream water, measured the olfactory responses of masu salmon and rainbow trout to bile acids in the stream waters, and estimated the contribution of bile acids to stream odors. Analyses of bile acids showed that the waters of six streams examined contained 0–7 cholic acid derivatives with a total concentration of from 0.296 to 5.11 nM. Application of natural waters to olfactory epithelium of masu salmon elicited large responses. The cross-adaptation experiments demonstrated that odors of natural stream water were discriminated by masu salmon. On the other hand, the artificial stream waters containing bile acids and salts elicited only small responses and were not discriminated. Thew rainbow trout olfactory system could not discriminate between the bile acids examined. The present results suggest that bile acids are not major components of the home stream odors for salmon homing. It can be considered that amino acids are major components of the home stream odors and have an important role for being discriminated by salmon olfactory systems rather than bile acids.

P65. Urine-induced second messenger accumulation in rat vomeronasal epithelium

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In rats, urine affects the timing of estrous cycles and accelerates sexual maturation. In a previous study, we found that introduction of urine from the male Wistar rat to the vomeronasal sensory neurons of the female Wistar rat increased impulse frequency under on-cell patch-clamp conditions. These responses were blocked by phospholipase C inhibitors and an IP_3-channel inhibitor. In addition, dialysis of 100 μM IP_3 into the sensory neuron induced inward currents under the voltage-clamp conditions. The results suggested that the urinary responses are mediated via the IP_3-mediated transduction pathway in rat vomeronasal sensory neurons. To test this hypothesis, we measured IP_3 and cAMP accumulation in response to urine in the vomeronasal membrane preparation of the female Wistar rat.

Application of urine did not induce any cAMP accumulation, although forskolin and GTP induced cAMP accumulation in vomeronasal membrane preparations of the rat, suggesting the existence of a functional adenylyl cyclase. In contrast, application of urine induced IP_3 accumulation in the membrane preparation in a dose-dependent manner. Treatment with pertussis toxin reduced IP_3 accumulation induced by urine. The present results support the idea that IP_3 acts as a second messenger in the reception of urinary pheromones in the rat vomeronasal organ.

P66. Zinc modulates the electro-olfactogram of the frog

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There are many previous reports on the influences of zinc to olfaction. Zinc difficiency causes olfactory loss but drop application of zinc ions to olfactory epithelium causes damage in experimental animals. We charged the ciliated side of olfactory mucosa with physiological saline with and without Zn^{2+} to investigate the effect of Zn^{2+} on olfaction. The frog olfactory mucosa was removed from the nasal cavity and set in the electro-olfactogram (EOG) recording chamber. When the mucosa was covered with (200 μM *n*-amylacetate stimulus) physiological saline containing Zn^{2+} (25 μM), the EOG was attenuated to 50.8 ± 17.6% (mean ± SD, $n = 4$). This effect occurs momentarily, and is quickly reversed when the solution is changed back to physiological saline without Zn^{2+}. Forskolin (2 μM) depolarized olfactory receptor cells and evokes the EOG-like response. This response was also attenuated by Zn^{2+}. These results are support the hypothesis that Zn^{2+} ions modulate the cAMP pathway of the olfactory receptor cell. It is suspected that the rapid effect of Zn^{2+} occurs by blocking the trasduction channels of the olfactory receptor cell.

P67. Space distribution of Cl^- in newt olfactory cells

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The Cl^- efflux has been proposed to be a part of the receptor current in vertebrate olfactory cells: these cells have Cl^- channels activated by Ca^{2+} that flows into the cell through the cyclic nucleotide-gated channels. Such a Cl^- movement should be dependent of concentration of Cl^- in and around the olfactory cells. In this report, we examined the intracellular Cl^- concentration ($[Cl^-]_i$) through olfactory cells using the fluorescent dye probe *N*-(6-methoxyquinolyl)-acetoethyl ester bromide (MQAE), the fluorescence of which is quenched by Cl^-.

We loaded the dye onto solitary olfactory cells which were enzymatically dissociated from newts. Their microfluorescence images were captured with a SIT-camera and recorded on a VCR. Calibration between the fluorescence intensity and $[Cl^-]_i$ on each cell using Cl^- standard solutions containing Cl^--ionophores indicated that the average $[Cl^-]_i$ in most parts of the cell is 30–40 mM, as reported previously. However, $[Cl^-]_i$ in the distal dendritic end of the cell (olfactory knob) was <10 mM, making a trough in the concentration in the cell. There seems to be a mechanism that lowers the $[Cl^-]_i$ at this site.

