ABSTRACTS

Twenty-ninth Japanese Symposium on Taste and Smell (JASTS XXIX)

Held in Tokyo, Japan, September 26–28, 1995

1. Recent progress in gustatory and olfactory receptor mechanisms

Kenzo Kurihara

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Recently, we examined responsiveness of a single olfactory neuron to odorants using patch clamp and ciliary recording techniques. The results obtained showed that a single olfactory neuron responds to many species of odorants and one species of odorant elicits responses in many neurons. These results are not consistent with the idea that each olfactory neuron has one or only a small species of receptors. We injected a solution containing high concentrations of cAMP and IBMX into an isolated turtle olfactory neuron. Application of an odorant to the neuron after the response to cAMP was adapted elicited a large response, suggesting that cAMP-independent pathway greatly contributes to generation of olfactory responses. G-protein coupled receptors (GCRs) were cloned from bovine taste tissues. The cloned GCRs belonging to olfactory receptor family. It is interesting to note that the GCRs were expressed not only in taste tissues, but also in kidney. It was reported that membrane-bound guanylyl cyclase is located on microvilli of rabbit taste cells. We identified novel membrane bound guanylyl cyclase from bovine taste tissues. There is a possibility that the guanylyl cyclase is a receptor for taste stimuli such as sugar. We found that a lipoprotein composed of phosphatidic acid and β-lactoglobulin selectively inhibits the frog taste nerve responses to bitter substances. The lipoprotein also inhibited bitter responses in humans. It was concluded that the lipoprotein binds to the hydrophobic sites on the taste cell membranes and prevents the binding of bitter substances to the receptor sites.

2. A key role of olfaction in aromatherapy

Shizuo Torii

Toho University, Japan

It is only in recent times that aromatherapy has been introduced into Japan. Japanese researchers have been interested in the psychological effects of aromas rather than the pharmacological benefits of essential oils. During the past 10 years, Torii at Toho University in co-operation with Takasago Research Center have been investigating the effect of odors on human moods and published a CNV study that suggested that odors of lavender, camomile, lemon and sandalwood were relaxing, while oils of jasmine, neroli, peppermint and rose were stimulating. They noticed that there were some disagreements with the traditional properties described in some references on aromatherapy. For instance, the odor of neroli produced a larger CNV amplitude, contrary to the supposed relaxing effect of oil of neroli. Their further study revealed that the odor of neroli produced both stimulating and relaxing effects. The subjective report revealed that subjects who perceived a floral note showed increased CNV amplitude, while subjects who perceived a citrus note showed decreased CNV amplitude. This finding suggests that perception of odor notes can play a key role in the observed CNV effects.

3. Patch-clamp study of taste transduction mechanisms

K. Sugimoto

Department of Neurobiology, Graduate School of Dentistry, Tokyo Medical and Dental University, Tokyo 113, Japan

For the purpose of investigating taste transduction mechanisms, voltage-gated and stimulus-induced ionic currents of isolated taste receptor cells were examined using whole cell patch-clamp recording in larval tiger salamanders, bullfrogs and ddY mice.

Voltage-gated Na^+ channels and delayed rectifying K^+ channels commonly exist in taste receptor cells of all these species, but inactivating K^+ channels (A channels) and voltage-gated Ca^{2+} channels have not yet been observed in mouse taste bud cells. In voltage-clamp configuration, salamander taste cells generated slow inward currents in response to 0.3 M NaCl at about resting potentials, which were reduced to 41% on average by addition of 250 μM amiloride. This result suggests that Na^{2+} ions may have passed through amiloride-sensitive Na^+ channels. Citric acid and acetic acid at 1 mM induced two components of inward currents in salamander taste cells:

one was the inward current shift resulting from K^+ channel block and the other was cation influx at holding potentials hyperpolarized over −40 mV. The cation influx seemed more important for transduction of acid taste. In isolated frog taste cells, 0.1–1.0 mM quinine-HCl strongly suppressed K^+ channels, resulting in depolarization of taste cells. Some mouse isolated taste bud cells elicited slow inward currents in response to monosodium L-glutamate at concentrations of 40–85 mM at a holding potential of −60 mV. The inward currents were reversed at about +10 mV, suggesting that cation-selective channels were activated by it.

Further ion-substituting and pharmacological experiments are needed to identify these stimulus-induced currents.

4. Vertebrate olfactory responses are not affected by changes in salt concentrations on the surface of olfactory epithelium

Takayuki Shoji

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Concentrations of Na^+ in fresh water and sea water are about 0.5 and 500 mM respectively, and hence salt concentrations in the medium at the olfactory epithelium are greatly changed when fish migrate between fresh water and sea water. I used the rainbow trout and chub salmon, and examined the adaptation mechanisms of the olfactory receptors to high concentrations of salts in sea water. Application of 500 mM NaCl to the olfactory epithelium elicited a large response in the olfactory nerve which was not adapted to a spontaneous level with time, while sea water elicited only a very small response. I found that the presence of 10 mM Ca^{2+} in sea water inhibited the response to 500 mM NaCl. I also examined whether the olfactory responses to amino acids were changed between fresh water and sea water. The magnitudes of the responses to the six amino acids examined were similar in artificial pond water and artificial sea water, indicating that a large change in NaCl concentration between fresh and sea water does not affect the responses to amino acids. I also examined whether turtle olfactory responses to odorants were abolished by elimination of salts on the olfactory epithelium. The magnitudes of odor responses were unchanged by perfusing a salt-free solution. It was concluded that olfactory responses in vertebrates are not affected by changes in salt concentrations on the surface of the olfactory epithelium.

5. Modification of neuronal responses of the amygdala and cortical gustatory cortex to taste stimuli after conditioned taste aversion in freely behaving rats

Yasunobu Yasoshima

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita, Osaka 565, Japan

To investigate functions of cortical gustatory area (CGA) and amygdala (Amy) in conditioned taste aversion (CTA), we recorded neuronal activities from these areas before and after conditioning in freely behaving rats. Before conditioning, no remarkable responses to the CS, 0.005 M sodium saccharin, in 54 CGA units was observed. Only one central amygdaloid (Ce) unit in 44 AMy units responded to the CS. At 10 min after a pairing of the CS with an i.p. injection of LiCl, we resumed recording activities to the CS intermittently for 6–7 h. No significant changes in response to the CS were observed in the Amy and CGA 10 min after the conditioning. We found long-term facilitatory responses to the CS in 5 basolateral amygdaloid (BLA), 1 Ce and 6 CGA units. These facilitatory effects lasted for about 6 h. We also found short-term facilitatory effects, which lasted for less than 90 min, in 8 CGA units, and short-term inhibitory effects lasting for about 2 h in 5 Ce units. These results suggest that neurons in the Amy and CGA play important roles in CTA formation.

6. Objective olfactory test from the aspect of clinical evaluation

Mitsuru Furukawa

Department of Otolaryngology, School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, 920, Japan

Most current psychophysiological tests are subjective. Considerable progress, however, is being made in both the development and understanding of some objective measures of olfaction such as odor-evoked potentials, which may soon be available for clinical use. Such tests may help determine whether the problem is traceable to the central nervous system or to the peripheral nervous system, whether the disorder is based on the olfactory nerve or trigeminal nerve, or whether it is attributable to sensorineural defect or airflow occlusion.

Clinical evaluation of a patient with a chemosensory disorder consists of four basic components: the history, physical examination, psychophysical assessment and medical imaging procedures. Each of the components provides valuable information on an individual basis, and an accurate anatomic and etiological diagnosis can be made for proper management and treatment.

7. Future goals for objective measurement and clinical testing methods of olfaction and taste in man

Mitsuo Tonoike

Life Electronics Research Center, Electrotechnical Laboratory, 3-11-46 Amagasaki, 661 Japan

Objective measurement and clinical testing methods of human olfaction and taste are one of the urgent and important problems in medicine. The aim of this research is to learn from the history of objective measuring methods, to analyse the present experiments and to make clear the theme of promoting clinical applications. Recent progress of many measurements and more powerful techniques of testing human olfaction and taste will give us a more precise and useful objective measuring method.

Through many discussions at this special symposium on clinical applications, we will find the most important answers to play a role in our study co-operation with medical doctors and fundamental researchers.

A typical method of objective measurement of olfaction and taste is the study of chemosensory-evoked responses and event-related potentials. Olfactory- and gustatory-evoked potentials have been debat-

able until now; however, many studies have recently been developed step by step. Our research group has tested olfactory-evoked potentials in two patients after removal of their olfactory mucosa. The results suggest the capability of clinical applications of chemosensory-evoked potentials.

We also analysed olfactory event-related potentials about 1 s from onset of odorant stimulation using a singular value decomposition (SVD) method and obtained the component of pleasant/unpleasant as the principal factor. These results suggest a future application in the form of new objective measurement and clinical testing.

8. Objective testing of olfactory function in human subjects

Toshihiko Kato, Yuuji Suoya, Takashi Mohri, Hirofumi Harada, Kimio Shraishi and Toyoji Soda

Department of Otorhinolaryngology, School of Medicine, Fukuoka University, Fukuoka 814-80, Japan

Odorant-evoked potentials (OER) were recorded from normal human scalp, and both the power spectra and the coherence spectra were measured in order to study EEG dynamics during odor administration. The results were as follows. (i) During pleasant odor stimulation with methyl-cyclopentenolone (B), δ and θ waves were reduced in the central and occipital regions. (ii) During unpleasant odor stimulation with scatol (E), α2 waves were activated in the frontal, temporal and occipital regions. (iii) During pleasant odor stimulation with B, coherence was decreased in the frontal region and increased in the temporal region. (iv) Odorant-evoked potentials in normal human subjects consisted of two positive peaks with a peak latency of about 350 ms (P350) and about 700 ms (P700), whereas such P350 or P700 peaks were not detected in anosmic patients. (v) The high potential of the OER P350 was elicited from the centro-occipital region of the scalp and the OER P700 was elicited from all over the scalp.

9. Olfactory-evoked potentials caused by electrical stimulation of the human olfactory mucosa

Tadashi Ishimaru, Takefumi Shimada and Mitsuru Furukawa

Department of Otorhinolaryngology, School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa 920, Japan

Studies of human olfactory-evoked potentials produced by odor continue to make advances. However, there are two problems with their clinical application. (i) Complicated odor stimulating devices. (ii) Too long duration of the stimulating odor pulse to allow detection of the potential of the olfactory bulb. To resolve these problems, we stimulated human olfactory mucosa with electrical pulses and detected changes in potential from the head.

A bipolar stimulating electrode was inserted in the olfactory cleft. Five active electrodes positioned laterally and frontally were referenced to each other. An ipsilateral ear-lap was used as earth. To reduce the voltage of background electroencephalograms, subjects slept with triclofos sodium and 300 trials were averaged.

Stimulation with 2 mA 500 μs 2 Hz evoked a change in potential with a negative peak of about 20 ms (mean of three human subjects). The amplitude of this change in potential was highest at the ipsilateral frontal position. Localization of the evoked potential confirmed that the source was the ipsilateral olfactory bulb. The latency of this peak was very similar to that of the olfactory bulb in rabbits, which we reported at the JASTS 1994 congress.

Research into olfactory-evoked potentials caused by electrical stimulation of human olfactory mucosa is still in its early stages, but will help us to diagnose olfactory dysfunction.

10. Objective evaluation of human olfactory dysfunction by event-related brain potential

Takuji Yamamoto

Ashikaga Institute of Technology, 268 Omae-cho, Ashikaga-shi, Tochigiken 326, Japan

From the literature on olfactory CNV and the study of olfactory-modulated CNV, objective evaluation of various olfactory dysfunctions was approached using the CNV as a possible parameter of an objective sign of olfactory perception and cognition.

The olfactory CNV, which was recorded by a simple reaction type paradigm with olfactory S1, revealed sensitivity to olfactory dysfunction at the perceptual level when a single olfactory stimulus served as S1. By using multiple olfactory stimuli for the S1 of the selective reaction paradigm, the olfactory CNV seems to yield possible discrimination of olfactory dysfunction at the cognitive level. In the case of olfactory modulated CNV, the hemispheric increase ratio of CNV basis wave was less prominent in the right hemisphere when olfactory stimulus evoked positive feeling, while the increased ratio was more prominent in the right hemisphere in the case of negative feeling, revealing different involvement of the brain hemispheres according to feelings.

From the results obtained in these two types of CNVs, the possible differential diagnosis between equivocal olfactory dysfunction is discussed. These kinds of procedures seem to yield a more effective method of objective evaluation of olfactory dysfunctions related to external olfactory stimuli.

11. Objective test using contingent negative variation elicited by electrical taste stimulation

Yoshiyuki Kitaoku

Department of Otolaryngology, Nissei Hospital, Japan

The warning stimulus was an electrical taste stimulation applied for 100 or 500 ms with a bipolar stainless steel electrode disc. The imperative stimulus was a pure tone of 1 kHz at 60 dB and the interstimulus interval between paired stimuli was 2000 ms. The contingent negative variation (CNV) was recorded with the stimulus at an intensity of 40 μA and the positive potential evoked by the electrical taste stimulation was recorded simultaneously at Fz, Cz and Pz sites.

Under co-operative conditions, the late component of CNV was recorded in all 25 subjects, while the evoked positive potential was recorded in 18 out of 25 at the Cz site and in 12 out of 13 at the Pz site.

To determine whether this examination could evaluate electrical taste objectively, we asked normal subjects to ignore the electrical taste stimulation and respond only to the pure tone. This experiment was performed by nine of the subjects who had an evoked positive potential under co-operative conditions. Although the amplitude of the evoked positive potential decreased, it was clearly detected in eight subjects. The late component of CNV was detected in three subjects. Thus, it was possible to evaluate electrical taste objectively by using the evoked positive potential as a sign of taste.

Finally, we explain the P300 components of the oddball paradigm in the electrical taste and pure tone stimuli.

12. Gustatory-evoked potential

Masashi Wada

Department of Oto-Rhino-Laryngology, National Center of Neurology and Psychiatry, Kohnodai Hospital 7-1, 1-chome Kohnodai, Ichikawa-shi, Chiba, Japan

A new method of applying quantitative gustatory stimuli is presented. Elastic material is attached to the tip of a 5 ml bottle containing a substance for gustatory stimulation. Evoked potentials to 20% glucose, and 3 and 10% sodium chloride using this new instrument are discussed. Summation starts 300 ms prior to the trigger signal, being the time taken for the button to be pressed after applying the bottle to the subject's tongue. The results show that the latency of the main positive wave was between 96 and 212 ms. There was no significant difference between responses evoked by the different stimuli.

13. Gustatory-evoked potentials for clinical examination

A. Ikui, H. Tomita, M. Ikeda and I. Ito

Department of Otolaryngology, Nihon University School of Medicine, 3-1 Ooyaguchi-kami-machi, Itabashi-ku, Tokyo, Japan

To cope with the recent increase in patients with taste disorders, new techniques for examining the extent and state of the disorder have been developed. Although useful in diagnosing patients, these methods are all subjective and ineffective in detecting psychosomatic disorders or stimulation. We developed an objective method of taste examination using taste solutions. When stimuli are applied every 5 or 10 min to the region innervated by the chorda tympani, responses consisting of positive and negative waves (about 20 μV), the latter of which were sometimes the only effect, were obtained from the ipsilateral temporal region and Cz in four out of 20 normal subjects. Bilateral stimulation of the area innervated by the chorda tympani induced only a response from Cz. These waves may be thought of as event-related potentials, because of their long latency and duration. Revised methods and electrical stimulation must be further explored in order to obtain stimulation-related potentials for clinical use.

14. Olfactory responses to cpt-cAMP and odorants after complete desensitization of cAMP-dependent pathways by dialysis of high concentration of cAMP into turtle olfactory cells

Makoto Kashiwayanagi and Kenzo Kurihara

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

The degree of contribution of the cAMP signal transduction pathway to odor responses was examined by recording current responses from isolated turtle olfactory cells under whole-cell voltage clamp conditions. The magnitude of response to cAMP dialysis from the patch pipette increased with increasing cAMP concentration and reached a plateau at 0.5 mM, suggesting that the cAMP-dependent pathway was completely desensitized by application of 1 mM cAMP. The magnitude of the response to 3 mM cpt-cAMP (a membrane-permeable cAMP analogue) after application of cAMP decreased with increasing cAMP concentration, and reached zero after 1 mM cAMP. This finding supports the above suggestion that the intracellular application of 1m M cAMP brings about complete desensitization of the cAMP-dependent signal transduction pathway. Extracellular application of 3 mM cpt-cAMP elicited no response after the cAMP signal transduction pathway was desensitized by dialysing 1 mM cAMP and 0.5 mM IBMX from the patch pipette into the cells, indicating that the cAMP pathway was completely desensitized. Application of an odorant cocktail induced a large inward current under these conditions. The present results suggest that the cAMP-independent signal transduction significantly contributes to generation of odor responses in the turtle.

