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ABSTRACTS

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I-1. Olfactory memory and its neural representation

E.B. Keverne

Sub-Department of Animal Behaviour, University of Cambridge, Madingley, Cambridge CB3 8AA, UK. email: ebkl0@cus.carn.ac.uk

Sheep form a recognition memory for their lambs based exclusively on olfaction, and the critical window for recognition is in the 4 h period post-partum. Removal of the lamb for the duration of this critical period results in rejection of all lambs. Positive recognition results in maternal acceptance of own lamb and aggressive rejection of strange lambs. We have investigated various regions in the brain that process olfactory stimuli in an attempt to understand the neural basis of olfactory recognition and the different behavioural responses that are generated to own and alien lambs.

The most thoroughly investigated region of the brain that plays a significant role in lamb recognition is the olfactory bulb. The olfactory bulb is an important interface between receptor neurons and the brain, as well as participating directly in the memory process. It analyses each input and then synthesizes its own message depending on what it has experienced with that odour previously. The self-organizing capacity of the bulb, with changes in sensitivities of some neurons to certain odours, serves as a repository of past associations. Any subset in this assembly can become rapidly stimulated, recruiting the rest of the bulbar network. These neural changes only represent the first stage in the processing of odours that underlie lamb recognition and how the rest of the brain handles this information is equally important.

The pyriform cortex appears not to be essential for the recognition of own lamb since acceptance proceeds normally even when the pyriform cortex is inactivated. However, the ability to discriminate strange lambs of the same breed is attenuated, but not that for lambs of a different breed. Although inactivating the pyriform cortex throughout the critical period for lamb recognition impairs this fine discrimination, even this is not permanent and discontinuation of pyriform cortex blockade results in a return to acuity and complete rejection of all but the lamb that was present during the critical period. Hence, the discrete critical period for recognition which is operative for the main olfactory bulb appears not to be required for the functioning of the pyriform cortex.

The medial prefrontal cortex receives a strong olfactory input in sheep and blockade of this region during the critical period for memory formation prevents rejection of strange lambs. However, aggressive rejection of strange lambs was immediately contingent on the withdrawal of medial prefrontal cortex tetracaine blockade, revealing that recognition had already occurred. These findings suggest that olfactory recognition memory *per se* does not require participation of the medial prefrontal cortex, but activation of this cortical region is necessary to override maternal acceptance and engage the motor activity that constitutes aggressive rejection of unfamiliar lambs.

T-1. Studies of physical responses to various taste stimuli in the human

T. Horio

College of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo, 665-0006, Japan

Physical responses to various taste stimuli studied in the human were as follows. (i) Relationships between taste stimuli and heart rate were evaluated. Heart rate increased by 7.1–13.6% for all the taste-stimuli after use as compared with pre-stimuli values. Heart rate reached its maximum with citric acid. Except for sucrose, increases in heart rate and the hedonic scale values of the taste solutions showed significant negative correlation. (ii) Electromyogram responses of the facial and chewing muscles induced by different taste stimuli were analysed. EMG responses of the corrugator supercilii, venter frontalis, orbicularis oculi, depressor anguli and digastricus muscles to capsaicin, tannic acid and citric acid showed larger amplitudes than to NaCl, MSG, homogentisic acid and sucrose. Increases in facial and chewing muscle EMG responses for the taste solutions had significant negative correlations to hedonic scale values for the taste solutions. (iii) The effects of physical exercise on preference for various sapid solutions was studied. After 30 min of exercise using a bicycle, preference scale values for sucrose and citric acid increased, whereas the values for NaCl, caffeine and MSG were not changed.

These findings indicate that the physical responses might be related deeply with hedonic tone.

T-2. Signal transduction mechanism in vomeronasal receptor neurons and properties of reciprocal synaptic currents in accessory olfactory bulb

Mutsuo Taniguchi

Department of Physiology, Kochi Medical School, Kochi 783-8505, Japan

Understanding the mechanisms of signal transduction in vomeronasal receptor neurons and of synaptic transmission in the accessory olfactory bulb (AOB) may provide insights into a better understanding of how pheromonal information is processed and integrated.

To investigate the role of the IP_3-mediated pathway in signal transduction, IP_3 was dialyzed into snake vomeronasal receptor neurons in slice preparations and the evoked currents were measured with whole-cell patch-clamp. Intracellular dialysis of

100 μM IP_3 evoked an inward current. Bathing the neurons in a 10 μM ruthenium red solution greatly reduced the IP_3-evoked inward currents. With an internal solution containing Cs^+, neither the Ca^{2+}-ATPase inhibitor, thapsigargin (1–50 μM), nor the Ca^{2+}-ionophore, ionomycin (10 μM), evoked a significant current, suggesting that IP_3 can elicit a current response in neurons without mediation by intracellular Ca^{2+}, stores. Extracellular application of chemoattractant for snakes evoked a large inward current. The reversal potential of the chemoattractant-induced current was similar to that of the IP_3-induced current. The present results demonstrate the existence of an IP_3-activated conductance in snake vomeronasal receptor neurons, supporting the idea that the IP_3-mediated pathway in snake vomeronasal receptor neurons is involved in the transduction of chemoattractant signals.

To investigate the properties of the synaptic transmission, studies were also conducted with mitral cells in slice preparations of the mouse (23- to 34-day-old BALB/c mice) AOB, where evoked synaptic currents were measured from mitral cells with the patch-clamp technique in nystatin-perforated whole-cell configuration. To evoke dendrodendritic inhibition, a depolarizing voltage step from –70 to 0 mV (5–20 ms) was applied to a mitral cell. Under control conditions, the voltage step evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs), which were greatly enhanced after the reduction of extracellular Mg^{2+}. In Mg^{2+}-free solution, the NMDA receptor antagonist D,L-APV, as well as an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, significantly reduced dendrodendritic inhibition. On the other hand, the non-NMDA receptor antagonist CNQX moderately blocked the IPSCs. In Mg^{2+}-containing solution, the mGluR2 antagonist LY341495 enhanced the IPSCs. The present results suggest that NMDA receptors and mGluR2 play an important role in reciprocal transmission between mitral cells and granule cells in the mouse AOB.

K-1. Study on bioactive substances in the rat brain after stimulation by a bitter taste

Yasuko Manabe

Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Injection of cerebrospinal fluid (CSF) from rat after intraoral stimulation of quinine–HCl (quinine CSF) into the fourth ventricle suppressed the intake of 5% sucrose solution in mice. We supposed that this suppression was induced by substances in the quinine CSF. We used Hydra bioassay, which utilizes pattern of a tentacle ball formation (TBF) of Hydra, to identify the releasing substance in the quinine CSF, and found diazepam-binding-inhibitor (DBI)-like peptide increased in the quinine CSF. Then, injection of the DBI-peptide fragment into the fourth ventricle also suppressed the intake of 5% sucrose, water and 0.9 mM quinine–HCl solution and the preference for 0.05% saccharine. DBI has been found to be the only endogenous ligand to benzodiazepine receptor. Pretreatment with flumazenil, a benzodiazepine receptor antagonist, antagonized this suppressive effect of DBI on the intake of 5% sucrose, suggesting that DBI acted through the benzodiazepine receptor. Furthermore, the taste reactivity test revealed that injection of the DBI-peptide fragment into the fourth ventricle increased aversive response in mice. These results suggest that DBI is related to taste aversion.

S1-2. Chemosensory function of the amphibian skin: transcellular and paracellular mechanisms

Stanley D. Hillyard, Karin Hoff and Takatoshi Nagai[1]

Department of Biological Sciences, University of Nevada, Las Vegas, NV 89154-4004, USA and [1]Department of Biology, Keio University School of Medicine, Yokohama 223-8521, Japan

Hoff and Hillyard (1993) used a behavior termed the water absorption response (WR) to assess toads' ability to detect the salt concentration of hydration. In this behavior, dehydrated animals press the ventral pelvic skin (seat patch) to a moist surface in order to absorb water. If amiloride was added to a surface moistened with a hypertonic NaCl solution, toads would initiate WR behavior in a significantly greater fraction of trials than with a NaCl solution alone. It was hypothesized that, like taste buds in the mammalian tongue, the toad skin epithelium serves a chemosensory function. In support of this hypothesis, Nagai *et al*. (1999) showed that exposure of the skin to hyperosmotic salt solutions induces a neural response in spinal nerves to the skin and that the neural response to NaCl solutions was partially reduced by amiloride. Using the lipophilic dye, diI, it was found that specific cells in the basal layer of the epidermis were innervated by branches of the spinal nerves. Sullivan *et al*. (2000) found that the amount of time that dehydrated toads spent on surfaces moistened with 250 mM salt solutions increased as a linear function of the mol. wt of the anion. These results suggest that at least one function of regulating occluding junctions in amphibian skin is to facilitate evaluation of hydration surfaces and challenge the current paradigm that the amphibian skin and bladder are consistently tight epithelia.

S3-1. Physical properties and palatability of foods

Yasuki Matsumura

Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Gokasho, Uji 611-0011, Japan

Physical properties of foods, such as texture and temperature, play important roles when human beings evaluate the palatability of foods. Unlike taste and flavor sensations, the textures of foods cannot be simply related to the amount of a single component, but are affected by interaction and macro- or microstructure formation of several components. The interaction and structure formation of food components are detected by change of physical parameters such as viscosity and elastic modulus of foods. Therefore, we can say that 'texture' means the sensation of such physical parameters of foods as perceived by human beings. Physical parameters of foods can be measured by rheological techniques. For liquid foods, the shear viscometer is very often used to assess fluid behavior. Many liquid foods are non-Newtonian, but show shear-thinning behavior. It has been shown that the sensory qualities of 'viscosity', such as 'thickness' and 'stickiness' of a liquid food, are closely related to viscosity as measured by the viscometer at a shear rate specific to the food. It is probable that human beings perceive viscosity of liquid foods by judging shear rates in their mouths. In this paper, I also point out the importance of oscillatory rheological measurements for assessing physical properties of sol, gel and solid foods.

S3-2. Palatability of food and fluid in the pharynx and larynx

Tomio Shingai and Jun-ichi Kitagawa[1]

Division of Chemoreception Science and [1]Oral Physiology, Graduate School of Medicine and Dental Science, Niigata University, Niigata 951-8514, Japan. e-mail: shingai@dent.niigata-u.ac.jp

Food and fluid entering the pharynx and larynx may stimulate a variety of sensory receptors in the regions. Although taste buds are mainly located in the oral cavity, they are also found extra-orally, in the pharynx and larynx. However, the physiological importance of taste receptors in the extra-oral region is not well understood. In the present study, we investigated the responsiveness of the superior laryngeal nerve (SLN) and the pharyngeal branch of the glossopharyngeal nerve (GPN-ph) to drinking fluids and taste stimuli. Nerve activities were recorded from the whole nerve of the SLN and GPN-ph in urethane-anesthetized rats. Fresh beer and 5% ethanol elicited marked responses in the both nerves. Soda water produced a transient response. Umami taste stimulation with 0.1 M MSG and 10 mM IMP produced a marked response, greater than that to water, in the GPN-ph. We found that long-chain fatty acids (oleic acid and linoleic acid) had potent excitatory effects on the GPN-ph. Triolein, which was used as a pure fat, and ethyl oleate had no effect on nerve activity. We also demonstrated that an i.v. injection of leptin (10 ng) suppressed the response to fatty acids without affecting responses to water and NaCl.

S3-3. Adipocytes and appetite regulation

Teruo Kawada[1,2], Nobuyuki Takahashi[2] and Thoru Fushiki[1]

[1]Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 and [2]Project of Molecular Mechanisms for the Regulation of Energy Metabolism and Adipocyte Functions, Bio-oriented Technology Research Advancement Institute, Tokyo 105-0001, Japan. e-mail: fat@kais.kyoto-u.ac.jp

Since animals are under constant threat of starvation, there is storage of energy sources inside the body for various activities. Therefore, animals exhibit highly sophisticated mechanisms for storing energy inside their bodies in adipose tissue. However, in humans it has been shown that fat cells (adipocytes), which compose adipose tissues and control differentiation and the extent of subsequent fat accumulation, are closely associated with the occurrence and advancement of various diseases resulting from obesity. Moreover, progress in biochemical studies with respect to adipocytes has in recent years rapidly clarified new functions and the differentiation mechanism of adipocytes. A particularly interesting point is the function of white adipocytes as 'secreting cells'. Various chemical factors, including cytokine groups such as TNF-α, are secreted from mature adipocytes filled with fat droplets, and secreted chemical factors strongly influence adipose tissues and the entire body. It is significant to clarify how factor groups secreted from such adipocytes are related to the occurrence of common diseases such as diabetes and arterial sclerosis on obesity. Physiologically active substances secreted from adipocytes are called 'adipocytokines'. Especially, leptin is an afferent signal from adipose cells to the brain in a homeostatic feedback loop that governs adipose tissue mass via appetite regulation and energy expenditure.

S3-4. Ghrelin: a novel orexigenic peptide from stomach

Masamitsu Nakazato[1], Noboru Murakami[2], Yukari Date[1,4], Masayasu Kojima[3], Kenji Kangawa[4] and Shigeru Matsukura[1]

[1]Department of Internal Medicine, Miyazaki Medical College, Miyazaki 889-1969, [2]Department of Veterinary Physiology, Miyazaki University, Miyazaki 889-2192, [3]Molecular Genetics, Institute of Life Sciences, Kurume University and [4]Department of Biochemistry, National Cardiovascular Central Research Institute, Osaka 565-8565, Japan

Ghrelin is an acylated peptide that stimulates the release of growth hormone from the pituitary. Ghrelin-producing neurons are located in the hypothalamus, whereas ghrelin receptors are expressed in various regions of the brain, which is indicative of central and as yet undefined physiological functions. Here we show that ghrelin is involved in the hypothalamic regulation of energy homeostasis. Intracerebroventricular injections of ghrelin strongly stimulated feeding in rats and increased body wt gain. Ghrelin also increased feeding in rats that are genetically deficient in growth hormone. Anti-ghrelin immunoglobulin G robustly suppressed feeding. After intracerebroventricular ghrelin administration, Fos protein, a marker of neuronal activation, was found in regions of primary importance in the regulation of feeding, including neuropeptide Y (NPY) neurons and agouti-related protein (AGRP) neurons. Antibodies and antagonists of NPY and AGRP abolished ghrelin-induced feeding. Ghrelin augmented NPY gene expression and blocked leptin-induced feeding reduction, implying that there is a competitive interaction between ghrelin and leptin in feeding regulation. We conclude that ghrelin is a physiological mediator of feeding, and probably has a function in growth regulation by stimulating feeding and release of growth hormone.

W1-1-1. Molecular genetic identification of a novel taste receptor T1R3

Michinori Kitagawa[1,2,4], Yuko Kusakabe[1,4], Hirohito Miura[1,4], Yuzo Ninomiya[2,4] and Akihiro Hino[1,4]

[1]National Food Research Institute, 2-1-12 Kannondai, Tsukuba-shi, Ibaraki 305-8642, [2]Institute of Molecular Embryology and Genetics, Kumamoto University, [3]Faculty of Dentistry, Kyushu University, [4] Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon Minato-ku, Tokyo 105-0001, Japan

A cDNA clone encoding a novel member of the putative taste receptor T1R family, designated *T1R3*, was isolated from circumvallate papillae of the mouse tongue using degenerate primers designed from the sequences of T1R and V2R. Reverse transcription-polymerase chain reaction analysis showed predominant expression of the receptor in circumvallate papillae. *In situ* hybridization analysis revealed that *T1R3* was expressed in a subset of taste receptor cells in taste buds and that the topographic distribution of *T1R3* in various taste papillae was different from those of the other T1R members. Two-color *in situ* hybridization showed that the signals for *T1R3* overlapped those for *gustducin* in fungiform papillae, though the signals for the both genes did not overlap in circumvallate and foliate papillae. Further, the expression of *T1R3* overlapped with that of *T1R2* in circumvallate and foliate papillae, and with *T1RI* in fungiform papillae, respectively. Genetic mapping of T1R3 with a mouse/hamster radiation hybrid panel located the gene on the distal end of mouse chromosome 4, correlated with the *Sac* locus affecting sweet sensitivity of mice.

Our results indicate that T1R3 may serve as the receptor for sweet perception in mice.

W1-1-2. Is *Drosophila Tre* identical to a gustatory receptor gene *Gr5a* or a G-protein receptor gene *CG3171*?

K. Isono, K. Ueno[1], M. Ohta, H. Morita and K. Yamamoto[2]

Graduate School of Information Science, Tohoku University, Sendai 980-8579, [1]Institute for Behavior Sciences, Gunma University School of Medicine, Maebashi 371-8511 and [2]Graduate School of Life Sciences, Tohoku University, Sendai 980-8578 Japan

Drosophila taste gene *Tre* controls gustatory sensitivity to a subset of sugars. Our previous analysis located the gene to either *CG3171*, encoding a rhodopsin family G-protein receptor, or an adjacent candidate chemosensory gene *Gr5a*, encoding a putative gustatory receptor. We showed that induced *Tre* mutations disrupt *Gr5a* gene organization and the expression of *Gr5a* mRNA in the taste receptor neurons. The *CG3171* gene, on the other hand, was shown to be ubiquitously expressed in various non-gustatory tissues and was not always disrupted by mutations in *Tre*. The coding sequences of these two candidate genes were compared among various strains carrying wild-type allele of Tre^{+} or a spontaneous mutation *Tre01*.

Polymorphic sites leading to amino acid changes in *CG3171* were not correlated with the gustatory phenotype. A single nucleotide polymorphism leading to an Ala2l8Thr substitution in the predicted second intracellular loop of *Gr5a* co-segregated with *Tre01*. Taken together, the mutation analyses support that *Gr5a* is allelic to *Tre*. Our results were inconsistent with the data described by Ishimoto *et al*. (2000, *Science*, 289: 116–119), who claimed that *CG3171* is expressed specifically in taste neurons and that *Tre* mutations can be rescued by inducing expression of *CG3171*.

W1-1-3. MSG-induced response and its enhancement by GW in mouse non-dissociated taste receptor cells

Takenori Miyamoto, Toshihiro Miyazaki[1], Yukio Okada and Kazuo Toda

Department of Oral Physiology and [1]Department of Oral Histology, Nagasaki University School of Dentistry, Nagasaki 852-8588, Japan

We examined electrical responses induced by monosodium glutamate (MSG), an umami substance, in taste receptor cells (TRCs) using the perforated patch clamp method with a localized stimulation procedure to avoid the contamination of MSG-induced responses other than umami responses. Apically applied MSG (0.03–0.3 M) induced inward current responses in 30% of TRCs and outward current responses in 26% of TRCs at a holding potential of –90 mV. Fifty per cent of the inward current responses were enhanced by 0.1 mM guanosine 5′-monophosphate (5′-GMP), an umami enhancer. On the other hand, outward current responses were not affected by 0.1 mM 5′-GMP and inhibited spontaneous firings under current clamp. The MSG-induced and 5′-GMP-enhanced current responses, which showed voltage-dependent inactivation at potentials more negative than –60 mV, were not blocked by the group III metabotropic glutamate receptor (mGluR) blockers, (*R*,*S*)-α-methyl-4-phosphonophenyl-glycine (MPPG). Immunohistochemical localization of a brain-derived mGluR4a, was observed in the multiple taste bud cells within a fungiform papilla, whereas one of the ionotropic GluRs, GluR4, was detected in only a few taste bud cells among many fungiform papillae. These results suggest that only inward current responses enhanced by 5′-GMP are related to the main part of umami response, and outward current responses play some modulatory role.

W1-1-4. Expression of neuro D in mouse taste buds

Yuko Suzuki

Department of Oral Anatomy, School of Dentistry, Health Sciences University of Hokkaido, Ishikari-Tobetsu 061-0293, Japan

Neuro D, a basic helix–loop–helix transcription factor, has been shown to play a role in the differentiation of neurons, olfactory cells and neuroendocrine tissues. Since the taste buds have characteristics of typical paraneurons, we examined the expression of neuro D in the taste buds of mice. By RT–PCR analysis, neuro D mRNA was found to be expressed in the epithelium of circumvallate papillae containing taste buds, but not in the lingual epithelium lacking them. Neuro D immunoreactivity was detected in a subset of taste bud cells in the circumvallate, foliate and fungiform papillae and in the soft palate. Cells expressing neuro D had a spindle-like shape, first appeared at postnatal day 3 and increased in number during postnatal development. After bisection of the glossopharyngeal nerves, neuro-D-expressing cells decreased in number at 4 days and disappeared from the trench wall of the circumvallate papillae by 14 days. A few neuro-D-expressing taste buds reappeared at postoperative day 28. Denervation and regeneration experiments showed that expression of neuro D in the taste buds was dependent upon gustatory innervation. Double immunolabeling with gustducin or with NCAM showed that neuro D-expressing cells did not express NCAM, but did express gustducin. These results suggest that neuro D is expressed in a differentiated cell type: in type II cells but not in type III cells.

W1-2-3. Study of electric response to sweet taste substances using taste sensor with lipid/polymer membranes

Masaaki Habara, Hidekazu Ikezaki[1], Kiyoshi Toko

Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka 812-8581 and [1]Anritsu Corporation, Kanagawa 243-8555, Japan

We had previously developed a multichannel taste sensor using lipid/polymer membranes which can detect basic taste substances in aqueous solution. The sensor has ‘global selectivity’, i.e. an intelligent sensitivity to chemical substances, which can classify five basic taste qualities of sourness, saltiness, sweetness, bitterness and umami taste. The sensor has been applied to many foods and drinks such as beer, coffee, tea, mineral water, sake, fruits and vegetables. Its discrimination ability, durability and sensitivity are superior to those of humans.

However, the sensor output for sweetness has hitherto been relatively low: one-fifth to one-tenth of that of basic taste substances, due to the nonelectrolytic nature of sweet stimuli. Therefore, it has

remained to us to obtain adequate sensitivity for sweet taste substances.