P68. Effect of niflumic acid on the electro-olfactogram of the bullfrog

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It is said that, when the vertebrate olfactory cells are excited with odorants, Ca^{2+} co-influxed with Na^+ through the cyclic nucleotide-gated channels activates the Cl^- channels to generate the inward electronic current which amplifies the depolarizing receptor current. We did a pharmacological study of the C^- component of the summated electrical response of the olfactory epithelium (electro-olfactogram: EOG) of the bullfrog. Olfactory epithelia were excised from the bullfrog's nasal cavities and continuously perfused in a recording chamber. Pulses of odorants dissolved in 0.8ml of perfusing solution were injected into the

perfusing flow by use of a six-port valve (flow rate: 5.0 ml/min). Using this system, we examined the effect of various concentrations of Cl^- and niflumic acid, the Cl^- channel blocker, in the perfusing solution. Our observations were as follows: the EOG amplitude was dependent on $[Cl^-]_o$, with the larger amplitude in the lower $[Cl^-]_o$; 0.5 mM niflumic acid reduced the EOG amplitude in any $[Cl^-]_o$ tested, with the larger reducing magnitude in the lower $[Cl^-]_o$; and 0.5 mM niflumic acid reduced the EOG amplitude in the Ca^{2+}-free conditions attained with 2 mM EDTA. These results suggest that niflumic acid inhibits not only the Cl^- channels but also the cAMP-activated channels if the opening of the Cl^- channels is not independent of the extracellular Ca^{2+}.

P69. Associative and discriminative odor learning in the rat

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We can recognize foods by their flavors, mixtures of odors and tastes. No systematic study, however, has been documented on this topic except for the 'taste-potentiated odor aversion'. We designed a new method to study an association between odor and taste in the rat. Male Wistar rats were deprived of water in their home cages and were allowed to drink in a circular open-field apparatus from small glass dishes. They were then exposed in their home cages and in the open-field apparatus freely to 0.005 M Na-saccharin and 0.0001 M quinine hydrochloride which contained either iso-amylacetate (5 μl/100 ml) or almond essence (5 μl/100 ml) for 2 days. They were then tested for odor preference in the open-field apparatus equipped with eight dishes (1 ml of test solution in each): four contained iso-amylacetate + water and the rest almond + water as test stimuli. Naive rats showed equal preference to the two test solutions, but trained rats preferred to drink water with the odor previously associated with saccharin. In a generalization test, the rats were exposed to the water which contained one of 16 odor stimuli. The rats who had acquired an aversion to the odor of iso-amylacetate avoided ingestion of water with a pineapple odor, whereas those who had acquired an aversion to the almond odor avoided peach and strawberry odors.

P70. The effect of bicuculline infusion into the olfactory bulb on olfactory learning in the young rat

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After odor exposure paired with foot shock, young rats show aversion to that odor. Noradrenergic activation through the locus coeruleus by somatosensory stimulation induces disinhibition of the mitral cells from the granule cells in the olfactory bulb. Therefore we infused bicuculline, a GABA antagonist, directly into the olfactory bulb to mimic the disinhibition of the mitral cells during odor exposure training on postnatal day (PND) 11. The drug was infused through cannulae implanted prior to training. Bicuculline (1.0 nmol/2 μl) was infused into each bulb during odor exposure. Subsequently these animals showed aversion to the same odor when tested on PND 12 although they did not respond to the infusion *per se*. However, a bicuculline infusion of 0.2 nmol/2 μl into the olfactory bulb induces preference for the odor. Since the olfactory bulb has an anatomical connection to the amygdala, these results suggest that excitation of the mitral cells in the olfactory bulb may modulate the emotional state.

P71. Differences in cognition to everyday odors between Japanese and Germans

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Experience with odors may influence their cognition. One of the few means of investigating this influence under the natural conditions of daily life is to make use of geographic differences in experience. Thus, it was the aim of our study to investigate the nature of experience-dependent differences in odor cognition by comparing the responses of Japanese and Germans to everyday odors. Forty Japanese and 44 age-matched German women smelled 18 odorants in squeeze bottles. Six were thought to be typically Japanese, six thought to be typically European and six thought to be international. Subjects were asked to rate each odor for intensity, pleasantness and familiarity, name it, and state whether it was from an edible substance or not.