15. Involvement of Ca^{2+}-calmodulin in the adaptation of rat olfactory receptor neurons

Shin-ichi Hirabayashi and Noriyo Suzuki

Animal Behavior and Intelligence, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

To test a hypothesis that Ca^{2+}-calmodulin negative feedback mechanism is involved in adaptation in vertebrate olfactory receptor neurons, we studied the inhibitory effect of calmodulin on single cyclic nucleotide-gated channel (CNG-channel) activities in different Ca^{2+} environments. Inside-out membrane patches were excised from rat olfactory receptor neuron somata with a patch pipette filled with zero Ca^{2+} solution and perfused with different Ca^{2+} solutions (3, 6, 10 and 50 μM Ca^{2+}) containing 10 μM cAMP on a holding potential of −60 mV in a microflow superfusion chamber. Addition of 250 nM calmodulin from bovine brain to 10 μM Ca^{2+} solution caused a remarkable decrease in open probability for the CNG-channels with a long latency (>30 s), while the unit conductance for the CNG-channels did not change significantly. Inhibition by calmodulin of the channel openings was released (disinhibition) quickly by the further addition of a calmodulin blocker, 2 μM mastoparan. Results suggest that Ca^{2+}-calmodulin feedback mechanism, in which Ca^{2+}-calmodulin lowers the affinity of binding sites of the CNG-channels for cAMP, may be one of the negative feedback pathways for the adaptation which is triggered by an intracellular increase in Ca^{2+} concentration followed by odorant stimulation.

16. Change of Cl^- concentration in mucus of frog olfactory epithelium induced by odorants

Ryoichiro Wakabayashi[1] and Tadashi Nakamura[1,2]

[1]Department of Applied Physics and Chemistry, Faculty of Electro-Communications and [2]Department of Information Network Science, Graduate School of Information Systems, The University of Electro-Communications, 1-5-1 Cyoufugaoka, Cyoufu, Tokyo 182, Japan

Previous reports suggested that Cl^- current through the Ca^{2+}-gated Cl^- channel amplifies the odor-induced cationic current. However, classical works by Takagi and his collaborators has shown that Cl^- flows into the supporting cells during positive electro-olfactogram (EOG) induced by the specific odorants. It would be useful to trace the movement of Cl^- in the olfactory epithelium during the transduction process and the generation of EOG. We tried simultaneous measurements of both EOG and concentration of Cl^- in the mucus ($[Cl^-]_o$) with Ag-AgCl electrodes and a Cl^--sensitive electrode, respectively.

Nasal cavities of bullfrogs were opened to have their epithelia exposed. The odor stimulation was accomplished by blowing saturated gas of *n*-amyl-acetate, geraniol, cineol or chloroform.

When negative EOG was induced by *n*-amyl-acetate, geraniol or cineol, $[Cl^-]_o$ increased transiently from the resting level of about 100 mM. On the other hand, the $[Cl^-]_o$ decreased transiently in parallel with the generation of positive EOG induced by chloroform, which implies Cl^- influx into the supporting cells, as Takagi *et al.* have suggested. However, we detected small $[Cl^-]_o$ increases before this decrease. Thus, so far all the odors tested induced an increase in $[Cl^-]_o$, in spite of the polarization of EOG induced by them. These results are consistent with the concept that Cl^- effluxes from the receptor cell during the transduction process.

17. Damped oscillatory field potentials and functional subdivisions in the guinea-pig accessory olfactory bulb

T. Sugai, M. Sugitani and N. Onoda

Department of Physiology, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan

Extracellular field potentials from guinea-pig accessory olfactory bulb (AOB) slices following electrical stimulation of the vomeronasal nerve layer (VNL) were recorded. A single shock of the VNL elicited a characteristic damped oscillatory field potential (DOFP) in the external plexiform layer (EPL). The DOFP consisted of a compound action potential followed by six or seven periodic negative peaks (n_1–n_7). The average frequency of the oscillation was 32 Hz. The n_1 wave seemed to be mainly related to the EPSPs generated in the apical dendrites of mitral cells. The n_2 and subsequent oscillatory waves seemed to be related to synchronous periodic activities in the EPL and mitral cell layer. These results suggest that the oscillatory activity in the AOB contributes to sensory information processing. In addition, the anterior VNL shock-elicited DOFPs were distributed only within the anterior AOB, but not in the posterior AOB and vice versa. Furthermore, damping factor in the decay function approximated with the negative peaks of the DOFP, was significantly smaller for the posterior than for the anterior AOB. These results suggest that the AOB consists of at least two functionally different regions along the anteroposterior axis.

18. Responses of the chorda tympani nerve to various sweeteners in the diabetic (*db/db*) mouse

Yoshihisa Imai, Yuzo Ninomiya, Kiyoto Nakashima[1], Toshiaki Imoto[2] and Yasushi Fukami

Departments of Oral Physiology and [1]Chemistry, Asahi University School of Dentistry, Hozumi, Motosu, Gifu 501-02 and [2]Department of Physiology, Tottori University Medical School, Yonago, Tottori 683, Japan

Sweet taste sensitivity in a genetic model of diabetes, the *db/db* mouse, was studied by examining chorda tympani nerve responses to various sweeteners. In this strain, a single major gene defect leads to the expression of diabetes and obesity. The chorda tympani responses to sucrose, saccharin, glycine, L-alanine, D-tryptophan in *db/db* mice showed greater relative magnitudes (about 1.5 times) of responses than those in lean control mice. Responses to other basic taste stimuli, such as NaCl, HCl and quinine HCl, and a sweet-tasting amino acid, D-phenylalanine, however, were not different in the two groups. Responses to these sweeteners in *db/db* and lean mice were suppressed by a sweet inhibitor, gurmarin, to about 70 and 50% of control, respectively. The magnitude of gurmarin-sensitive and -insensitive component of responses to each sweetener was compared separately. It was found that the greater response to sweeteners in *db/db* mice was evident only in their gurmarin-insensitive component and not in the gurmarin-sensitive one. These results suggest that the *db* gene may act on a common factor(s) involved in taste transduction through the gurmarin-insensitive sweet receptor component in taste cells and in the stimulus-secretion coupling in pancreatic B cells of *db/db* mice.

19. Role of saliva in maintenance of taste sensitivity: analyses of rat chorda tympani response

Ryuji Matsuo

Department of Oral Physiology, Faculty of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565, Japan

Removal of the submandibular and sublingual salivary glands decreases taste responsiveness of the chorda tympani nerve in rats, suggesting a role for saliva in maintenance of taste sensitivity. To evaluate water (the principal constituent of saliva) and salivary ions for the taste maintenance, taste responses of the chorda tympani nerve were compared in normal, desalivated (1 week after removal of the submandibular–sublingual complexes) and water or salivary ion-supplied rats. Water or salivary ions (60 mM $NaHCO_3$ and KCl) was injected into the mouth of desalivated rats for 1 week at a rate of 0.6 ml/h. The following results were obtained. (i) Response magnitudes to NaCl, sucrose, HCl and quinine in desalivated rats were about 50, 40, 20 and 20% respectively of those in normal rats. (ii) Water-supplied salivated rats showed about 20% increase in NaCl, HCl and quinine responses, whereas sucrose response was slightly smaller than those in normal rats. (iii) Salivary ion-supplied rats showed about

20% decrease in NaCl, HCl and quinine responses, whereas sucrose response increased to about 150%. These results indicate that salivary water and ions can change taste sensitivity, and are important in maintaining normal taste sensitivity.

20. A model of taste cells of the rat: multisensitivity and non-linear responses of taste cells to mixed taste stimuli

Yoshiki Kashimori, Masayoshi Natio[1] and Takeshi Kambara

Department of Applied Physics and Chemistry, University of Electro-Communications, Chofu, Tokyo 182 and [1]Advanced Research Laboratory, Hitachi Limited, Hatoyama, Saitama 350-03, Japan

Coupling between ionic flows through different kinds of channels affects essentially the responses of multisensitive taste cells to single and mixed taste stimuli. The mechanism of taste transduction is studied by using a realistic model of the multisensitive cells of the rat. The transduction pathways considered are amiloride sensitive Na^+ channels for NaCl and HCl, and cAMP-mediating K^+ channels for sucrose. Contributions of paracellular pathways and taste insensitive ionic channels are also studied. We use two types of quantities which characterize the properties of responses to the mixture: one describes an additive effect and the other a mixed effect. Whether the response of a cell to a binary mixture becomes synergetic or antagonistic can be estimated systematically based on a pair of response tendency of the cell induced by individual components of the mixture. The response tendency means depolarization or hyperpolarization. Whether the response is enhanced or suppressed by adding a new taste to the original one is also answered reasonably based on the pair of single response tendency.

21. Activation brain areas induced by drinking in rats using magnetic resonance imaging

Eiichi Tabuchi[1,2], Takashi Yokawa[1], Takashi Kondo[1], Taketoshi Ono and Kunio Torii[1,3]

[1]Torii Nutrient-stasis Project, ERATO, R&D Corporation of Japan, Yokohama 221, [2]Department of Physiology, Toyama Medical and Pharmaceutical University, Toyama 930-01 and [3]Central Research Laboratory, Ajinomoto Corp. Inc., Yokohama 244, Japan

The spatial and temporal dynamics of activated areas in the brain induced by drinking behavior were investigated by magnetic resonance imaging in awake rats. During ingestion of glucose solution, the signal intensity, in T2*-weighted magnetic resonance imaging, which increases in areas with high consumption of oxygen (oxyhemoglobin), was elevated in most brain areas measured. It increased significantly in the lateral hypothalamic area, ventromedial nucleus of the hypothalamus, central nucleus of the amygdala, the piriform cortex and CA1 subfield of the hippocampus. The intensity in the hypothalamus during drinking increased continuously and was maximum. After drinking, it tended to return gradually to the control level, although remained high in some brain areas. Temporally, the signal intensity was changed most critically at 2 min during 3 min drinking and increased again 20 min after stop licking in some areas, especially in the hypothalamic areas. These findings indicate that the above-mentioned brain regions co-operate and work in parallel during drinking behavior, and that the hypothalamus may play a central role in the behavior including the component of reward value (satisfaction) or movement, such as drinking.

22. Facilitation of extracellular acetylcholine release in the gustatory cortex of salt-deprived rats in response to concentrated NaCl

Tsuyoshi Shimura and Takashi Yamamoto

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

When animals are deficient in body sodium, they consume considerable quantities of concentrated salty solutions that are normally avoided at such high concentrations. Compared with lesion and electrophysiological studies on salt appetite, neurochemical information about this phenomenon is quite limited. Since we recently reported that aversive taste stimuli facilitate extracellular acetylcholine (ACh) release in the insular gustatory cortex (CGA), in the present experiment we measured ACh levels in the CGA in response to normally aversive concentrated (0.5 M) NaCl in freely moving, salt-deprived rats. Acute deprivation of body sodium by injections of a natriuretic drug, furosemide, reliably increased ingestion of concentrated NaCl in salt-deprived rats. ACh levels in response to concentrated NaCl in salt-deprived rats were significantly higher than those in control rats. Behavioral analyses revealed a highly positive correlation between ACh levels and overt activity of animals after taste stimulation in salt-deprived rats. The magnitude and duration of ACh release, however, were larger in animals that received an aversive taste stimulus in the previous study. Thus, although ACh in the CGA is involved in both ingestive and aversive taste responses, it is suggested that ACh release in the CGA is more closely related to behavioral expression of aversive taste stimuli.

23. Somatotopy and cytoarchitecture of the maxillary barbel lobule of the facial lobe in the channel catfish *Ictalurus punctatus*

Sadao Kiyohara[1] and Thomas E. Finger[2]

[1]Kagoshima University, Japan and [2]University of Colorado, Denver, CO 06510, USA

Purpose. The primary taste center of the facial taste system, facial lobe (FL) of catfishes has a distinct somatotopical map. The entire body surface of the catfish is represented in the FL, the barbels and trunk-tail being sharply defined in the lobule structures extending caudorostrally in the FL. The FL possesses at least three types of neurons: small, medium and large. This study was undertaken to reveal the detailed somatotopy and cytoarchitecture of olfactory lobule (MXL) in the FL of channel catfish.

Methods. Juvenile freshwater catfish, *Ictalurus punctatus*, were used. Maxillary barbels receive innervation from two distinct nerve branches: a large branch from the maxillary nerve and a smaller branch from

the mandibular nerve. To trace the peripheral distribution of the two branches, carbocyanine dye (DiI) was applied to the peripheral cut end of either branch in fixed barbels. The central projections of the two branches were examined with transganglionic tracing techniques of horseradish peroxidase or fluorescent dextran amines. To locate ascending and descending second order neurons in the MXl, DiI or fluorescent dextran amines were applied, respectively, to the ascending secondary tract or descending secondary tract in fixed brains or Ringer-perfused brains *in vitro*.

Results. The large and small branches are distributed, respectively, through the rostral and caudal portions of the maxillary barbel. The large branch terminates throughout the MXL, while the small branch projects to the dorsolateral margin of the MXL. Application of the tracers to the distal portion of the barbel results in labeling only the rostral portion of MXL. These results show that the proximal-distal axis of the maxillary barbel is represented along the posteroanterior axis of the MXL, the rostrocaudal axis being represented ventrodorsally in the MXL. Application of the tracers to the ascending secondary gustatory tract labeled medium neurons scattered throughout the MXL; application of the tracers to the descending secondary gustatory tract labeled large neurons. Large neurons were much scarcer than the medium-sized neurons.

24. Phagocytosis by supporting cells in the olfactory epithelium after bulbectomy

Yuko Suzuki and Masako Takeda

Health Sciences University of Hokkaido, Ishikan-Tobetsu, Japan

Macrophages are known to be phagocytes in the olfactory epithelium. The participation of other cells types in phagocytosis in association with the process of cell death was examined in the olfactory epithelium after unilateral bulbectomy of neonatal mice and rats. The TUNEL method revealed that the process of olfactory cell death consists of acute and chronic periods. The number of apoptotic cells on the operated side peaked at 1 day and decreased rapidly at 3 days after bulbectomy. Cell death increased in number again at the end of the first post-operative week and had increased 2- to 4-fold by 2 months. Electron micrographs of day 1 showed that many supporting cells contained apoptotic bodies, large phagosomes and cell debris in the cytoplasm. Supporting cells were very active in phagocytosis throughout the post-operative days. Immunohistochemistry using a monoclonal anti-macrophage antibody (OX42) showed that macrophages were present in the olfactory epithelium of the operated side at day 1; however, the number of macrophages was relatively small and returned to control levels at 7 days and longer post-operation. The result indicates that that both supporting cells and macrophages are phagocytes in the olfactory epithelium after bulbectomy of neonatal animals. Bulbectomy of newborn animals caused olfactory cells to have a short life-span, which might be related to phagocytosis by supporting cells.

25. A comparative immunohistochemical study between stages of development and regeneration in the rat vomeronasal organ

Toshiya Osada[1,2], Atsushi Ikai[2], Masumi Ichikawa[3] and Richard M.Costanzo[1]

[1]Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0551, USA, [2]Department of Biological Sciences, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midoriku, Yokohama 227 and [3]Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183, Japan

In order to study neurogenesis of the vomeronasal chemosensory neurons, immunocytochemical studies were undertaken to find cell markers that can distinguish different stages of the sensory cell development. Results from this study suggest that chemosensory cells can be classified into four stages. In the first stage, cells are nestin single positive. Nestin single positive cells were observed at both early stages in development and at 6–10 days after recovery from nerve transection. In both cases, the number of cells and the thickness of the neuroepithelium increased with time. During the second stage, cells were nestin and neural cell adhesion molecule (N-CAM) double positive. These cells are thought to represent pre-immature neurons. In the third stage, cells are N-CAM single positive which corresponds to immature neurons. N-CAM positive cells were observed at E17 (embryonic day 17). After nerve transection of vomeronasal neurons, the number of newly generated N-CAM positive cells increased beginning at day 8. In the final stage of development, cells were olfactory marker protein (OMP) and N-CAM double positive indicating that they were mature neurons. Mature neurons were first observed at E17. The classification of cells into four stages of development may facilitate studies of neurogenesis and regeneration in the chemosensory system.

26. Human taste bud cells contain immunoreactivity for serotonin and CCK-8

S. Takami, M. Yamagishi[1], Y. K. Caise[2], M. L. Getchell[3] and T. V. Getchell[3,4]

Department of Anatomy, Kyorin University, School of Health Science, [1]Department of Otolaryngology, Ina Hospital, Saitama, Japan, [2]Department of Biology, University of Kentucky, [3]Department of Surgery and [4]Department of Physiology and Sanders-Brown Center on Aging, University of Kentucky College of Medicine, Lexington, KY, USA

Human taste buds were investigated immunohistochemically by using antisera to serotonin and cholecystokinin-octapeptides (CCK-8). Human circumvallate and folliate papillae were collected from four Alzheimer's disease (AD) patients and two AD controls at autopsy, fixed with Zamboni's fixative for 2 h and processed for cryostat sectioning. Fifteen-μm thick sections were immunostained using the standard streptavidin-biotin-peroxidase complex method. Serotonin-immunoreactive taste bud cells with a bipolar shape were demonstrated in all taste papillae from all subjects, suggesting that human taste receptor cells utilize serotonin as a neurotransmitter. The mean number of serotonin-immunoreactive taste bud cells in the AD's foliate papillae (0.7 per single taste bud section) was significantly decreased compared with that of an AD control (2.8) ($P < 0.01$). A small subset of taste

bud cells exhibited CCK-8 immunoreactivity. In both circumvallate and foliate papillae, the mean number of CCK-8 immunoreactive taste bud cells was much decreased in AD patients when compared with that in AD controls, although a significant difference was detected only in circumvallate papillae (1.1 for AD control versus 0.1 for AD patients, $P < 0.01$). These results suggest that down-regulation of synthesis of serotonin and CCK-8 has taken place in AD that may cause dysfunction of taste perception at the level of taste receptor cells.