In this study, we have developed an almost ten times higher sensitivity for sweet taste substances compared to a conventional lipid/polymer membrane. We used 4.5 mg of negatively charged lipid (2C16) and changed the quantity of positively charged lipid (TDAB). The optimum value was obtained at ~1.25 mg TDAB. This means that the mixed quantity of TDAB has a influence on the response and thus balanced charge density is required to improve the sensitivity for sweetness.

W1-2-4. Observation of fungiform papillae by contact endoscope

Atsushi Negoro, Masanori Umemoto, Megumi Fujii, Keijiro Fukazawa and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya City, Hyogo 663-8501, Japan

A contact endoscope (Contact Micro-Rhinoscope®) was used to observe a microscopic construction of superficial layers of the vocal cord epithelium and the nasal mucosa. We tried using this scope in the observation of fungiform papillae, and examined a correlative of taste disorder.

This scope clearly showed that terminal blood vessels run around fungiform papillae and that blood runs very fast in these vessels. We were able to record this phenomenon using a video system. In the case of patients with a normal sense of taste, terminal blood vessels ran clearly in the fungiform papillae. But, in the case of patients with disordered taste, blood vessels were shorter than normal and became thin at the ends of ducts. On the other hand, we stained superficial layers of the tongue with 1% methylene blue. There were many epithelial cells stained blue, which had small and round nuclei. In the same way as with vocal cord and nasal mucosa, this scope may give superior performance in the diagnosis of tongue lesions. In the future, we want to examine correlation of taste disorder and the ends of blood vessels in fungiform papillae and determine the utility of this device in oncology.

W1-3-1. Effects of orexin-A on the intake of taste solutions in rats

Yuuichi Furudono and Takashi Yamamoto

Division of Behavioral Physiology, Department of Behavioral Sciences, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Japan 565-0871

Orexin-A and -B are known to increase food intake. Orexin-containing neurons are specifically localized within and near the lateral hypothalamus. These neurons innervate the nucleus of the solitary tract (NTS), parabrachial nucleus (PBN) and amygdala (Amy) which are related to taste function. These findings suggest that orexins play some role in the drinking of sapid solutions. Therefore, we examined effects of centrally administered orexin-A on the intake of taste solutions. Rats were anaesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with a guide cannula into the left lateral ventricle. Rats received microinjections of either orexin-A (3 nmol) or vehicle under water deprived or replete conditions. Immediately after the microinjection, rats were allowed to drink water, 0.1% saccharin or 3×10^{-4} M quinine for 3 h. Although orexin-A increased the intake of the three fluids under the deprived condition, the effect was most dominant in saccharin intake. In the replete condition, only the intake of saccharin was increased. These results suggest that orexin-A is concerned with a craving for sweet taste.

W1-3-2. Effects of olfactory experiences on appetite in the blowfly, *Phormia regina*

Mamiko Ozaki, Teruhiko Takahara, Kyoko Nakamura, Mayako Kakiuchi and Ryohei Yamaoka

Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Using D-limonene and some other monoterpenes, we examined their oral toxicity for the blowfly, *Phormia regina* and found the following order of toxicity: D-limonene > L-limonene > citral > toluene. When the taste sensilla of the blowfly were touched with D-limonene, the fly exhibited aversive behavior accompanied by vomiting and excretion.

Vapors of these chemicals did not show severe effects on the life time period of the fly. However, their odors or even the memory of the odors obviously decreased the sensitivity of the proboscis extension reflex to sucrose. We also measured monoamines in the brain and found some differences between the flies thus reduced in their appetite and the control flies showing normal appetite.

For survival, the flies, when exposed such dangerous odors, may reduce their appetites. Even if they touch the toxic chemicals, the fifth cells in the taste sensilla responding to them would prevent the flies from eating them.

W1-3-3. Effects of black tea on the central and autonomic nervous systems

Fusae Nishimura, Hideaki Kajiura, Kumiko Shimamoto, Yoko Kusumi, Yuko Yotsumoto[1] and Masahiro Arima[1]

Central Laboratories for Key Technology, Kirin Brewery Co. Ltd, 1-13-5, Fukuura, Kanazawa-ku, Yokohama, 236-0004 and [1]Research and Development Laboratory, Kirin Beverage Corp., 1620, Kurami, Samukawa-machi, Koza-gun, Kanagawa, 253-0101, Japan

Psycho-physiological effects of ingestion of black tea were investigated using healthy young volunteers. EEG spectral analysis revealed that relative power of slow alpha significantly decreased and that of beta increased for 30 min after ingestion of tea drink. Such changes in EEGs were not observed after ingestion of control (sucrose) solution with the same concentration as the tea drink. Furthermore, relative power of slow alpha significantly decreased and that of fast alpha increased immediately after ingestion of the tea drink or presentation of the odor of black tea, suggesting that the flavor of black tea itself had significant effects. EEG desynchronization with increase in beta power suggests that tea ingestion might increase arousal and attentional level, while increase in fast alpha power might be related to increased intelligent activity in the brain. On the other hand, power spectral analysis of heart rate variability revealed that high-frequency power of heart rate variability significantly increased with decrease in heart rate for ~30 min after tea ingestion. However, such changes were not observed after ingestion of control solution. The results suggest that ingredient(s) in black tea drink may induce relaxation of the autonomic nervous system through inhibition of the sympathetic nervous system.

W1-3-4. Importance of oral sensory function and taste stimuli for aged patients with neurological disorders: a case report of the terminal oral cancer patient

Masaaki Takita, Noriyoshi Nishikawa, Hiroyuki Kyomoto, Yoshimi Matsuda and Seiji Takada

Saiseikai Nakatsu Hospital, Osaka Department of Oral Surgery, 10-39 Shibata 2-chome, Kitaku, Osaka 530-0012, Japan

A 78-year-old man with advanced neurological disorders (cerebral infarction, senile dementia and Parkinson's disease; Yahl classification 5) and progressive gingival cancer in the end stage was referred to our hospital for terminal care. The patient's airway was significantly depressed by tumor growth with secondary edema and his consciousness was drowsy. Systematically, by palliative chemotherapy (antibiotics and low dose cisplatin combined with 5-fluorouracil), his consciousness level was improved and locally due to oral health care, he was enabled to eat soft meals (rice gruel) with help from his wife without problems swallowing. Pleasure and/or aversion responses to food taste [palatability of seaweed boiled down with soy including umami taste, aversion to monotonous and/or dull sour taste of *umeboshi* (pickled plum)] were observed as facial expressions. He received oral nutrition based on his taste preferences for 3 months until disease progression caused swallowing problems, followed by stomach tube until the endpoint, without parenteral nutrition. His survival period was 6 months. It is suspected that even in aged patients with cortical brain disorders, taste preferences based on oral sensory and midbrain function are present and that personal taste preferences induce positive emotions and have beneficial psychoneuroimmunological effects, even during the terminal course. Also, from the perspective of bioethics, oral sensory function (chemical senses) and personal taste preferences should be considered important as an expression of personal respect, even for handicapped patients.

W2-01. Aversive effects of various chemicals on crow

Seiji Morimoto[1], Tetsuya Mori[2], Kenzo Kurihara[1] and Tadashi Kashiwagura[1,2]

[1]*Environmental Sciences, Graduate School and* [2]*Department of Bioscience and Biotechnology, Faculty of Engineering, Aomori University, Aomori 030-0943, Japan*

Recently, the numbers of crow have greatly increased in Japanese cities, which has brought about various serious problems: crows often attack humans and scatter garbage. Attempts to repel crows have been made using a number of methods based on their visual or auditory senses. These methods cannot, however, be used for a long time because of the learning abilities of crows. In the present study, we searched for chemicals which cause aversive effects in crows, since aversive action to chemicals seems to be instinctive. In the experiments, dog foods were used as bait for crow. Control samples consisted of the dog food alone and test samples consisted of the dog food plus a test chemical. Aversive effects were evaluated by weighing the dog food eaten by crows. Various odorants such as camphor, naphthalene, menthol and limonene showed appreciable aversive effects. Aversive effects of skins of grapefruit seem to be due to limonene in these skins. It is unknown yet whether aversive effects of *wasabi* and garlic come from their odorant or taste components. Further, more quantitative experiments are needed.

W2-02. Blockade of chemical communication by an aromatic environmental pollutant

Takashi Shiraiwa and Makoto Kashiwayanagi

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Many classes of environmental pollutants are found at significant levels in the environment. As for the reproductive field, environmental pollutants which disrupt the endocrine system have been shown to be endowed with hormone/antihormone activity. Gonadal functions of various animals are regulated by pheromones whose production is modulated by hormones. The purpose of this study was to explore effects of 3-methylcholanthrene (3-MC), which is widely distributed in a variety of products, on excretion of pheromones from male mice. On day 1 after the i.p. dosage of 3-MC, the density of Fos-immunoreactive cells, which is correlated with cellular activity, in the accessory olfactory bulb of female mice after exposure to urine excreted from treated males was lower than that after exposure to urine from non-treated males. SDS analyses were performed on the urine on day 1 after the 3-MC treatment. The level of proteins, including major urinary proteins (MUPS), in urine from treated males was similar to that from non-treated males, suggesting that 3-MC lowers pheromonal activity in urine without significant changes in urinary MUPs on day 1. It is likely that environmental pollutants interfere with chemical communications between males and females.

W2-03. The mGluR2 agonist DCG-IV facilitates olfactory learning in young rats

Fumino Okutani and Hideto Kaba

Department of Physiology, Kochi Medical School, Nanko-ku, Kochi 783-8505 and CREST, JST, Japan

Post-natal day (PND) 12 rats before eye opening show aversion to the odor which has been exposed with foot shock for 30 min on the previous day. The mechanism underlying this olfactory conditioning is thought to be disinhibition of the mitral cells from the granule cells by somatosensory-stimulation-induced activation of noradrenergic inputs to the olfactory bulb (OB). We previously reported that intrabulbar infusion of a GABA receptor antagonist, bicuculline, through the cannulae implanted on PND 10 facilitates olfactory aversive learning. It is also known that metabotropic glutamate receptors (mGluRs) play a role in synaptic plasticity. *In situ* hybridization and immunohistochemical studies revealed that mGluR2 was richly expressed in the external plexiform layer and granule cell layer of the OB. An *in vitro* electrophysiological study showed that mGluR2 activation of the granule cells decreases GABA release from the cells at the dendrodendritic synapses. To determine the role of OB mGluR2 in olfactory aversive learning, we infused an mGluR2 agonist, DCG-IV, into the OB during odor exposure on PND 11. DCG-IV infusion facilitated olfactory aversive responses without foot shock in a dose-dependent manner. These results suggest that mGluR2 activation disinhibits the mitral cells by reduction of GABA release from the granule cells, inducing plasticity at dendrodendritic synapses between the mitral and granule cells.

W2-04. Relaxation effects associated with different coffee bean aromas

Yoshihiko Koga, Chizuru Konagai[1], Mayuko Hamada[1] and Hideo Kurosaki

Department of Neuropsychiatry, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 112-8611 and [1]Department of Food and Nutrition, Japan Women's University, Tokyo 112-8681, Japan

We used electroencephalographic analysis to clarify differences in effects on mental functions induced by the aroma of various types of coffee beans. Ten right-handed healthy females (20–24 years old) participated in this study. We used six types of coffee beans—Brazil Santos, Guatemala, Blue Mountain, Mocha Mattari, Mandheling and Hawaii Kona—which had been medium roasted and medium ground. Just prior to the EEG measurement, the ground coffee (0.5 g) was mixed with hot water (98°C, 5 ml) in a test tube. These samples were randomly presented under the subject's nose. We recorded the eyes-closed EEG from 19 electrode sites and calculated the power spectrum at each site using FFT analysis. The alpha activities induced by the coffee aroma of each variety were compared. The aroma of Guatemalan beans significantly increased the amount of alpha waves as compared with that of control (no odor) and four other kinds of coffee beans, excepting Blue Mountain, in the higher frequency range of alpha waves. The aroma of Mandheling tends to be associated less alpha activity as compared with the control. Results from this study suggest that the relaxation effects differ among different types of coffee beans.

W2-05. Effects of jasmine tea odor on the autonomic nervous system

Naohiko Inoue, Kyoko Kuroda, Akio Sugimoto[1], Takami Kakuda[1] and Tohru Fushiki

Laboratory of Nutritional Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 and [1]Itoen Ltd, Central Research Institute, 21 Mekami, Sagara-cho, Haibara-gun, Shizuoka-ken 421-0516, Japan

The effects of jasmine tea odor on the autonomic nervous system were investigated by power spectral analysis of heart rate variability. The physiological and psychological effects of odors are thought to be the results of interaction between the odor compounds and emotional effects depending on preference. We divided 10 healthy volunteers into two groups with high or low preference for jasmine tea odor. We used the weak and normal odors of jasmine tea. The normal odor was from jasmine tea prepared by adding 25 g jasmine tea leaves to 1 l of freshly boiled water. The weak odor was from jasmine tea diluted at 20 times. In the experiment with weak odor, there was no difference in autonomic nervous activities between the high and low preference groups. A decrease in heart rate and an increase in parasympathetic nervous activity were observed in the both groups. In the experiment with the normal intensity of odor, a decrease in heart rate and an increase in parasympathetic nervous activity were observed in the high preference group, but an increase in sympathetic nervous activity was observed in the low preference group. From these results, we suggested that the odor of jasmine tea activated the parasympathetic nervous system, but in the case of the higher intensity of odor, preference for the jasmine odor affected the autonomic nervous system.

W2-07. The brain regions activated by the evaluation of the pleasantness of odors

Nobuyuki Sakai[1,2], Akira Takahashi[1,3], Tatsu Kobayakawa[1], Yasushi Yamauchi[1], Sumio Imada[4] and Sachiko Saito[1]

[1]National Institute of Advanced Industrial Science and Technology (AIST), [2]Japan Science and Technology Corporation, [3]Shizuoka University and [4]Hiroshima Shudo University, Japan. e-mail: nob-sakai@aist.go.jp

To clarify the brain mechanisms involved in the processing the pleasantness–unpleasantness of odors in humans, we investigated human brain activities during smelling of odors utilizing functional magnetic resonance imaging (fMRI). The participants were asked to smell three kinds of odor (triethyl amine, rose P and citral) and were also asked to evaluate the intensity and the pleasantness (or unpleasantness) of each olfactory stimulus during our acquisition of echo-planar images of the brain. The hedonics of the stimuli was evaluated on a scale from –3 (extremely unpleasant) to +3 (extremely pleasant) with the decimal. Thus, the data obtained from conditions in which the participants rated the presented olfactory stimuli as unpleasant (evaluated score < 0) were processed together as the 'negative' condition. The data obtained from conditions in which the participants rated the presented stimuli as pleasant (evaluated score > 0) were processed together as the 'positive' condition. The cerebellum, the insular cortex and the prefrontal cortex (Broadmann area 46) showed correlative activity with the olfactory stimulation. The correlated activity was found in the higher visual cortex (Broadmann area 18) in the 'negative' condition, whereas the amygdala showed correlated activity in the 'positive' condition. These results are discussed together with those of preceding studies.

W2-08. Comparative study of the T&T olfactometer and the odor stick identification test for patients with olfactory disturbance

T. Miwa, I. Horikawa, T. Ishimaru, S. Hatanaka, M. Furukawa, S. Saito[1] and Y. Takashima[2]

Department of Otolaryngology, Kanazawa University, Kanazawa 920-8640, [1]National Institute of Advanced Industrial Science and Technology, Tsukuba 386-8567 and [2]Takasago International Co., Tokyo 144-8721, Japan

The comparison between thresholds for the Japanese standardized olfactory test (T&T olfactometer) and identification rate for the odor stick identification test for Japanese was investigated in 60 patients, ranging in age from 17 to 78 years, who have olfactory impairment. Both the detection and the recognition thresholds were measured using five odors with the T&T olfactometer. The identification rate from 13 odors familiar to Japanese was measured by odor stick identification test in which odorants were encapsulated. There exists a definite inverse correlation between recognition threshold with the T&T olfactometer and the identification rate with the Japanese stick test. Stratification of patients into five levels of olfactory function along the spectrum from anosmia to normosmia is routinely decided by the recognition threshold determined by T&T olfactometer in Japan. Although

there were good inverse correlations in both test results, the next range of olfactory disturbance (e.g. normal and slightly impaired) was not discriminated by the Japanese stick test. We conclude that the Japanese stick test is more useful for initial physical examination than for close examination in hospital.

P1-01. Relationship between the gamma band oscillation and intravenous olfaction

Sachiko Hatanaka, Tadashi Ishimaru, Tsuyoshi Yata, Toshiaki Tsukatani, Isao Horikawa, Takaki Miwa and Mitsuru Furukawa

Department of Otorhinolaryngology, School of Medicine, Kanazawa University, Kanazawa 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan

We reported that spontaneous electroencephalograms (EEGs) of gamma band oscillation increase when intravenous olfaction with thiamine propyldisulfide (Alinamin®, Takeda Pharmaceutical Co., Osaka, Japan) occurs. In this study, 173 subjects were examined using the intravenous olfaction test with EEG recordings. The gamma band oscillations were surface integrated. The integrated values of EEGs (IEEGs) before and after Alinamin® injection were compared. IEEGs of intravenous olfactory sensitive subjects were statistically larger than those of non-sensitive subjects. Injection of Alinamin® sometimes causes vascular pain. Influence of vascular pain on IEEGs was investigated. The relationship between vascular pain and IEEG was not significant. We also observed increases in IEEG induced by intravenous olfaction in rabbits with a similar protocol. The gamma band oscillations seemed to be induced by intravenous olfaction.

P1-02. Near infrared spectroscopic response of the human olfactory cortex activated by the T&T olfactometer

Tadashi Ishimaru, Tsuyoshi Yata, Sachiko Hatanaka, Kumiko Iwahashi-Horikawa, Isao Horikawa, Toshiaki Tsukatani, Takaki Miwa and Mitsuru Furukawa

Department of Otolaryngology, School of Medicine, Kanazawa University, Kanazawa 920-0934, Japan.
e-mail: ishimar@orl.m.kanazawa-u.ac.jp

The activities of bilateral orbitofrontal cortexes stimulated by the T&T olfactometer were investigated by near infrared spectroscopy (NIRS). Two odorants, β-phenyl ethyl alcohol (A) and isovaleric acid (C) were chosen as stimulants from five odorants of the T&T olfactometer. Ten healthy subjects were examined and odorant-elicited NIRS responses were detectable from five subjects. Increases in the concentration of oxyhemoglobin were observed after 10 s stimulation of odorant A or C. Placebo stimulation did not elicit changes in oxyhemoglobin concentration. Differences in increases of oxyhemoglobin concentration between odorous and placebo stimulation were significant. The significant increasing period of oxyhemoglobin concentration of the right side was longer than the left side ($P < 0.05$). The NIRS technique is considered to be a powerful new method for the investigation of human olfaction.

P1-03. The relation between the T&T olfactometer and the odor stick identification test for Japanese in patients with olfactory disorder

Megumi Fujii, Keijiro Fukazawa and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, 1-1 Mukogawa, Nishinomiya, Hyogo 663-8501, Japan

The relation between the T&T olfactometer and the odor stick identification test for Japanese in patients with olfactory disorder was studied. There was a statistically significant correlation between the average recognition thresholds of the T&T olfactometer and identification rates of odor stick identification test, but, in patients with anosmia, the identification rates varied from 7.7 to 38.5% (average 19.25%). A further study with more cases is necessary before clinical use of this test clinically. The test is also particularly useful as a screening test, because of ease of use and lack of the spread of odor.

P1-04. The relationship between the T&T olfactometer/the intravenous Alinamin® test and CC-SIT

Keijiro Fukazawa, Megumi Fujii, Sadamu Takayasu and Masafumi Sakagami

Department of Otorhinolaryngology, Hyogo College of Medicine, 1-1 Mukogawa-cho Nishinomiya, 663-8501 Hyogo, Japan

The relationship between the T&T olfactometer/the intravenous Alinamin® test and CC-SIT (cross-cultural smell identification test) was studied in 112 patients with olfactory disorder. Hyposmia/anosmia occurred in 56 patients due to rhino-sinusitis, in 41 patients after a common cold, in three patients after head trauma and in the others for unknown reasons. Spearman correlations were found between the scores on a 12-item CC-SIT and T&T detection and recognition threshold values. Significant correlations were also found between the CC-SIT scores and the individual smell function with VAS (visual analogue scale) scores. In the patients with anosmia (>5.6 points with the recognition threshold of the T&T olfactometer), the CC-SIT scores of 85.5% of patients were <7 points. The mean score of patients who were positive for the Alinamin® test was 6.85 ± 2.45 (SD) and the mean score of patients who were negative for the Alinamin® test was 5.15 ± 2.43 (SD). The CC-SIT is useful for evaluation of smell function in identification.

P1-05. Study of 13 odors of the odor stick identification test on patients with olfactory disturbance

Miki Shino, Emiko Suzuki, Enatu Shibuya, Norihiko Asahina, Harumi Suzaki, Sachiko Saito[1] and Yasuhiro Takashima[2]

Department of Otorhinolaryngology, Showa University, Tokyo 142-8666, [1]Neuroscience Research Institute, Tsukuba 305-8566 and [2]Takasago International Corporation, Tokyo 108-0074, Japan

A new stick-type odor identification test has been developed in Japan. Thirteen odors familiar to Japanese people are used in the 'odor stick identification test'. In this study, 91 patients with olfactory disturbance, ranging in age from 15 to 82 years, were

examined with the odor stick identification test and the results analysed. There was significant correlation between the number identifying correctly in the odor stick identification test and the average of the recognition threshold in T&T olfactometry, which is the standardized olfactory test in Japan. As for the rate of correctly identifying the odors, the curry-odor was highest (48%), while the Japanese cypress odor was lowest (11%). It was observed that many patients confused the rose odor with the perfume odor, the putrid odor with the sweat socks odor, and the wood odor with the Japanese cypress odor.