Significant differences were found between the two groups on all measures. Better performance by the Japanese in providing appropriate descriptors for 'Japanese' odorants and by the Germans for 'European' odorants supported the pre-selection of stimuli as culture-typical. Particularly clear differences between the two populations were found in pleasantness ratings. In general, a positive relationship was found between pleasantness and judgement of stimuli as edible, suggesting that culture-specific experiences—particularly of foods—may influence odor perception significantly. Significant differences were also found between the two groups in intensity ratings for some odorants. These differences raise the possibility that experience may even influence such basic aspects of odor perception as stimulus intensity.

P72. Experimental results of gas–liquid chromatography and sensory evaluation for aroma compounds

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We reported that with the free energy variations of dissolution $\Delta\Delta G$, estimated from the variable temperature experiments involving log γ, the relative retention values correspond to the sample–liquid stationary phase interaction in the gas–liquid chromatography of mono-substituted benzene derivatives and are <15 kJ/mol. The regression analysis based on the weak molecular interaction was carried out using three type descriptors, σ_a, σ_{bd} and σ_{ms}, representing the dispersion (E_{dis}), polar (E_p) and

charge-transfer interaction (E_{CT}) energies respectively. The relation is given by

$$\Delta\Delta G = a\sigma_a + b\sigma_{bd} + c\sigma_{ms} + d \quad (1)$$

The regression analysis of $\Delta\Delta G$ values using terpene and its analogues and β-cyclodextrin were done and equation (2) was obtained:

$$-\Delta\Delta G = 8.852(0.885)\sigma_a + 1.380(0.386)\sigma_{bd} - 4.373(0.687) \quad (2)$$

$$n = 25,\ r = 0.978,\ F = 240.2,\ \mathrm{SD} = 0.236$$

The interaction is mainly expressed by the values of E_{dis} and in addition E_p must be considered for ketone, ether, etc. However, we cannot evaluate the configuration of the optical isomers which have serious differences for the odor.

Sensory evaluation of essential oils (lemon, orange and lavender) could not indicate the differences in the mental work. We discussed the working efficiency of the fragrance using Uchida–Kraepelin's test and suggested that the tendency to drop the ability to concentrate was observed in the order lemon, orange and lavender. The influence by lavender was the difference in behavior of dropping and depended on her felling of the fragrance.

P73. Reproducibility and reliability of the sensory evaluation scales for flower odor

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In order to select sensory evaluation terms for flower odors for use by ordinary people, we performed a psychological experiment using 17 natural flowers in a previous study. As a result, we could construct 16 pairs of scales for flower odor evaluation. In the present study we performed a psychological experiment using these scales and 30 flowers, and examined the reproducibility and reliability of both scales and odors. Finally, we extracted 13 scales for flower odor evaluation after removing three scales that had low reproducibility and reliability.

P74. Odor analysis in a car's cabin

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Various odors, produced from interior parts and materials streamed into a car's cabin from ambient air and from the driver and passenger(s), coexist in a car's cabin. These odors are important parameters for safety and comfortable driving. We studied these odors in the car's cabin by instrumental analysis and sensory evaluation.

By odor analysis in a new car's cabin, it was apparent that a masking effect by aromatic hydrocarbon groups controlled unpleasant odors which were caused by organic nitrogen compounds. The mechanism of odor production was studied in respect of the dusty odor from the car cabin's air conditioner. The dusty odor from the air conditioner was found to contain the corrosion products of the evaporator (aluminum) by instrumental analysis and sensory evaluation. The dusty odor was stronger in high humidity than in a dry atmosphere.

We also noticed vehicle-emitted volatile hydrocarbons, NO_x, etc. as streaming odors. Odorants peculiar to ambient air in a driving area were picked up. These odorants were found to contain butane exhausted from LPG taxis at Nagoya station area, and NO_x and decane exhausted from diesel engines in En Mt. tunnel.

P75. Odors of new cars (II)

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The odor in the cabin of a vehicle is one of the most important factors for the customer's satisfaction. The odor of a new car is caused by a complex mixture of various compounds evolved from interior parts and materials. To identify the odor and the compounds responsible for the odor, sensory evaluations and instrumental gas analysis were carried out. In sensory evaluations, odor intensity, pleasantness and odor characters were evaluated for 73 new cars. The compounds of the odor of 17 new cars were analyzed with GC and GC-MS using adsorbent tubes and canisters.

From the principal components analysis of sensory evaluation results, odor characters were classified into six types: irritative, aromatic, fishy, amine, leather and sour. Cluster analysis was used to classify new cars with their odor characteristics.

From the instrumental gas analysis, >200 compounds were characterized as components of the odor. The correlation between the odor types and the odor compounds identified the compounds responsible for the each type of odor. These results will be used for the improvement of the odor of new cars.