Supported by NIH grants NIDCD-00159 (T.V.G.) and NIDCD-01715 (M.L.G.).

27. Responses to alkali-metal cation of salt receptor cell and water receptor cell of the fleshfly *Boettcherisca peregrina*

Shinobu Goshima, Tomoki Kazawa, Midori Murakami and Hiromasa Kijima

Department of Physics, Faculty of Science, Nagoya University, Nagoya 464-01, Japan

Dose–response relationships to alkali chlorides of salt receptor cells and water receptor cells of labellar chemosensory hair of the fleshfly *Boettchrisca peregrina* were studied.

As for water receptor cells, shapes of dose–response curves and concentration range at which water receptor cells responded to alkali chlorides were quite different among different alkali chlorides. The hypothesis that the water receptor cell is an osmometer cannot explain these results. We analysed dose–response relationships of water receptor cells using normalized responses and relative concentrations for KCl, NaCl or LiCl. Hill coefficients and Beidler constants were different among these salts. If water receptor cells are an osmometer, Hill coefficients and Beidler constants at the same concentration should be identical even if different salts are used. Dose–response curves for salt receptor cells were also different if different salts were used. The order of sensitivity to salts between salt receptor cells and water receptor cells was similar when LiCl, NaCl or KCl were used as stimulants. Similar mechanisms may underlie the alkali metal cation reception by both the salt receptor cells and water receptor cells.

28. Quantitative analysis of concentration–response relationships and the dissociation constant of a stimulant–receptor complex in the sugar taste receptor of the fly

M. Ozaki and T. Amakawa[1]

Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560 and [1]Department of Natural Environment, Faculty of Human Development, Kobe University, Nada, Kobe 657, Japan

In taste systems, stimulant–receptor interaction triggers transduction and prescribes sensitivity. However, most taste substances have such low affinities with their receptors that they are easily washed away by saliva, and the affinities difficult to measure. Here, we calcuate the dissociation constant of a stimulant–receptor complex by means of electrophysiological changes in the concentration–response relationships between non-adapted and adapted states of the sugar receptor cell of the fly. In the non-adapted state, the electrophysiological mid-point concentration was six times lower than the dissociation constant. As the cell adapted, however, the mid-point concentration increased, approximating the dissociation constant. The dissociation constant of the sucrose–receptor complex was determined at 140 mM, which corresponds to 1.2 kcal of free energy change for the complex formation. This method is practicable for evaluating stimulant–receptor interactions under physiological conditions.

29. The effects of olfaction on taste

K. Tonosaki and H. Uebayashi[1]

Department of Veterinary Physiology, Faculty of Agriculture, Gifu University, Gifu 501-11 and [1]Department of Oral Physiology, School of Dentistry, Asahi University, Gifu 501-02, Japan

In most mammals, except human beings, the alimentary canal is a solid crossing (two-level crossing) with the trachea at the pharynx. In human beings, the alimentary canal is a grade crossing with the trachea. For this reason, most mammals are obligatory nasal breathers; they can breathe air through the nose and simultaneously swallow food through the mouth. Human beings cannot breathe and swallow simultaneously. Apart from these reasons, it is generally accepted that the taste of turnip is indistinguishable from that of apple or potato when a head cold blocks olfaction in man. Researchers have reported rats and mice being unable to find food following acute anosmia. Recently, it has also been reported that non-taste factors need to be considered in taste discrimination studies. The experiments reported here briefly describe the effect of anosmia on taste discrimination. Since animals have strong taste preferences, it is possible to test the efficiency of their sense of taste using two-bottle preference and lick sensor tests as tools in behavioral studies. The data suggest that anosmic mice can hardly discriminate among five taste stimuli (sucrose, NaCl, HCl, QHCl and distilled water).

30. Facial expression induced by taste stimuli in adults

T. Horio and Y. Kawamura

Faculty of Nutrition, Koshien University, Takarazuka 665, Japan

In order to evaluate the patterns of facial expression evoked by taste stimuli, electromyograms (EMG) of facial and chewing muscles were recorded in 14 healthy university students. Rating scale tests for hedonic tone for the taste solutions used were also performed. Sucrose, NaCl, citric acid, quinine-HCl, monosodium glutamate (MSG), capsaicin, tannic acid and homogentisic acid were used as the test solutions.

Capsaicin, tannic acid and citric acid induced larger amplitudes of EMG responses than the other taste solutions in *Musculus corrugator supercilii*, *M. orbicularis oculi*, *M. risorius*, *M. masseter* and *M. temporalis*.

The EMG activities of *M. corrugator supercilii* and *M. orbicularis* showed phasic patterns for capsaicin, tannic acid and citric acid. However, NaCl, MSG, homogentisic acid and sucrose induced small and tonic patterns of EMG responses.

The EMG amplitude of venter frontalis and *M. depressor anguli oris* were almost similar in all taste solutions used.

These results suggest that the EMG activities of facial and chewing muscles show large responses to disliked tastes and weak responses to less preferred tastes. However, the EMG activity for citric acid was larger than that for quinine-HCl, while citric acid was preferred over quinine-HCl. Thus EMG activity of facial muscles and chewing muscles is not always consistent with the character of hedonic tone.

31. Effects of odor and personality traits on the tracking task

Kumiko Yamada and Tomoyuki Yoshida[1]

Maki Psycho-educational Counselling Center and [1]Physioinformatics Laboratory, Human Informatics Department, National Institute of Bioscience and Human Technology 102, 1-10-6 Asagayakita, Suginami, Tokyo 171 and 1-1 Higashi, Tsukuba, Ibaraki 305, Japan

Effects of odor during tracking tasks were examined in relation to personality traits. Using the Maudsley Personality Inventory, 12 extrovert and 12 introvert students were selected. In each group, six subjects were exposed to the odor of peppermint while the remaining six were not exposed to the odor during the task.

The following results were obtained.

i. In the extrovert group, task performance was significantly better in the presence of odor.

ii. The increase in heart rate when dealing with an emergency was less in the presence of odor.

iii. When EEGs were analysed, alpha waves during rest with eyes closed before the start of the task were significantly stronger in subjects not exposed to the odor.

iv. The increase in awareness level when the subjects dealt with an emergency was particularly marked in subjects not exposed to the odor. After the emergency was handled, in the introvert group, the awareness level tended to remain elevated in subjects not exposed to the odor.

These results indicate that the odor of peppermint tended to suppress autonomic activities when dealing with an emergency and that it also favorably affected task efficiency in the extrovert group.

32. Measurement of the liking for odors using startle eyeblink reflex

Miyuki Nakamura, Hajime Nagai, Masashi Nakagawa and Fumio Yamada[1]

Institute for Fundamental Research, Suntory Ltd, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka and [1]Laboratory of Psychophysiology, Osaka Prefectural College of Nursing, 3-7-30 Habikino, Habikino-city, Osaka, Japan

The purpose of this study was to investigate whether the subjective feeling of pleasantness or unpleasantness provoked by odors would modulate the startle eyeblink reflex.

Subjects were 24 volunteers, 12 males and 12 females, mean age 24.5 years (range 18–36). Subjects received the startle noise pulse during odor stimulation. The onset of odor exposure was automatically set at the initiation of inspiration. Startle pulse was presented 1.5 or 2.0 s after the odor presentation via headphones. The intensity of the startle noise was set at 110 dB (A). The noise duration was 50 ms. The exposure duration of odor was 5 s, and just after the offset of odor the air without smell was presented to wash out the preceding smells. Three categories of odors, i.e. pleasant, unpleasant or neutral, were selected among 13 odors used in earlier research. Integrated EMG of orbicularis oculi, 20–80 ms after the onset of startle stimulus, was assessed for statistical analysis.

The results showed that the magnitude of EMG activity during pleasant odor conditions was lower than in the neutral condition. On the other hand, EMG magnitude during the unpleasant condition was higher than in the neutral condition. It is suggested that startle eyeblink reflex would be a good index of subjective feeling of pleasantness and unpleasantness.

33. Effect of verbal labels on recognition memory of familiar odors

Saho Ayabe-Kanamura, Tadashi Kikuchi and Sachiko Saito[1]

Institute of Psychology, University of Tsukuba, Tsukuba, Ibaraki 305 and [1]NIBH, AIST, MITI, Higashi 1-1, Tsukuba, Ibaraki 305, Japan

The experiment investigated the effect of verbal labels on recognition memory for familiar odors. Forty odor sources were selected from the 'odor classification of Japanese people'. Thirty-two subjects learned 20 odors. Subjects in the control group learned the odors without accompanying verbal labels, while subjects in the other group learned the odors with accompanying verbal labels. A recognition memory test was administered 15 min and 1 week after the learning phase. On the recognition test, subjects were required to recognize 10 learned odors from 10 unlearned odors. The results were as follows: (i) in general, recognition performances for familiar odors were higher than for unfamiliar odors; (ii) experimentally presented verbal labels did not facilitate recognition performance of familiar odors after a short retention interval, whereas they were useful for recognition memory of familiar odors after a 1 week retention interval; and (iii) recognition performance decreased over retention interval and in particular that of familiar odors without labels decreased remarkably.

34. Evaluation of odor quality using a semantic differential method for five odorants of a T & T olfactometer—the case for using concrete adjectives

Syunshiro Ohnishi, Atsushi Shinkawa, Masahito Katto, Fuminori Hamanaka, Yutaka Kurioka, Michiaki Kawasaki[1] and Yasuhiro Takashima[1]

Department of Electrical Engineering, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577 and [1]Takasago International Co., 1-4-11 Nishi-hachiman, Hiratuka, Kanagawa 254, Japan

Using a semantic differential method, odor quality was evaluated for five odorants of a T & T olfactometer described in an earlier paper, in which 25 adjectives proposed by Yoshida were used. The first principal component was 'comfortable–uncomfortable' for every subject. The second and third components were 'deep', 'lively' and

'sweet'. Characters of these two components were different for different subjects. A question arises whether these three components are real odor quality. In this paper, odor quality of the five odorants is evaluated using concrete adjectives proposed by Shimoda *et al.* In the same manner, odor quality is evaluated for five odorants and principal component analysis is conducted. The following conclusions were obtained.

(i) The first component is 'rotten smell', the second 'burst smell' and the third 'floral odor' for three subjects.

(ii) On the principal component analysis using abstract adjectives, characters of the second and third principal components are different for different subjects. On the other hand, they were the same for three subjects in the analysis using concrete adjectives.

(iii) Odors A, B and D can be discriminated using the first, second and third principal component scores, but odors C and E cannot.

Proper adjectives should be added so that odors C and E can be discriminated.

35. Applying multi-channel taste sensors for quality control of soft drinks

Aoi Itami, Yoshiharu Shirasu and Hidekazu Ikezaki[1]

R&D Laboratory, Kirin Beverage Corporation, 1620 Kurami Samukawa-cho, Koza-gun, Kanagawa-Pref. 253-01 and [1]Anritsu Corporation, Japan

We have indices such as brix, acidity and pH to control the quality of soft drinks. However, we cannot strictly control the quality of products without evaluation by actual tasting.

Although sensory evaluation can detect minute differences that a chemical analyser cannot, its drawback lies in the difficulty in quantifying the results of tasting and the large variance in accuracy among individuals.

The multi-channel taste sensor SA401, which was developed by Anritsu Corporation, can simulate the sense of taste, utilizing a change in electric potentials of artificial bilayer lipid membrane. The system may have the ability to quantify taste.

We report the results of application of the taste sensor to quality control of oo-long tea products.

36. Investigation of saliva viscosity in human taste (second report)

I. Ohsawa, K. Hara, K. Koike and M. Gotoh

Department of Oral Diagnosis, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiiyoda, Tokyo 101, Japan

The authors considered that sensitivity of taste had much in relation to saliva. We investigated the relationship between taste sense and saliva volume, pH or viscosity, and reached the conclusion that the higher the viscosity of saliva the better taste sensitivity. However, this conclusion differed from previous reports. We re-examined the relationship between taste sensitivity and viscosity of saliva from the viewpoint of thixotropy hysteresis.

Subjects were 29 male students who attended Nihon University School of Dentistry. We collected saliva secretions for 15 min by holding a piece of paraffin wax in the mouth. We used a rotational viscometer (type E) with the cone at 0.48°, measured in one action of a cycle with 13 share rates: 7.5/s, 18.75/s, 37.5/s, 75/s, 150/s, 375/s, 750/s, 375/s, 150/s, 75/s, 37.5/s, 18.75/s and 7.5/s. We divided them into three groups by the hysteresis loop; viscosity curve in increasing share rate was lower than in decreasing, i.e. thixotropy, increasing was higher than decreasing, i.e. rheopexy, and increasing was crossed decreasing.

Taste sensitivity was worse by saliva in thixotropy and better in rheopexy. This result suggested that the easier it was to destroy the structure of saliva, the better taste sensitivity.

We measured the yield value of the method by Casson. There was no relationship between Casson yield value and taste sensitivity in rheopexy saliva, but in thixotropy saliva the smaller Casson yield value showed the worst taste sensitivity. These results seemed to indicate that the easier it was to destroy the saliva structure, the higher the quality of saliva and the better the taste sensitivity.

37. The therapeutic efficacy of zinc picolinate in patients with taste disorders

F. Sakai, S. Yoshida, S. Endo and H. Tomita

Department of Otolaryngology, Nihon University School of Medicine, Tokyo, Japan

We studied the therapeutic efficacy of zinc picolinate in patients with zinc-deficient and idiopathic taste disorders. The efficacy was assessed in a double-blind study. Our findings were: (i) there was a significant difference between the zinc picolinate and placebo groups in the grade of improvement in the filter-paper disk method; (ii) there was a significant difference between the zinc picolinate and placebo groups in the concentration of zinc in serum; (iii) there was no significant difference between the two groups in the degree of total subjective recovery and the grade of improvement in the whole mouth method; and (iv) we concluded that the administration of zinc picolinate is effective in patients with zinc-deficient and idiopathic taste disorders.

38. Taste and smell disorders following upper respiratory tract infection

K. Shimada, H. Ogino, T. Meteani[1], I. Takemoto[2] and I. Koizuka[3]

Department of Otolaryngology, Otemae Hospital, Osaka, [1]Department of Otolaryngology, Itami City Hospital, Itami, [2]Department of Otolaryngology, Osaka University Medical School, Suita and [3]Department of Otolaryngology, St Mananna University School of Medicine, Kawasaki, Japan

Smell and taste disorders following influenza-like upper respiratory tract infection were referred to as postinfluenza-like hypogeusia and hyposmia (PIHH) by Henkin in 1975. In this study, we examined differences in the prognosis between olfactory disorder and taste disorder in patients following upper respiratory tract infection (URI).

From 1992 to 1994, ninety-four patients complaining of smell disorder following URI visited our clinic. Twenty-four out of 94 patients also complained of taste disorder. In this study, we investigated 72 patients with smell disorder (Group S) and 20 patients with smell and taste disorders (Group B). No significant differences were found in the background history, such as age distribution, sex difference and duration of disease, between the two groups. No patients showed

pathological findings of the olfactory cleft from fiberscopic observation or radiological examination. They were therefore diagnosed as having a smell disorder caused by damage to the olfactory nerve and/or central part of the olfactory system. No pathological findings in the gustatory system were found in the patients in Group B. Forty-one per cent of the patients in Group S showed subjective improvement in olfaction. Thirty-five per cent of them also showed objective improvement in olfaction as evaluated by a T&T olfactogram, whereas the patients in Group B showed 22 and 29% improvement, respectively. Subjective and objective improvement rates of taste disorders in patients in Group B were 94 and 33%, respectively.

39. Drug-induced olfaction and taste disorders. Statistics in our clinic

Tsunemasa Aiba, Midori Sugiura[1], Junko Mori[2] Kenta Tomiyama and Yoshiaki Nakai

Department of Otolaryngology, Osaka City University Medical School, [1]PL Hospital and [2]Tane Hospital, 1-5-7 Asahimachi, Abeno-ku, Osaka 545, Japan

Cases of drug-induced olfaction and taste disorders in Osaka City University Medical School Hospital Olfaction and Taste Clinic were investigated. In the past two and a half years, we have experienced 13 cases of drug-induced olfaction disorder (2.8% of olfaction clinic patients) and 23 cases of drug-induced taste disorder (13% of taste clinic patients). In addition, 41 and 39 patients, respectively, were suspected of having drug-induced disorders.

Tegafur and its derivatives are common causes of olfactory disorder, while drugs for hypertension, cardiac disease, nervous disease, psychosis, gout and anti-cancer chemotherapy are common causes of taste disorder. Serum zinc values of the patients were relatively low, but there was no significant differences in serum zinc among causative agents. On T&T olfactometry, olfactory function was found to be severely damaged in cases of olfactory disorder, while for the filter paper disk method relatively mild damage to taste function in gustatory disorder. The rate of improvement was about 60% for both olfactory and taste disorders.