P1-06. The study of drug-induced sensorineural olfactory disorder due to fluorouracils

Y. Tokuhara, K. Matsumoto, T. Aiba[1], K. Amano, K. Hashimoto and H. Yamane

Department of Otolaryngology, Osaka City University Medical School and [1]Department of Otolaryngology, Osaka City General Hospital, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan

We investigated 37 patients diagnosed with sensorineural olfactory disorder due to fluorouracils. This disorder was more frequent in females. Their most common disease was breast cancer, followed by gastric cancer, colon cancer and uterine cancer, in that order. The duration from the start of taking drugs till onset of olfactory disorder ranged from 2 to 78 months. Anosmia or severe olfactory disorder was found in 94.6% of the patients. The improvement rate was 54.8%. This disorder tended to develop earlier in patients with low blood zinc (<80 μg/dl). It is suggested that a longer period of follow-up would be desirable.

P1-07. Olfactory function measured by odor lipsticks: changes with age on identification, perceived intensity and hedonics

Sachiko Saito[1], Yuka Masuda[1,2], Tatsu Kobayakawa[1], Naomi Gotow[1], Saho Ayabe-Kanamura[1,3], Naomi Naito[4], Miyako Mise[4], Yukiko Yoshida[4] and Yasuhiro Takashima[5]

[1]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 386-8567, [2]Shinshu University, Ueda 386-8567, [3]University of Tsukuba, Tsukuba 305-8572, [4]National Institute of Technology and Evaluation, Tokyo 151-0066 and [5]Takasago International Co., Tokyo 144-8721, Japan

Odor identification ability, perceived intensity and hedonics were measured by the odor stick identification test for 191 participants ranging in age from 20 to 86 years. The odor stick identification test consisted of 13 odor lipsticks presenting odors familiar to Japanese people. As for the results, odor identification rate and perceived intensity did not changed in those <60 years old, but declined step by step in those >60 years old. People >60 years old ('old people') showed a decline of identification rate and perceived intensity compared with people <60 years old ('young people') in most odors. Old people felt hedonics in weaker degrees than young people in several odors, including offensive and dangerous odors found in everyday life such as 'gas for cooker', 'sweat socks' and 'putrid smell'. Old people and young people showed similar familiarity for the 13 odors presented in this test. These results indicate that the olfactory function of old people might be different from that of young people, not only in identification and perceived intensity but also in terms of hedonics. These results also demonstrated the validity of this format as an odor identification test for different generations.

P1-08. Evaluation of odor quality for ten odorants from the T&T olfactometer using concrete terms for odor description

Hideko Kawaki, Shunshiro Ohnishi[1] and Yutaka Kurioka[1]

Research & Development Institute for Pharmaceutical Science, Department of Electric Engineering, Faculty of Science and Technology, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, 577-8205 Japan. e-mail: kawaki@msa.kindai.ac.jp

We previously introduced the terms 'rotten', 'burnt' and 'floral' from 28 odor-descriptive terms of ten standard odorants (A–J) using semantic differential (SD) methods with a student panel. Then, we again analysed to use the extracted 30 terms from 28 and Osajima's 54 terms by a large group of students. Non-estimated terms were 'milk', 'butter', 'meat', 'blood', 'hot-spring' and 'spice' smells. An individual variation was estimated by 'moldy', 'wood', 'metal', 'sunshine' and 'almond' smells. It is suggested that the smell is reflected by a lifestyle memory of, for instance, a meal. We carried out principal component analysis on our extracted 30 terms and estimated the principal elements of 1st–5th, which were 'floral', 'sour and rotten', 'peppermint', 'sweet and burnt' and ' petroleum'. These elements are suited to Amoor's classified terms. Consequently, we suggest that 'floral', 'rotten', 'peppermint' and 'burnt' are basic odors.

P1-10. The double effect of 1-menthol on brain activity

Tomoyuki Yoshida, Masa Nagano[1] and Hiromi Fujiwara[2] and Akio Fukumashi[2]

Department of Kansei Information, Faculty of Human and Social Environment, Hiroshima International University, [1]Product Development Department of Health & Beauty Business HQ Sunstar Inc., Tokyo 108-0073 and [2]Product Development Department of Health & Beauty Business HQ. Sunstar Inc., Osaka 569-1195, Japan

We examined 30 male subjects for the effects of menthol shampoo, estimated from the change of period-fluctuation rhythm of the alpha wave at the right and left frontal regions. The subjects were divided into two groups. The first group used menthol shampoo for washing hair and the second used non-menthol shampoo. The fragrance of shampoo was the same in each case. The subjects faced the experiment, after the sensor of a simple EEG rhythm measuring device was fixed to right and left frontal parts of the head (Fp1 and Fp2). Initially, the frontal brain wave was measured in the rest condition. After that, the sensor was removed and subjects left the experimental room and ran lightly along the road for ~3 km. As soon as they returned to the room, frontal brain waves were measured using the same EEG rhythm measuring device; the subjects then washed their hair with shampoo. Finally, frontal brain waves were measured again. We found that the fluctuation-rhythm in right frontal brain heightened, which indicates relaxation of the brain when menthol shampoo was used in comparison with non-menthol shampoo, and suggests that menthol shampoo may have a double effect, i.e. a refreshing and a comforting effect.

P1-11. Compound effect of odor and audio-visual information on a comfortable feeling

Tomoyuki Yoshida, Masao Kubota[1] and Ryoichi Komaki[1]

Department of Kansei Information, Faculty of Human and Social Environment, Hiroshima International University, Hiroshima 724-0695 and Kanebo Ltd Cosmetics Laboratory, Kanagawa-ken 250-0002, Japan

The purpose of this study was to examine whether the compounding of odor and audio-visual information could evoke a more comfortable feeling in comparison with the condition that only odor was given. Thirty-two subjects took part in the experiment. Each subject was seated on a chair in a shielded room. In the front, a video monitor and odor-presenting tube were set. The odor of the beach was composed using the HSG method. Subjects experienced six kinds of experimental condition for 3 min. Those were: the rest (r); video film of a wave approaching a beach only (C); only odor (P); P + the visual scenery (P + V); P + the sound of a wave approaching a beach (P + S); and P + the normal video watching (P + T). The period-fluctuation rhythm of the α wave of right and left frontal regions (Fpl and Fp2) was measured in the eye-closed state for the r, P and P + S conditions. The period-fluctuation rhythm was firstly measured with eyes opened for the P + V and P + T conditions, and the rhythm in with eyes closed was measured after the video was finished (V2 and T2). We found that the rhythmicity heightened in the T2, P + S and V2 conditions. The result suggests that the compound of odor and audio-visual information should be more effective in generating a comfortable feeling when an odor was continuously presented after the odor-matched audio-visual condition.

P1-12. The effect on EEG of the aroma from soybeans after heating

Chizuiru Konagal, Mayuko Hamada, Yoshihiko Koga[1] and Van Chuyen Nguyen

Department of Food and Nutrition, Japan Women's University, 2-8-1 Mejirodai, Bunkyo-ku, Tokyo 112-8681 and [1]Department of Neuropsychiatry, Kyorin University School of Medicine, Japan

Soybeans are often heated during food processing, with a large number of products being generated during this process. These individual components are believed to have various physiological activities. In this study, we investigated the effect on electroencephalography (EEG) of the aroma of soybeans heated to various temperatures. We prepared slight, medium and deep heated soybeans and, in order to investigate the relationship of the amino-carbonyl reaction products upon EEG, we also prepared soybeans heated after immersion in distilled water or 0.2 M fructose–glycine solution. Fourteen healthy females (right-handed, 21–26 years old) participated in this study. We recorded EEGs from 19 electrode sites and calculated the power spectrum of the alpha activity at each site using FFT analysis. We compared the amount of alpha waves among samples in the frequency range from 9.5 to 10.0 Hz. Results showed that the amount of alpha waves induced by the aroma of medium heated soybeans was significantly greater than that induced by control (no odor), slight and deep heated soybeans. In addition, when soybeans were heated after immersion in fructose–glycine solution, the alpha waves increased, as compared with those that had been immersed in distilled water. These results suggest the possibility that amino-carbonyl reaction aroma products increase brain alpha waves.

P1-13. Psycho-physiological influence of odors stimulation on elderly adults

Byung Chan Min, In Hyeng Kang, Sun Young Lee[1], Eun Jung Sung, Soon Cheol Chung[2], Se Jin Park, Chul Jung Kim and Kazuyoshi Sakamoto[3]

Ergonomic Laboratory, Korea Research Institute of Standards and Science, [1]Department of Architecture, Jinju National University, [2]Department of Biomedical Engineering, College of Medicine, Konkuk University and [3]Department of Systems Engineering, University of Electro-Communications, Ergonomic Laboratory

Odor is something that we can find everywhere in our daily life. However, unlike the visual sense and auditory senses, the psycho-physiological influences of which are clarified and about which knowledge is well accumulated, research on the sense of odor is lagging behind. There are many kinds of odor and gender, personality, age, environmental conditions, etc. are factors which affect sensitivity evaluation by humans of an odor. In this research, the results of both measurement of physiological signals (e.g. ECG and GSR) and subjective evaluation were synthetically compared between age groups. Subjects were 16 young and 18 elderly persons with normal sense-of-smell function. Six odor stimuli were used for the experiment: basil, jasmine, lavender, lemon, sketole and ylang oil (all 100%). The results were as follows. In the elderly age group, 'evaluating' was mentioned as an evaluation factor of odors from subjective evaluation, and lavender, lemon and ylang were obtained as a large odor of 'activation'. From the results of measurement of a physiological signal, these odors were checked by GSR as the upward tendency and by R–R interval as the downward tendency. However, this relevance was not seen in the young age group. The results of both the measured physiological reaction to the odor stimulus and the subjective evaluation were not necessarily completely in agreement. It was found that the study using the psycho-psychological technique was effective in evaluating the like–dislike tendency for young and aged persons.

P1-14. Effects of odorant inhalation on plasma cortisol levels in humans

T. Tsuchiya, K. Inoue, M. Tanida, J. Hosoi, I. Horii, K. Shoji[1], S. Haze[1], H. Fukui[1], I. Tsukui[2], M. Fukumoto[2] and Y. Miura[2]

Shiseido Life Science Research Center, 2-12-1 Fukuura, Kanazawaku Yokohamashi 236-8643, [1]Shiseido Products Research Center and [2]Japan Aeromedical Research Center, Japan

We previously reported that some odorant inhalations can produce sedative effects and that some odorant inhalations have an inhibitory effect on stress-induced cutaneous responses, some of which were inhibited by the pre-administration of a glucocorticoid receptor antagonist. These findings suggest that odorant inhalation produces changes in the hypothalamus–pituitary– adrenocortical (HPA) axis, resulting in changes in skin functions. Thus, we investigated the effects of inhalation of sedative-producing odorant on the stress-induced response of cortisol levels in circulating blood and saliva levels in humans.

Plasma and salivary cortisol levels increased by ~35 and 22% 30 min after the start of the Stroop color-word test (Jensen *et al.*, 1966, *Acta Psychol.*, 25: 36), whereas during odorant inhalation there were no increases. This difference was statistically significant ($P < 0.05$). Immediately after the cessation of the interview and mental calculation test, cortisol levels increased markedly (85 and

110%) and odorant inhalation reduced these responses, but the differences were not significant ($P > 0.05$).

From these findings, we suggest that some olfactory inputs are useful to prevent stress responses in the HPA axis in humans.

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P1-15. Research on the human sense of smell

Satomi Kunieda and Kiyohito Sawano

Takasago International Corp., 1-4-11 Nishi-Yawata, Hiratsuka, Kanagawa, Japan

The goal of this experiment was to examine the sensitivity of human odor by investigating 280 persons, aged 6–76 years old, selected at random from among visitors to the Hiroshima Health Science Museum. The panel smelt six kinds of odors and responded concerning the intensity, liking and discernment of each odor.

The six types of odors which were shown to the panel were either a single substance or a mixture of two substances; the odors were familiar smells of food which is popular with the Japanese. These were processed into the seal sheet and they were devised so that a representative panel would not have any difficulty smelling the odor.

It was suggested from the result using ANOVA that perceived intensity of and/or preference for odors are influenced strongly by an aging factor. Moreover, the ability to discern odors may not be a problem of only the sensitivity accompanied by aging, but may also be related to the experience or study effect, etc., since the distribution in the rate of correct answers was seen over the range of younger and older ages.

P1-17. Assessment of preference for fragrances using facial EMG

H. Sugano and Y. Tsuda

MOA Health Science Foundation, 162-1 Ohkuma, Kasuya-machi, Fukuoka 811-2302, Japan

It is well known that the facial muscles can reflect emotional stress. To evaluate the preference for fragrances physiologically, we studied the EMG of the facial muscles—the corrugator supercilli, levator labii superioris and zygomaticus major—for different odors. It was found that the activity of these muscles correlated well with the stated preference of the subject, since odors that are not preferred resulted in a decrease in activity of the corrugator muscles, and preferred odors increased the activity of the levator. The corrugator muscle was more sensitive to the preference of odor. Although both corrugator muscles reacted more sensitively than the other muscles, the right-hand corrugator showed more significant sensitivity than the left. Although the preference for odors varies from person to person due to a variety of factors, our experience has shown that the odors of fruit, such as lemon and orange, and the scent of the beach appear to provide the greatest stress reduction effects.

P1-18. Chemical structure–odor relationships of 'green odor' affected by terminal functional groups

Kota Sano, Jun Suzu and Akikazu Hatanaka[1]

Fragrance Laboratory, Soda Aromatic Co. Ltd, Tokyo and Yamaguchi University, Yamaguchi, Japan

Chemical structure–odor relationships of 14 esters such as 3*Z*-hexenyl esters and 2*E*-hexenyl esters related to 'green odor' with six-carbon aliphatic compounds were investigated. Sensory odor characteristics of 21 compounds (seven 3*Z*-hexenyl esters, seven 2*E*-hexenyl esters, *n*-hexane, *n*-hexanol, *n*-hexanal, 3*Z*-hexenol, 3*Z*-hexenal, 2*E*-hexenol and 2*E*-hexenal) were evaluated by score sheets with a six-point scale using eight sensory descriptive terms—grassy-leafy green (GLG), vegetable green (VLG), fruity (FRT), sweet (SWT), fresh (FRS), spicy (SPC), oily-fatty (OLF) and herbal (HRB)—and the data from sensory evaluation were statistically analysed using principal component analysis. From the results of sensory evaluation and principal component analysis, it was found that structure of terminal functional groups and unsaturated bonds caused some unique and interesting aspects in green odor.

P1-19. Biogeneration of seaweed-like odor

Yoshihiko Akakabe, Kenji Matsui and Tadahiko Kajiwara

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan. e-mail: akakabe@agr.yarnaguchi-u.ac.jp

The characteristic odor components of the marine green alga *Ulva pertusa* were (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrienal, (8*Z*,11*Z*)-8,11-heptadecadienal, (8*Z*)-8-heptadecenal and pentadecanal. In particular, (8*Z*,11*Z*,14*Z*)-8,11,14-heptadecatrienal was important for the typical seaweed odor. When long-chain saturated and unsaturated fatty acids were incubated with crude enzymes of the marine green alga *Ulva pertusa*, the corresponding (*R*)-2-hydroperoxy acids were found to have high enantiomeric excess (>99%). We suggested that 2-hydroperoxylation of long-chain fatty acids proceeds enantiomerically to afford (*R*)-2-hydroperoxy acids that will decompose into the above-mentioned aldehydes. In a similar administration experiment, (*R*)-2-hydroperoxyhexadecanoic acid was obtained from the incubation of palmitic acid with crude enzymes of marine algae and terrestrial plants. Thus, we found that not only marine algae but also higher plants are capable of enantioselective 2-hydroperoxylation of long-chain fatty acids.

P1-20. QCM gas sensor with plasma-polymerized, molecular-recognition film prepared using a plasma CVD method

S. Sanada, Y. Hamaguchi, K. Nobuyama, E. Kusano, A. Kinbara, H. Nanto, T. Matsumoto[1], S. Kurosawa[1] and K. Tanabe[1]

Advanced Material Science R&D Center, Kanazawa Institute of Technology, 3-1 Yatsukaho, Mattou, Ishikawa 924-0838 and [1]National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Umezono, Tsukuba 305-8563, Japan

In order to develop a sensor system for environmental monitoring, chemical sensors using QCM coated with acrylate film with different functional groups, methacrolate film with different functional groups and styrene film as a molecular recognition membrane were prepared using plasma-polymerized CVD method. It was found

that the sensor response for various gases strongly depends on the functional group of the molecular recognition membrane of the sensor. The styrene-film-coated sensor exhibited no selectivity for a specific gas and responded to various gases. It was also found that the styrene-film-coated sensor in conjunction with principal component analysis is useful for identification of gases in environmental monitoring.

P1-21. Deodorant effect monitoring of the oxygen cluster for harmful gases using gas sensors

Y. Takayama, T. Kobayashi, H. Kasahara, T. Oyabu, E. Kusano, A. Kinbara and H. Nanto

Advanced Material Science R&D Center, Kanazawa Institute of Technology, 3-1 Yatsukaho, Mattou, Ishikawa 924-0838, Japan

The deodorant effect of harmful gases such as formaldehyde, ammonia and carbon monoxide, as well as odors from cigarettes, using oxygen cluster was monitored using a commercially available gas sensor array. It was found that the oxygen cluster is very effective in deodorizing harmful gases and that the gas sensor array is useful for monitoring the deodorant effect. It was also found that the system using the oxygen cluster and gas sensor array is useful for deodorant effect monitoring of odors from cigarettes.

P1-22. Deodorizing activity of constituents extracted from black tea

Miyuki Masutani, Keiko Saito and Kazuhiro Hirota

Faculty of Engineering, Department of Systems Engineering, Okayama University, Okayama 700-8530, Japan. e-mail: hirota@cc.okayama-u.ac.jp

The deodorizing activity of constituents of a black tea was studied in the gas phase using quartz-crystal microbalances (QCM) coated with constituents of the tea. The constituents were extracted with various solvents. The QCM, which was coated with constituents to prepare a sensing film, was exposed to single-odor substances: alcohols, aldehydes, ketones, esters and amines. The resonance frequency changes of the QCM responsible for the adsorption of odorants on the film were measured. Partition coefficients of odorants were calculated from the changes and were used to evaluate deodorizing activity. Among 13 odorants tested, furfural, *n*-amyl acetate and *n*-hexyl acetate showed high coefficients of 1.7×10^{-4}, 1.7×10^{-4} and 3.7×10^{-4}, respectively, when ethyl acetate was used for the extraction. Ammonia also showed a high magnitude of 3.5×10^{-4} in the use of 50% methanol–water as the solvent. Other odorants—methanol, acetone, 3-pentanone, ethyl acetate, pentane and hexane—showed much lower coefficients in any of the solvents 50% methanol–water, methanol, acetone and ethyl acetate. The deodorizing activity is discussed on the basis of constituents responsible for the nature of solvents used for the extraction.

P1-23. Sensing of odor using quartz-crystal microbalances coated with plant lipids

Keiko Saito, Miyuki Masutani and Kazuhiro Hirota

Faculty of Engineering, Department of Systems Engineering, Okayama University, Okayama 700-8530, Japan. e-mail: hirota@cc.okayama-u.ac.jp

A lipid was isolated by the extraction of dried fruits of *Lyceum chinense* (*kuko-mi*) with acetone, saponified with potassium hydroxide and purified by chromatography with a silica-column and thin-layer plate. The lipid was used as a sensing film for an odorant-detection device consisting of a quartz-crystal microbalance (QCM). The QCM, coated with the lipid, was exposed to a single-odor substance—alcohols or esters—in the gas phase, and the resonance frequency changes of the QCM were measured. The selectivity in odorant detection was evaluated from the magnitude of the changes. It was found that the magnitude increased with increases in the carbon number of alcohols when the liquids methanol, ethanol, 1-propanol and 1-butanol were used as the sources of odors. Such a phenomenon was observed in the measurements of acetic acid esters carrying ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-hexyl and *n*-octyl groups. The structure of the lipid used was not determined.

P1-25. Toward selection of standard odorous molecules. 3. Calculation of molecular frequency and examination of odor evaluation method

Fumiko Yoshii, Toshio Ogino[1], Yoshiaki Yamada[2], Takashi Hoshi[3], Masayoshi Ando[3] and Hisahiro Hagiwara

Graduate School of Science and Technology, [1]Faculty of Education and Human Sciences, [2]Graduate School of Medical and Dental Sciences and [3]Faculty of Engineering, Niigata University, Niigata 950-2181, Japan. e-mail: fuyoshii@mb.infoweb.ne.jp

Our primary goal is to select standard odorous molecules for olfactory experiments as well as for odor evaluation, based on molecular properties. In this study, 24 compounds involving C, H and N atoms only, were selected and their inelastic tunneling spectra (IETS) were calculated using Gaussian98 with B3LYP, 6-31G(d) and MP2, 6-31G(d), according to Turin's new theory of olfaction (Turin, 1996, *Chem. Senses*, 21: 773–791). Five compounds (pyridine, piperidine, 2-methylpyrazine, 6-methylquinotine and indole) among the 24 compounds were selected based on the result of the IETS calculation, and the odors were evaluated by human subjects. Odor similarity judgements by direct pair-wise comparison and odor-quality evaluation using Harper's odor descriptors were utilized for the evaluation. Though the agreement was not perfect, the odor similarity of the five compounds judged by the descriptive analysis showed the same tendency as the similarity of the IETS of the five compounds. This suggests that the selection of molecules based on molecular frequency and successive olfactory evaluation would be useful to select standard odorous molecules.

P1-26. An attempt at evaluation of fish freshness using potentiometric gas sensors

Hirotoshi Tanaka, Koji Shimada[1], Noriaki Kaneki[1], Tadashi Ide and Yasukazu Asano[2]

Department of Applied Chemical Engineering and [1]Department of Computer Science and Systems Engineering, Muroran Institute of Technology and [2]Hachinohe National College of Technology, 27-1 Mizumoto-cho, Muroran, 050-8585, Japan

We have attempted the development of a gas sensor of potentiometric type by applying an oxidation–reduction system. Using commercial ammonia and hydrogen sulfide gas electrodes together with the oxidation–reduction system of gas electrode, the sensor system has been constructed for the measurement of odor. Our present system is able to collect more information, due to its potentialities of covering both positive and negative data of changes in potential compared to conventional gas sensor systems e.g.

of metal semiconductor and quartz-resonance type. We have attempted to evaluate fish freshness using the sensor. Fish samples were salmon, sardine and scallop, and the responses of such a composite sensor system were measured to the components of a smell derived by letting them deteriorate. In this study, changes in potentials of samples with the passage of time were examined to each electrode, and the smell changes during putrefaction of the same samples were also examined with sensory evaluation. Comparison was made with the responses of sensors and sensory evaluation.

