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# ABSTRACTS

## The 32nd Annual Meeting of the Japanese Association for the Study of Taste and Smell (JASTS XXXII)

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### P2. Fine structural aspects of the vallate taste buds of rats with a taste disorder induced by tetracycline

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Fine structural aspects of the effect of tetracycline on the vallate taste buds of the tongue were studied in the rat using an electron microscope. A taste disorder was induced in rats by i.p. injection of tetracycline for 2 weeks and the degree of that disorder was assessed by a two-bottle preference test. In rats with the taste disorder, the following pathological changes were observed. Some clear cells showed positive acid phosphatase activity. A part of the mitochondria in the clear cells showed vacuolate or vitreous changes. Many taste bud cells (the clear and dark cells) possessed secondary lysosome-like dense bodies and vacuoles. These dense bodies and vacuoles were not uniform in number, size and electron density, and some deposits, vacuoles and organelles in dark cells were also surrounded by intermediate filaments. In addition, some dense bodies in the dark cells showed positive acid phosphatase activity. The dark cells contained debris derived from degenerated clear cells with dense deposits, suggesting that lysosomal activity in the dark cells is stabilized with intermediate filaments. However, after stopping the administration of tetracycline for 2 weeks rats recovered from the taste disorder. In these rats, the structure of the vallate taste buds was normal and many synapse-like structures were frequently observed on them.

### P3. Gustatory function measured by electrogustometry and the filter-paper disk method in middle ear diseases

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The chorda tympani nerve (CTN) is frequently damaged in various inflammatory middle ear disorders. However, there are a few cases where patients complain of taste disturbance.

To examine the function of CTN in various grades of middle ear inflammation, the threshold of the taste function was measured preoperatively by electrogustometry (EGM) and the filter-paper disk method (FPD).

Subjects were 131 ears of 126 patients with various middle ear disorders. We classified diseases from the standpoint of otorrhea; non-inflammatory diseases such as post-traumatic perforation (10 ears): normal; chronic otitis media without otorrhea (18 ears): COM(–); chronic otitis media with otorrhea at the time of operation (35 ears): COM(+); cholesteatoma without otorrhea (18 ears): chole(–); cholesteatoma with otorrhea (24 ears): chole(+); operated ears in which the CTN was detected (9 ears): P.O.(N+); operated ears in which the CTN was not detected (17 ears): P.O.(N–).

The mean thresholds of normal, COM(–), chole(–) and P.O.(N+) in EGM were within the normal range (<8 dB). Those of COM(+) and chole(+) were >8 dB and that of P.O.(N–) was almost scaled out. In FPD, an abnormal threshold was detected in only P.O.(N–). There was no difference in the threshold among four kinds of tastes (sweet, salty, sour and bitter).

In conclusion, abnormal thresholds depended on the presence of otorrhea. Even if the patients showed an abnormal threshold of EGM, no patient complained of subjective taste disturbance.

### P5. Importance of taste stimuli for cancer (oral) patient care. Case reports

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#### Case one

An 83-year-old woman with terminal stage oral cancer complained of anorexia; however, she continued oral nutrition. She exhibited pleasure responses to Japanese foods, including umami substances. Her terminal suffering was palliated without opioid therapy, and laboratory data were better close to death, compared with other terminal oral cancer patients treated with parenteral nutrition (serum TP: 6.8 g/dl; serum ALB: 3.6 g/dl; RBC: $3.54 \times 10^6/mm^3$, as against $5.3 \pm 0.7$ g/dl, $2.7 \pm 0.3$ g/dl, $2.318 \pm 40.7 \times 10^6/mm^3$; each example, $n = 4$, mean ± SD).

#### Case two

A 57-year-old man who underwent radical surgery (tongue cancer stage IV) received nutrition by a nasopharyngeal feeding tube due to postoperative swallowing disorders. Feeding fluids were made from vegetables, fish and meat, and umami flavor was added at the patient's request. Despite the loss of oral function, he remembered the taste of food, and demonstrated a good psychological condition. He has been followed without any sign of recurrence for 3 years. Eating habits (taste stimuli as individual liking taste) induce positive emotions, and can markedly improve cancer patient care.

## P6. Evaluation of olfaction after total laryngectomy

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Olfactory disturbance is common after larygectomy. This phenomenon is explained by the fact that the patient breathes directly through the tracheostoma and the airway of the nasal cavity is separated from the lower respiratory system. However, there have been few reports regarding the olfactory function following laryngectomy.

In this study, we investigated the olfactory function of 18 patients who had undergone total laryngectomy. We evaluated the olfactory function by using a 'Jet Stream Olfactometer (JSO)' and a venous intra-olfaction test before operation and at 3, 6 and 12 months after the operation.

JSO is a new method for measuring the olfactory function in patients with a tracheostoma by blowing the odor into the nasal cavity.

The olfactory function of almost all the patients was worse at 3 months after the operation than before the operation. However, it was improved in some patients at 6 months after the operation.