Over 130 kinds of drugs have already been reported as causing olfaction and taste disorders. Long-term and various other types of administration of drugs are important risk factors for these disorders. Physicians should attend to the possible occurrence of olfaction or taste disorder as a side-effect of therapeutic drug administration.

40. Evaluation with the Smell Identification Test (SIT) international version of treatment of odor dysfunction

Toshi Matsuda and Shunkichi Baba

Department of Otorhinolaryngology, Nagoya City University, School of Medicine, Kawasumi 1, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan

Forty patients (17 men and 23 women) who had odor dysfunction caused by paranasal diseases, nasal allergies or post-upper respiratory infections were treated with nasal drops of 0.1% betamethasone solution, systemic clarithromycin therapy for sinusitis and systemic Azelastin hydrochloride therapy for nasal allergies. Smell sensitivity of each patient before and after treatment was evaluated with the Smell Identification Test (SIT) international version and the intravenous smell test as objective tests. At the same time, a questionnaire about smell sensitivity was performed as a subjective test for each patient and the results were quantified. The intravenous test smell test using Alinamine (prosultiamine, garlic-like smell) is most widespread and well known as a smell sensitivity test in Japan. Improvement of smell sensitivity after 3 months treatment was represented by the score of the questionnaire and significantly correlated with the score of the SIT. No significant correlation was observed between the questionnaire and the intravenous test. We conclude that the SIT international version reflects smell sensitivity more precisely than the intravenous smell test and is a useful tool in the otorhinolaryngology clinic. However, SIT has several problems, e.g. odorants of the SIT are not familiar to the Japanese population, especially the aged.

41. Estimation of the gustatory area of human cerebral cortex with measurement of evoked magnetic fields

Tatsu Kobayakawa, Sachiko Saito, Saho Ayabe-Kanamura, Hisashi Ogawa, Hiroshi Endo, Yoshiko Yamaguchi, Toru Kumagai and Tsunehiro Takeda

National Institute of Bioscience and Human-Technology, University of Tsukuba, Kumamoto University, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan

Only a few experiments have been reported about the gustatory-evoked potential of humans, because gustatory stimuli are liquids and hence their presentation is difficult to control. Needless to say, no study on gustatory-evoked magnetic fields (GEMs) have been reported. We have therefore developed a new gustatory stimulation apparatus which has about 20 ms rise time of the stimulus. Using this apparatus and a 64-channel whole-cortex SQUID system, we have measured GEMs and estimated the source of gustatory field current in the human cerebral cortex.

We used 0.003 M saccharin and 1 M NaCl as the tastants. Forty trials were presented to each subject. The duration of each stimulus was 400 ms, then deionized water was presented for rinsing for about 30 s.

Two dipoles were estimated with both saccharin and NaCl. They seemed to be located near the somatosenosry area. In the case of NaCl, cortex activity was observed after presentation of the tastant. In the case of saccharin, however, it was observed 130 ms after presentation. On the other hand, the difference in reaction time of NaCl and saccharin is about 110 ms. The difference in the activity latencies of these tastants is in agreement with our findings on the reaction times.

42. Distribution of intracerebral electric source of alpha waves against odor stimuli

Tomoyuki Yoshida and Hiroko Yano[1]

Physioinformatics Laboratory, Human Informatics Department, National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba, Ibaraki 305 and [1]Kobayasi Pharmaceutical Co. Ltd, R&D Department, 13-35 3-chome, Mitsuyaminami, Yodogawa-ku, Osaka 254, Japan

In this study, an experiment was conducted for the purpose of examining the distribution of intracerebral electric source of alpha waves against odor stimuli. EEGs against four kinds of odor (sandalwood, terpen, peppermint oil and perilla aldehyde) were unipolar leads and recorded from 21 points on the scalp with a reference to the right earlobe to presume alpha waves electric source with off-line. After measuring the form of their heads, four subjects were given odor at a distance of 30 cm from the nose from an odor presenting machine which was set in a sealed room, alternately sending and intercepting odor infiltrated onto filter paper by a wind at 2 min intervals. The alpha waves recorded were analysed over 500 ms, 2 s after the odor presentation. The results of this experiment indicate that electric volume at the frontal region tends to increase compared with when odor was intercepted, and at the frontal region electric source occurs in the right hemisphere at higher frequency than in the left hemisphere. This phenomenon is discussed from the viewpoint of negative mood evoked by odors.

43. Comparison of odor event-related potentials in two different stimulation methods

H. Kida, I. Koizuka[1], K. Shimada[2], R. Seo[3], H. Yano[4] and M.Tonoike[5]

Toyonaka Hospital, Oka-uenotyo, Toyonaka City 560, [1]St Marianna Medical University, [2]Otemae Hospital, [3]Kouseinenkin Hospital, [4]National Osaka Minami Hospital and [5]Life Electronics Research Center, Osaka ETL, Japan

In 1966, Finkenzeller reported a chemosensory-evoked potential (CSEP) for the first time. Since then, several stimulating methods to evoke CSEP have been reported. Therefore, the shape of potentials and latency obtained by each method often seemed different. We have applied a blast method, in which a pulse of odor gas is administered in the nasal cavity directly via a tube in the nostril. Kobal has reported another method, an isolation technique, in which subjects are asked to close their epipharynx to isolate the nasal cavity from the respiratory tract, then a constant air stream is directed into one nostril together with a pulse of odor gas.

In this study we compared the two methods in seven normal subjects. One was the blast method, the other the isolation technique where an odor pulse was created by pouring gas into a constant air stream.

Responses obtained by each method showed very similar wave form but different latencies. They consisted of a fuge positive peak which rose steeply and fell very slowly, with a small negative peak sometimes coming just before the positive peak. Peak latency was almost 80 ms later by the flow method. This may be because of the length of the stimulation, or in the flow method odor might be spread more extensively in the nasal cavity than in the blast method because of the air stream. In both methods, air by itself produced no response.

When subjects were instructed to ignore the odor stimuli by giving them a task, such as reading a book, no EEG responses were obtained.

In the present study we found no differences in the responses obtained by each stimulation method. Responses obtained by each stimulation method are thought to be intrinsic, so-called olfactory event-related potentials.

44. Measurements of olfactory event-related magnetic fields using a whole-cortex type biomagnetometer system

Mitsuo Tonoike, Yoshiaki Adachi, Masamine Takebayashi, Atsushi Maeda[1], Hideto Kawai[1], Isao Kaetsu[1], Shinichi Yoshimura[2], Izumi Koizuka[3] and Noriaki Kishi[4]

Life Electronics Research Center, Electrotechnical Laboratory, Amagasaki 661, [1]Faculty of Science and Technology, Kinki University, Higashi, Osaka 577, [2]Asuka Electric Co. Ltd, Osaka 531, [3]St Marianna Medical School, Kawasaki 216 and [4]Nippon Denso Co. Ltd, Kariya 448, Japan

The aim of this study was to measure and analyse odor event-related magnetic fields using our whole-cortex type biomagnetometer system. This MEG system is a 122-channel first order planar type SQUID gradiometer (Neuromag-122 system) set up in a four-layer magnetically shielded room (MSR) in ETL-LERC. Amyl acetate (about 1%) was administered to either the right or left nostril of a subject's nose under synchronization with his respiration using an attached mask and optical fiber sensor. The odorant gas of 300 ms duration was administered to the subject in the MSR by changing the action of air pressure valves by operating the electric valves of the olfactory stimulator near the outside wall of the MSR.

Six healthy volunteers (male subjects) participated in our olfactory MEG experiments. Noise from the valves was masked by using earplugs and presenting white noise to the subjects. Eye movements and blinking were prevented by having the subjects stare at a fixed point on the wall in front of them.

Clear responses of olfactory event-related magnetic fields were obtained symmetrically from both sides near the forehead area in all subjects. From estimations using the two-dipole method, two dipoles were located almost symmetrically on both sides, but with a slight dominance of the ipsi-lateral side in a fairly deep region near the forehead area.

45. Event-related potentials as a measure of the effect of the odor of coffee on human brain function

Kazumi Nakagawa, Yoshihiko Koga, Kenichi Takemasa, Akiko Fujimura[1] and Shizue Ohno[1]

Department of Neuropsychiatry, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181 and [1]Department of Clothing, Faculty of Home-Economics, Japan Women's University, 2-8-1 Mejirodai, Bunkyo-ku, Tokyo 112, Japan

To investigate changes in brain function induced by the odor of coffee, ERPs were measured in 40 healthy students inhaling the odors of

coffee, lavender oil, lemon oil and isovaleric acid. Distilled water was used as an odorless control. ERPs were recorded from four scalp sites (Cz, Pz, P3, P4) while the subjects performed a visual oddball task.

An odor × sex × electrode site analysis of variance was performed on the peak latency value. The analysis revealed no significant differences among the samples, although a significant interaction was found between odor and sex ($F = 3.5407$; $P < 0.01$). An odor × sex × electrode site analysis of variance for peak amplitude showed significant F values for odor ($F = 5.9379$; $P < 0.01$), sex ($F = 6.8616$; $P < 0.01$) and interaction between odor and sex ($F = 2.7505$; $P < 0.05$). The P300 amplitude of female subjects when inhaling the odor of coffee was significantly higher than for isovaleric acid and lavender oil, but this result was not obtained in male subjects. The P300 amplitude of the females was significantly higher than that of the males for all odors except lavender oil.

It is suggested from these results that each odor affects the visual information in a different way, depending on the sex of the subject.

46. Coherence analysis of EEG changes during odor administration in humans

Hirofumi Harada, Kimio Shiraishi, Toshihiko Kato and Toyoji Soda

Department of Otorhinolaryngology, School of Medicine, Fukuoka University, Fukuoka 814-80, Japan

The purpose of this pilot study was to observe EEG changes during odor administration using coherence analysis.

The subjects were 10 normal adults. Simultaneous recordings of 16 EEG channels with and without odor administration were stored on analogue tape for further processing. EEG signals were analysed by signal analyser. Coherence spectra were calculated between all possible channel pairs on the scalp. The amount of data was reduced by extracting broad band coherence values for five frequency bands: delta (2–3.9 Hz), theta (4–7.9 Hz), alpha 1 (8–9.9 Hz), alpha 2 (10–12.9 Hz) and beta 1 (13–17.9 Hz). Coherence values extracted from the EEG of control recordings and recordings during odor administration were compared to evaluate significant differences.

In the theta and beta 1 bands, significant changes of EEG coherence during control recordings were recognized. Then we excluded these frequency bands from examination.

During odor stimulation, coherence in the delta band decreased in frontal regions and coherence in the alpha 1 and alpha 2 increased in the frontal, occipital and temporal regions.

47. Information carrier with regard to gustatory sense generation and transduction effects

Kurose Isoji

Orta University, 4-19-11-2 Minamiosawa Hachioji, Tokyo 192-03, Japan

Water is a chemically unique, special substance in that the water molecule represents a dipolar molecule. The fact that water has a di-electric constant of 80 is common knowledge. At normal room temperature, water as a dipolar molecule is structurally composed to about 60% of a dipolaron. In an electric field, in particular, it is transformed to di-electric dipolarons to almost 100%. The mixed aqueous solutions needed for the electrode system are such that their di-electric constant is set to fix their static potential. Under these conditions, transduction takes place to permit the reception responsible for the generation of gustatory perception. It also has the ability to dissolve other substances, a behavior which can also be attributed to its di-electric constant of 80. Another special property of water which deserves particular mention is that it permits d.c. power generation. The discovery that water is a d.c. generator is of the greatest importance in checking the action of water.

48. Development of salt taste responses in the bullfrog

T. Kumazawa, T. Sasaki, J. Tanaka and H. Simizu

Department of Environmental Engineering, Saitama Institute of Technology, 1690 Fusaiji, Okabe, Saitama 369-02, Japan

To learn whether salt taste responses change during development of the gustatory system, we recorded integrated responses and neural impulses in bullfrogs (*Rana catesbeiana*) aged 1–2 weeks and over several months after metamorphosis from tadpole to frog. The integrated responses were recorded from the whole glossopharyngeal nerve. The neural impulses were recorded from the single taste disks using a suction electrode. The integrated responses elicited by salts such as NH_4Cl, NaCl, KCl, $CaCl_2$ and $MgCl_2$ displayed both the phasic and the tonic components in mature frogs, whereas only phasic components were elicted by salts in immature frogs. On the other hand, salts induced two kinds of impulses, small and large, in mature frogs, whereas mainly small impulses were observed in immature frogs. The discharges of the small impulses were transient in both development stages of frogs while those of the large impulses lasted during salt stimulation in mature frogs. These results suggest tht the phasic and tonic components of the integrated responses to salts recorded from whole glossopharyngeal nerve of frogs were caused by the generation of small and large impulses, respectively.

49. Dependence of temporal pattern of taste nerve response on quality and intensity of taste stimuli

Akira Tsuboi, Yoshiki Kashimori and Takeshi Kambara

Department of Applied Physics and Chemistry, University of Electro-Communications, Chofu, Tokyo 182, Japan

The afferent taste nerves respond to a variety of taste stimuli with variation in the rate of their discharge pattern. Using a microscopic model of peripheral taste receptive system, we studied the mechanism through which the distribution patterns of interspike intervals in the nerves are induced by taste stimuli. The model consists of three main parts: a taste receptor cell, a synaptic connection and a primary peripheral neuron (afferent taste neuron). The calculated patterns for salty, sour and sweet stimuli may reproduce well the characteristic features of the observed patterns. The calculated results suggest the following property of temporal pattern of taste nerve response. The distribution pattern of interspike intervals induced by NaCl and HCl tends to become exponential with increasing stimulus strength. The

distribution pattern is apt to become bimodal and changes from bimodal to gamma with decreasing stimulus strength. The distribution pattern may encode stimulus strength for salty and sour stimulation. On the other hand, the distribution pattern is bimodal for sucrose stimulation and quite insensitive to the concentration of sucrose. The distribution pattern for sweet stimulus may encode taste quality.

50. Responses to salts in the chorda tympani nerve of the rat: effects of amiloride and La ions on responses to Na salts in the regenerated nerve after nerve transection

Yasuyuki Kitada and Yoshihiro Mitoh[1]

Department of Physiology and [1]Research Service of Oral Function, Okayama University Dental School, 2-5-1 Shikata-cho, Okayama City 700, Japan

After chorda tympani nerve (CT) transection, taste buds degenerate and disappear. Given time, however, taste buds reappear in fungiform papillae after regeneration of the cut nerve. In the present study, recovery of Na salts response from the regenerated CT after unilateral transection of the CT lingual nerve in adult rats was investigated using amiloride. Ratios for all responses were calculated relative to the 0.5 M NH_4Cl response. Taste responses from the regenerated CT were first obtained 33 days after sectioning. The responses to 0.3–0.5 M NaCl and Na acetate from the regenerated CT were about 50% lower than in controls on days 3–35 and were very close to those in controls on days 38–45. Results of amiloride treatment revealed that amiloride-sensitive components in the regenerative CT were very small on days 33–35 and abruptly increased thereafter. It appears that recovery of the Na salts responses in the regenerated CT resembles the developmental increase in response sensitivity of the CT to Na salts. La ions, a tight junction blocker, at 2.5 mM did not affect amiloride-insensitive components in either the regenerated CT in operated rats or the intact CT in control rats. This suggests that paracellular pathways between taste cells are not involved in amiloride-insensitive sodium taste reception in rats.

51. Comparison of taste responses of the chorda tympani nerve between two strains of mice which have different preference for ethanol

Hideaki Kajiura[1], Yoshihisa Imai, Yoshiko Yokomukai[1] and Yuzo Ninomiya

Department of Oral Physiology, Asahi University School of Dentistry, 1851-1 Hozumi-cho, Motosu-gun Gifu 501-02 and [1]Central Laboratories for Key Technology, Kirin Brewery Co., Ltd, 1-13-5, Fukuura Kanazawa-ku, Yokohama, Kanagawa 235, Japan

To help understand the factors affecting preferences for beer, two strains of mice which differ in their acceptance for ethanol were used as a model. The responses from the chorda tympani nerve of the mice were recorded when various taste substances, including ethanol and isohumulones (main bitter components of beer) were applied to the tongue. The results showed that the neural response to isohumulones occurs not only during stimulation (on-response), but also when stimulant is rinsed off (off-response). The threshold of the on-response to iso-humulones was about 100-fold lower in the mice which reject ethanol (BALB/c) (about 0.2 mg/l) than the ones which accept ethanol (C57Bl/KsJ) (about 20 mg/l). The addition of 4% ethanol to the isohumulones seemed to produce a synergistic effect in the off-response in BALB/c mice. Furthermore, after adaptation to ethanol, the response to sucrose was suppressed in C57Bl/KsJ, but not in BALB/c mice. Our results suggest that ethanol may cause sweet sensation in C57Bl/KsJ mice and bitter sensation in BALB/c mice.