Consequently, having been applied to measure volatile aroma compounds of fish to evaluate fish freshness, the system was able to detect characteristic signals from individual electrodes with regard to kinds of fish and their diurnal quality change.

P1-27. Research on the taste change phenomenon using a taste sensor

Tatsuhito Arimura, Sou Takagi, Munehiro Iwakura, Kiyoshi Toko and Toshiaki Imoto[1]

Graduate School of Information Science and Electronic Engineering, Kyushu University, Fukuoka 812-8581 and [1]Faculty of Medicine, Tottori University, Tottori 683-0826, Japan

A multichannel taste sensor is composed of several types of lipid membranes transforming information of taste substances into electric signals, which are input to a computer. We detected suppressive effect of bitterness by phospholipids and also suppressive effect of sweetness by gymnemic acid and gurmarin using this taste sensor. First, we measured bitter substances (quinine and tannin acid) and sweet substances (aspartame, L-alanine and glycine). Then, these bitter substances and sweet substances were measured after part of lipid membranes of the taste sensor was soaked in each of phospholipids, gymnemic acid and gurmarin.

The magnitude of response pattern of the taste sensor for bitter substances decreased for the higher phospholipid concentration when the lipid membranes were soaked in phospholipids. The response pattern for sweet substances when the membranes were soaked in gymnemic acid was greatly different from that when the membranes were not. The pattern for sweet substances in the case of gurmarin almost did not change from the original pattern. From this result we find that a taste sensor can detect the suppressive effect of bitterness by phospholipids, while improvements of membrane material and measurement method are necessary for the detection of suppressive effect of sweetness.

P1-28. Astringent evaluation method using a quartz-crystal microbalance

Hirotaka Kaneda, Junii Watari, Masachika Takashio and Yoshio Okahata[1]

Brewing Research Laboratories, Sapporo Breweries Ltd, Yaizu 425-0013 and [1]Department of Biomolecular Engineering, Tokyo Institute of Technology, Yokohama 226-8501, Japan. e-mail: hirotaka.kaneda@sapporobeer.co.jp

It was found that the adsorption of astringent tannins on lipid membrane significantly increases in the presence of peptides (proteins) such as gelatin, bovine serum albumin (BSA), salivary proline-rich protein repeat sequence, or poly-L-proline, while NaCl, tartaric acid, quinine sulfite, sucrose and glutamic acid do not show this tendency. The adsorption rates of red wines on lipid membrane in the presence of BSA showed a significant correlation with their astringent intensities in a sensory evaluation. It seems that the adsorption of complexes of tannins with several proteins on the oral lipid membrane could be concerned in the astringent sensation and could be applied for evaluation of astringency of beverages.

P1-29. Evaluation of TIMP-1 levels in whole saliva of patients with taste disorder, before and after therapy

Mitsunori Monya, Atsuko Igarashi, Masumi Asatsuma, Naoko Kato and Shuichi Nomura[1]

Dysphagia Rehabilitation and [1]Oral Health in Aging and Fixed Prosthodontics, Niigata University Graduate School of Medical and Dental Sciences, Gakkocho 2-5274, Niigata City 951-8514, Japan

We have already reported that TIMP-1 levels in whole saliva is significantly lower in the diseased group than in the control group. In this study, we determined whether TIMP-1 levels in whole saliva of patients with taste disorder was different before and after therapy. Stimulus saliva samples were collected after 5 min from seven patients (aged 52–82 years, mean age 70.7 years) before and after therapy. Control samples (healthy subjects) were obtained from 40 healthy volunteers (aged 61–78 years, mean age 67.7 years). Immediately after collection, the samples were quickly frozen until assay; they were centrifuged at 12 000 r.p.m. for 5 min to remove the precipitate just before the assay. TIMP-1 level in the supernatant was measured using the sandwich enzyme immunoassay (sandwich-EIA) system kit. Sera were obtained from patients with taste disorder. The all patients with taste disorder use the zinc internal treatment only.

The results indicate that TIMP-1 levels in whole saliva samples after therapy (233.2 ± 57.9 ng/ml) were significantly higher ($P < 0.05$) than those before therapy (153.1 ± 35.8 ng/ml). The post-treatment levels were very near those in normal subjects.

We suggest that the TIMP-1 level in whole saliva is connected with taste disorder and may offer a useful method for diagnosis of this condition.

P1-30. Evaluation of TIMP-1 levels in saliva from patients with primary Sjögren's syndrome

Masumi Asatsuma, Atsuko Igarashi, Mitsunori Monya, Naoko Kato and Syuichi Nomura[1]

Dysphagia Rehabilitation and [1]Oral Health in Aging and Fixed Prosthodontics, Niigata University Graduate School of Medical and Dental Sciences, Gakkocho 2-5274, Niigata City 951-8514, Japan

Decreased salivary secretion is one of the causes of taste disorder. Sjögren's syndrome, an autoimmune disease of unknown aetiology, is characterized by xerostomia as a result of decreased lachrymal and salivary secretion caused by destruction of the glands by an as yet unknown mechanism.

TIMP (tissue inhibitor of metalloproteinases) is the major endogenous regulator of MMP (matrix metalloproteinase) activities in tissue remodeling, repair and destruction. In this study, we

determined TIMP-1 levels in saliva of patients with Sjögren's syndrome.

Stimulus saliva samples were collected after 15 min from 30 primary Sjögren's syndrome (26–78 years old, mean age 54.5 years) and 62 control samples (healthy subjects 24–75 years old, mean age 57.1 years). The saliva samples were quickly frozen until assay and they were centrifuged at 12 000 r.p.m. for 5 min to remove the precipitate just before assay. The level TIMP-1 in the supernatant was measured using the sandwich enzyme immunoassay (sandwich-EIA) system kit. This data showed that TIMP-1 levels are significantly higher in the diseased group than in the control group [485.09 ± 258.04 ng/ml (mean ± SD) versus 279.14 ± 117.51 ng/ml]. Further studies are necessary to establish TIMP-1 levels in saliva of those with primary Sjögren's syndrome.

P1-31. Taste disturbance on single taste solution

Kohji Matsumoto, Tsunemasa Aiba[1], Yasutake Tokuhara, Kaori Amano, Kazuya Hashimoto and Hideo Yamane

Department of Otolaryngology, Osaka City University, Medical School and [1]Department of Otolaryngology, Osaka City General Hospital, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. e-mail: kmatsu@med.osaka-cu.ac.jp

We investigated 83 patients who visited our taste clinic. For only one taste solution, they recognized level 5 solution or did not recognize it. The most commonly disordered taste was sourness, followed by sweetness, saltiness and bitterness, in this order. This disorder was more frequent in females. Patients with low serum zinc level often showed improvement of gustatory function. Among patients with mild disorder, 25% were those with single taste disorder. Thus, single taste disorder was generally a disorder with less severity, but it was indicated that the mechanism of recognition was different among various basic tastes.

P1-32. Taste function in old patients with middle ear diseases

Mieko Sone, Atsushi Negoro, Megumi Fujii, Masanori Umemoto and Masafumi Sakagami

Department of Otolaryngology, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan

Taste function of healthy people is believed to deteriorate in the old age, but it is still unknown whether taste function of old patients with middle ear diseases deteriorates or not. Subjects were 72 ears of 70 patients >60 years old, who underwent middle ear surgery during the previous 4 years. Their taste functions were measured by electrogustometry (EGM) 2 days before surgery and these were compared with patients of different aged groups who underwent surgery during the same period. The average EGM thresholds of non-inflammatory diseases were 0 ± 7.1 dB in the 0–20 years group, 3.3 ± 9.5 dB in the 21–40 years group, 8.9 ± 12.5 dB in the 41–60 years group and 12.1 ± 12.2 dB in the >60 years group. The average thresholds of chronic otitis media were 0 ± 6.5, 3.6 ± 13.8, 12.5 ± 13.5 and 13.1 ± 14.2 dB, respectively. The average thresholds of cholesteatoma were 4.3 ± 12.1, 13.5 ± 14.0, 14.8 ± 15.2 and 21.7 ± 14.6 dB, respectively. These findings suggested that old patients with middle ear diseases had lower taste function than younger patients, which is consistent with the results of healthy subjects.

P1-33. Morphological investigations of radiation induced taste disorders

Hiroaki Kanazawa, Chiyo Kawaura and Mitsuhiko Homma

Nagoya University, School of Health Science, Department of Radiological Technology, 1-20 Daiko minami 1-chome, Higashi-ku, Nagoya 461-8673, Japan

Major salivary glands and taste buds of rats were studied with the light and electron microscopy after cobalt 60 gamma radiation with 40 Gy as one dose on their head and neck area. After 4 days, parotid and submandibular glands were atrophied, but the sublingual gland was not damaged. Over the same time, tongue epithelium was thinned because epithelial basal cells were damaged by radiation, but taste buds retained their normal shapes. At the seventh day, bacilli and neutrophils increased on tongue epithelium and they destroyed epithelium and made pus that buried the fossa of circumvallate papilla. At the top of taste buds, taste hairs were affected. These findings suggested that direct radiation attack on the taste buds cells was not the main cause of taste disorders after radiation. Radiation mainly influenced salivary glands and tongue epithelium. For the former, the decrease of serous saliva secretion induced change of the environment of the mouth encouraging infections to occur; for the latter, turnover of surrounding tongue epithelium stopped. Secondary infections and the collapse of epithelium surrounding taste buds induced damage of taste buds cells. In the end, taste loss occurred.

P1-34. On the evaluation of taste for disease patients by evoked potential

Yuki Naito, Masato Yagi, Takuya Ikeda[1], Fang Yan[1], Shiho Morooka[1], Kenichi Kaneko[2], Byung-Chang Min[3], Kunihiro Onishi[1] and Kazuyoshi Sakamoto[1]

Musashino Red Cross Hospital, 1-26-1 Kyounann-cho Musashino-shi Tokyo 180-8610, Japan, [1]University of Electro-Communications, [2]Fuji University and [3]Korea Research Institute of Standards and Science. e-mail: naitoy@musashino.jrc.or.jp

Taste disorder subjects were investigated by both measurement of evoked potential and using questionnaires, comparing them with normal taste subjects. Solutions of four taste qualities were sucrose as sweet, sodium chloride as salty, acetic acid as sour and quinine–HCl as bitter. The quantity applied to the subjects' tongues was 10 µl. The evoked potential was measured by Neuropack 8. The locations for the evoked potential system were Cz, Al and Fpz. The position stimulated on the tongue was the area controlled by the chorda tympani. The disorders of subjects were sweet, sour and all tastes. The results of normal subjects showed a negative peak (NI) around 100 ms and a positive peak (P2) around 150 ms; the difference between NI and P2 gave >20 µV, while the disordered subjects for specified taste quality (sweet and sour) showed a smaller evoked potential compared with the normal subjects. The disorder subjects for all the tastes, however, presented continuously various levels of the potential, and the pattern was the different from that of the normal subjects. In the questionnaire, the disordered subjects did not correctly the taste of the solution, and the degree depended on the subjects. As a conclusion, the disordered subjects showed the above-mentioned features in the evaluation of the evoked potential and the questionnaire, and these features were different from that of the normal subjects.

P1-35. Pleasure of eating and life—investigation of patients' eating, taste and masticatory function by questionnaire at a dental clinic

Yayoi Takita and Masaaki Takita[1]

Private Dental Practice, Nagoya, Chikagai-nai, 6-9, Tsubaki-cho, Nakamura-ku, Nagoya 453-0015 and [1]Department of Oral Surgery, Saiseikai Nakatsu Hospital, 10-39 Shibata 2-chome, Kita-ku, Osaka 530-0012, Japan

Patients' eating, taste and masticatory function or comments on pleasure of eating and life were added to the investigation items of an questionnaire on Japanese terminal care performed at a dental clinic (The Sixth Japanese Society for Palliative Medicine, 2001) and significant answers were obtained from the perspective of a study on taste.

The subjects were 109 patients (74 males and 35 females) with a mean age of 60.3 ± 12. 1 (SD) years. Seventy per cent of the subjects wore dentures. The response rate was 87% and the patients' answers consisted of 45 comments (48%) related to instinct and emotion (eating = living, pleasure of eating is the last privilege, becoming unable to eat suggests the end of life, etc.), 28 comments (30%) related to the relationship between oral sensory function and encephalic function (taste and dental health or pleasure of eating even at advanced age, biting = activation of the brain, eating foods in season, chewing sufficiently, etc.) and nine comments (10%) related to environmental and ethical problems (Japanese satiety with rich food and decline in public morals, greenhouse vegetables have no natural taste, etc.), showing the profoundness of the answers to this item.

P1-36. Relation of acceptable level of sweetness and amount of intake to sweet food liking

Eiko Sadaoka, Sayuri Akuzawa, Shigeru Sawayama, Fumiko Iida[1] and Shizuko Yamaguchi

Department of Nutritional Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502 and [1]Department of Food and Nutrition Science, Faculty of Home Economics, Japan Women's University, 2-8-1 Mejiro-dai Bunkyo-ku, Tokyo 112-8681, Japan

Pleasant sweetness level and eatable amount of sweet food differ between individuals. The purpose of this study was to clarify the relation of these factors to the liking for sweet foods, using sensory evaluation and a questionnaire. Three hundred and eighty-nine university students served as subjects. On the questionnaire given before the sensory evaluation, subjects rated their desires to eat various sweet foods on various occasions, such as at breakfast, before sleep, with meal, as a meal substitute, etc. The names of the foods were selected from three types of foods: for high carbohydrate intake, such as cakes; for enjoying sweetness with low carbohydrate intake, such as jams; and just for improving flavor, such as plum pickles. The ratings were made on 7-point scales (7 = extremely want to eat, 1 = never want to eat). In sensory evaluation, subjects were given two 5 g pieces of chocolate and asked to rate the pleasantness of sweetness on a 5-point scale (1 = too sweet, 5 = less sweet). After eating, subjects were asked how many more pieces of chocolate they would be able to eat. The pleasantness level of sweetness was highly related to the amount that could be eaten. The results were significantly related to the desire for sweet foods for high carbohydrate intake on any occasion, but not related to foods eaten just for improving flavor.

P1-37. Enhancement of sucrose sweetness with soluble starch

Norikazu Kanemaru, Shuitsu Harada and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

The taste-modifying effects of soluble starch (acid-modified starch) were investigated in humans. Various concentrations of sucrose (Suc), six sweeteners, NaCl, quinine HCl (QHCl) and citric acid (Cit) were dissolved in either distilled water (DW) or starch solution (test solution). The solutions were presented to naive subjects, each of whom was requested to compare the taste intensity between the standard and test solutions based on a scale ranging from +3 (enhanced) to –3 (inhibited). Sweetness was enhanced with Suc at 0.1–1.0 M dissolved in soluble starch (0.125–4.0%) compared with Suc in DW. Similarly, five other different products of soluble starch at 0.25 and 4.0% showed enhancement of sweetness for 0.3 and 1.0 M Suc. Sweetness enhancement did not occur with 0.43 M fructose, 0.82 M glucose, 0.82 M sorbitol, 0.0037 M aspartame, 0.0042 M saccharin-Na or 0.016 M cyclamate. Soluble starch did not affect the saltiness of NaCl (0.01–0.3 M), the bitterness of QHCl (0.00003–0.001 M) or the sourness of Cit (0.0003–0.01 M). These results suggest that the sweetness-enhancing effect of soluble starch on Suc might not depend on the taste transduction mechanism, but on the molecular interaction between Suc and soluble starch.

P1-38. Taste interactions between various amino acids and Na/KCl

Misako Kawai, Yuki Hayakawa and Yoichi Ueda[1]

Central Research Laboratories and [1]Seasoning Research and Development Department, Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki-shi 210-8681, Japan. e-mail: misako-kawai@ajinomoto.com

We examined taste interactions between moderate concentrations of amino acids (AAs) and 50 mM NaCl or KCl by means of sensory evaluation. Changes in taste were observed as follows: (1) sourness reduction with Na/KCl, without pH-change—acidic AAs; (2) bitterness reduction with NaCl—bitter AAs with hydrophobic or basic side chains; (3) umami enhancement with NaCl—several umami AAs; and (4) sweetness enhancement with NaCl—sweet AAs. We then focused on case (4) above and measured sweetness of mixed solutions of Gly (50, 100 and 200 mM) and NaCl (12.5, 25, 50, 100 and 200 mM) using the sucrose-point of subjective equality (PSE) method. The mixed solution became sweeter than the unmixed Gly solution at any tested concentrations by 10 mM PSE when 12.5 mM NaCl was added. On the other hand, when a higher concentration of NaCl was added, sweetness enhancement occurred relative to both concentrations of Gly and NaCl, while KCl barely enhanced sweetness. Dilute saline and sucrose have been reported to share a receptor system for sweetness (Bartoshuk *et al.*, 1978, *Physiol. Behav.*, 21: 609–613). It has also been reported that sweetness is enhanced additively when sucrose and Gly are mixed (Yamaguchi *et al.*, 1970, *Agric. Biol. Chem.*, 34: 187–197). Thus, the sweetness enhancement that occurred at low NaCl concentrations independent of Gly concentration might occur additively. At higher NaCl

concentration, NaCl might act as an enhancer of nerve response, as seen in the phenomenon of the canine nerve (Ugawa and Kurihara, 1994, *Am. J. Physiol*., 264: R1071–R1076), and magnify the sweet sensation.

P1-39. Taste characteristics of model low-salt soy sauce

Rie Kuramitu

Department of Chemistry, Faculty of General Education, Akashi National College of Technology, Uozunii, Akashi 674-8501, Japan. e-mail: kuramitu@akashi.ac.jp

The object of the present study was to create a novel low-salt soy sauce model by using salt-substituting substances. Although the taste of soy sauce is composed mainly of the umami taste of monosodium glutamate and the salty taste of NaCl, soy sauce contains many sorts of amino acids. After succeeding in simplifying the amino acids composing soy sauce, I added salt-substituting substances to this solution in amounts corresponding to 16.3% NaCl. When 50% of the NaCl was replaced by a salt-substituting substance, the mixed solution had the desired saltiness, but in regard to likeness to soy sauce, it was considerably different from a solution in which solely sodium chloride was added. In the case of 33% replacement of both glycine ethylester hydrochloride and KCl, taste characteristics were identical with those of NaCl. Lysine hydrochloride also showed a high similarity in quality with NaCl. These results are in accordance with the experimental results on securing saltiness by using pre-soy sauce. A heating test was conducted. Heating did not cause distinct changes in taste and flavor of the synthetic soy sauces. From these results I have designated the synthetic soy sauce obtained by simplifying the amino acid composition of commercial soy sauce as 'model soy sauce'.

P1-40. A new approach to consumer preference for tea beverages utilizing a neural network model and cluster analysis

Gakuro Ikeda, Mayumi Hioki, Yasuyuki Sagara and Hajime Nagai[1]

Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657 and [1]Research Institute for New Product Development, Suntory Ltd, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan

In recent years, the demand for sugar-free tea beverages, such as oolong and green teas, has increased in Japan. In this work we developed a system to estimate consumer preference and trend for those tea beverages. This system utilized multivariate analysis and a neural network model with conventional sensory evaluation methods. First, sensory evaluation and instrumental analysis were performed on sugar-free tea samples, such as oolong, green and barley teas. In the sensory evaluation, the strength of taste, flavor and preference were estimated in category scales for each sample. In instrumental analysis, ingredients of samples were determined by means of chromatography in addition to pH, soluble solid content and color. The system (i) classifies consumers according to sensory evaluation data by the mean of cluster analysis and (ii) shows three-dimensional maps of the characteristics in taste recognition and preference estimation. A neural network model was applied to determine correlation among ingredients, tastes and preferences. The results indicated clear differences among clusters in taste sensitivities for amino acids contained in tea beverages. Additionally, tastes and flavors favored by each cluster were shown on the maps.

P1-42. Relationship between eating behavior and heart rate in the human

T. Horio

College of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo 665-0006, Japan

Relationships between eating behavior and heart rate were evaluated in 17 healthy university students. The test materials were citric acid, curried rice, Japanese noodles and a rice ball.

Heart rate increased by 10.2–16.7% for taste stimuli and during eating behavior as compared with pre-stimuli values. The response of heart rate to citric acid before taking a meal was not different from that after taking the meal. The increase rate of heart rate in taking curried rice was larger than that in Japanese noodles, followed by that in a rice ball.

These findings show that heart rate increased during eating behavior and that the increase rate differed with the nature of the meal.

P1-43. The effect of CRF antagonist on saccharin intake behavior

Ryoji Fukabori and Takashi Yamamoto

Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Corticotrophin releasing factor (CRF) is known to regulate food intake through various pathways and strongly induce POMC gene expression. Alpha-melanocyte stimulating hormone (alpha-MSH) processed from POMC product is a food regulation factors and it is thought that POMC neurons release alpha-MSH in hypothalamus when CRF is activated. Our previous report showed that the expression of the POMC gene in the hypothalamus is increased by non-caloric sweet taste stimulation with saccharin. This result suggests that CRF is activated by taste stimulation. The present study aimed to examine whether sweet taste stimulation activates the CRF pathway. An intracerebroventricular (i.c.v.) injection of 0.5 μg CRF remarkably decreased saccharin consumption for 30 min in food- and water-deprived rats. On the other hand, an i.c.v. injection of 5 μg of a CRF antagonist (an alpha helical CRF9-41) also decreased saccharin consumption in a dose-dependent manner. However, the same dose of CRF antagonist did not change the consumption of aversive fluid (30 μM quinine). These results suggest the existence of two independent pathways activated by CRF. One is the suppression pathway, where alpha-MSH is known to suppress food intake; the other is the rewarding pathway, where beta-endorphin is known to enhance palatability of food.