P76. Psychophysiological influence of eau de cologne in the office work setting

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The psychophysiological influence of fragrance was studied in a simulated office work experiment. Fifty female college students (aged 20.5 ± 1.3 years, mean ± SD) with informed consents were requested to collate a document displayed on a notebook computer with the original printed on paper, as quickly and accurately as possible. Salivary cortisol (s-CS), mental state and work performance were measured or evaluated during the experiment. Two different types of documents and three kinds of the eau de cologne were combined to make up the experiment. One cologne contained citrus-herbal essence, another had a floral-woody essence and the third was a placebo without fragrance but containing the same amount of alcohol as the other two. The subjects kept working for 140 min after ea sufficeint period of practice.

The concentration of s-CS, one of the so-called 'stress

hormones', increased in the fragrant environment in this study. Other effects suggested from the mental state and work performance were different, depending both on the type of the fragrance and the quality of each document.

P77. Effect on humans of inhalation of essential oil of linalool: sensory evaluation and physiological measurements using optically active linalool

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In this study, the effect on humans of inhalation of essential oil of linalool was examined in terms of sensory evaluation and conventional forehead surface electroencephalography (IBVA-EEG, Psychic Lab. System) measurements using optically active linalools, (*R*)-(−)-, (*S*)- (+)- and (*RS*)-(±)-forms. (*R*)-(−)-linalool with specific rotation of $[\alpha]_D = -15.1°$ was isolated by repeated flash column chromatography from lavender oil, while (*S*)-(+)-linalool with $[\alpha]_D = +17.4°$ and (*RS*)-(±)linalool with $[\alpha]_D = 0°$ and content of (*R*)-form 50.9% and (*S*)-form 49.1% were obtained from coriander oil and commercial linalool, respectively, by use of the same method. As a result, the effect of (*RS*)-(±)-linalool after hearing environmental sounds, which was quite similar to that of linalool in the sensory test with much more favorable impressions than before work, appeared to be identical to that observed with (*R*)-(−)-linalool accompanied with a considerable tendency of a decrease in beta waves as compared with before work with a non-smell blank as control. On the contrary, the feature in the sensory test was just the reverse when mental work was assigned to subjects instead of hearing an environmental sound as the contents of work, but the effect of (*RS*)-(±)-linalool with agitated inclination also seemed to be explained by (*R*)-(−)-linalool, which was accompanied by the same tendency of a decrease in beta waves as observed in (*R*)-(−)-linalool after hearing environmental sounds. Therefore, it may be suggested that the decrease in beta waves once caused agitated inclination after mental work and once caused favorable impressions after hearing environmental sounds.

P78. MEG activities in the human brain by an olfactory oddball paradigm

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We measured activities in response to odorant stimuli with MEG. Two odorant stimuli, amylacetate (sweet, banana-like odor) and isovaleric acid (stinking sweat odor), were used in an oddball paradigm. Namely, one of the odors was the rare stimulus (target) and the other the frequent stimulus (non-target). The stimuli had a duration of 300 ms and were delivered into either the right or the left nostril in one session of the experiment. The delivery was synchronized with the subject's inspirations, which were chosen randomly at a rate of every 3–12 respirations. The responses were averaged over ~40 times. During recordings, subjects were instructed to count the number of presentations of the target. White noise was applied to both ears for masking the sound from the switching valves. The response had three peaks: M1, M2 and M3. The M1 peak had latencies between 190 and 250 ms. Since M1 decreased when the stimulus had less pressure, we assume this is due to trigeminal stimulation. M2 had latencies of ~310–420 ms: we assume it is an olfactory response. M3 had a latency between 490 and 650 ms, which is close to that observed by Kettenmann *et al.*, and was estimated to originate from the superior temporal lobe. We considered M3 to reflect cognition.

P79. An analysis of the event-related potentials elicited by mismatch between an odorant stimulus and a word stimulus in a pleasant or unpleasant situation

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We examined whether an odorant stimulus that deviates from a semantic context or the emotion such as pleasantness/unpleasantness can elicit the event-related potentials (ERPs) as a word or the emotion. ERPs were recorded while 14 students judged the veracity of a simple statement (e.g. Lemon / is / pleasant.) presented with the order of subject (S), object (O) and verb (V), which is normal in Japanese grammer with an S–O–V paradigm. Three kinds of experiment were tested. In these experiments a real odor or a word of pleasantness/unpleasantness was given as subject (S) and/or as object (O). Four different odors were administered randomly using four types of Japanese statements (true-affirmative, false-affirmative, true-negative and false-negative). The students judged the statement's truth or falsity by pushing a button. In each condition, a late negative potential was elicited by the object (O) stimulus when it was mismatched with the subject (S). In addition, the negativities elicited by the incongruous odorant stimulus and word or the emotion of pleasantness/unpleasantness had the same morphology and scalp distribution. These results indicated that a physical stimulus such as a real odor or the emotion of pleasantness/unpleasantness which deviates from a semantic context or the emotion can elicit the negative potentials as the same N400 component as a color patch already used by J. Katayama and A. Yagi.