52. Influence of bilateral lesions of parabrachial taste areas on ingestive behavior in rats

Yozo Miyaoka, Tomio Shingai, Yoshihiro Takahashi and Yoshiaki Yamada

Department of Oral Physiology, Niigata University School of Dentistry, Niigata 951, Japan

Changes in food intake (FI), water intake (WI), excretion in the feces and urine (E), and body weight were measured in eight Wistar male rats during 4 weeks before (1 week) and after (3 weeks) bilateral microlesions (10–20 μA, 12–15 s; $n = 6$) of the parabrachial taste areas (PTAs). Two rats were used as sham-lesioned animals. Responses to the four basic taste stimuli applied to the tongue were recorded in all of the eight rats to identify the PTAs for the lesions and sham-lesions. A sustained decrease in FI was observed in three of the six lesioned rats in which the lateral parabrachial nucleus was lesioned. However, there was no noticeable changes in WI and E after the lesions in the three rats. Decreases followed by increases in WI and E also were observed in a lesioned rat in which a more rostrolateral portion of the lateral parabrachial nucleus was lesioned. Transient, not sustained, decreases in FI and WI were produced in the other two lesioned rats. In the two sham-lesioned rats, there were no consistent changes in FI, WI and E. The present results suggest that PTA neurons may play a role in regulation of ingestive behavior in rats.

53. Optical and electrophysiological recordings of monosodium glutamate-induced responses in mouse taste cells

Yukako Hayashi[1,2], M. Muz Zviman[2], Joseph G. Brand[2,3], Diego Restrepo[2,4] and John H. Teeter[2,4]

[1]Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan, [2]Monell Chemical Senses Center, Philadelphia, PA 19104, [3]Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, and the Veterans Affairs Medical Center, Philadelphia, PA 19104, and [4]Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104, USA

In addition to its role as a neurotransmitter, glutamate produces responses in the taste system associated with umami taste. Recordings from planar lipid bilayers containing membrane fragments from vallate and foliate taste papillae of C3H (glutamate-taster) mice, suggested that the taste response to monosodium glutamate (MSG) may be mediated by cation channels directly gated by MSG (Kumazawa

et al., 1994, *AChemS* Abstr.). We have used whole-cell patch-clamp recordings from isolated mouse taste cells and ratiometric imaging of the calcium indicator, fura-2, in isolated taste buds to characterize responses to MSG and other glutamate receptor agonists. Cells from vallate and foliate taste buds induced inward currents at −80 mV (2/7) by rapid applications of 10 mM MSG from a puffer pipette. Using digital deblurring of fura-2 measurements from cells in isolated taste buds, 1 mM MSG elicited increases in $[Ca^{2+}]_i$ in 21% (11/51) of the cells examined. The glutamate receptor agonist, NMDA (1 mM), produced increases in $[Ca^{2+}]_i$ in five of 61 taste cells, while the metabotropic glutamate receptor agonist, AP-4, elicited decreases in $[Ca^{2+}]_i$ in 12 of 69 cells, sometimes in the same cells responding to NMDA, indicating independent mechanisms. These results indicate that glutamate responses in taste cells may be mediated by several independent pathways.

54. Taste responses and action potentials of mouse taste bud cells in peeled tongue epithelia

Hidemasa Furue and Kiyonori Yoshii

Kyushu Institute of Technology, Kawatsu Iizuka 820, Japan

Electrophysiological responses of taste bud cells were investigated under whole-cell voltage-clamp or current-clamp conditions in peeled mouse tongue epithelia. The basolateral and receptor membranes of the taste bud cells were perfused with a saline solution and stimulating solutions such as deionized water or 200 mM NaCl, respectively. Each taste bud cell protruded an apical portion (θ~1.5 μm × 30 μm) from round soma (~10 μm) to taste pore. The resting potentials were ~−50 mV and the input resistances were ~5 GΩ. The taste bud cells elicited various voltage-dependent ionic currents. Almost all measured taste bud cells elicited TTX-sensitive transient inward currents and action potentials on depolarization. Even repetitive firing was recorded from a taste bud cell. The application of 200 mM NaCl to the receptor membranes adapted to deionized water elicited slow inward currents the estimated reversal potentials of which were more than 30 mV. The blocking effect of 20 μM amiloride on the NaCl-induced inward current was different from cell to cell. These results indicated that the peeled tongue epithelia supplied intact taste bud cells and suggest that action potentials contribute taste signal transduction from the receptor membrane to basolateral membrane through the long apical portion.

55. Analysis of salt-responses by whole-cell recordings from non-dissociated mouse taste cells

Takenori Miyamoto, Yukio Okada and Toshihide Sato

Department of Physiology, Nagasaki University School of Dentistry, 1-4-1 Sakamoto, Nagasaki 852, Japan

Recordings obtained from isolated taste cells using whole-cell clamp method are possibly contaminated with several side-effects unless application of taste stimuli is exactly restricted to the apical membrane area to solve this problem. We performed patch-clamp recordings from non-dissociated mouse taste cells within the taste bud while applying a taste stimulus to the pore. Under this condition, we could simultaneously accomplish whole-cell recording, visualization of taste cell morphology, localized taste stimulation and maintenance of micro-environment around the mouse taste organ. We could confirm the presence of amiloride-sensitive current through the apical receptive membrane by the localized application of 50 μM amiloride to the taste pore. The amiloride-sensitive current showed an inwardly rectifying property in 70% of the taste cells examined. Rapid responses to 0.5 M NaCl were also obtained with this method. Fifty per cent of the responses consisted of generation of the inward current at negative potential level and suppression of the outward current at the positive potential level (Type I). The rest of cells examined showed either generation of the inward current (Type II) or suppression of the outward current (Type III). The suppression of the outward current may be one of the candidates for amiloride-insensitive pathway of salt-signal transduction.

56. Effect of vasopressin (AVP) on the membrane properties of frog taste cell

Yukio Okada, Rie Fujiyama, Takenori Miyamoto and Toshihide Sato

Department of Physiology, Nagasaki University School of Dentistry, Nagasaki 852, Japan

Arginine vasopressin (AVP) enhanced the neural responses elicited by NaCl and HCl in the frogs, suggesting that taste response could be modulated by the hormone. In the present experiment, we examined that the effect of AVP on the membrane properties of taste cells isolated from bullfrog using a perforated whole-cell patch-clamp technique. Isolated frog taste cells were classified into two types of rod and wing cells. AVP (40 mU/ml) induced three kinds of responses in rod cells: appearance of inward current (four in 14 cells), decrease of outward current (three in 14 cells) and increase of outward current (two in 14 cells). The hormone had little efficaciousness against the membrane properties of wing cells. Ionomycin (Ca-ionophore, 3 μM) induced inward current at the membrane potential of −50 mV in rod cells. 8-CPT-cAMP (0.3 mM) inhibited outward current at positive membrane potential in some rod cells, while the drug increased the current in other cells. The results suggest that the receptor protein for AVP-related peptide may exist in rod cells and that AVP may increase intracellular Ca^{2+} or cAMP level in rod cells.

57. Two types of mouse single chorda tympani fibers with amiloride sensitivity differing in temperature dependency of NaCl responses

Yuzo Ninomiya, Yoshihisa Imai and Yasushi Fukami

Department of Oral Physiology, Asahi University School of Dentistry, Hozumi, Motosu, Gifu 501-02, Japan

Inhibitory effects of amiloride on salt responses and its temperature dependency were examined by recording responses of mouse single chorda tympani fibers. Single fibers responded to NaCl were divided into two groups depending on their sensitivity to amiloride (amiloride-sensitive and insensitive types). Eleven out of 21 fibers sampled were amiloride-sensitive and 10 fibers were amiloride-insensitive. Eleven

amiloride-sensitive fibers were further divided into two groups according to their temperature dependency. One group (HT type: $n = 8$) showed responses to NaCl greater at around 24°C than at around 12°C, and the reverse was true for the other group (LT type: $n = 3$). Ten amiloride-insensitive fibers showed greater NaCl responses at higher temperatures. These results suggest that there exist at least two different types of amiloride-blockable sodium pathways in the mouse taste cell whose temperature dependence in sensitivity to NaCl were different.

58. The reducing effect of Ca^{2+} ion or mixtures of stimulants on the variance of the taste transduction current on the fleshfly

Tomoki Kazawa, Shinobu Goshima, Midori Murakami and Hiromasa Kijima

Department of Physics, Faculty of Science, Nagoya University 464-01, Japan

The fluctuation of the receptor current in the flesh fly *Boettcherisca pergina* was examined, when sucrose and Ca^{2+} ion were applied.

The variance of the fluctuation caused by application of 50 mM sucrose was reduced about 30% when $CaCl_2$ 5 mM was added to stimulants compared to the variance when only sucrose was applied. This effect was observed in the wide range of sucrose concentration from 3 to 200 mM. Sucrose in the higher Ca^{2+} concentration reduced the fluctuation to a larger extent.

This effect was compared to the change of fluctuation in the process of the adaptation. The net fluctuation caused by sucrose for 4 s starting 1 s after the onset of stimulus was about 25% larger than the fluctuation during the following 4 s.

This adaptation process was concluded to be caused by the inactivation of the transduction ion channels, because (i) the reduction of the response was about the same as the reduction of the variance of the fluctuation and (ii) this effect was observed in the wide range of sucrose concentration, too.

The possibility was suggested that three fluctuation reducing-effects, (i) in the mixture of sugar receptor stimulants, (ii) in the presence of Ca^{2+} with stimulants and (iii) by adaptation, may have the same origin, that is, the inactivation of the transduction ion channels.

59. Disruptive effects of inhibitors of protein kinase C injected into the amygdala or insular cortex in conditioned taste aversion in the rat

Yasunobu Yasoshima, Yuri Takahashi and Takashi Yamamoto

Department of Behavioral Physiology, Osaka University, Suita, Osaka 565, Japan

To study the possible involvement of neuronal protein kinase activity in conditioned taste aversions (CTAs), we injected protein kinase C (PKC) inhibitors, polymyxin B (PMXB) or H-7, into both sides of the amygdala (Amy) or insular cortex (IC) in the rat. Immediately after the rats ingested 0.005 M sodium saccharin (conditioned stimulus, CS), PMXB or H-7 was injected into the Amy or IC. With a 30 min interval, they received an i.p. injection of LiCl (unconditioned stimulus, US; 0.15 M, 2% of b.wt). The injections of PMXB or H-7 disrupted CTA acquisition significantly in comparison with the injections of the vehicle in control rats. However, PMXB infusion into the vicinity of Amy was ineffective and infusion of HA1004, which has little effects on PKC, into the Amy did not prevent CTA acquisition. CTA acquisition was not blocked by infusion of PMXB 4 h after CS-US pairing into the Amy or IC. When PMXB was infused into the Amy 30 min before the first postconditioning test, no disruptive effect was observed on CTAs. These results suggest that neuronal protein kinase activity in the Amy and IC participates in the brain mechanism of CTA acquisition.

60. Taste reactivities and EMG activities of masticatory muscles during ingestion of food with different tastes in the rat

Hiroyuki Sogo, Noritaka Sako and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Science, Osaka University, Suita, Osaka 565, Japan

It is known that rats show the ingestive and/or aversive behaviors in response to intra-orally administered taste solutions (Grill and Norgren, 1978). However, the effects of tastes on feeding behavior is not well analysed yet. In the present study, food-deprived Wistar male rats ate test foods made up by mixing powdered chow with one of the liquids such as distilled water, 1 M sucrose, 0.02 M saccharin-Na, 0.2 M NaCl, 20 mM HCl, and 1 or 5 mM quinine-HCl. The behavioral study revealed that rats ate a smaller amount of food containing 5 mM quinine-HCl with longer time than foods containing other tastes. Gaping, one of the aversive behaviors, was frequently observed when the rats ate food containing quinine. In the EMG study, the activities of temporalis, masseter and digastric muscles were recorded. The magnitudes of burst discharges were smaller for quinine-adulterated food than for other foods. The masticatory cycle during eating quinine-adulterated food was faster than that for other foods. The present study concludes that rats show altered eating behavior and masticatory EMG activities exclusively when they eat food with quinine taste.

61. Electrophysiological and behavioral studies on taste effectiveness of propylene glycol

Noritaka Sako and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, Suita Osaka 565, Japan

Three experiments were designed to study the gustatory effects of propylene glycol (1,2-propanediol; PG). In Experiment 1, a two-bottle preference test was performed between 0.01–10 M PG and distilled water. The rats preferred 0.1–1 M PG, but showed aversion to 3–10 M. In Experiment 2, the rats which acquired conditioned taste aversion to 1 M PG also avoided 0.5 M sucrose and 10 mM quinine-HCl. However, the aversion was not generalized to NaCl and HCl. In Experiment 3, neural responses to gustatory stimuli were recorded chorda tympani nerve. The stimuli included a fixed concentration

(1 M) of methanol, ethanol, ethylene glycol, 1-propanol, 2-propanol, 1,3-propandiol, glycerin and PG. The responses to alcohols with two alcoholic hydroxyl groups such as ethylene glycol, 1,3-propandiol, glycerin and PG were larger than those to other alcohols. The responses to these alcohols were suppressed to about 20% by treatment of the tongue with 2% pronase E, a proteolytic enzyme. The present study suggests that PG has a taste similar to the taste of both sucrose and quinine, and its relevant receptor may be comprised of a protein.

62. Fiber types of the nerves which supply the oropharyngolarynx and the cardiovascular responses in rats

T. Hanamori, T. Kunitake and H. Kannan

Department of Physiology, Miyazaki Medical College, Miyazaki 889-16, Japan

The effect of repetitive electrical stimulation at 50 Hz for 20 s of the lingual branch of the trigeminal nerve (LN), chorda tympani (CT), lingual-tonsillar branch of the glossopharyngeal nerve (LT-IXth), pharyngeal branch of the glossopharyngeal nerve (PH-IXth) and superior laryngeal nerve (SLN) on the changes in arterial blood pressure (BP) and heart rate (HR) were investigated in anesthetized and paralysed rats. The conduction velocities of the components in the compound action potentials were also investigated to know the relationships between the fiber types and the cardiovascular responses. Electrical stimulation of the nerves except the CT elicited a tachycardia and an increase of BP. The cardiovascular responses were related to the component-2 in the compound action potentials in respective nerves. The mean conduction velocities of the component-2 among the nerves were between 9.5 and 17.0 m/s. Activation of the fibers with faster (component-1) or slower conduction velocities (components 3 and 4) did not seem to evoke the cardiovascular responses. These results suggest that the pain and taste fibers of A-delta fibers contribute to elicit the cardiovascular responses.

63. Modulative effects of acetylcholine on cortical taste neurons in rats

K. Hasegawa and H. Ogawa

Department of Physiology, Kumamoto University School of Medicine, Kumamoto 860, Japan

Acetylcholine receptors are widely distributed throughout the cerebral cortex in rats. It is reported that acetylcholine modulates neural responses in visual and somatosensory cortices (Sillito and Kemp, 1983; Lamour *et al.*, 1982). Transplantation experiment of the gustatory cortex suggests importance of acetylcholine in the cortex in rats (Lopez-Garcia *et al.*, 1990). To examine modulatory effects of acetylcholine on cortical taste neurons, we recorded taste neurons from the cortical taste area (CTA) with multibarreled micro-electrodes in urethane anesthetized SD-strain rats, and iontophoretically applied to them acetylcholine and its antagonists (atropine, pirenzepine as muscarinic antagonists and mecamylamine as a nicotinic antagonist). Acetylcholine did not affect spontaneous discharges in most taste neurons (~85%). However, it affected the response profiles of about 50% of the taste neurons to the four basic taste stimuli and even altered the best stimuli: acetylcholine increased taste responses in 23.6% of the taste neurons tested, but decreased them in 22.2%. Effects of muscarinic and/or nicotinic antagonists on taste responses were opposite to acetylcholine action in most of the neurons tested. It is suggested that CTA neurons have muscarinic and/or nicotinic receptors and that acetylcholine acts as a neuromodulators through second messengers in some CTA neurons.

64. Application of patch clamp method to sensory processes of fly labellar taste cells

Midori Murakami, Tomoki Kazawa, Shinobu Goshima and Hiromasa Kijima

Department of Physics, Nagoya University, Japan

Although the transduction mechanisms of the fly taste receptor have been studied intensively, it has been difficult to apply the patch clamp method, since the fly taste receptor cells are surrounded by the cuticle layer. We cut the labeller taste hair of the pupa just before eclosion in Ca-free Ringer solution, the sensory dendrites grew out from the cut end 30 min after cutting and swelled after 6 h. The diameter of the swelled dendrite was about 5–10 μm, so they were suitable for application of patch clamp technique.

In cell-attached mode, the spontaneous firing of impulses were observed, but single channel currents triggered by sugars in pipette solution were not observed. In whole-cell configuration, the impulses were found both in voltage- and current-clamp mode. In former, positive current injection also generated trains of voltage spikes. Upon puffing sucrose and K^+ ion mixture toward outer membrane, inward current was evoked. Injection of cyclic GMP from the patch-pipette also induced inward current. These results suggest that the fly taste transduction may involve the cascade mediated by second messenger, cGMP, as well as the receptor-channel complex.

65. Involvement of Gq type of G protein in the taste system of the fly

W. Idei, M. Ozaki and F. Tokunaga[1]

Departments of Biology and [1]Earth and Space Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

In the taste organ of the blowfly, *Phormia regina*, we previously reported that IP3 or DG affects the sugar taste response, suggesting an involvement of Gq type of G protein in this cell.