P1-44. Some analyses of drinking behavior in rats with a system monitoring fluid intake

Tadashi Inui, Miki Watanabe and Takashi Yamamoto

Division of Behavioral Physiology, Department of Behavioral Sciences, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan

We analysed drinking patterns of sapid solutions in rats by using a

monitoring system. This newly developed system is characterized by: (i) short sampling time (1 min); (ii) long-time continuous measurement (4 days); (iii) simultaneous presentation of two fluids; and (iv) changing left–right alternations of positions for two fluids with a preset interval. In the present experiments, we examined the circadian rhythm of water intake, the preference behavior to water and NaCl, and the erects of furosemide on the intake of NaCl. Intraperitoneal injection of furosemide is known to produce salt appetite and increase the intake of NaCl. Rats drank water almost exclusive in the dark cycle, with the two peaks of intake at 19:00–20:00 h and at 4:00–5:00 h. When water and NaCl were presented, rats preferred water to 0.2 M NaCl, whereas there was no significant difference between water and 0.1 M NaCl. The rats treated with furosemide preferred 0.2 M NaCl to water soon after presentation of these fluids, but the total intake was greater for water than for 0.2 M NaCl during the 12 h dark period. On the other hand, the preference for 0.1 M NaCl over water was observed throughout the dark period. These results indicate that the internal level of Na ions and the hedonics of NaCl taste regulate salt preference.

P1-45. Characteristics of salt intake behavior in Na_V2 gene deficient mice

Tsuyoshi Shimura, Uno Watanabe, Eiji Watanabe[1], Masaharu Noda[1] and Takashi Yamamoto

Laboratory of Behavioral Physiology, Graduate School of Human Science, Osaka University and [1]Division of Molecular Neurobiology, National Institute of Basic Biology, 1-2, Yamadaoka, Suita, Osaka 565-0871, Japan

Na_V2, a subfamily of voltage-gated sodium channels, is suggested to be involved in body-fluid homeostasis. Previous studies have revealed that Na_V2 gene deficient mice show abnormal intakes of hypertonic saline under water-depleted condition. To further clarify the role of Na_V2, we examined salt intake behavior under water-replete conditions using the traditional two-bottle preference test. Na_V2-null mice showed higher preference for NaCl solutions (75, 150 and 300 mM) compared with control mice. They consumed significantly larger quantities of NaCl at those concentrations than controls did. However, their preferences for other tastes (sucrose, saccharin, HCl and quinine) are almost the same as those in controls. Transections of either the chorda tympani or glossopharyngeal nerve had little effect on NaCl intake in Na_V2-null and control mice. Sections of both nerves also did not change preferences for NaCl in either group of mice. Although sections of the supralaryngeal nerve slightly reduced NaCl intake in Na_V2-null mice, the preference ratio for NaCl in Na_V2-null mice decreased to the level of controls after transactions of both the supralaryngeal and glossopharyngeal nerves. These results suggest that the abnormal higher intake of NaCl in Na_V2-null mice is partially mediated by taste nerves innervating the pharynx and larynx.

P1-46. Increase in taste discrimination among essential amino acids in C57BL mice fed L-lysine-deficient diet

K. Nakashima, H. Katsukawa, T. Sugimura, D. Sugita[1] and Y. Ninomiya[1]

Asahi University, Gifu 501-0296 and [1]Kyushu University, Fukuoka 812-8582, Japan

Our previous studies using a conditioned taste aversion (CTA) paradigm indicated that taste discrimination between L-lysine (Lys) and L-arginine (Arg) was not evident in mice fed a normal diet. However, we also showed that preference for Lys in a single-bottle test increased when mice were fed a Lys-deficient diet. They consumed moderate concentrations of Lys solution, which otherwise are aversive. This deficiency-induced craving for Lys solution may be accompanied by increase in taste discrimination between Lys and other amino acids. The present study examined this possibility by comparing behavioral discrimination in a CTA test among essential amino acids in mice fed control and Lys-deficient diets. A conditioned aversion to Arg generalized to Lys in normal mice fed the control diet, but not in the mice fed the Lys-deficient diet. On the other hand, when the glossopharyngeal nerves were sectioned, an conditioned aversion to Arg generalized to Lys and L-histidine in mice fed control and Lys-deficient diets. These findings suggest that increases in both acceptability of Lys and behavioral discrimination among essential amino acids play important roles in protecting the animal from nutritional deficiency, and that taste information conveyed by the glossopharyngeal nerve seems to play a major role in recognition of nutritional deficiency.

P1-47. Newly developed system available for analysing feeding and drinking behavior in mice

Takuya Itou[1,2], Nobuko Miyake[2], Toshiaki Ishii[1,2], Tadashi Takewaki[1] and Masakazu Nishimura[1,2]

[1]Department of Pathogenic Veterinary Science, The United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193 and [2]Department of Pharmacology, University of Obihiro School of Veterinary Medicine, Obihiro 080-8555, Japan

Appetite should be estimated with regard not only to food consumption, but also to behavior in searching for food. We developed a task–feast system (TFS) to analyse both food- and water-oriented behavior in mice. Two areas for feeding and drinking were placed 180 cm above a square acrylic cage and could be accessed by climbing wire-net cylinders 8 cm in diameter. The top 3 cm long area of each was termed 'task–feast-domain for food' (TFDF) or 'task–feast-domain for water' (TFDW). Both the frequency and duration of trips to the TFDF and TFDW were automatically watched for 24 consecutive hours. Ten mice were adapted to the system for 30 days. The mice were deprived for food on day 29 and recovered on day 30. Behavioral profiles at days 28–30 were compared. Food-deprivation increased the frequency but decreased the duration of trips to the TFDF and refeeding increased the duration for TFDF. These changes in behavioral profiles may be useful for analysing appetite.

P1-48. Recognition of fatty acids by *Caenorhabditis elegans*

Shinya Matsumoto, Shoko Sawano and Tomohiko Mori

Graduate School of Agriculture, Kyoto University, Gokasho Uji 611-0011, Japan

The chemotaxis behavior of *Caenorhabditis elegans* toward various fatty acids was investigated. Oleic acid (C 18:1), linoleic acid (C 18:2), γ-linolenic acid (C 18:3) and arachidonic acid (C 20:4) were dissolved in ethanol and spotted on a chemotaxis plate (0.25% Tween 20, 10 mM MOPS, pH6). Plain ethanol was spotted on the same plate as the control. Young adult N2 worms were washed with S basal buffer and spotted at the center of the plate, where the

distances from the two spots were roughly equal. The numbers of worms near the spots were counted after incubating the plate at 20°C for 30–60 min. The ratio of the numbers of worms located near the fatty acid spot and those near the control spot was calculated in % and statistically analysed for significance. The worms were shown to be attracted when 20 mM linoleic acid was spotted. On average, 74% of worms were attracted to the spot, the number being statistically significant at $P < 0.01$ (number of trials = 15). However, when 1, 10, 50 and 100 mM linoleic acid was spotted, no significant attraction was observed. No obvious attraction was observed when other fatty acids—oleic, γ-linolenic and arachidonic—were used in this assay.

P1-49. The palatability of fat substitute in the aspect of the oral cavity in mice

Ayako Suzuki, Masahiro Imaizumi, Tohru Fushiki

Laboratory of Nutritional Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kitashirakawa, Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

We examined whether a liquid-type fat substitute, sorbestrin, consisting of sorbitol fatty acid esters with low energy (1.5 kcal/g), is palatable or not, by means of the two-bottle choice test or the conditioned place preference (CPP) test. Mice took sorbestrin at the same level as corn oil in the short-term, two-bottle choice test, indicating that sorbestrin was acceptable in the oral cavity. But they did not continue taking it in the long-term test. And sorbestrin didn't act as the reinforcer in the CPP test, but mice with 0.1 ml corn oil in their stomach just before the conditioning to simulate postprandial effects showed reinforcing effects on taking sorbestrin in the CPP test. These results suggest that the postprandial effects of sorbestrin are involved in the preference to lipid.

P1-50. The relationship between aversive sensation of taste and DBI

Yasuko Manabe, Taro Toyoda, Kyoko Kuroda, Masahiro Imaizumi, Takashi Yamamoto[1] and Tohru Fushiki

Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 and [1]Laboratory of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka 565-0871, Japan

We have reported that diazepam binding inhibitor (DBI) is released in the rat brain after stimulation of a bitter fluid (Manabe *et al.*, 2000, *Chem. Senses*, 25: 739). To examine the relationship between DBI and aversion to various kinds of fluid intake, we injected the DBI peptide fragment into the fourth ventricle of mice. Injection of the DBI peptide fragment suppressed the intake of 5% sucrose as a sweet taste, water and 0.9 mM quinine–HCl solution as a bitter taste and preference for 0.05% sodium saccharine solution. Administration (i.p.) of flumazenil, a benzodiazepine receptor antagonist, 20 min before the injection of the DBI peptide fragment antagonized these suppressive effects of DBI on fluid intake and preference, suggesting that the effect of the DBI peptide fragment acted through benzodiazepine receptors. Furthermore, the injection of the DBI peptide fragment into the fourth ventricle increased the aversive response to 0.9% NaCl solution in a taste reactivity test in mice. These results suggest that DBI in the brain is related to aversive feeling.

P1-51. Participation of visual cues in the gustatory discrimination of *Drosophila melanogaster*

Hiromi Naritsuka, Satoko Narikawa, Masayuki Koganezawa and Ichiro Shimada

Department of Developmental Biology and Neuroscience, Graduate School of Life Science, Tohoku University, Sendai 980-8578, Japan

Acute differential sensitivity has been revealed in the feeding behavior of *Drosophila melanogaster*. Learning and memory processes have been suggested to participate in the behavior. We examined how a visual cue affected differential sensitivity in feeding behavior. Feeding tests were carried out in dark and light conditions using micro-test plates with 60 wells. One of the paired concentration levels of sucrose solutions, mixed with the blue food dye (visual cue) and the other without dye were made up in 1% agar and the two solutions were applied symmetrically to the wells. Blue food dye itself did not affect ingestion in either dark or light conditions. The ingested volume of the higher concentration of sucrose was, however, significantly larger in the light than in the dark, while that of the lower concentration was smaller in the light than in the dark. Thus, a blue color cue in light conditions enhanced discrimination of different concentration levels of sucrose solutions. Such an enhancement was observed under conditions where 12, 18 and 30 out of 60 wells were filled symmetrically with 50 and 100 mmol/l sucrose solutions (6, 9 and 15 wells each). The maximum enhancement was obtained with 18 out of 60 wells filled.

P1-52. Acquisition and characteristics of conditioned temperature aversion

Noritaka Sako, Ritsuko Ohashi, Nobuyuki Sakai[1], Kun-Hisian Yang and Tadataka Sugimura

Department of Oral Physiology, Asahi University School of Dentistry, Gifu 501-0296 and [1]Japanese Science Technology Co. (AIST), Tsukuba 305-8566, Japan

Sensations elicited by chemical and physical properties of drinking water are important in ingestive behavior. In the present study, in order to examine the characteristics of temperature as conditioned stimulus, we conducted behavioral experiments in Wistar male rats using a conditioned food aversion technique. Results were as follows. (i) Rats aversively conditioned to 5 or 40°C distilled water did not avoid four basic taste stimuli at 5 or 40°C. (ii) Rats aversively conditioned to 5 or 40°C 0.1 M sucrose did not avoid distilled water adjusted to the same temperature as the conditioned stimulus, but they generalized sucrose with all tested temperatures. (iii) When the bilaterally denervated (both chorda tympani and glossopharyngeal nerves) rats were conditioned to 5 or 40°C 0.1 M sucrose, they generalized four basic taste stimuli adjusted to same temperature of the conditioned stimulus. (iv) The rats with bilateral lesions of the amygdala could not acquire the conditioned temperature aversion. These results suggest that rats take taste as priority over temperature in ingestive behavior and that the amygdala is important in the acquisition of conditioned temperature aversion.

P1-53. Behavioral studies on association learning of taste and smell in rats

Wenzhong Bai and Takashi Yamamoto[1]

College of Life Science, Hebei Normal University, Shijiazhuang 050016, P.R. China and [1]Laboratory of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka 565-0871, Japan

Associative learning between taste and odor was studied in Wistar male rats. They were trained to drink 0.5 mM Na-saccharin and 0.2 M NaCl which contained low concentrations of odor stimuli such as strawberry essence, grape essence, almond essence or iso-amylacetate. After the 6 day training session, they were tested for odor preference by exposing two kinds of waters, each of which contained one of the odors with the conventional two-bottle preference test. The results were as follows. (i) Rats preferred to drink water with the odor previously associated with saccharin. (ii) When the rats were put into sodium deficiency by injections of a diuretic, furosemide, they preferred water with the odor previously associated with NaCl. (iii) Rats conditioned to avoid saccharin with the conventional taste aversion paradigm rejected water with the odor previously associated with saccharin. These findings suggest that odors can work as an effective cue to search preferable, needed or harmful foods on the basis of odor–taste association learning acquired after mere exposure to these flavored foods.

P1-54. Computer-assisted analysis of reduced glutathione-induced response of *Hydra*

Kazumitsu Hanai; Koji Takeyama[1], Akira Tsukada[1] and Kazuo Sasaki[1]

Department of Physics, Kyoto Prefecture University of Medicine, Kyoto 603-8334 and Faculty of Engineering, Toyama University, Toyama 930-8555, Japan

Hydra shows tentacle ball formation as a response to *S*-methyl-glutathione. The response is sensitively modified in the presence of various biologically active substances in a specific manner for each substance. Computer-assisted image analysis was introduced to determine the modification in an objective way. Seven image parameters (area, boundary length, diameter, etc.) and 12 image moments up to the 4th order ($x^p y^q$, $p + q \leq 4$) were calculated for all images of *Hydra* behavioral response captured as a series of digital images every 2 s using custom-made software on MacOS. The raw image moments were transformed into 12 invariant moments (M1–M12, invariant for translation, size and rotation). Auto-covariance functions were then calculated for all these image data and an autoregressive model with the minimum AIC (Akaike information criterion) was estimated by the Yule–Waker method. High frequency components of the power spectra in several image parameters from response in the presence of AFGF were larger than those from control response. The statistical significance was estimated by comparing AIC values: one was obtained under the assumption that both belonged to a different set of experiments and another was obtained under the assumption that all the data belonged to a single set of experiments. The AIC difference was large enough to conclude that both the responses are different.

P1-55. Laryngeal afferent-mediated gastric relaxation and c-fos expression in the dorsal motor nucleus of the vagus in rats

Motoi Kobashi, Mamoru Yanagihara[1], Masatoshi Mizutani[1] and Ryuji Matsuo

Department of Oral Physiology, Graduate School of Medicine and Dentistry, Okayama University, Okayama 700-8525 and [1]Department of Physical Education and Welfare, Okayama Prefecture University, Soja 719-1197, Japan

Relaxation of the stomach is achieved by inhibiting cholinergic myenteric neurons that connect with vagal preganglionic neurons. In addition, non-cholinergic and non-adrenergic (NANC) myenteric neurons that were activated by the excitation of vagal preganglionic neurons induce relaxation. The present study aimed to identify the vagal preganglionic and myenteric neurons responsible for the laryngeal afferent-mediated relaxation of the proximal stomach. Both the administration of water into the larynx and the electrical stimulation of the superior laryngeal nerve (SLN) evoked relaxation in the proximal stomach. Relaxation evoked by the administration of water disappeared after the i.v. injection of atropine methyl nitrate, a peripherally acting muscarinic antagonist. Relaxation evoked by the electrical stimulation of the SLN was diminished in magnitude, but still observed after the injection of atropine. The excitation of neurons in the dorsal motor nucleus of the vagus (DMV) was detected by c-fos expression after electrical stimulation of the SLN. Abdominal-projecting neurons were identified after i.p. injection of fluorogold. The intermittent electrical stimulation of the SLN evoked c-fos expression in the abdominal-projecting DMV neurons. The water-response of the proximal stomach is mainly achieved via cholinergic myenteric neurons; however, activation of the other sensory fibers in the SLN might induce gastric relaxation via both the cholinergic and NANC myenteric neurons.

P1-56. Modulation of water response in the superior laryngeal nerve in rats

Yuka Kajii, Tomio Shingai[1], Jun-ichi Kitagawa[2], Yoshihiro Takahashi[2], Yo Taguchi and Yoshiaki Yamada[2]

Division of Pediatric Dentistry, [1]Chemoreception Science and [2]Oral Physiology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata 951-8514, Japan

It is known that water is a most effective stimulus for elicitation of the swallowing reflex in the pharyngolaryngeal region and that water-sensitive fibers in the superior laryngeal nerve (SLN) are responsible for the initiation of the reflex. Recently, some reports have suggested that substance-P-containing nerve fibers in the laryngeal mucosa are associated with the elicitation of the swallowing reflex. In the present study, we examined the effect of substance P on both the water response in the SLN and on the water-induced swallowing reflex in the larynx. We also examined the effect of quinine hydrochloride (QHCl) on the activity of the SLN, since we had previously observed that the swallowing reflex was suppressed after application of QHCl. Nerve activity was recorded from the whole nerve of the SLN of urethane-anesthetized rats. The water response of the SLN was enhanced after intravenous injection of substance P (1.4 μg). Water-induced swallowing was also facilitated after injection of substance P.

Application of QHCl on the laryngeal mucosa markedly inhibited both the spontaneous activity and water response in the SLN. Our findings indicate that the sensitivity of water fibers in the SLN is closely associated with substance P.

P2-01. Primary structure of pheromone binding protein occurring in an antenna of *Ascotis selenaria cretacea*

Hayaki Watanabe, Nami Miura, Takashi Miyamoto, Ryoichi Sato and Tetsu Ando

Department of Environment—Symbiotic Production Systems, Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan. e-mail: hayakin8@cc.tuat.ac.jp

Antennal mRNA was isolated from 100 males of *Ascotis selenaria cretacea* (Lepidoptera: Geometridae), which responded to the epoxyalkenyl sex pheromone secreted by the female moths. A cDNA clone encoding a pheromone binding protein (PBP) was multiplied by a reverse transcription–polymerase chain reaction (RT–PCR) utilizing specific primers, which were deduced from sequences of PBPs previously reported for other lepidopterous species. From fragments of the cDNA, the total DNA sequence was determined using the RACE method. This sequence shared some characteristics of PBPs from male moths, i.e. positional conservation of cysteine residues and major hydrophobic domains. Furthermore, for the antenna and other parts of the males, RT–PCR was performed by specific primers. From the results, cDNA only amplified from antenna. This result indicates that the isolated cDNA specifically expresses in antenna.

P2-02. Cloning G protein alpha subunit cDNA from the antennae of the adult male silkmoth (*Bombyx mori*)

Nami Miura, Masashi Iizuka and Ryoichi Sato

Graduate School of Bio-Application and Systems Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan

In insects, rapid, transient increases in inositol triphosphate (IP_3), cGMP, diacylglycerol (DAG) and Ca^{2+} are detected on stimulation with pheromones or non-pheromonal odorants. This suggests that heterotrimeric guanine nucleotide binding proteins (G proteins) may transduce some odorant responses in insects. Amino acid sequence comparisons show that the different G protein alpha subunits fall into four classes, which are conserved in animals.

In order to study olfactory signal transduction in insects, we explored G protein alpha subunit expression in the olfactory organ of the silkmoth (*Bombyx mori*). We used an RT–PCR approach to amplify G protein alpha subunits from male silkmoth antenna mRNA. The primers were derived from amino acid sequences of conserved regions in vertebrate and insect G protein alpha subunits. As a result, we obtained cDNA clones encoding three classes of alpha subunits: Go, Gq and Gs. RT–PCR experiments showed that these G protein alpha subunit mRNAs were present in a variety of tissues.

P2-04. Functional database system of olfactory receptors

Kazunori Miyazaki and Satoshi Itoh

Advanced Materials and Devices Laboratory, Corporate Research & Development Center, Toshiba Corp., Kawasaki, Kanagawa, Japan

We have developed a functional database system for olfactory receptors (OR) which is constructed semi-automatically and self-organizably. This database system has gathered the nucleotide sequences and amino acid sequences from public databases, i.e. GenBank at NCBI and Olfactory Receptor Database (ORDB) at Yale University. Since these extracted data were described by using the hypertext markup language (HTML), we have changed them to data described by using extensible markup language (XML), which is an emerging standard for data interchange on the World Wide Web. Since XML documents are textual data, they are able to be used independently from any applications. And they are highly flexible with respect to the addition of new element data. We analyse the XML data on olfactory receptors using predictive tools and add the result to them as additional data. In this study, we chose SOSUI developed by Mitaku of Tokyo University of Agriculture and Technology as a predictive tool. SOSUI is able to predict transmembrane domains of transmembrane proteins. Using it, we analysed olfactory receptor amino acid sequences extracted from the XML data and added the data of predicted transmembrane domain structures, which would include binding sites for odorants, to our database for olfactory receptors.

P2-05. Chloride concentration change induced by furosemide in bullfrog olfactory cilia

Mika Yoshioka and Tadashi Nakamura

Department of Network Science, University of Electro-Communications, Chofu-si, Tokyo 182-8585, Japan

When vertebrate olfactory cells receive odorants at their cilia, inward Na^+ and Ca^{2+} flows are generated. Then, Cl^- channels on cilia are also activated by those Ca^{2+} flows to induce outward Cl^- flow, which amplifies the inward receptor current. In this mechanism, we may expect a molecule(s) regulating the intracellular Cl^- concentration—$[Cl^-]_i$—such as a Cl^- pump or Na^+–K^+–$2Cl^-$ symport in the cell. Here, we examined effects of ethacrynic acid and furosemide, the inhibitors for the pump and the symport respectively, on the $[Cl^-]_i$ at cilia. While solutions of those chemicals were applied to the isolated bullfrog olfactory receptor cells, $[Cl^-]^i$ was monitored by the use of MQAE, the fluorescent Cl^- probe. We observed that both chemicals at millimolar level induced the transient decrease of $[Cl^-]_i$. In addition, when those chemicals at millimolar level were applied to the isolated newt olfactory epithelium, electro-olfactograms were generated. Therefore, these chemicals are most likely to stimulate the receptor cells through the same pathway as odorants. While other effects of ethacrynic acid than decreasing $[Cl^-]_i$ are unclear, 1 mM furosemide induced the increase following the transient decrease of $[Cl^-]_i$. These results suggest that the furosemide-sensitive Cl^--symport contributes to the regulation of $[Cl^-]_i$.