P80. Study of a sensing film of lipid blended with PVC for an odor sensing system

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Since the development of an odor sensing system is required in many fields, we have developed a system using a Quartz Crystal Microbalance (QCM) array and neural-network pattern recognition.

Since it is important to obtain stable sensor responses, a sensing

film of lipid blended with PVC has been studied here. In this study, 10 MHz AT-cut QCMs were used. First, we studied the characteristics of PVC (polyvinyl chloride) and four plasticizers (DBP, DOP, TCP and DOPP), analyzing the pattern of QCM sensor responses by multivariate analysis such as principal-component analysis. We found that the pattern of DOPP (dioctyl phenyiphosphonate) is very different from that of PVC. Thus, we adopted DOPP as the plasticizer and made a lipid film blended with PVC. A stable sensor response was obtained using a lipid film blended with both PVC and DOPP.

P81. Measurement of coffee aroma by odor sensors

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Various types of odor sensors, including the metal oxide semiconductor type, QCM type and conducting polymer type, have been developed. As part of this effort to investigate the application of these odor sensors in the quality and process control in the production of food and drink, two experiments in the measurement of the aroma of brewed coffee were conducted.

One was in the discrimination of different brands of brewed coffee by conducting polymer type odor sensors (Aromascan). The differences among three kinds of regular coffee brewed from different beans and instant coffee were discriminated by analyzing the response pattern of 32 sensors.

The other was in the detection of deterioration of coffee during heated storage. Coffee incubated at 80°C for 0, 0.5, 1, 1.5, 2, 2.5 and 3 h was measured by metal oxide semiconductor-type odor sensors. The response of two sensors, among the 15 sensors in the odor sensing system, changed according to storage time.

As a result, it has turned out to be possible to use odor sensors for brewed coffee by selecting adequate sensors and adjusting conditions for the purpose. Odor sensors are also likely to be suitable as a substitute or supplement for the cup test currently used in quality and process control.

82. Social medicine and odor hygiene studies on the relationship between odor perception and environmental background factors

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To elucidate the effects on odor perception of different environmental background factors (temperature, atomospheric pressure, light intensity, O_3, CO_2, O_2, CO, content, flammable gas content, humidity, loudness, UV-light intensity and air velocity), odor information was detected with an SnO_2-type sensor simultaneously with the environmental factors by means of a 12-pen multichannel recording system. The system was equipped with adequate sensors to measure changes in environmental background factors.

Signals from the odor sensor were influenced specifically by changes in air velocity, but not by other factors such as CO_2 and O_3 content, temperature and loudness. The simultaneous recording of different environmental factors by a multichannel recording system was found to be useful for the analysis of human odor perception.

P83. Effects of β-cyclodextrin on gurmarin inhibition of sweet taste responses in mice

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Cyclodextrins (CDs) have the remarkable ability to form inclusion complexes with a wide variety of guest molecules, including amino acids with aromatic rings. In the present study, possible influences of CDs on the effect of a sweet response inhibiting peptide, gurmarin, were examined. Responses of the mouse chorda tympani nerve to sucrose were suppressed to ~50% of control by treatment of the tongue with 30 μg/ml gurmarin. Rinsing the tongue with 15 mM β-CD after gurmarin facilitated the recovery of sucrose responses, whereas that with the same concentration of α- and γ-CD did not affect it. Treatment with a mixture of 30 μg/ml gurmarin with 15 mM β-CD suppressed sucrose responses to ~80% of control, suggesting the reduction of suppressive effects of gurmarin on sucrose responses by mixing with β-CD. Such reduction of effects of gurmarin was not clearly observed in the case of mixtures with α- and γ-CD. Gurmarin includes L-tyrosine and L-tryptophan as constituents whose aromatic rings locate outside of its three-dimensional chemical structure, which may allow the formation of inclusion complexes with β-CD. Taking this into consideration, it is probable that the observed reduction of effects of gurmarin is due to steric hindrances in gurmarin, formed by β-CD, that may interfere with binding with sweet taste receptors.