By northern hybridization with partial cDNA sequence derived from the labellum of the blowfly, we showed the expression size of the Gq protein in the taste organ to be 3 kbp. Besides the labellum, other parts of tissues indicated this size of expression. Additionally, a large amount of expression was shown especially in the head, but its size was smaller than 2 kbp.

This result suggested that the Gq protein was generally expressed in various tissues. Thus, we investigated whether the Gq protein was actually involved in the taste organ or not, using the antiserum against the *Drosophila* Gq protein, which can cross react with the *Phormia* Gq protein. Immunoblot of the protein extract from isolated labellar taste sensillum showed a clear reaction signal with the antiserum. It indicated that there is a 3.9 kDa Gq protein in the isolated taste

sensilla. The taste sensillum includes the sensory processes of the four functionally different taste cells, but does not include the cell bodies and axons. Thus, it was strongly suggested that the Gq protein functions in the receptive region of the taste cells.

66. Direct transfer of taste-receptor proteins by artificial boundary lipid-containing liposome

Mikihiko Nakamura, Kaoru Tsujii and Junzo Sunamoto[1]

Kao Institute for Fundamental Research, Tsujii Laboratory and [1]Division of Synthetic Chemistry & Biological Chemistry, Graduate School of Engineering, Kyoto University, Japan

This work first provides that epithelial membrane proteins can be directly transfered from animal intact tissue to liposome. Bullfrog tongues were treated with a specially modified liposome that consists of an artificial boundary lipid, 1,2-dimyristoylamido-1,2-deoxyphosphatidylcholine (DDPC, 40 mol%) and an ordinary phospholipid, 1,2-dimyristoylphosphatidyl-choline (DMPC, 60 mol%), as well as that consisting of DMPC alone. Glossopharyngeal nerve responses of the treated tongue were then measured to five taste stimuli (NH4Cl, L-Ala, sucrose, L-Leu and quinine hydrochloride). The DDPC/DMPC liposomal treatment caused remarkable changes of the taste nerve responses, but the DMPC liposome did not. On gel electrophoretic analysis of the treated two kinds of liposomes, some protein bands were observed on only DDPC/DMPC liposome portion.

67. Use of sweet taste inhibitory peptide, gurmarin to find candidates of the sweet taste receptor in rats

Toshiaki Imoto and Yuzo Ninomiya[1]

Department of Physiology, Faculty of Medicine, Tottori University, Yonago 683 and [1]Department of Oral Physiology, Asahi University School of Dentistry, Hozumi, Gifu 501-02, Japan

Gurmarin, a sweet taste inhibitory peptide from leaves of *Gymnema sylvestre*, is thought to manifest the effect by interacting with the sweet taste receptor(s) on apical membrane of the taste cell. In order to find candidates of the receptor, we tried to detect proteins in taste papillae of the rat tongue that have affinity for biotinylated gurmarin, which could enable us to utilize high detection sensitivity of chemoluminescence (CL) through peroxidase-coupled streptoavidin. Fungiform papillae and circumvallate papillae were isolated from anesthetized rat tongues together with epithelial tissue containing no taste papilla and frozen by immersing them into liquid nitrogen. SDS gel electrophoresis was performed on water soluble and SDS soluble fractions prepared from the homogenates of each tissue, followed by Western blotting on a nitrocellulose membrane. Chemoluminescence detection showed at least three positive bands which were commonly observed in SDS soluble fractions of both fungiform and circumvallate papillae, and not in the control epithelium. Taking detection limit of CL into consideration, the amount of these proteins were estimated to be less than several tens of pg per taste bud. The present study would afford a prospect of isolation and identification of the sweet receptor proteins by biochemical approach.

68. G-proteins expressed in carp taste bud cells

Yuji Amagai, Mitsuru Tsuji, Takayuki Marui, Mamiko Ozaki[1] and Fumio Tokunaga[2]

Department of Physiology, Ohu University School of Dentistry, Koriyama 963 and Departments of [1]Biology and [2]Earth and Space Science, Faculty of Science, Osaka University, Toyonaka 560, Japan

Carp has a highly sensitive taste system, and its barbels and lips have a large number of taste buds. So, the carp is suitable for studies on taste signal transduction. It has been shown that vertebrate taste cells express a G-protein α-subunit, α-gustducin, which is closely related to a visual G-protein, α-transducin. Recently, α-transducin itself was also shown to be involved in gustatory transduction mechanism. In an attempt to detect cDNA of G-protein α-subunit in the carp taste cells, poly $(A)^+$ mRNAs were obtained from the barbels and lips, and were used for templates for RT-PCR. Primers having sequences of conserved regions of G-proteins were used. The PCR resulted to amplify DNA fragments of about 300 bp. Analyses of nucleic acid sequences and deduced amino acid sequences showed that they are related to partial sequences of two types of G-protein, G_i and G_s. The sequence of the G_i type resembled that of α-gustducin. *In situ* hybridization with antisense RNA probe synthesized with the G_i type DNA showed reactions exclusively within taste bud cells. These results strongly suggest that the G_i type clone can be a partial nucleic acid sequence of a putative gustducin-like G-protein of the carp taste cells.

69. Mechanism of suppression of bitter taste responses by phosphatidic acid-containing lipoproteins adsorption on frog tongue surface and hydrophobic model membranes

Yoshihisa Katsuragi, Yoko Sugiura, Kazuya Otsuji and Kenzo Kurihara[1]

Kao Corporation Food Products Research Laboratories, Kashima 314-02 and [1]Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Previously, we found that a lipoprotein composed of phosphatidic acid (PA) and β-lactoglobulin (LG) selectively suppressed the taste responses to bitter substances in the frog and humans. In the present study, we found that the PA-LG having the high inhibitory activity is adsorbed on the frog tongue surface while lipoproteins having no activity are not adsorbed. We also examined adsorption of lipoproteins on model lipid membranes coated on a quartz-crystal microbalance by measuring its frequency changes. The lipoproteins having the inhibitory activity were strongly adsorbed on the hydrophobic lipid membranes, while the lipoproteins having no inhibitory activity were little adsorbed on the membranes. There was a close relationship between inhibitory activities and adsorption ability to the hydrophobic membranes. It seems that receptor sites for bitter substances on the

taste cell membranes are hydrophobic and those for other taste stimuli such as salts, acids and sugars are hydrophilic. Hence, the binding of PA-LG to hydrophobic sites of receptor membranes will lead to selective inhibition of bitterness.

70. Expression of cDNA encoding a sweetness-inducing protein, miraculin, in yeast

Satoru Nirasawa, Yutaka Masuda, Kazuyasu Nakaya[1] and Yoshie Kurihara

Department of Chemistry, Faculty of Education, Yokohama National University, 156 Tokiwadai, Hodogaya-ku, Yokohama 240 and [1]Laboratory of Biological Chemistry, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 240, Japan

The DNA encoding pre-miraculin (*MIR*) and matured miraculin (*MIRPCR*) were cloned into the expression vector, pYPR3831 and pYE RNAP-Rh. These miraculin expression vectors, pYMI-1, pYMI-4 and pYMI-5, contain *GAL* promoter, *MIR*, *GAPDH* promoter, *MIRPCR* and *GAPDH* promoter, RNase Rh signal sequence, *MIRPCR*, respectively.

Plasmids pYMI-1, pYMI-4 and pYMI-5 were transformed into yeast strain EH13-15 by the lithium acetate procedure. Yeast transformants were grown on SD medium at 30°C for 48 h. After culture, yeast EH13-15/pYMI-1 was induced by galactose at 30°C for 3 h. The cells were lysed and the extract was checked for protein. Miraculin was not detected in cell extracts and mediums on SDS-PAGE gel following Coomassie Brilliant Blue R staining. The presence of miraculin in the yeast EH13-15/pYMI-1 extract was confirmed by immunoblotting using anti-miraculin serum. Northern blot analysis showed that the band of miraculin mRNA was detected in yeast EH13-15/pYMI-1 and EH13-15/pYMI-5. However, miraculin mRNA was slightly expressed in yeast EH13-15/pYMNI-5.

The sweetness-inducing activity of miraculin in the yeast EH13-15/pYMI-1 extract was assayed by human sensory test. The yeast extracts which were purified by ammonium sulfate fractionation showed no sweetness-inducing activity.

71. Effects of feeding on capsaicin-containing diet on components of rat saliva

Hideo Katsukawa and Yuzo Ninomiya

Department of Oral Physiology, Asahi University School of Dentistry, Hozumi, Motosu, Gifu 501-02, Japan

Application of capsaicin to the tongue and palantinal mucosa causes salivation and neonatal exposure to the drug decreases amounts of substance p in the salivary gland. We investigated effects of feeding on a diet supplemented with capsaicin on components of submandibular saliva of the rat. Male Wistar rats were fed on diets containing 0.0001–0.05% capsaicin (cap-diet) for 3–7 days before assay. Diet intake on the first day of feeding was less in 0.01 and 0.05% cap-diet groups than in control-diet group. Such intake of 0.0001 and 0.001% cap-diet group was similar to that of control-diet group. On the third day, diet consumption in 0.01 and 0.05% cap-diet groups returned to a level of control-diet group. Cystatin-like substance was noticed on the basis of chromatographic and electrophoretic data to be induced in submandibular saliva 3 days after the beginning of the feeding on cap-diet. Induction of this peptide by cap-diet was not mimicked by systemic adminstration of capsaicin. After bilateral dissection of the glossopharyngeal nerve, diet intake decreased in 0.001–0.05% cap-diet groups. Cystatin-like substance was decreased in these groups. The results suggest that oral sensation is involved in induction of cystatin-like substance and that changes in components of submandibular saliva enable animals to ingest capsaicin-containing diet.

72. *C-fos* expression in the brain stem neurons in response to intragastric infusions of various taste solutions in the rat

Kosuke Sawa, Nobuyuki Sakai and Takashi Yamamoto

Department of Behavioral Physiology, Faculty of Human Sciences, Osaka University, 1-2 Yamadaoka, Osaka 565, Japan

We have previously shown that ingestions of taste solutions induce the expression of a proto-oncogene, *c-fos*, in a chemotopic pattern within the parabrachial nucleus (PBN) of the rat (Yamamoto *et al.*, *Physiol. Behav.*, 56, 1197–1202, 1994). The present study was conducted to examine the pattern of *c-fos* expression in the PBN by post-ingestional factors. Therefore, an aliquot of 7.5 ml of each taste solution was intragastrically infused, and the expression of *c-fos* was immunohistochemically detected by a conventional LSAB method. Most remarkable *c-fos* expression was induced in the external lateral subnucleus (els) of the PBN by infusions of LiCl and ethanol which are known to be unconditioned stimuli in conditioned taste aversions. Carbohydrates including polycose and sugars such as glucose, fructose, galactose, sucrose and maltose induced *c-fos* expression in the dorsal lateral subnucleus (dls) as well as or stronger than in the els. Saccharin, glycine and alanine, ingestions of which induced *c-fos* in the els, did not induce *c-fos* in the dls, indicating the existence of gustatory inputs, as well to the dls. The present results showed that the dls received gustatory and post-ingestional (visceral) inputs, and suggest that the dls may play a role in conditioned taste preferences.

73. Immunohistochemical localization of serotonin, protein gene product 9.5 and neuron-specific enolase in developing mouse vallate papillae

M. Kudoh

Department of Biology, Fukushima Medical College, Hikariga-oka, Fukushima 960-12, Japan

Immunoreactivity to serotonin, protein gene product (PGP 9.5) and neuron-specific enolase (NSE) was surveyed immunohistochemically in the mouse vallate papillae with a confocal laser scanning microscope. Serotonin-like immunoreactivity was confined to several cells in the taste bud obtained from the mouse treated with 5-HTP. These immunoreactive cells, presumably type III cells, were slender in shape, extending apically from the base to the taste pore, and such profiles were quite similar to those of monoamine-containing fluorescent cells.

PGP 9.5-like and NSE-like immunoreactivity was recognized in some taste bud cells supposed to be type III cells, and also in intragemmal, as well as subgemmal nerve fibers. These findings suggest that serotonin may be useful as a good marker to type III cells and that either PGP 9.5 or NSE may be available for a marker both to taste bud cells and to nerve fibers. In infant mice, PGP 9.5-like and NSE-like immunoreactivity appeared in several clusters of the cells, presumably the progenitor of taste buds, in the apical epithelium of undifferentiated vallate papillae at 1–2 days after birth, while serotonin-like immunoreactivity appeared within taste buds at 4–5 days when taste perception was considered to be functional by completion of taste pores.

74. Developmental change in distribution and morphology of taste buds over the oral cavity in mammals

Keiko Yamaguchi, Shuitsu Harada and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, Kagoshima 890, Japan

The soft palate and the tongue were examined histologically in the rat (SD), mouse (C3H), gerbil (Mongolian), marmoset and cat at different age. A distribution map of taste buds on the soft palate was reconstructed and the number of taste buds was counted in each animal. A number of taste buds observed on the soft palate at birth was 113 for a rat, 81 for a mouse and 165 for a marmoset, while that was 14 for a gerbil and only eight for a cat. Although there existed species difference among those animals for the total number and distribution of taste buds, the number of those on the soft palate increased more promptly with increase of age than those on fungiform papillae, then reached to the maximum until 3 weeks for the rat and mouse, and until at least 2 months for the marmoset. The rate of the number of taste buds with taste pore at birth was 57.5% for a rat, 48.1% for a mouse and 82.4% for a marmoset at birth. On the other hand, the rate on fungiform papillae was 9.0%, 18.2% and 60.1%, respectively. These observations suggest that the function of the soft palate might play an important role as a source for gustatory input on the early stage of development in mammals.

75. Daily periodicity of urinary marking behavior in mice

Tsuneo Hatanaka

Department of Biology, Faculty of Education, Chiba University 1-33 Yayoi-cho, Inage-ku, Chiba 263, Japan

Mice deposit urine marks on their home range or novel environments, their marking patterns varying according to their sex, social rank, experience and reproductive state. In order to investigate whether the urinary patterns are reflected by locomotor patterns, motility of the mouse urinary marking behavior in an experiemental field with a previously deposited urine mark of other mouse was observed. The coordinate of mouse head and tail was corrected at interval of 1/3 s over 10 min and the various parameters were calculated. Moreover, in order to analyse the most active marking behavior, the amount of marking at four different times of the day (early morning, daytime, late evening and midnight) was compared.

Male mice showed a daily periodicity in urine marking, while female mice did not so obviously. The previously deposited urine marks of other mice, decreased the male's marking during the day, but in the dark period, the urine spots of females facilitated the male's marking. There was no sexual difference in the motility (total distance, speed, active period, etc.), although the marking patterns were evidently different between males and females. The previously deposited marks in the experimental field suppressed the activity regardless of sex. So it is concluded that there is no relation between the motility and the marking pattern.

76. Activation of chloride conductance in the olfactory cilia by various divalent cations

Hirokatsu Kobayashi, Tomo-Oki Satoh and Tadashi Nakamura

Department of Applied Physics and Chemistry, Faculty of Electro-Communications and Department of Information Network Science, Graduate School of Information Systems, University of Electro-Communications, Chofu, Tokyo 182, Japan

It was previously demonstrated that Ca^{2+}-gated chloride channels in vertebrate olfactory receptor cilia carry a part of the transduction current amplifying the odor response. However, this channel itself has been rather less studied. We examined the capacity of divalent cations to activate the Cl^- conductance using the ciliary patch preparations from frog olfactory cell. Ba^{2+} and Mg^{2+} were tested among the alkali earth metals, to which Ca^{2+} belongs. The conductance was activated by Ba^{2+} with a dose dependency ($K_{1/2}$: ~1 mM), but not by Mg^{2+}. Reversal potential of Ba^{2+}-gated conductance was dependent of the gradient of Cl^- concentration across the patch membrane, which could be fitted to the Nernst equation. In addition, it was blocked by niflumic acid, the chloride channel blocker. Thus, we confirmed that the Ca^{2+}-sensitive chloride channels were activated by Ba^{2+}. We also found that other divalent cations such as Mn^{2+} or Ni^{2+} could activate the conductance as Ba^{2+}. It is difficult to suppose that these divalent cations have the physiological role to increase the conductance. The present results may rather aid in studying the olfactory transduction by calling the further cautions to the use of divalent cations in experiments.

77. Change of Cl^- concentration by odor stimulation in the olfactory receptor cell

Hiroshi Kaneko[1], Nobuteru Nishida[2] and Tadashi Nakamura[1,2]

Department of Information Network Science, Graduate School of Information Systems and [2]Department of Applied Physics and Chemistry, Faculty of Electro-Communications, The University of Electro-Communications, 1-5-1 Chofugaoka, Chofu, Tokyo 182, Japan

It has been reported that the Cl^- efflux to the vinicity of the olfactory cell through Ca^{2+}-sensitive channels in the olfactory cilia amplifies the depolarizing current in the odor response. This result provides a rather unique example among the Cl^--channels studied some of which

have been reported to lead to Cl^- influx giving hyperpolarization to cells with low concentration of intracellular Cl^- ($[Cl^-]_i$). For the suggested role of the olfactory Cl^--channels, the cell requires to have high $[Cl^-]_i$. Although the $[Cl^-]_i$ was estimated by reversal potentials of the odor-responses, it is important to measure the $[Cl^-]_i$ directly.