P2-06. The culturing of newt olfactory receptor neurons

Naofumi Matsumoto and Tadashi Nakamura

Department of Applied Physics and Chemistry, University of Electro-Communications, 1-5-1 Chofugaoka, Chofu, Tokyo 182-8585, Japan

Although cultures of olfactory receptor neurons have been reported, most of them used mammal cells. We supposed that amphibians might provide good cells suitable for culture as they have been used in many studies of tissue-regeneration. Here, we tried 'explant culture' of newt olfactory receptor neurons following a report by MacDonald *et al*. (1996).

Olfactory receptor epithelia were dissected from animals, disinfected with antibiotics and then chopped into very small pieces (~1 × 1 mm^2). They were plated on plastic culture dishes coated with fibronectin that contained a diluted (70%) MEM solution including 10% fetal bovine serum and 100 μM ascorbic acid. On days 5–10 from the plating, cells migrated from the tissue pieces to surrounding areas to form flat cell sheets. On days 10–15, bipolar cells appeared and stayed near the edges of those sheets.

On about day 30, we examined whether these cells could respond to odorant stimuli by monitoring the Ca^{2+} concentration in the cells with a fluorescent probe (Fura2). We found that those bipolar cells, as well as fresh isolated olfactory cells, responded to the stimuli. Thus functional olfactory receptor neurons were detected in the 1 month old 'explant culture' of the olfactory epithelia.

P2-07. Odorant discrimination between enantiomers of carvones in mouse olfactory receptor neurons

Junzo Hirono and Takaaki Sato

Life Electronics Laboratory, National Institute of Advanced Industrial Science and Technology (AIST), 3-11-46 Nakoji, Amagasaki 661-0974, Japan

R(–)-Carvone [*R*(–)C] and *S*(+)-carvone [*S*(+)C] were recognized as spearmint and caraway odor, respectively. We examined the odor discrimination ability of mouse olfactory receptor neurons (ORNs) to carvone isomers and two series of *n*-aliphatic odorants, *n*-fatty-acids and *n*-aliphatic-alcohols, in three different concentrations (100, 10 and 1 μM) using a tissue-printing method and a Ca-imaging assay with fura-2. About 10% of 2740 tested ORNs responded to either of the carvones at 100 μm. We classified the odor responsiveness of individual ORNs based on their sensitivities to two enantiomers in the responsive concentration or the response amplitudes at the lowest responsive concentration. Eighteen per cent of 221 carvone-responsive ORNs (Car-ORNs) were *R*(–)C-preferred ORNS, while 25% were *S*(+)C-preferred. The other Car-ORNs were sensitive similarly to both of enantiomers (non-discriminative Car-ORNs). A quarter of Car-ORNs also responded to *n*-aliphatic odorant(s), but the dominant of them were non-discriminative Car-ORNs. The group of non-discriminative Car-ORNs may contribute to a common part of odor quality between *R*(–)-carvone and *S*(+)-carvone and each of the groups of *R*(–)C- or *S*(+)C-preferred ORNs may play a unique part in the respective odors. In this manner, the receptor combinatorial code enables us to discriminate optical isomers from each other.

P2-08. Oscillatory current responses of olfactory receptor neurons to amino acids in the rainbow trout

Koji Sato and Noriyo Suzuki

Animal Behavior and Intelligence, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan. e-mail: suzuki@sci.hokudai.ac.jp

Oscillatory activities, so-called 'waves', have often been observed to superimpose on EOG responses and impulse responses of olfactory neurons to odorants in many vertebrate species. To elucidate the origin of oscillations, we studied the oscillatory properties of current responses of isolated olfactory receptor neurons (ORNs) of the rainbow trout to an amino acid mixture (L-Glu, L-Arg, L-Ala and L-Nva), using whole-cell voltage-clamp techniques. Current responses of ciliated and microvillous ORNs had a phasic time course with fast rise and slower decay phases. The decay phases were fitted well by double exponential functions. The mean root mean square (RMS) of residual noises subtracted from the fitted exponential functions increased with an increase of stimulus intensity. In high ranges of mean RMS, the noises turned into oscillations. The mean RMS values were correlated with those of the initial response peaks (n = 23, r = +0.783), indicating that the oscillations occurred when ORNs were stimulated by odorants in high intensity. The continuous wavelet analysis using the Gabor function revealed that the mean median frequency of oscillations was 1.921 ± 0.495 Hz (mean ± SD, n = 62). There was no significant difference in oscillation frequency between those of ciliated and microvillous ORNs, and between different perfusion conditions with standard and Na^+-free (choline) Ringer's solutions (Mann–Whitney U-test, $P > 0.3$, $P > 0.5$), but there was a slight difference in oscillation frequency between different holding potential conditions of negative (–60 mV, 1.917 ± 0.258 Hz, n = 71) and positive (+20 mV, 1.595 ± 0.159 Hz, n = 13) potentials (Mann–Whitney U-test, $P < 0.01$). The oscillations of current responses were simulated well by the solutions of simultaneous differential equations based on the assumption that the current response is directly related with intracellular second messenger cAMP and its production and hydrolysis are regulated by intracellular Ca^{2+} (Cooper *et al*., 1995). Our results suggest that the oscillations of current responses are due to the oscillatory properties of intracellular cAMP and Ca^{2+} concentrations.

P2-10. Localization of noradrenergic fibers and receptors in the neuronal circuits of an accessory olfactory bulb

Shu Takigami[1,2], Tomoyuki Miyashita[3] and Masumi Ichikawa[1,2]

[1]Department of Developmental Morphology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, [2]CREST of Japan Science and Technology Corporation, Kawaguchi and [3]Department of Molecular Physiology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan

Female mice form memory for pheromones of the partner stud during mating. This memory is established in the neuronal circuits of the accessory olfactory bulb (AOB). The formation of the memory requires both vomeronasal inputs of pheromones and secretion of noradrenaline by the vaginal stimulus. The termination of nor-

adrenergic fibers and the localization of noradrenergic receptors in the AOB have hitherto been unknown. In this study, we examined a detail of the noradrenergic system in the neuronal circuits of the AOB. Immunoreactivities against tyrosine hydroxylase or neuropeptide Y were recognized in the granule cell layer and the mitral/tufted (M/T) cell layer. Also, immunoreactivities for dopamine beta hydroxylase (DBH) were observed mainly on the M/T cells. Using electron microscopy, we found immunoreactivities for DBH in the neuronal terminals around the M/T cells. *In situ* hybridization and immunohistochemistry for alpha 2c noradrenergic receptor clarified that the receptors were expressed in the M/T cells but not in the granule cells. These results suggest that noradrenaline will have an effect on the M/T cells in the formation of memory for pheromones.

P2-11. Blockage of glutamate receptors modulates reciprocal synaptic currents measured from mitral cells in the mouse accessory olfactory bulb in slice preparations

Mutsuo Taniguchi and Hideto Kaba

Department of Physiology, Kochi Medical School, Kochi 783-8505 and 2CREST, Science and Technology Corp., Saitama 332-0012, Japan

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

AOB slices were prepared from 23- to 34-day-old BALB/c mice. To evoke dendrodendritic inhibition, a depolarizing voltage step from –70 to 0 mV (5–20 ms) was applied to a mitral cell. Under control conditions, the voltage step evoked $GABA_A$-receptor-mediated inhibitory postsynaptic currents (IPSCs), which were greatly enhanced after the reduction of extracellular Mg^{2+}. In Mg^{2+}-free solution, the NMDA receptor antagonist D,L-APV, as well as an agonist for group II metabotropic glutamate receptors (mGluR2/mGluR3), DCG-IV, significantly reduced dendrodendritic inhibition. On the other hand, the non-NMDA-receptor antagonist CNQX moderately blocked the IPSCs. In Mg^{2+}-containing solutions, the mGluR2 antagonist LY341495 enhanced the IPSCs. The present results suggest that NMDA receptors and mGluR2 play important roles in reciprocal transmission between mitral cells and granule cells in the mouse AOB.

P2-12. Synaptic plasticity in the mouse accessory olfactory bulb

Guang-Zhe Huang and Hideto Kaba

Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505 and CREST, JST, Japan

When female mice are mated, they form a memory of the pheromonal signal of their male partner. The neural changes underlying this memory occur in the accessory olfactory bulb (AOB) and involve neurochemical and morphological changes at the reciprocal dendrodendritic synapses between mitral and granule cells. We analysed synaptic transmission and its plasticity in slice preparations of the mouse AOB using field potential recording. Stimulation of mitral cell axons evoked two negative field potentials recorded in the AOB external plexiform layer. The second potential was blocked by CNQX, but not by AP5, indicating non-NMDA-receptor mediation of monosynaptic activation of the granule cell dendrites. The first potential was resistant to CNQX and AP5, but it was eliminated by TTX, which is indicative of antidromic activation of the mitral cells. Spaced and long-term θ frequency stimulation (10 Hz) effectively induced long-term potentiation (LTP) of the second field potential. The LTP was blocked by AP5. Noradrenaline (NA) enhanced the induction of LTP. NA-induced enhancement of LTP was blocked by phentolamine, but not by propranolol. These results indicate that relatively low frequency stimulation induces NMDA-receptor-dependent LTP at the mitral-to-granule-cell synapses and that LTP induction is enhanced by the activation of α-adrenergic receptors.

P2-14. Effect of intrabulbar infusion of a MAP kinase inhibitor on olfactory learning in young rats

Jing-Ji Zhang, Fumino Okutani and Hideto Kaba

Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505 and CREST, JST, Japan

MAP kinase (MAPK) has been demonstrated to regulate the transcriptional factor CREB in the hippocampus during learning. In this study we examined whether MAPK is involved in CREB phosphorylation during olfactory learning. After the pairing of an odor and foot shock on postnatal day (PND) 11, young rats show aversion to the odor in the odor preference test on PND 12. The MAPK inhibitor PD-098059 was continuously infused into the olfactory bulb (OB) during a 30 min training session. On the following day, rats spent significantly longer time over the odor zone than control animals that had received vehicle (DMSO). We also analysed phospho-CREB (PCREB) by immunohistochemistry. Pups were bilaterally infused with DMSO or PDO98059 into the OB and killed 1 h later. The number of PCREB-positive cells increased in several cell types, especially in the granule cells of the OB in the vehicle-infused group that received odor–shock presentation. MAPK inhibitor infusion, however, decreased the number of pCREB-positive cells in the OB after odor and shock stimulation. These data indicate that MAPK regulates CREB phosphorylation on olfactory learning in young rats.

P2-15. Visualization of odor-evoked oscillation in insect antennal lobe

Koutaroh Okada, Hiroyuki Ai[1] and Ryohei Kanzaki

Institute of Biological Science, University of Tsukuba and [1]Division of Biology, Department of Earth System Sciences, Fukuoka University, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

Odor-induced oscillatory signals have been reported in odor discrimination in various insects. To understand dynamic olfactory coding in the first-order olfactory center, the antennal lobe, localization of oscillatory signals was investigated using an optical recording technique with a voltage-sensitive dye. We present here the first report of visualization of the spatial distribution pattern of odor-induced oscillations in the bumblebee antennal lobe. The oscillatory signals were evoked in the antennal lobe by odor stimulation. The peak frequency varied from 17.5 to 30.5 Hz in 14 different animals. However, the oscillatory frequencies in different trials in the same animal were almost identical. The oscillations disappeared following treatment with TTX. Analysis of the odor-induced optical responses by a maximum entropy method allowed visualization of oscillatory regions in the antennal lobe. The oscillatory signals were usually localized in regions of the antennal lobe

that were the same size as individual glomeruli. Our results suggest that glomerular structures may be functional units of odor processing from the viewpoint of odor-induced population responses, the oscillations.

P2-16. The property of odor-induced oscillatory local field potentials in piriform cortex of isolated guinea pig whole brain with the nose

Takaaki Sato, Ichiro Takashima[1], Riichi Kajiwara[1], Kiyo Murano[1], Kaoru Tsukada[1] and Toshio Iijima[1,2]

Life Electronics Laboratory, National Institute of Advanced Industrial Science and Technology (AIST), Amagasaki, Hyogo, [1]Neuroscience Research Institute, AIST, Tsukuba, Ibaraki and [2]Tohoku University, Sendai, Miyagi, Japan

Odor discrimination initiates at the odorant receptor in receptor neurons and terminates in the central nervous system. The receptor combinatorial coding for odors with overlapping in odorant–structure tuning specificities has been proposed as a principle for odor discrimination by Malnic *et al*. (1999). On the other hand, the function of the piriform cortex which is the largest olfactory cortical area, has not been well known at the system level. In order to investigate the odor-induced activities in isolated brain in a respiration-free condition, we have developed the preparation of isolated whole brain with half of the nose intact. In the isolated whole brain with the nose, odor-induced oscillatory local field potentials in dorsal anteriorpiriform cortex were observed. The dominant frequency was ~10 Hz. High correlation coefficients were obtained in the response profiles of the initial 0.4 s among the same odor. The relatively high correlation coefficients which were obtained for the response profiles of ~2 s between some of different odors might be attributed to the order of the stimulation, suggesting a function of association among successively detected odors in anterior piriform cortex.

P2-17. Analysis of odor responses in dogs using electroencephalography

Yoshiyuki Hirano, Yasutake Shimizu and Keiichi Tonosaki

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

Many studies have been reported concerning olfaction in dogs, and their olfactory abilities have been used in many fields. However, trainers and researchers were unable to avoid great suffering to the animals in these experiments, because simple methods to measure olfactory ability have not been established. The purpose of this study was to examine olfactory characterization of dogs using electroencephalographic recording. Electroencepahlography in dogs indicated that slow waves decreased and rapid waves increased during stimulation with an odorant. These results suggest that rapid waves of electroencephalographic activity are important in determining a dog's olfactory ability. Electroencephalographic recording is effective in diagnosing anosmia in the dog and is less stressful than a behavioural experiment.

P2-18. Expression of the amiloride-sensitive epithelial sodium channel in mouse fungiform and circumvallate papillae

Chiharu Sadamitsu[1,2], Keiko Yasumatsu[1], Noriatsu Shigemura[1] and Yuzo Ninomiya[1,2]

Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582 and [2] Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon Minato-ku, Tokyo 105-0001, Japan. e-mail: csadamit@dent.kyushu-u.ac.jp

It is proposed that amiloride-sensitive epithelial Na^+ channels (ENaCs) are involved in taste signal transduction for Na^+ salts. Electrophysiological studies in C57BL mice demonstrated that responses to NaCl are inhibited by amiloride in the chorda tympani (CT) nerve, but not in the glossopharyngeal (IXth) nerve, suggesting lack of amiloride sensitivity (AS) in the posterior tongue region innervated by IXth nerve. The AS also differs among inbred mouse strains. The BALB and 129 strains did not clearly show the AS even in the anterior tongue innervated by the CT. In the present study, by using *in situ* hybridization (ISH) and RT–PCR techniques, we examined expression of three subunits of ENaC in the anterior (fungiform papillae, FP) and posterior (circumvallate papillae, CP) parts of the tongue in C57BL and BALB mice. ISH analysis suggests that in both C57BL and BALB mice, signals for the α-subunit were detected either in the FP or in the CP, whereas those for β- and γ-subunits were detected in the FP but not in the CP. RT-PCR analysis showed that all three subunits was expressed both in the FP and CP in C57BL mice, whereas no clear expression of β- and γ-subunits was observed in the CP of BALB mice. These results suggest that expression patterns for the ENAC subunits were not necessarily comparable with tongue regional and mouse strain differences in the AS.

P2-19. Recovery of sensitivities to gurmarin and amiloride after crushing of the mouse chorda tympani nerve

Keiko Yasumatsu[1], Hirofumi Kojima[1], Chiharu Sadamits[1,2] and Yuzo Ninomiya[1,2]

[1]Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582 and [2] Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon Minato-ku, Tokyo 105-0001, Japan

Previous electrophysiological studies demonstrated that single fibers of the mouse chorda tympani (CT) nerve responding to sucrose or NaCl are classified into two different types according to their sensitivities to a sweet response inhibiting peptide, gurmarin—gurmarin-sensitive (GS) and -insensitive (G)—or a sodium channel blocker, amiloride—amiloride-sensitive (AS) and -insensitive (AI). This suggests a possibility that there are at least four different types of taste receptors (GS, GI, AS and AI) on the anterior two-thirds of the tongue innervated by the CT. The present study investigated reappearance of these receptors after the CT nerve was crushed by examining responses of regenerated CT to various taste and electrical tongue stimulations and their inhibition by gurmarin and amiloride in C57BL mice. At ~2 weeks after the nerve crush, no significant responses to taste stimuli were observed in the CT. At ~3 weeks after the crush, responses to sucrose and NaCl reappeared. In almost all cases, NaCl responses

were not inhibited by amiloride (AI), whereas in some but not all cases sucrose responses were suppressed by gurmarin (GS). At ~4 weeks after the crush, NaCl responses appeared to be AS. After >1 month, the CT showed sensitivities to gurmarin or amiloride at similar levels to those shown by intact animals These results suggest that the time sequences in recovery of the four different types of receptors is somewhat different, and GI and AI types reappeared earlier than GS and AS types in mouse taste cells after CT nerve crush.

P2-20. Effects of Ni^{2+} on the voltage-dependent inward and outward currents in frog taste disc cells

Kazuhisa Okuda-Akabane, Yukiko Iwabuchi and Yasuyuki Kitada

Department of Oral Physiology, School of Dentistry, Iwate Medical University, Morioka 020-8505, Japan

It has been reported that Ni^{2+} has an enhancing effect on the salt response of the glossopharyngeal nerve in frogs. Frog taste disks contain excitable cells, rod and wing cells. We investigated the effects of Ni^{2+} on the voltage-dependent currents recorded from the taste disc cells in bullfrogs, *Rana catesbeiana*, using the whole-cell patch-clamp technique. Voltage-dependent fast transient inward (Na^+) currents followed by long lasting outward (K^+) currents were recorded by depolarizing pulses under the voltage-clamp mode from enzymatically isolated rod and wing cells or from cells in slice preparations of the taste organ. $NiCl_2$ at 1.0 mM prolonged the time to peak Na^+ currents ~1.5-fold and increased the duration of Na^+ currents ~3-fold without affecting the peak amplitudes of Na^+ currents. Ni^{2+} also affected K^+ currents. That is, in the case of cells showing inactivating component of K^+ currents, Ni^{2+} at 1.0 mM extended the time of both the rise and decline of transient K^+ currents. The present results suggest that Ni^{2+} modulates the gating properties of voltage-dependent Na^+ and K^+ channels in frog taste disc cells and increases the time to open and close the channels.

P2-21. Organic acids reduce the amplitude of the impulses from the salt receptor cell of the blowfly, *Phormia regina*

Yoshihiro Murata[1], Naoko Kataoka-Shirasuge[2] and Taisaku Amakawa[1,3]

[1]Department of Life Science, Graduate School of Science and Technology, Kobe University, [2]Division of Life Environment, Faculty of Human Development, Kobe University and [3]Division of Sciences for Natural Environment, Faculty of Human Development, Kobe University, Kobe 657-8501, Japan

The amplitude of impulses recorded from the labellar taste cells of the fly by the tip recording method determines where the impulses came from of the four types of chemoreceptor cells. However, the impulses induced by NaCl plus organic acids could not be identified because the amplitude significantly differed from that of any chemoreceptor cell, though we suggested in a previous report that they came from the salt receptor cell. In the present paper, first we confirmed that the impulses induced by NaCl plus organic acids come from the salt receptor cell using PER tests and electrophysiological experiments with the tip recording method. Then we analysed the impulse shape by superimposing the impulses obtained by the tip recording method to see why the amplitude of the impulses induced by NaCl plus organic acids was different from that of the salt receptor cell. We hypothesize that the inward current induced by the organic acids at the tip of a chemosensillum may reduce the amplitude of the impulses from the salt receptor cell, as only the positive phase of the impulse was reduced compared to that of the salt receptor cell.

P2-22. Sugar specificity profile of the *Drosophila* sweet-taste receptor TRE

Hiromi Morita, Kohel Ueno[1], Masayuki Ohta and Kunio Isono

Graduate School of Information Sciences, Tohoku University, Sendai 980-8579 and [1]Institute for Behavior Sciences, Gunma University School of Medicine, Maebashi 371-8511, Japan

The *Drosophila* taste gene *Tre* is identical to *Gr5a*, a member of the *Drosophila* candidate gustatory receptor genes (Grs) that belong to a novel seven-transmembrane receptor family. The receptor TRE is known to control taste sensitivity to trehalose in gustatory sensory neurons. To know whether TRE is also involved in the sensitivity to other sugars or not, we compared quantitatively the gustatory sensitivity to various saccharides between wild-type and the mutant flies of *Tre* using a feeding test. The null mutant of *Tre* showed a significant decrease in sensitivity, not only to trehalose but also to a limited subset of sugars. The difference spectrum of wild-type minus mutant gave the sugar specificity profile of TRE. The mutant, on the other hand, showed almost normal sensitivity to a different subset of sugars. A total of seven Grs homologous to *Tre* are known to be located on the third chromosome. Therefore it is suggested that some of these *Tre* subfamily members may also encode sweet taste receptor(s) for those sugars in *Drosophila.*

P2-23. Triterpenoidsaponin stimulated sugar taste receptor cells of the blowfly *Phormia regina*: effect of G protein inhibitor on the sugar response

Arifa Ahamed, Seiji Tsurumi[1] and Taisaku Amakawa[2]

Graduate School of Science and Technology, Kobe University, [1]RI Research Centre, Kobe University and [2]Faculty of Human Development, Kobe University, Kobe 657-8501, Japan

The chemosensilla on the fly labellum are highly specialized for sugar, salt and water, respectively. Previously we introduced chromosaponin I (CSI) and glycyrrhizin (GL) as sweet substances for the blowfly, *Phormia regina*. Application of these saponins induced feeding responses as well as impulses of the sugar taste receptor cell in the LL-type sensillum. We show here the involvement of G-protein-mediated cascade in the CSI- and GL-responses, as well as in the sugar response. CSI directly activates the sugar signal transduction cascade after penetrating through the membrane. On the other hand, GL exerts dual effects to stimulate the sugar signal transduction, possibly by directly activating it inside the cell and also by interacting with the pyranose site. A non-hydrolyzable G protein inhibitor—guanosine 5′-*O*-(2-thiodiphosphate), (GDPβS), markedly decreased the response of the sugar receptor cell to sucrose and fructose as well as the responses to CSI and GL, suggesting that the sugar response induced by the pyranose or furanose receptor sites as well as the sugar transduction cascade directly activated by triterpenoidsaponins is at least partly mediated by G protein.

P2-24. Expression of leptin receptors (Ob-Rb) and STAT3 mRNA in mouse taste buds

Noriatsu Shigemura[1], Hirohito Miura[2,3], Akihiro Hino[2,3] and Yuzo Ninomiya[1,2]

[1]Section of Oral Neuroscience, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, [2]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon Minato-ku, Tokyo 105-0001 and [3]National Food Research Institute, Ibaraki 305-8642, Japan. e-mail: shigemura@dent.kyushu-u.ac.jp

Leptin is a hormone that regulates food intake, energy expenditure and body wt. Our previous studies demonstrated that the hormone suppresses responses of peripheral taste nerves to sweet substances in mice. We proposed that leptin activates outward K^+ currents of sweet-sensitive taste cells through its receptor Ob-R, and leads to hyperpolarization of the cells which results in decrease in their excitabilities. In this study, we investigated molecular mechanisms of leptin effects on taste receptor cells by examining expression of mRNA encoding Ob-R isoforms (a–e) and a transcription factor, STAT3, in taste receptor cells by use of RT–PCR and *in situ* hybridization analysis. RT–PCR analysis showed that Ob-Rb and STAT3 mRNA was expressed in the tissue containing the fungiform (FP) and circumvallate papillae (CP), but not clearly in the tongue epithelium (ET) without taste buds. Expression for Ob-Ra, Ob-Rc and Ob-Rd was not specifically detected in either the taste papillae or the ET. No clear expression for Ob-Re was observed in any of the three tissues. Consistently, *in situ* hybridization analysis showed that Ob-Rb signals were detected in some taste cells, but only slightly if at all in ET cells. STAT3-positive cells were predominantly observed in taste buds. These results suggest that Ob-Rb and STAT3 may be involved in the signal transduction cascade for the leptin action on taste receptor cells.

P2-25. Inhibitory effects of leptin on sweet taste: behavioral response analyses in the obese diabetic *db*/*db* and *ob*/*ob* and lean control mice

Rie Ohta[1,2], Kiyohito Nakasima[3], Noriatsu Shigemura[1], Kiyosi Koyano[2] and Yuzo Ninomiya[1,4]

[1]Section of Oral Neuroscience, [2]Section of Removable Prosthodontics, Graduate School of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, [3]Department of Chemistry, Asahi University School of Dentistry, 1851-1 Hozumi, Motosu-gun, Gifu 501-0296 and [4]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon Minato-ku, Tokyo 105-0001, Japan

The hormone leptin, primarily produced by adipocytes, is known to regulate food intake, energy expenditure and body wt. Leptin acts by binding to specific 'obese receptors' (Ob-R). Our previous studies demonstrated that the *db*/*db* mouse, which has defects in Ob-Rb, shows greater neural responses to sweet substances than normal lean mice. Subsequent studies suggested that this may be caused by their lacking leptin inhibition on sweet taste responses which normal lean mice possess. In the present study, we behaviorally examined leptin inhibition of intakes for sweet solutions in Ob-Rb-deficient (*db*/*db*), leptin-deficient (*ob*/*ob*) and normal lean mice. The results showed that i.p. injection of leptin significantly suppressed intake of various concentrations of sucrose–quinine and saccharin–quinine mixtures at 10–60 min after the injection in *ob*/*ob* and lean mice, but not in *db*/*db* mice. In an experiment using a conditioned aversion paradigm, we found that mice conditioned to avoid sweet substances increased intake of lower concentrations of sucrose and saccharin after the leptin injection, indicating increases in aversion thresholds for sucrose and saccharin. These results are quite consistent with previous results from electrophysiological studies. The inhibitory effect of leptin on sweet taste responses is thereby further confirmed by the present behavioral response analysis.

P2-26. Behavioral genetic analysis of sweet taste responses in mice

Tohru Suzuki[1,3], Noriatsu Shigemura[l], Yuko Kusakaba[2,3], Akihiro Hino[2,3] and Yuzo Ninomiya[1,3]

[1]Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University, Fukuoka 812-8582, [2]Molecular Engineering Laboratory, National Food Research Institute, Ministry of Agriculture, Forest, and Fisheries, Tsukuba 305-8642 and [3]Bio-oriented Technology Research Advancement Institution, Saitama 331-0044, Japan

Previous studies have shown that there were large differences in taste preference between C57BL/6 (B6) and 129/J (129) inbred strains of mice. The aim of the present study was to characterize differences in behavioral responses of B6 and 129 mice and their F2 progeny to various sweet-tasting molecules and a sweet-response inhibitor, gurmarin. For this purpose, we used a behavioral test counting the number of licks per 10 s for mixtures of various concentrations of sweeteners (12 sweeteners) with 3 mM quinine hydrochloride. The results suggest that B6 mice made significantly more licks for saccharin, maltose and several amino acids than 129 mice do. In addition, the behavioral responses to sucrose were suppressed by treatment of the tongue with gurmarin in C57BL mice, whereas no such sweet-suppressive effect of gurmarin was observed in 129 mice.

By using various polymorphic markers, several recombinants for T1R1-3 genes were segregated among F2 progenies. These animals showed wide ranges of responsiveness to various sweeteners and gurmarin. It is necessary to add more data to examine possible correlations between allelic differences in T1R genes and phenotypic differences in responses to sweeteners and the sweet inhibitor.

P2-27. Differences of saccharin responses between C57BL/6NCrj and BALB/cAnNCrj mice

Seong-Hee Oh[l,2] and Akihiro Hino[2]

[1]Japan Science and Technology Corp., Kawaguchi Center, Building 1-8, Kawaguchi, Saitama 332-0012 and [2]National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan

The differences of saccharin responses of two mouse strains (C57BL/6NCrj and BALB/cAnNCrj) were examined with whole-cell patch-clamp across concentrations of sodium saccharin (1, 10 and 20 mM). Under voltage clamp (holding potential = –80 mV), bath application of 1–20 mM saccharin elicited two types of responses (inward and outward) in both strains. The total responses (inward and outward) of saccharin in C57BL mice showed approximately twice the percentage of those of BALB mice in 1 and 10 mM concentrations, but responses of both strains in 20 mM saccharin showed similar percentages. Independently, the rate of

inward current response also showed the same tendency for total responses. However, the rate of outward current response showed the same level in all three concentrations. These results suggest that two mechanisms are involved in the transduction of saccharin at least, and the differences of saccharin responses in both strains across concentrations are related to inward current response.

P2-28. Taste nerve responses to polysaccharides in rats

Yasutake Shimizu, Maki Sakurai and Keiichi Tonosaki

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-1193, Japan

We examined the possible involvement of gustatory nerves in starch detection in rats. This was accomplished by the electrophysiological recording of chorda tympani nerve (CTN) and glossopharyngeal nerve (GLN) responses, as well as behavioral experiments using a conditioned taste aversion technique. Starch clearly elicited the CTN responses. The starch-evoked CTN responses were substantially abolished by dialysis of starch solution or by application of the sodium channel blocker amiloride. Thus, it seems likely that these responses may not be generated by starch itself, but by ionic contaminants such as Na^+. This was consistent with behavioral observations, in which bilateral transection of the CTN did not influence starch avoidance in rats conditioned to reject starch. In contrast, starch generated the GLN response even though it was dialyzed before application. The increase in GLN activity in responses to starch would participate in starch detection, since bilateral transections of the GLN attenuate the avoidance of starch conditioned to be aversive. It is therefore concluded that starch induces the GLN, and the signal mediated through the GLN can be used for starch detection.

P2-29. Studies of umami synergism using ionotropic glutamate receptor agonists

Keiko Iseki, Yukako Hayashi and Tomohiko Mori

Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan. e-mail: iseki@food2.food.kyoto-u.ac.jp

Monosodium glutamate (MSG) elicits a unique taste in humans, called 'umami', and there exists a remarkable synergism between MSG and IMP or GMP in human. We have already shown that the responses both MSG and a mixture of MSG and IMP were divided into three types (transient inward current, sustained inward current and outward current) and that the amplitudes of both the transient and sustained inward currents were much larger in the mixture than with MSG alone. It is likely that ionotropic glutamate receptors are involved in these inward currents. We report here responses of mouse taste cells to some stimulant solutions using: the glutamate receptor agonists and nucleotide, NMDA (1 mM) and KA (1 mM); a mixture of NMDA (1 mM) and IMP (0.5 mM); and a mixture of KA (1 mM) and IMP (0.5 mM). The response was measured under whole-cell voltage-clamp (–80 mV).

The mixture of NMDA and IMP induced two different types of responses (transient inward and outward currents), similar to NMDA alone. The amplitudes of both the transient and outward currents were almost the same as with NMDA alone. KA also induced transient inward and outward currents in taste cells, while the mixture of KA and IMP induced outward currents. These results suggest that ionotropic glutamate receptors are not involved in umami synergism transduction. Further studies will be needed to examine the transduction mechanisms involved in umami and umami synergy.

P2-30. Expression of brain-mGluR4 and taste-mGluR4 in rat gustatory papillae

Takashi Toyono, Yuji Seta, Shinji Kataoka[1] and Kuniaki Toyoshima

Department of Oral Anatomy and Neurobiology, Kyushu Dental College, Kitakyushu and [1]First Department of Oral and Maxillofacial Surgery, Kyushu Dental College, Kitakyushu, Japan

Taste-mGluR4 is a truncated variant of brain-expressed mGluR4a (brain-mGluR4) and is known to be a candidate for the receptor involved in the umami sense of umami sensation. Although the expression patterns of taste- and brain-mGluR4 mRNAs have been revealed, no mention has so far been made of the expression of these two mGluR4 proteins in taste tissues. In order to examine the expression of taste- and brain-mGluR4 proteins in rat taste tissues, we used a specific antibody for mGluR4a, which shared C-terminus of both taste- and brain-mGluR4, for immunoblot analysis and immunohistochemistry.

The immunoblot analysis showed that both brain- and taste-mGluR4 were expressed in taste tissues. The immunoreactive band of brain-mGluR4 protein was much stronger than taste-mGluR4 protein. In cryosections of fungiform, foliate and circumvallate papillae, the antibody against mGluR4a gave intense labelling of the taste pores and taste hairs in all taste buds of gustatory papillae examined. The cytoplasm of taste bud cells below the taste pore and surrounding keratinocytes did not show any significant affinities for the antibody.

The results of the present study strongly indicate that, in addition to taste-mGluR4, brain-mGluR4 may function as a receptor for glutamate, i.e. umami taste sensation.

P2-32. Roles of G protein on denatonium signal transduction in mouse taste cells

Shoko Sawano, Yukako Hayashi, Eri Seto and Tomohiko Mori

Graduate School of Agriculture, Kyoto University, Kyoto 611-0011, Japan

We investigated roles of G protein in denatonium signal transduction in mice. We have previously demonstrated that both inositol-1,4,5-triphosphate (IP_3) and cyclic nucleotide (cNMP) were involved in denatonium signal transduction. Increases of IP_3 and cNMP levels were induced by activation of effectors—phospholipase C (PLC) and phosphodiesterase (PDE)—so G protein is thought to play an important role in denatonium transduction. In this study, roles of G protein were examined by the whole-cell patch clamp technique in isolated taste cells of mice. With GDP-beta-S (0.5, 1 mM) as an inhibitor of G protein, the responses to denatonium stimuli decreased in some cells, but did not decrease in the other cells. When 8-Br-cGMP (1 mM) as a permeable cNMP analog, thapsigargin (2 μM) as an IP_3-independent intracellular calcium releaser and U73122 (10 μM) as a PLC inhibitor, were added to cells unaffected by GDP-beta-S, responses to denatonium stimuli decreased in all of them.

The results obtained suggest secondary messenger systems

unaffected by GDP-beta-S are involved in denatonium signal transduction.

P2-33. Taste responses in the facial gustatory system of goldfish, *Carassius auratus*

Takayuki Marui and Tomoko Matsushita

Ohu University School of Dentistry, Department of Oral Physiology, 31-1 Misumidou, Tomita, Koriyama, Fukushima 963-8611, Japan

Goldfish (*Carassius auratus*) have been used in research several scientific fields, e.g. feeding behavior, central taste pathways, visual sense and so on. However, there have been no report concerning taste receptive mechanisms in this species. Thus, we report here histological structures of taste organs (taste buds) and taste sensitivities for amino acids, their analogues and four basic taste substances with electrophysiological techniques.

Amino acids are highly effective stimuli for the goldfish gustatory system. Thresholds for L-alanine, betaine, L-proline and L-cysteine ranged between 10^{-5} and 10^{-8} mol/l. The response spectrum to amino acids in this species is species-specific among those tested in other fish species. Taste responses to amino acids were loosely stereospecific, e.g. L-isomers (alanine, arginine and proline) were stimulatory, but the enantiomers showed approximately half that stimulatory effect. Both L and D isoforms of cysteine were equally stimulatory. Protons are a strictly effective stimulus for the system, but sugars were not. Quinine is stimulatory for the system, but caffeine is not.

P2-34. Construction of the subtracted cDNA library for gene expression profiling of the taste bud

Kim Mi-Ryung[1], Yuko Kusakabe[1,3], Hirohito Miura[1,3], Rika Hashimoto[1], Mizuho Tagami[1], Yuzo Ninomiya[2,3] and Akihiro Hino[1,3]

[1]*National Food Research Institute, 2-1-12 Kannondai, Tsukuba 305-8642,* [2]*Faculty of Dentistry, Kyushu University and Bio-oriented Technology Research Advancement Institution (BRAIN), Tokyo 105-0001, Japan*

To develop the cDNA microarray, a cDNA library was constructed from the epithelium of mouse circumvallate papillae. The cDNA library was normalized and subtracted with the cDNA library of tongue epithelium that did not include taste buds, to increase the population of taste-bud-specific cDNA clones. The efficiency of the normalization and subtraction was evaluated by colony hybridization using housekeeping genes, β-*actin* and *glyceraldehyde-3-phosphate dehydrogenase* (*G3PDH*), as a mixed probe. The representations of β-*actin* and *G3PDH* in the subtracted library were reduced to 1/8 of that in the original, suggesting that taste-specific genes might be enriched in the subtracted library. One hundred and sixty clones randomly picked up from the subtracted library were sequenced. Blast search revealed that 82% of the clones exhibited high similarities to known genes, including the sequences related to neurotransmitter release and cell death pathways which might play roles in taste signal transduction and in taste cell-turnover, respectively. *In situ* hybridization showed that a clone coding a death-associated protein and a clone having no significant similarity were expressed in a subset of taste bud cells. The cDNAs of the subtracted library are arrayed to be used as a cDNA chip.

P2-35. The expression of fatty acid transporters in rat tongue epithelium

Akiko Soma, Shinya Matsumoto and Tomohiko Mori

Graduate School of Agriculture, Kyoto University, Gokasho Uji 611-0011, Japan

To address the possible involvement of the putative fatty acid transporters in dietary fat/fatty acid recognition in the oral cavity, we examined the expression of the fatty acid transporters in rat tongue epithelium by RT–PCR. Tongue epithelia containing circumvallate and foliate papillae were prepared from male Wistar rats. The epithelia in the vicinity of both types of papillae, which do not contain taste buds, were also obtained as controls. Total RNA was prepared from the tissues and used as the template for the reverse transcription reaction, followed by PCR using specific primers for rat FAT (fatty acid transporter), VLACS (very-long-chain acyl CoA synthetase) and FATP (fatty acid transport protein). The primers specific to gustducin α-subunit and β-actin were also used. The specific expressions of FAT and VLACS were observed in both circumvallate- and foliate-papillae-containing epithelium, but not in the corresponding controls. In the circumvallate papillae epithelium, the expression of FATP was observed with no expression in the control epithelium, as in the case of FAT and VLACS. However, in the foliate papillae epithelium, the expression of FATP was confirmed in both the papillae-containing epithelium and the control epithelium. The expression of gustducin α-subunit was only observed in the papillae-containing epithelia and of the β-actin in all epithelium samples.

P2-36. Immunohistochemistry of tyrosine hydroxylase and dopamine β-hydroxylase in the frog taste organ

Hiroshi Ando, Naokazu Asanuma and Katsuhiro Inoue[1]

Department of Oral Physiology and [1]*Department of Oral Anatomy, Matsumoto Dental University School of Dentistry, Shiojiri 399-0781, Japan. e-mail: andohiroshi@po.mdu.ac.jp*

So far, pharmacological and histochemical studies have suggested several candidates, including noradrenaline and serotonin, for the neurotransmitter between taste cells and the glossopharyngeal nerve in frogs. However, precise identification of the transmitter has not yet been achieved.

This study was undertaken to examine histochemically the existence of tyrosine hydroxylase (TH—enzyme for L-dopa synthesis from tyrosine) and dopamine β-hydroxylase (DBH—enzyme for noradrenaline synthesis from dopamine) in taste discs of the frog *Rana catesbeiana.*

Antibodies against TH and DBH were provided by Dr Ikuko Nagatsu, Fujiita Health University. No TH immunoreactivity was observed in the taste disc, although TH-like immunoreactive cells were observed in the adrenal gland, showing that the above antibody was effective in frog tissues. In contrast to TH, we observed DBH-like immunoreactive cells in the taste disc. Those cells were located at the middle and lower layers of the taste disc. The cells had apical processes reaching the free surface of the disc, and basal processes. That DBH exists in the taste disc, probably in the taste cells, supports the argument that noradrenaline works as a neurotransmitter in taste reception. The lack of TH immuno-

reactivity in the taste disc may imply that taste cells uptake L-dopa or dopamine for noradrenaline synthesis.

P2-37. Developmental changes of the number of taste bud cells immunoreactive for protein gene-product 9.5 and gustducin in mouse vallate papillae

Akiko Fukunaga, Hiroshi Uematsu, Kirio Kawai[1] and Kumiko Sugimoto[2]

Section of Gerodontology, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8549, [1]Department of Anatomy (Division II), School of Dental Medicine, Tsurumi University, Yokohama 230-8501 and [2]Section of Molecular Neurobiology, Graduate School, Tokyo Medical and Dental University, Tokyo 113-8549, Japan

It is known that the neural responses to NaCl and sweet and bitter stimuli gradually increase during the 2 weeks after birth in the rat. To investigate the underlying structural mechanisms for this development, we have investigated the developmental changes in the number of taste bud cells and the cells immunoreactive for taste-cell-specific proteins, protein gene-product 9.5 (PGP 9.5) and gustducin in vallate papillae of ddY mice aged from 1 day to 12 weeks. No taste bud structure and no immunoreactive cells were observed in the epithelial tissue of vallate papillae until the mice were 3 days old. On post-natal day 5, small numbers of taste buds were observed and only a few taste bud cells demonstrated immunoreactivity for either PGP 9.5 or gustducin. Thereafter, the number of taste bud cells and immunoreactive cells increased remarkably during the second week of post-natal life. The structure of the taste buds and the ratio of cells immunoreactive for both PGP 9.5 and gustducin in single taste buds reached the adult level by 3 weeks old. These results suggest that the taste responses of the glossopharyngeal nerve may be poor until 1 week after birth and that the responses may develop rapidly during the second week after birth and reach the mature level by 3 weeks old in the mouse as well as the rat.

P2-38. Comparison of the expression of *Mash-I* with taste-reception-related genes *gustducin* and *T1R2* in taste buds

Yuko Kusakabe[1,3], Hirohito Miura[1,3], Rika Hashimoto[1], Chiaki Sugiyama[l], Yuzo Ninomiya[2,3] and Akihiro Hino[1,3]

[1]National Food Research Institute, 12-1-12 Kannondai, Tsukuba 305-8642 [2]Faculty of Dentistry, Kyushu University and [3]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon, Minato-ku, Tokyo 105-0001, Japan

Taste bud cells have a limited life span and are continuously replaced, as are other epithelial cells. Although there is evidence that taste buds may arise from the local epithelium, taste receptor cells have neuronal properties. This implies there must be a critical stage during which the epithelial precursor cells for taste receptor cells start to exhibit neural properties during the differentiation of the taste receptor cells. In the nervous system, the expression of neural-specific transcription factor *Mash-I* is transient and precedes neuronal differentiation. Therefore, we examined the expression of *Mash-I* in the epithelium of circumvallate papillae to clarify the localization of the precursor cells with neural properties; we observed that the expression is restricted to taste buds. Two-color *in situ* hybridization showed that the signals for *Mash-I* did not overlap those for taste-receptor-cell-specific genes such as *gustducin* and *T1R2*. In the process of development and regeneration of taste buds, the expression of *Mash-I* preceded that of *gustducin* and *T1R2*. These observations suggest that *Mash-I* could be a candidate for the marker of immature taste receptor cells.