Here, we report preliminary results of the direct measurement of resting $[Cl^-]_i$ and change of $[Cl^-]_i$ induced by odorants. We loaded Cl^--sensitive fluorescent dye, N-(6-methoxyquinolyl)-acetoethyl ester (MQAE), to olfactory cells isolated from newts. Fluorescence images of the cells were recorded with a SIT-camera and their fluorescence data were later analysed. To measure the resting $[Cl^-]_i$, perfusate was changed to standard solutions of various Cl^--concentrations containing Cl^--ionophore, tributyltin (20 μM). The resting $[Cl^-]_i$ was found to be about 105 mM. On the other hand, stimulation of the cells were performed by exchanging the perfusate to that containing odorants. Then, $[Cl^-]_i$ was found to decrease, prominently in the knob. These results are consistent with the previous reports that Cl^- effluxes through the Ca^{2+}-sensitive channels in the olfactory knob or in the cilia.

78. Identification of protocerebral olfactory and mechanosensory neurons in the male cockroach, *Periplaneta americana*

H. Ai, J. Inouchi and K. Nagasaka

Department of Insect Physiology and Behavior, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305, Japan

Numerous olfactory receptor cells populate the antennal flagella, including sensitivity to food odors and pheromone. The receptor cells project axons into the brain via antennal nerve and these fibers terminate in the deutocerebrum (DC). Olfactory information is processed in the DC by neurons with axons projecting from the DC to several regions of neuropile in the protocerebrum (PC), including the calyces of the ipsilateral mushroom body and the lateral PC. In the present study, we have identified three types of olfactory neurons and three types of mechanosensory neurons in the PC. The first type of olfactory neuron shows excitation that responds to *n*-hexyl alcohol, *n*-heptanoic acid, cinnamaldehyde, citral, terpineol and santalol. The second type shows excitation that responds to only terpineol. The third type shows excitations that responds to only cinnamaldehyde. The first and second types show excitation that responds to wind stimulation to the antenna. Arborizations of these neurons invade both lobus lateralis protocerebralis (LLP) and alpha-lobe of mushroom body. In contrast to these ramifications, arborizations in the superior median protocerebrum (SMP) have a beaded appearance and might be the output arborizations of the neurons. The third type shows no response to wind stimulation to the antenna. Arborizations of the neuron invade the stork of mushroom body, and arborizations in the alpha- and beta-lobe have a beaded ramification. The neurons which have no response to odor stimulation and have an excitation or an inhibition that respond to wind stimulation to the antenna have no or sparse arborization in the LLP and have arborizations with varicosities in both the alpha- and beta-lobe of the mushroom body. The neuron which has excitation to both wind stimulation to the antenna and light stimulation to the eyes has dense arborizations in the alpha-lobe and has wide arborization with varicosities in both the SMP and LLP.

79. Odor discrimination of 'cAMP-' and 'IP_3-increasing' odorants in the turtle olfactory organ

Makoto Kashiwayanagi, Kouhei Inamura, Fumiko Nagasawa and Kenzo Kurihara

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

The ability of the turtle olfactory system to discriminate between various cAMP- and IP_3-increasing odorants in the olfactory bulb was examined by the cross-adaptation technique and analysed by a multidimensional scaling. The mean values of the degree of discrimination among the IP_3-increasing odorants were higher than those among the cAMP-increasing odorants, and were similar to those between cAMP- and IP_3-increasing odorants. The degrees of discrimination among the IP_3-increasing odorants at 25°C were greater than those at 40°C, while those among the cAMP-increasing odorants at 25°C were similar to those at 40°C. The present results suggest that the features of the receptors of cAMP-increasing odorants are different from those which respond to IP_3-increasing odorants. Analysis by multidimensional scaling suggested that the difference in second messenger pathways is not related to detecting odor quality in the turtle olfactory system.

80. Carnosine evoked inward current in organotypic slice cultured neuron in the rat olfactory bulb

Kentarou Kanaki, Sachiyo Kawashima, Makoto Kashiwayanagi and Kenzo Kurihara

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

A high concentration of carnosine (β-alanyl-l-histidine) exists in olfactory epithelium and olfactory bulbs. It was reported that depolarization released carnosine from mouse olfactory bulb synaptosomes depending on external calcium ions, suggesting a possibility that carnosine is a neurotransmitter between olfactory receptor neuron and neuron in the olfactory bulb. However, no experimental result clearly indicating that carnosine is a neurotransmitter has been reported.

In the present study, we examined the effect of carnosine in organotypic slice cultured neurons in the rat olfactory bulb using a whole-cell recording technique. There are neurons with diameter of 20–30 μm located at near the edge of slice. Depolarizing voltage steps induced fast transient inward and slow sustained outward current in these neurons. We assumed these cells as mitral/tufted cells, and used them primarily in the present experiments. Application of 5 mM carnosine to the neuron induced three types of excitatory response. In 25 of 126 neurons, it induced large inward currents with increasing conductance. The magnitude of currents varied from −10 to −1240 pA (−292 ± 61 pA, mean ± SE). In six neurons, it induced small inward currents. The magnitude of currents varied from −30 to −140 pA (−80 ± 19 pA, mean ± SE). In eight neurons, it induced inward currents without changing conductance. The magnitude of currents varied from −22 to −310 pA (−96 ± 33 pA, mean ± SE). These results suggest a possibility that carnosine is a neurotransmitter and/or modulator of the olfactory receptor neuron.

81. Enhancement of turtle olfactory responses to forskolin and odorants by increasing mucosal Na^+ concentration

Kouhei Inamura, Makoto Kashiwayanagi and Kenzo Kurihara

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Effects of an increase in mucosal Na^+ concentration on the turtle olfactory bulbar responses to forskolin and odorants were examined. The response to 0.01 mM forskolin that response via cAMP-gated channels was greatly enhanced by addition of high concentration of NaCl or Na gluconate to normal Ringer's solution, but was unchanged or enhanced only slightly by addition of high concentrations of choline Cl or mannitol. On the other hand, the degree of enhancement of the response to cAMP-increasing odorants such as citralva, eugenol and geraniol by addition of Na^+ was much smaller than that of forskolin, suggesting that the responses to odorants include a component mediated via the cAMP-independent pathway. The enhancement of the responses to IP_3-increasing odorants varied among odorants and their concentrations. The odor discrimination function of the olfactory system in high-[Na^+]containing solution was similar to that in normal Ringer's solution.

82. Analysis of oscillation in the guinea-pig accessory olfactory bulb slice with optical imaging technique

N. Onoda, T. Sugai and M. Sugitani

Department of Physiology, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan

Recently, we found a damped oscillatory field potential in response to a single shock of the vomeronasal nerve layer (VNL) in sagittal slices of the guinea-pig accessory olfactory bulb (AOB). In order to confirm the above finding, we tried to visualize the spatio-temporal pattern of signal propagation in the AOB using optical imaging method. Excitation elicited by VNL shock spread the same layer and invaded the glomerular layer. About 17 ms after the stimulus onset, active region started shifting into the external plexiform and mitral cell layers (EPL/MCL). Then, the oscillation superimposed on a large and long lasting activity, occurred in the EPL/MCL. The cycle of an oscillatory response varied from 3 to 5, with a frequency range of 15–50 Hz, which was similar to that for the oscillatory field potentials. Thus, optical imaging not only confirmed the oscillation, but also revealed signal propagation in the AOB. Furthermore, the spread of excitation to anterior VNL shock was restricted within the anterior AOB, whereas posterior VNL shock-evoked oscillatory activity spreading only in the posterior AOB. Thus, the result coincided with our electrophysiological mapping data, indicating that the AOB consists of at least two functionally different regions with a boundary.

83. Changes in taste perception of bitterness following long- and short-term mental workload (I)

Takako Inui and Masashi Nakagawa

Suntory Ltd, Institute for Fundamental Research, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

We reported the evaluation of taste sensations by using time-intensity method in 1994 that the taste sensation of bitterness was reduced after the mental workload.

In this study, the taste sensation of bitterness after long- and short-term mental workload were tested by using time-intensity method and quantitative analysis of residual amount of bitter compound in human saliva. Quinine sulfate (1.82×10^{-5} M) was used as bitter tasting sample.

Subject performed mental tasks for 40 min by personal computer as long-term workload and 5 min mental arithmetic under uncomfortable noise from headphone as short-term workload.

TI evaluation showed that after long-term mental workload, the sensation of bitterness was reduced as same as before experiment, and that after short-term mental workload, the sensation of bitterness was enhanced.

After long-term mental workload, residual quinine in saliva was more content than before workload. On the other hand, after short-term mental workload, residual quinine in saliva was less content than before workload.

Consequently, results showed good conformity with TI evaluation and quantitative analysis of bitter compound.

84. Measurement of cytosolic Ca^{2+} concentration change in human and rat taste bud cells induced by gustatory stimulation of the restricted receptive membrane alone

R. Fujiyama and T. Sato

Department of Physiology, Nagasaki University School of Dentistry, 1-7-1 Sakamoto, Nagasaki 852, Japan

With isolated taste cells it is too difficult to chemically-stimulate a restricted receptive membrane area. Restricted gustatory stimulation of apical receptive membrane of taste cells is important for basic understanding of taste transduction mechanism. To elucidate the cytosolic Ca^{2+} dynamics in taste buds in response to taste stimuli, we used Fura-2 imaging of the lingual epithelial sheets containing some fungiform papillae in rats and humans. By using the *in situ* lingual epithelial sheets which enabled to separately stimulate the apical receptive membrane side and basolateral membrane side, we were able to observe tastant-induced changes of the cytosolic Ca^{2+} concentration (Ca_c) under the normal physiological condition. In rat taste buds, sucrose and NaCl did not increase Ca_c in many taste buds. Some taste buds responded to sucrose and/or NaCl. The lingual epithelial sheets obtained from humans were too small to separately stimulate the apical membrane side and the basolateral membrane side. Unavoidably, we tried to stimulate both sides of the epithelial sheet with sucrose. One fungiform papilla responded to sucrose. We need further experiments with human taste buds.

These results suggest that in rats sucrose and NaCl increase Ca_c in differential cells in a taste bud or increase Ca_c in the taste buds of different fungiform papillae.

85. The masking effect of salt and sugar to tasty materials

Teruko Ito

Seitoku University, 531 Sagamidai, Matsudo-shi, Chiba-ken, Japan

This paper investigates where if some additives are added in one's favorite, human can feel the taste more intensively or weakly contraries.

In this paper, I describe the masking effect caused by the addition of salt and sugar to tasty materials. I examined the effect of salt and sugar added first in the adequately diluted solution of vinegar, MSG and coffee, then in some Japanese traditional soups such as konbu, niboshi, katsuo-bushi and shiitake soup.

The tasters were 20 young women and 20 young men. Three cups of sample solution (2.5 ml) of different concentration without additives were given. After drinking they checked each concentration. Consequently, the same three samples with additives were given, respectively. These results were analysed by means of Kendor's method.

I found that salt and sugar act as a masking agent to vinegar, MSG solution and all Japanese traditional soups except born soup, but sugar doesn't affect the taste of coffee.

86. Detection thresholds of MSG and four basic taste substances in single and mixed solutions

Shizuko Yamaguchi, Sachiko Kanno and Toshiro Haga[1]

Ajinomoto Co., Inc., Tokyo Science University, 1-1 Suzuki-cho, Kawasaki, Kanagawa 210 and [1]1-3 Kagurazaka, Shinjuku-ku, Tokyo, 162 Japan

Detection thresholds of MSG was determined as compared with those of sucrose, NaCl, tartaric acid and quinine sulfate. The measurement was done in simple aqueous solutions or in other taste substance solutions. The method was based on the descending method of limit. A series of triangle tests was conducted for the wide range of concentrations from clearly detectable to an undetectable level for each test substance. For data analysis SAS PROBIT was used and the group's thresholds were calculated as those concentrations for which the probability of correct identification is 66.7% that corresponds with true detection probability of 50%. The thresholds obtained were generally low and close to the minimum values compared with those appeared in literature. The threshold of MSG was not largely affected by other taste substances except IMP, which remarkably lowered the threshold of MSG due to the synergistic effect. Reversely, the thresholds of sucrose, NaCl and quinine sulfate were not largely affected either by both MSG and IMP but that of tartaric acid was considerably raised by both MSG and IMP, because of the change in pH. The results suggested that umami is independent from the other basic tastes.

87. Sensing system to measure putrid level of food using gas sensors and psychological data

Yoshiko Yamaguchi and Sachiko Saito

National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, 1-1 Higashi, Tsukuba-City, Ibaraki 305, Japan

We obtained, from 19 housewives, the psychological sensory data (i.e. intensity of odor, pleasantness of odor, irritation caused to eyes, and causing a headache, nausea and so on) and the psychological judgement for 15 odors of foods (beef, tuna and onion) varied in five putrid levels. As a result of analysis, the relationship between the sensory data and the judgement of the putrid level was found to be different depending on the kind of food. Then, the relationship is represented using neural networks which are prepared for each food. For these 15 food odors, we also obtained the response data of five gas sensors; four of which are crystal resonators covered lipid bimolecular membrane and the other, a metal-oxide semiconductor gas sensor. Eleven effective parameters were selected from sensors' response, and the relationship between these sensor's data and the above-mentioned psychological sensory data was represented using another neural network. Combining these neural networks makes it possible to construct a sensing system to measure the putrid level using gas sensors. The result was good enough to judge the putrid level of food.

88. Behavioral and neurophysiological study on the effect of capsaicin ingestion on the sodium chloride preference in rats

Atsuko Goto, Michio Komai, Hitoshi Suzuki[1] and Yuji Furukawa

Laboratory of Nutrition, Faculty of Agriculture, Tohoku University, Sendai 981 and [1]Ishinomaki Sensyu University, Ishinomaki 986, Japan

Capsaicin is a major component of red pepper and causes irritation sensation in the mouth. One of the physiological actions of capsaicin has been shown to reduce NaCl preference in our laboratory, but little is known about the effect of capsaicin on a basic taste reception by the taste nerve such as chorda tympani nerve. The present experiment was demonstrated in order to clarify the effect of capsaicin on NaCl preference and on the chorda tympani nerve sensitivity to NaCl in SD rats.

Experiment 1: the effect of capsaicin administration through oral cavity as a dietary component. Four-week-old, male SD rats were used for the preference test. The dietary protein (purified egg protein) level was set at 5% (5P) and 10% (10P), and the rats were fed the diets to rats with [+CAP (+0.014%)] or without capsaicin (four experimental groups totally). Each group consists of five rats, reared together in a big wire-mesh cage with four bottles of test solutions (deionized water, 0.5, 0.9 and 1.4% NaCl solutions). After finishing the preference test, the rats were used for the chorda tympani nerve recordings.

Experiment 2: the effect of intragastric administrations of capsaicin. Six weeks old, male SD rats were used for the preference test. The rats were fed the 5P and 10P diets, and administered 6% HCO-60 solution intragastrically with (3 mg/rat/day) or without capsaicin.

NaCl preference test and the chorda tympani nerve recordings were performed as Experiment 1.

The results obtained were as follows: (i) both dietary- and intragastrically-administered capsaicin had a reducing effect equally on the preference for NaCl solutions particularly under the 5% protein diet; (ii) it was suggested that capsaicin administration increased the chorda tympani nerve sensitivity to NaCl solutions.

These observations suggest that capsaicin may alter the physiological condition of NaCl balance in the body and the chorda tympani nerve sensitivity without relating to the burning sensation in the mouth.

89. Behavioral and neurophysiological study on the taste preference alteration by forced-swimming fatigue

Kazunori Morimoto[1], Michio Komai[1], Kazumi Osada[1,2], Kenji Tsunoda[2], Hitoshi Suzuki[3] and Yuji Furukawa[1]

[1]Laboratory of Nutrition, Faculty of Agriculture, Tohoku University, Sendai 981, [2]Taisho Pharmaceutical Co., Ltd., Tokyo, 171 and [3]Ishinomaki-Senshu University, Ishinomaki 986 Japan

It is generally considered that the physiological condition of animal body affects the taste preference. For example, physical fatigue causes an alteration in the taste preference of sour taste etc. in human and animals. Taste signals in the oral cavity could have a fundamental role to maintain homeostasis of animal body through controlling fluid or nutrient balances. In this study, we adopted the forced-swimming in rats as a human fatigue model, and tried to clarify the effect of physical fatigue on the taste preference and the reception of sour and bitter tastes.

Six weeks old, male SD rats were used for the preference test and were finally killed in the electrophysiological study that was performed using the chorda tympani and glossopharyngeal nerves. After the acclimatization for 5 days, the rats were divided into two groups, the control and the fatigue. The fatigue rats were initially placed into a big pail filled with water (30°C ± 2°C) for 1 h, and then they were forced into swimming with putting on a weight of 5–8% of the body weight. The control rats were kept in a cage without drinking water during the forced swimming of the fatigue group. Two bottle (the test solution and water) preference test was undertaken for 22 h everyday.