P2-39. Shh and Ptc are associated with taste bud maintenance in the adult mouse

Hirohito Miura[1,4], Yuko Kusakabe[1,4], Yuzo Ninomiya[2,4], Jun Motoyama[3] and Akihiro Hino[1,4]

[1]National Food Research Institute, 2-1-12 Kannondai, Tsukuba-shi, Ibaraki 305-8642, [2]Faculty of Dentistry, Kyushu University, [3]Molecular Neuropathology Group, Brain Science Institute, The Institute of Physical and Chemical Research (RIKEN) and [4]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon, Minato-ku, Tokyo 105-0001, Japan

In mammals, the taste buds are maintained by continuous cell proliferation, even in adults. Denervation causes the disappearance of taste buds, indicating that the taste neurons trophically maintain the taste buds by inducing cell proliferation. Analysis following bromodeoxyuridine uptake has demonstrated that epithelial cells around taste buds are proliferating and enter taste buds when they stop dividing. We found that the receptor for Sonic Hedgehog (Shh), Patched1 (Ptc), was expressed in the cell proliferating zone around taste buds in adult mice. In contrast, the expression of *Shh* was detected within basal cells of taste buds. The expression of both *Shh* and *Ptc* in the tongue was restricted to the epithelium containing taste buds and was not detectable in underlying mesenchyme. The crushing of the taste nerves caused the loss of *Shh* and *Ptc* expression within and around the taste buds, respectively, before the degeneration of taste buds. Our observations suggest that Shh and Ptc are associated with taste bud maintenance in the adult mouse. This is the first report that the taste nerves control gene expression in the cell proliferating zone around taste buds.

P2-40. Induction of novel salivary proteins by a diet containing theobromine in mice

Ayako Yamada[1,2], Daigo Sugita[1,3], Hideo Katsukawa[4], Tadataka Sugimura[4], Minoru Nakata[2] and Yuzo Ninomiya[1,5]

[1]Section of Oral Neuroscience, [2]Pediatric Dentistry, Graduate School of Dental Science, Kyushu University, Fukuoka 812-8582, [3]Lotte Co. Ltd, Central Laboratory, Saitama 336-0027, [4]Department of Oral Physiology, School of Dentistry, Asahi University, Hozumi 501-0296 and [5]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon, Minato-ku, Tokyo 105-0001, Japan

Saliva contains many proteins, the physiological roles of which are unknown. In rodents, several salivary proteins (e.g. proline-rich proteins and cystatins, etc.) are known to be induced by specific chemical substances in diet (e.g. tannic acids, etc.). In a previous study we examined if synthesis of novel salivary proteins would be induced by a diet containing theobromine, which is a component of the seed of cacao and tastes bitter to humans, and found

unusual proteins in the submandibular saliva which were not detected in the control. In this study we used BALB and C57BL mice, and examined possible strain differences in the salivary protein induced by theobromine diet. Analyses using electrophoresis (SDS-PAGE and 2D-PAGE) indicated that an unusual protein with the mol. wt of ~40 kDa was induced in the submandibular saliva of BALB mice fed with 0.5 and 1.0% theobromine diet, whereas no such protein was detected in the saliva of C57BL mice. This suggests the existence of strain differences in the effects of theobromine on induction of salivary protein, which may be due to possible differences in peripheral taste sensitivity to theobromine between the two mouse strains.

P2-41. New protein components of rat submandibular saliva induced by a diet containing theobromine and treatment with a β-agonist, isoproterenol

Daigo Sugita[1,2], Ayako Yamada[1,3] and Yuzo Ninomiya[1,4]

[1]Section of Oral Neuroscience, Faculty of Dentistry, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, [2]Lotte Co. Ltd, Central Laboratory, 3-1-1 Numakage, Saitama 336-0027, [3]Section of Pediatric Dentistry, Faculty of Dentistry, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582 and [4]Bio-oriented Technology Research Advancement Institution (BRAIN), 3-18-19 Toranomon, Minato-ku, Tokyo 105-0001, Japan

Irritating dietary substances such as tannin, papain and capsaicin have been reported to alter the morphology of salivary glands and their secretions. We investigated possible effects of dietary methyl xanthines (MXs), theobromine (TBR), theophylline and caffeine, on rat submandibular saliva. New theobromine-induced salivary proteins (p*I* ~4.5, mol. wt 15 kDa) were found in a 2D-PAGE gel. The electrophoretic properties of these proteins were similar or identical to those of rats chronically treated with isoproterenol (IPR). The induction of these proteins in rats fed TBR-containing diets is stronger than in rats fed other MX-containing diets. Additionally, MXs induce other salivary proteins (mol. wt ~25 kDa) which are not found in the IPR-treated group. These results suggest that in rats the TBR-containing diet can induce new protein components in submandibular saliva and that synthesis of the components may be mediated not only via the pathway involving β-adrenoreceptors, but also via other unknown pathways.

P2-42. Capsaicin-containing diets and salivary cystatin

H. Katsukawa, K. Nakashima[1], K.-H. Yang, R. Ohashi, Y. Ninomiya[2] and T. Sugimura

Departments of Oral Physiology and [1]Chemistry, Asahi University School of Dentistry, 1851 Hozumi, Motosu, Gifu 501-02 and [2]Sections of Oral Neuroscience and Pediatric Dentistry, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

When rodents are given capsaicin in their diet for several days, it is shown to induce cystatin-like substances in their submandibular saliva. Yet the physiological role of salivary proteins has not been thoroughly investigated. Salivary cystatin in the rat submandibular glands is known to be induced by chronic treatment with the sympathetic beta-agonist isoproterenol (IPR). In the present study, therefore, possible roles of salivary proteins in the food intake of animals were examined by comparing consumption of the capsaicin diet in rats with and without IPR pretreatment (0.1 and 5.0 mg/kg, 5 days). Electrophoretic analysis performed prior to feeding trials revealed that the group pretreated with 5.0 mg/kg IPR had large amounts of cystatin in the saliva compared with the group pretreated with 0.1 mg/kg IPR and the control group. The group treated with 5.0 mg/kg IPR showed greater consumption of a diet containing 0.05% capsaicin than the other groups until the 3rd day of trials. Bilateral removal of the submandibular and sublingual glands neutralized such effects of IPR. Induction of salivary cystatin by IPR treatment was not mimicked by systemic and intragastric administration of capsaicin. These results suggest that cystatins are included in salivary proteins induced by capsaicin and contribute to enhanced ingestion of the capsaicin diet. Induction of salivary cystatins may be triggered by irritation of the oral mucosa by capsaicin.

P2-43. Salivary protein induction by capsaicin-containing diet and its related area in the central nervous system in rats

Yong Shang[2], Kazuo Mishima[1], Daigo Sugita[1,3], Hideo Katsukawa[4], Minoru Nakata[2] and Yuzo Ninomiya[1,5]

[1]Section of Oral Neuroscience, [2]Section of Pediatric Dentistry, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, [3]Lotte Co. Ltd, Central Laboratory, Saitama, [4]Department of Oral Physiology, Asahi University School of Dentistry, Gifu and [5]Bio-oriented Technology Research Advancement Institution (BRAIN), Tokyo 105-0001, Japan

It has been reported that diet containing capsaicin (CAP) can induce novel proteins in rat submandibular saliva. This salivary protein induction is abolished by denervation of the glossopharyngeal (IXth) nerve, suggesting participation of chemical signals conveyed by the glossopharyngeal nerve into the salivary protein induction. Our recent study demonstrated that bilateral lesion of the second taste relay nucleus, the parabrachial nucleus (PBN), caused disappearance of the protein induction. In this study using rats, we examined effects of lesion of the ascending tract (AT) from the PBN upon the protein synthesis to clarify whether input of the sensory information into the upper CNS would be necessary for the protein induction or if the same information could work via a brain stem reflex arc. The AT is reported to be situated ventral to the midbrain central gray and just lateral to the medial longitudinal fasciculus. We lesioned the AT just caudal to the level including the rad nucleus from which the AT bifurcates into a separate way. Rats appeared well although tired and without any paralysis after the anesthesia used in the lesioning operation. Using electrophoresis, we found the novel protein band in saliva of both normal and lesioned CAP-fed rats; the saliva of the latter corresponded with that of cystatin S-like protein. Therefore, unilateral or bilateral lesion of the AT did not influence protein synthesis. These results suggest that sensory information may not always necessarily be sent to the CNS above the PBN and processed there; it may be that the brain stem reflex arc including the PBN is the main mechanism for the production of the new protein. However, further studies are necessary to investigate the precise route and its function in the production of the protein.

P2-44. Reduction of CA II expression in submandibular glands of severe zinc-deficient rats

Tomoko Goto, Michio Komai, Hitoshi Shirakawa and Yuji Furukawa

Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan

It appears that carbonic anhydrase (CA), a zinc metalloenzyme, is involved in gustation because hypogeusia has been reported as a side-effect by patients undergoing therapy for glaucoma and high-altitude sickness with CA inhibitors. We have reported that the reception of carbonated water through the lingual trigeminal nerve requires the participation of CA in rats. We have also reported that zinc deficiency decreases sensitivities of the lingual trigeminal nerve to carbonated water and of the chorda tympani nerve to taste stimuli in rats. Moreover, we have confirmed that CA activities of the tongue epithelium and the submandibular gland are significantly lower in zinc-deficient rats than in control rats. Therefore, we investigated the effects of zinc deficiency on CA (CA II and CA VI) expression in the submandibular gland of the rat. Male Sprague–Dawley rats, 4 weeks old, were divided into three groups (Zn-deficient, low-Zn and pair-fed). After administration of the experimental diet for 42 days, the submandibular gland was excised. Expression of CA II (mRNA and protein) in the submandibular gland was significantly lower in Zn-deficient and low-Zn rats than in pair-fed rats, although there was no difference among expression of CA VI. These results suggest that reduction in CA II expression may cause reduction in CA activity in the submandibular gland of Zn-deficient rats.

P2-45. Projections from the facial lobe in the brainstem of goatfish

Masato Kirino, Yoko Sakata, Masanori Kasai and Sadao Kiyohara

Department of Chemistry and BioScience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

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

In this study, efferent neurons in the FL and their projections to the brainstem were examined using isolated, paraformaldehyde-fixed brains and nerve-tracing techniques with the carbocyanine dye, DiI. Applications of DiI into the cut surface of the ascending secondary gustatory tract result in retrograde labeling of neurons in the dorsal FL. Labeled neurons were found ipsilaterally and had an oval or fusiform cell body measuring ~30 × 15 μm. They were located mainly in the superficial and deeper layers. Medium neurons located in the intermediate layer were not labeled, suggesting these neurons are intrinsic. Following injection of DiI into the dorsal FL, projecting axons arising from the dorsal FL were heavily labeled. They could be traced to terminate mainly the following targets: (i) superior secondary gustatory nucleus; (ii) inferior secondary gustatory nucleus; (iii) spinal trigeminal nucleus; and (iv) reticular formation near the nucleus of facial motor neurons. As the facial motor neurons innervate muscles controlling the movement of barbel, the present result show the distinct reflex connection of the barbel taste system in the medulla.

P2-46. Innervation of taste buds in the oro-pharyngeal epithelium of carp, *Cyprinus carpio*

Yoko Sakata, Junzo Tsukahara and Sadao Kiyohara

Department of Chemistry and BioScience, Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan.
e-mail: gsbsakat@chem1.sci.kagoshima-u.ac.jp

This study was performed to reveal innervation of taste buds inside the mouth of carp using isolated, paraformaldehyde-fixed tissues and nerve tracing techniques with the carbocyanine dye, DiI. DiI was applied to the peripheral cut stump of the nerve dissected in the oral or pharyngeal tissues. After a diffusion period of 20–90 days, the tissue was sectioned on a vibratome in various planes and examined with a standard epifluorescence microscope or laser scanning confocal microscope.

In the oral region, many epidermal ridges run rostrocaudally. The small nerve bundles located between the dermis and epidermis run under the tops of epidermal ridges sending two to five strands toward the surface at regular intervals (200 μm). Each strand terminates in a taste bud. This result shows that one longitudinally running bundle innervating the oral cavity has a receptive field extending rostrocaudally rather than mediolaterally.

In the pharyngeal cavity the palatal organ is well developed. This organ has a convoluted surface forming many dumpling-like protrusions with a diameter of 100–200 μm. Each protrusion receives one small nerve bundle. This bundle repeatedly ramifies to innervate taste buds located in the protrusion. The number of taste buds located in one protrusion ranges from 10 to 30. This result suggests that one functional unit of fibers in the palatal organ has a small receptive area comprising 10–30 taste buds and projects somatotopically to the vagal lobe to make a finely tuned reflex connection.

P2-47. The effect of taste stimulation on histamine release in the anterior hypothalamus of rats

Tomoko Ishizuka, Yada Treesukosol[1], Yumiko Yamamoto, Takashi Yamamoto[1] and Atsushi Yamatodani

Department of Medical Physics, School of Allied Health Sciences, Faculty of Medicine and [1]Department of Behavioral Physiology, Faculty of Human Science, Osaka University, Osaka 565-0871, Japan

We previously showed that i.p. injection of leptin, a satiety factor secreted from white adipose tissue, facilitated hypothalamic neuronal histamine release by peripheral signal inputs via the chorda tympani (Morimoto-Ishizuka *et al*., 2001, *Neurosci. Lett*., 300: 107–110). This finding suggests that the histaminergic system is activated by peripheral inputs through the chorda tympani. Thus, we studied the effect of gustatory stimulation of the anterior part of the tongue innervated by the chorda tympani on the hypothalamic histamine release using *in vivo* microdialysis in anesthetized rats. Application of a mixture of four basic taste solutions composed of 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl and 0.02 M quinine–HCl to the anterior tongue increased histamine release by 130% of the basal release, and the enhancement was partly abolished by bilateral dissection of the chorda tympani.

When each of the four basic taste solutions was examined, 0.1 M NaCl caused the largest histamine release of all. These results suggest that taste information via the chorda tympani activates the histaminergic system.

P2-48. An analysis of neuron activities during ingestion of taste solutions in behaving rats

Yumie Kanamori, Tsuyoshi Shimura[1], Tohru Fushiki and Takashi Yamamoto[1]

Laboratory of Nutrition Chemistry, Division of Food Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502 and [1]Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan

To determine the functional significance of brain regions which are involved in taste-guided behavior, activities of single neurons were recorded in freely moving Wistar rats during licking of distilled water and taste solutions. Taste stimuli were 0.1 M sucrose, 5 mM saccharin, 0.1 mM quinine, 0.01 M HCl and 0.1 M NaCl. Of 20 neurons in the ventral tegmental area (VTA), five increased their activities just before ingestion of the solutions, regardless of the taste qualities. Of 12 neurons in the basolateral amygdaloid nucleus (BLA), three increased their activities during licking of all solutions. Only one neuron in the BLA selectively increased its firing during licking of sucrose, saccharin and water. Of 16 neurons in the cingulate cortex (Cg), four increased and four decreased their activities during licking of all solutions. These data suggest that the VTA is concerned with the motivation to ingest of liquids. While the BLA seems to be related to taste functions, the Cg appears to be involved in the motor response of ingestion.

P2-49. Centrifugal influence on taste responses in the parabrachial nucleus

Ken'ichi Tokita, Tsuyoshi Shimura and Takashi Yamamoto

Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, 1-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Neuroanatomical studies indicate that parabrachial nucleus (PBN) receives descending fibers from gustatory forebrain structures such as the gustatory cortex (GC) and the central nucleus of the amygdala (CeA). We examined the influence of electrical stimulation in ipsilateral GC or CeA on taste responses in the PBN to clarify the function of these descending inputs. Rats were separated into an experimental group that had acquired a taste aversion to NaCl and a control group that had not. The taste stimuli presented were sucrose, NaCl, HCl and quinine–HCl. A 10 train of 10 Hz pulses was applied to the GC and CeA during the period of taste stimulation. We recorded 15 units for each group. In the control group, GC stimulation facilitated three cells and inhibited one, and CeA stimulation inhibited eight cells In the experimental group, GC stimulation facilitated three cells and inhibited eight, and CeA stimulation facilitated three cells and inhibited 10. In both groups, CeA stimulation affected more cells than did GC and its effect was inhibitory in many cases. More cells of the experimental group were modulated by stimulation in both GC and CeA than those of the control group. These results demonstrate that the GC and the CeA modulate PBN taste neurons in different fashions and that conditioned taste aversion influences its forms of modulation.

P2-50. Neuronal connection between the insular cortex and the amygdala in rats: an electrophysiological study

Takamitsu Hanamori

Department of Physiology, Miyazaki Medical College, Miyazaki 889-1692, Japan

We have reported that the neurons in the posterior insular cortex (posterior to the region where the chorda tympani projects) respond to gustatory, visceral and nociceptive stimuli. Anatomically, many studies have demonstrated that the insular cortex has connections with the amygdala. The amygdala is considered to have an important role in affective conditioning. In the present study, we recorded the extracellular neural responses from the posterior insular cortex following electrical stimulation of the amygdala, using a tungsten electrode. In the posterior insular cortex, 16 neurons were identified as responding to electrical stimulation of the amygdala. Of the 16 stimulation sites in the amygdala, nine were found in the basolateral nucleus, four in the basomedial nucleus, two in the central nucleus and one in the lateral nucleus. Electrical stimulation of the amygdala evoked synaptic potentials and/or action potentials in the posterior insular cortex neurons with a mean latency of 15.3 ms (n = 16, range = 2.9–88.5). Most of the neurons recorded in the present study were also responsive to electrical stimulation of the superior laryngeal nerve (SLN). Interactions between amygdala and SLN inputs were observed. These data suggest that connections between the insular cortex and the amygdala may have an important role in affective conditioning.

P2-51. The central pathway for thermal sensation from the tongue in rats: the relay in the thalamus and its connection

Tomio Hayama, Rumi Ishimoto, Kana Urata[1] and Hisashi Ogawa

Department of Physiology, Kumamoto University School of Medicine, Department of Medical Technology, College of Medical Science, Kumamoto University, Kumamoto 860-0811, Japan

We electrophysiologically located neurons responding to innocuous thermal stimulation of the anterior tongue in the boundary region between the posteromedial ventral thalamic nucleus and its parvicellular part. Then, a neuronal tracer—wheatgerm agglutinin-conjugated horseradish peroxidase—was injected there. We saw anterogradely labeled terminals and retrogradely labeled cell bodies in a rostrocaudally extended zone in the ipsilateral insular cortex. Both kinds of labels were located in the boundary between the granular insular cortex (GI) and dysgranular insular cortex (DI), and in the ventral portion of the GI. The retrogradely labeled cell bodies were also found contralaterally in the boundary between the parabrachial nucleus (PB) and trigeminal main sensory nucleus (PV), the dorsal portion of the PV and the superficial layer of the dorsal portion of the caudal subnucleus of the spinal trigeminal nucleus (spVc). The present finding, together with our previous reports on neuronal connections of the spVc and the boundary between the PB and PV, indicates that thermal information originating from the tongue is conveyed through the spVc (i) by way of the boundary between the PB and PV and/or (ii) directly to the thalamic relay, and finally to the boundary portion of the GI and DI.

P2-52. Responsiveness of taste relay nuclei neurons to a mixture of the four basic tastants in rats

K. Hasegawa, T. Hayama, S. Ito, T. Nomura, T. Imoto and H. Ogawa

Department of Physiology, Kumamoto University School of Medicine, Honjo 2-2-1, Kumamoto 860-0811, Japan

To clarify the taste coding mechanism in the gustatory pathway, we studied the responsiveness of 52 neurons in the solitary tract nucleus, 18 in the parabrachial nucleus and 40 in the thalamic taste relay nucleus (VPMpc) of the anesthetized rat to a mixture of the four basic tastants and how the mixture was represented in these nuclei. The mixture yielded response suppression more frequently than response facilitation in all the nuclei studied. The correlation between the responses to the mixture and those to NaCl was highly significant in all the nuclei, but it was slightly lower in the VPMpc. Cluster analysis revealed four to six clusters of neurons in every nucleus. All the clusters containing NaCl-best neurons responded to the mixture, except for VPMpc, where two kinds of NaCl-clusters were found, one responsive to the mixture, but another non-responsive.

Multidimensional scaling showed that the mixture was located outside the tetragonal made of the four basic tastes but near NaCl, except for the VPMpc where the mixture was found far from the tetragonal. Thus, it is indicated that information about the mixture is carried by various groups of neurons, but that of the mixture may be processed differently in the VPMpc.

P2-53. A further study on columnar organization of mechanoreceptive neurons in cortical taste areas in rats

Xiaodong Wang and Hisashi Ogawa

Department of Physiology, Kumamoto University, School of Medicine, Kumamoto 860-081 1, Japan. e-mail: xiaodong-wang@excite.co.jp

Previously we presented evidence that mechanoreceptive neurons with receptive fields (RFs) in the oral cavity are arranged in a columnar fashion in the cortical taste area (CTA) in rats (Ogawa and Wang, 2000). In the present study we further recorded mechanoreceptive and/or taste neurons at 50 or 100 μm along the track, penetrated perpendicularly in rats. Neurons with inhibitory RFs were often found in the infragranular layer. Three types of mechanoreceptive neurons with RFs in the oral cavity were found, as previously: those with RFs only in the oral cavity (type 1); those with RFs both in the oral cavity and on the lip (type 2); and with RFs in the oral cavity and on the external surface of the body, such as ear-flap or tail (type 3). The size of the columnar organization was the largest (75–300 μm) in type 3 neurons; it was smaller in other two types. Taste neurons recorded had mechanoreceptive RFs of type 3 neurons. In some cases two neurons sharing the best stimulus were recorded successively, but in most cases two successive taste neurons did not share the best stimulus. It is possible that taste neurons are arranged in a column with a very small diameter within the large column of mechanoreceptive neurons.

P2-54. Optical recording in the rat gustatory cortex

Tatsuko Yokota

Department of Physiology, School of Dentistry and Medicine, Aichi-Gakuin University, Nagoya 464-0037, Japan. e-mail: tyokota@dpc.aichi-gakuin.ac.jp

The spatiotemporal pattern of neural activity in the gustatory cortex was examined using optical imaging with a voltage-sensitive dye (di-2-ANEPEQ) in anesthetized rats. Anodal electrical currents applied on the tongue evoked optical signals and field potentials with similar peak latencies: 15–45 and 17–37 ms, respectively. The optical signals were evoked consistently over the cortical region where taste neurons had been identified electrophysiologically. These optical signals evoked by anodal currents probably resulted from activation of taste receptors, a phenomenon called 'electrical taste'. Peak latencies (8–10 ms) of optical signals evoked by cathodal currents were shorter than those evoked by anodal currents. These short latencies suggest that the cathodal currents directly activated nerve fibers, including mechanosensitive and taste afferents.