First of all, there was no difference in the distilled water preference between the fatigue and the control groups, which means that we can compare the preference for taste solutions between the two groups impartially. In the sour taste preference test, the fatigue rats preferred citric acid (1 mM) and ascorbic acid (5 mM) solutions than did the control rats, but didn't prefer HCl solutions (1 mM) compared with the control group. As for the bitter taste preference, the fatigue rats preferred caffein solution (5 mM) very much, but contrary for urea solution (0.3 M) compared with the control group. The taste nerve responses to all the stimuli of basic tastes including sour and bitter tastes did not change at all between the two groups.

These results suggest that the required sour and bitter substances after the forced swimming fatigue are different within the same kind of taste, probably due to the change of endogenous requirement. From the results of the taste nerve responses, the change in the mechanism of peripheral reception site may not contribute much to the changed taste preference due to the fatigue. Rather, the changes in the vagus nerve or much more central nervous system may contribute much to this phenomenon.

90. Dual wavelength differential optical recording of rat cortical activities

M. Yanaura, S. Shiono, M. Nakashima and M. Tanifuji[1]

Advanced Technology R&D Center, Mitsubishi Electric Corp., Hyogo 661 and [1]Department of Information Science Fukui University, Fukui 910, Japan

Simultaneous optical recording with two wavelengths has the potential advantage of achieving real-time imaging of neural activities, since it allows efficient noise reduction and selective signal amplification. In the optical system two CCD cameras were used to visualize the same cortical surface in two wavelengths. The combination of band-pass filters with 570 and 600 nm was chosen to detect intrinsic hemoglobin signals depending on the cortical activities. We applied the optical recording system to measure the cortical activities of rat gustatory area responding to taste stimulation.

A SD strain rat anesthetized with urethane was maintained at surgical level. The animal was placed in a non-traumatic head holder and its gustatory cortex whose dura remained intact was exposed to measure the optical signals. Taste organs located at tongue and oral cavity were stimulated by the three types of taste solutions; 1 M sucrose, 0.5 M NaCl and 25 mM HCl.

We successfully obtained the optical signals responding to the taste stimulation from the gustatory cortex. Significant changes in the optical signals were observed at the area of about 4 × 3 mm dorsal to rhinalis sulcus. Its extent was a little wider than that reported by the electrophysiological results (Yamamoto, 1987). About 30% of the area responding to the taste stimulation was different among sucrose, NaCl and HCl.

91. Changes in taste perception of bitterness following long- and short-term mental workload. II

M. Nakagawa, T. Inui and M. Ozaki[1]

Sensory Science, Suntory Ltd., Institute for Fundamental Research, Shimamoto-cho, Mishima-gun, Osaka 618 and [1]Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

It has been widely discussed that mental conditions influence taste sensations. We focused on the relationships between mental workloads and bitter sensation.

By using the time-intensity method, we reported in 1994 that the bitter sensitivity to quinine sulfate is reduced after a mental workload inducing deep exhaustion. Besides this type of task, i.e. the 'long-term mental workload', we tested the 'short-term mental workload', the different type of task evoking irritation. The long-term workload reduced the bitter sensitivity, but the short-term workload did not or rather enhanced it. This result may be reasonably explained, if the saliva includes a sort of bitter substance-carrier, which can remove the quinine molecule from the receptor membrane surface of taste cells and if its content would increase after the long-term workload.

Thus, we investigated the alteration in the salivary protein composition and found a protein which was apparently increased after the long-term workload. In most cases, the protein content was transiently increased after the workload and reduces to the control level within several minutes. The salivary content of this protein may alter in a mental condition-dependent manner. At the present time, however, it is not examined whether this protein has the binding ability for the bitter substances or not.

92. Mapping of the electrogustometric thresholds in the oral cavity

Sohei Endo, Shin Suzuki and Hiroshi Tomita

Nihon University School of Medicine, Department of Otorhinolaryngology, 30-1 Oyaguchi, Itabashi-ku, Tokyo 173, Japan

The purpose of this study was to clarify the distribution of the taste thresholds in the oral cavity. The electrogustometric thresholds of five healthy young non-smoking females were measured at about 40 points on the tongue and palate. The points measured were located on the tongue or the palate at every 1 cm on the foliate and on the circumvallate papilla. The thresholds were obtained through stair-case method. The thresholds at the tongue tip were lowest, followed in ascending order by those in the tongue margin, the tongue base and the palate.

93. Depressing activity of the hydra R4 response which increases after food intake in human serum and the activity from adrenal medulla

Kazumitsu Hanai

Department of Physics, Kyoto Prefectural University of Medicine, 13 Nisitakatsukasa-cho, Kyoto 603, Japan

We reported increased activity depressing Hydra R4 response in human serum and rat cerebrospinal fluid after food intake, and this activity was similar to acidic fibroblast growth factor. To examine further this point, here, we tried to find another substance with an activity specifically to depress the R4 response of Hydra. Adrenal medulla contained potent activity to depress the R4 response. Then, we tried to purify this active principle from bovine adrenal medulla. Hydrochloric acid extract was applied to a heparin column after neutralization. After extensive wash, the heparin-binding protein was eluted from the column with a linear gradient from 0.15 to 2 M NaCl. Then, the eluate was further fractionated with reverse-phase HPLC. Among fractionated proteins, H31-40.6 protein had an activity to depress only the R4 response of Hydra. The amino acid sequence analysis of this protein showed that the sequence was exactly that of high mobility group protein I or another name, amphoterin, which had been isolated as a protein with a neurite outgrowth promoting activity from the brain. Now, we are planning to examine further whether amphoterin is participated in the regulation of food intake or not.

94. Relationship between serum levels of trace metals and dysgeusia

Hiroshi Ohshiro, Tatsuya Fukuiwa, Koji Deguchi, Shigeru Furuta and Masaru Ohyama

Department of Otolaryngology, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890, Japan

Severe zinc deficiency is known to produce taste deficits. Although zinc is an important cofactor for a number of metalloenzymes and is also required for metabolism of nucleic acids and synthesis of protein, its specific function in taste is not known. To investigate the relationship between trace metals and taste function, 157 patients who visited in Kagoshima University Hospital were administered four taste function tests and laboratory tests for serum metals (zinc, iron and copper). The gustatory function tests consist of electrogustometry, taste threshold test by whole mouth method, taste sensitivity test and salty taste threshold using papers with salty crystals. Serum zinc and serum iron decreased as one growth older. No correlation found between mean values of serum elements and probable etiologies of gustatory dysfunction. However, a ratio of zinc deficiency in patients with common cold, drug induced taste dysfunction, and psychogenic factors was lower than that in unknown origin patients or normal subjects. Those results indicate that taste dysfunction may be occurred by a variety of causes in addition to the latent deficiency of trace metal.

95. Evaluation of lingual papilla lesions by using a videomacroscope

Masami Tanaka, Minoru Ikeda and Hiroshi Tomita

Department of Otorhinolaryngology, Nihon University School of Medicine, 31-1, Kamimachi, Ohtaniguchi, Itabashi, Tokyo 173, Japan

By using a videomacroscope (Nihon Kohden, Tokyo), we investigated lingual papillae of healthy persons without a smoking habit and healthy persons with a smoking habit, Sjögren's syndrome patients and zinc-deficient taste disturbance patients. On the built-in monitor, tongue surface was monitored by 10 times magnification, fungiform papillae and filiform papillae by 50 times, and the state of terminal vessels within fungiform papillae by 100 times.

Videomacroscope can easily produce highly magnified pictures of lingual papillae, and realizes detailed evaluation such as blood flow evaluation in the blood capillary within lingual papillae. At present, features of a specific disease cannot be described by using these pictures. In future, we plan to categorize morphological features of lingual papillae observed on a videomacroscope and hope to use them in the evaluation of lingual papilla lesions in systemic disease patients.

96. A clinical analysis of the patients with taste disorders at our university hospital

Norihisa Hamada, Sohei Endo, Shin Suzuki and Hiroshi Tomita

Nihon University, School of Medicine, Department of Otorhinolaryngology, 30-1 Oyaguchi, Itabashi-ku, Tokyo, Japan

We investigated the incidence of taste disorders, by age mainly, in 2278 patients (871 males and 1407 females) who visited our Taste

and Smell Clinic during the 10 years, between 1981 and 1990. In comparison to the population of Tokyo and its environs by age group, our patients showed a higher incidence of taste disorders along with their age increases. In addition, although the incidence has been thought to be high in females, it was mainly due to the large number of female patients having mild symptoms. There were no gender differences in the incidence of moderate or severe disorders. In the analysis of the disease etiologies, the incidence of flavor disorders, and post-influenza-like hypogeusia and hyposmia (PIHH) were high in middle age, and the incidence of drug-induced dysgeusia, zinc-deficient dysgeusia, dysgeusia caused by systemic diseases and dysgeusia caused by oral diseases, increased along with age. Therapeutic efficacy in the treatment of taste disorder decreased with increased age, regardless of the severity during their first hospital visit.

97. Screening of effective fragrances for the work: sensory test

N. Sugimoto, Y. Sugawara[1], K. Tamura, Y. Yamanishi, T. Masujima, T. Aoki[2], T. Sumida[3] and Y. Itakura[3]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, [1]Department of Health Science, Hiroshima Women's University, Hiroshima 734, [2]Suzugamine Women's College, Hiroshima 733 and [3]Wakunaga Pharmaceutical Co., Ltd., Hiroshima 729-64, Japan

We have reported that the subjective evaluation for fragrances before and after works changes by the contents of the work. To investigate the change of the evaluation, we conducted sensory test of the fragrance before and after the work by SD method.

Fragrances used in this experiment were lavender oil, rosemary oil and linalool oil. Kraepelin mental performance test (mental work), exercise of stepping up and down, and hearing environmental sound were adopted as the work. Sensory test of the fragrance was conducted before and after the work. Thirteen pairs of adjective words were used for evaluating the fragrance and its statistical significance of the change were estimated by *t*-test.

In the case of the linalool oil, the estimation of the fragrance increased significantly in items of impressions which is calm, light, natural, elegant and pleasant after hearing environmental sounds, but decreased after mental work. Result of linalool oil after hearing sound should present that the fragrance has something needs for the work. These results show that we can detect effective fragrances for various works by this sensory test.

98. Screening of effective fragrances for the work: sensory evaluation and physical measurements

K. Tamura, Y. Sugawara[1], N. Sugimoto, Y. Yamanishi, T. Masujima, T. Aoki[2], T. Sumida[3] and Y. Itakura[3]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, [1]Department of Health Science, Hiroshima Women's University, Hiroshima 734, [2]Suzugamine Women's College, Hiroshima 733 and [3]Wakunaga Pharmaceutical Co., Ltd., Hiroshima 729-64, Japan

We reported that the impressions of the fragrance was different before and after the work. In this report, differences in sensing fragrance before and after the work were investigated by sensory test. In order to estimate any statistical significance of sensory evaluation of the fragrance before and after the work, physical measurements were carried out in detail by using conventional electroenphalograph (IBVA EEG).

Lavender oil, rosemary oil and linalool oil were used in this study. The fragrance was presented before after the work, and the forehead surface potential wave (EEG) were measured simultaneously. After FFT analysis, the effects of fragrances were estimated. This EEG was also measured during mental work, exercise, as well as hearing sound and then examined in relation to the contents of the work.

In the case of linalool oil, IBVA EEG was almost correlated with results of sensory test after mental work. We have also tried to measure skin resistance and a fingertip temperature in addition to IBVA EEG. Total results showed that screening of effective fragrance for the work can be evaluated by using sensory test and physical measurement together.

99. Research for the relationship between odor quality and molecular size

Fumiko Yoshii and Shuichi Hirono

Department of Physical Pharmacy, School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo, 108, Japan

Standard odorants are necessary for more precise and reliable odor quality estimation. Twenty-four substances are presented in ISO 5496:1992(E), for initiation and training of assessors in detection and recognition of odors. We calculated the size of conformers of the 24 substances and tried to find a relationship between odor quality and molecular size. At first, conformational analysis of each substance was done by random search using program package, Sybyl (Ver. 6.1a). SiliconGraphics Iris Indigo XS24 was used for the calculation. Then, the size of the obtained conformers was approximated by circumscribing box that circumscribes the van der Waals surface of conformers. As a result, 20 substances out of 24 were quite flexible and the relationship between odor quality and molecular size was not clear. The 20 substances are also not adequate as standards, because molecules with high flexibility are considered to interact with receptor in more complicated manner and to show complicated odor quality. The sizes of other 30 rigid molecules were calculated as same as above. We presented 34 molecules that include benzaldehyde, thymol, methyl anthranilate, 2-methyl isoborneol, heliotropin, skatole and celestolide as standards for odor quality estimation.

100. An examination for elucidating complex odors.

K. Sakakibara, M. Hattori, K. Kaitani, I. Sakata[1], T. Tanaka[1] and S. Satoh[1]

Toyota Central R&D Laboratories, Inc., Nagakute, Aichi, 480-11 and Toyota Motor Corp., Toyota, Aichi, 471, Japan

To reduce diesel exhaust odor, it is required not only measuring odor intensity but also evaluating irritation and odor characters, and considering the chemical species responsible for the odors. Sensory evaluations and chemical analyses were carried out for these require-

ments. Eight direct injection engines were operated at the constant speed of 50 km/h or under idle conditions. For sensory evaluations, diesel exhaust gas was diluted to one-tenth with odor-free air. Odor intensity, irritation and odor characters were evaluated by 6–9 panels. The similarity of the exhaust gases to some standard gases were also evaluated. Chemical species in the exhaust gases were analyzed with gas chromatography-mass spectrometry etc.

Irritation were able to discriminated by non-parametric tests. The principal components analysis of odor character scores showed that the exhaust odors were expressed with burnt-dusty, aromatic-sweet and sour. In the evaluation of the similarity, it was found that the exhaust gases were rather similar to nitrogen dioxide, tridecane or acetaldehyde. The similarity evaluation is considered to be a useful method of determining the chemical species responsible for the odor. In the multiple regression analysis, irritation was able to be described by the expression with the concentration of nitrogen dioxide and aldehydes-ketones ($R = 0.86$).

101. Effect of experience of odors on odor preference in 2-year-old children

Chikahiro Fujiwara, Sachiko Saito, Tatsu Kobayakawa, Masako Fujimoto, Natsu Kumada and Saho Ayabe-Kanamura[1]

Psychoinformatics Laboratories NIBH, AIST, MITI, Higashi 1-1, Tsukuba, Ibaraki 305 and [1]Institute of Psychology, University of Tsukuba, Ibaraki 305, Japan

How is preference of odors formed? We tried to make a experiment related to this theme for 2-year-old children, because few experiments have been reported preference to odors of this age. As a pleasant odor condition, Phenylethyl alcohol (Rose P) was filled in a box size of 1 m^3 and as an unpleasant odor condition, Skatol was filled in another same size box. In the first experimental stage, a child watched an animated movie presented in the box with his/her mother, and then again he/she did in another box. Then the child's mother asked to her child of which box he/she was fond. At this stage, there was no difference of preference between two odors. The second experimental stage was performed 1 month after the first stage, in the same procedure. During this 1 month, children were exposed to Rose P several times a day. We found that the young children having fewer times of exposure to Rose P showed less preference to odor of Rose P than they having many times exposure to it. It suggested that experience of odors may have some effect on odor preference of young children.

102. Characteristics of olfaction in 7–9-year-old children

Sachiko Saito, Saho Ayabe-Kanamura[1] and Tatsu Kobayakawa

NIBH, AIST, MITI, Higashi 1-1, Tsukuba, Ibaraki 305 and [1]Institute of Psychology, University of Tsukuba, Ibaraki 305, Japan

We have already reported a smell test (STAUTT3) for Japanese people in *JASTS '94*, and we also have reported characteristics of olfaction in the aged people using this same test (*AChemS '95*). In our study, we applied this test to 7–9-year-old children and examined identification, rated intensity and pleasantness-unpleasantness for 22 odors in Japanese life. These children showed a significantly smaller percentage of identification of odor quality, but a larger degree of odor intensity than did adults. These characteristics of children's olfaction were different from those of the aged people in that both the percentage of identification and intensity are smaller than the adults. We suppose that the characteristics of olfaction in children come from less experience of odors, whereas those of the aged people come mostly from a functional decline of their sensory organs. We also found that children found odors to be more unpleasant than did adults and the aged people.

103. Risk factors in patients with anosmia

S. Furuta, T. Tateyama, D. Seki, K. Deguchi and M. Ohyama

Department of Otolaryngology, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima, 890, Japan

Twenty-four per cent of 428 patients with smell disorder reported total loss of smell function (anosmia) in our clinic. The study group of patients with anosmia was composed of 53 male and 47 female (mean age 55.2 years). About 8% of patients associated partial taste loss. A variety of primary probable etiologies were associated with anosmia. Sinusitis, the common cold and head trauma accounted for 80% of cases. To investigate a risk factor for anosmia, the subjects excepting head trauma and congenital anosmia were classified into three groups by a probable etiology, such as sinusitis, the common cold and others. Patients with the common cold were older than that in other groups. Male with smoking is dominant in sinusitis group. Since many patients had combined diseases, the medication induced impairment may occur on olfactory epithelium. Those results indicate that anosmia may reveal a direct disturbance by infection or trauma besides indirect factors such as aging, smoking, medication and the trace metals.