## P7. Effect of endoscopic endonasal sinus surgery for chronic sinusitis with special regard to the influence of the extent of the disease on olfactory disturbance

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An effect of endoscopic endonasal sinus surgery for chronic sinusitis with special regard to olfactory function was studied. Eighty-three patients (56 males and 27 females) with chronic sinusitis were evaluated. The extent of the disease with special regard to the posterior part of paranasal sinus was determined by CT scans and rigid endoscopy. Olfactory clefts and olfactory mucosa were observed by nasal endoscopy. Olfactory mucosa were classified into four groups: normal (50 patients), slightly edematous (four patients), severely edematous (15 patients) and polyposis (14 patients). Twenty-five patients (50%) with normal olfactory clefts had normal posterior paranasal sinuses, whereas only three patients (9%) in the other groups had a normal one. Olfactory tests were performed both pre- and post-operation with a T&T olfactometer for 44 patients. Post-operative improvements of olfactory function were found in ~60%. Normosmia was post-operatively achieved in 32% of the patients with normal olfactory clefts, but in none of the patients in the other groups. It can be said that the extent of sinusitis in the posterior part of the paranasal sinus correlates with olfactory dysfunction.

## P8. Odor aversion learning in rats

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Through previous studies on odor aversion learning where an odor stimulus is paired with internal malaise, it has been suggested that the difference in conditioning procedures affects the efficacy of this learning. Therefore, we examined the strength of odor aversion in Wistar rats when a cotton soaked with an odor stimulus (0.001% isoamyl acetate) was presented in front of the nostril during licking of a liquid (Aroma condition) or the same stimulus was dissolved in the liquid (Flavor condition). The liquid was either distilled water or 0.05% sodium saccharin. Soon after 6 min exposure of the odor stimulus, 0.15 M LiCl (2% body wt) was injected i.p. When the odor stimulus was presented with water, stronger odor aversions were attained in the Flavor condition than in the Aroma condition. When the odor stimulus was presented with saccharin instead of water, odor aversions became attenuated in the Aroma condition, while they were similarly firmly established in the Flavor condition. There results suggest that the establishment of odor aversions depends on how the odor stimulus is applied and whether or not the gustatory stimulus coexists.

## P9. Phylogenetic comparison of the dependency on olfactory and visual cues among primates.

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The dependency between olfactory and visual cues was investigated in the situation of foraging on primates. Four species were used: chimpanzees (*Pan troglodytes*), tufted capuchins (*Cebus apella*), black lemurs (*Eulemur macaco*) and brown lemurs (*Eulemur fulvus*). Subjects were first trained to discriminate between two types of jelly foods which were manipulated by odor and color, or odor and shape. After the subjects had learnt to discriminate, they were tested for the discrimination among all combinations, i.e. all four types of jelly foods which were manipulated by odor and color, or odor and shape. The order in which the animals picked up the jelly foods, and the responses to each type of jelly food were recorded. Every species showed dependency on the olfactory cue. On the other hand, the visual cues were not used by all species and the manner they were used was different among the species. Chimpanzees depended on both of olfactory and visual cues, although they use visual cues primarily. Capuchins used a visual cue or color to explore targets first, but an olfactory cue to finally select foods. Lemurs depended on only an olfactory cue. Consequently, this study can conclude that the olfactory cue has an important role for these four species to select foods.

## P10. The difference of urinary marking behavior in mouse strain

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Mice deposit urinary marks on their home range, and their marking patterns vary according to their sex, social rank, age, reproductive stage and experience, and the odor of previously deposited urine marks. In order to ascertain that the relation between urination patterns and various conditions is universal among all mice, the urinary marking patterns of C3H strain mice were observed and compared with those of BALB/c strain that have been investigated previously. The activity of C3H mice was about twice as high as that of BALB/c mice, but the change in total

amount of urination of C3H mice according to mouse development was similar to that of BALB/c mice. The number of deposited urine marks of copulated C3H males decreased and reached the female level according to aging, although copulated BALB/c males increased the number of urine marks at puberty stage and showed a typical dominant male urinary pattern. Sexually naive grouped C3H males and copulated C3H females countermarked previously deposited urine spots from females like BALB/c mice, but copulated C3H males lost interest in females' urine. Also, C3H mice did not show delicate variations in their marking response to the surroundings' odor like BALB/c mice.

## P11. Influence of contact or odor of female mice on sperm activity and reproductive organs in male mice

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Male mice were kept in pairs and were divided into the following three housing conditions. Group 1, with female group: females were individually kept in wire mesh cages and were placed on top of males' cages. Group 2, with female bedding group: males were provided with bedding soiled by females periodically. Group 3, control group: males were kept without any stimuli from females. Social dominance of males was determined with intruder tests, which were conducted three times per week from 8 to 15 weeks of age. At 15 weeks of age sperm activity, weight of reproductive organs and levels of testosterone and corticosterone were measured and were compared between the dominant and subordinate male mice and also between the three housing conditions. It was revealed that the sperm motility (% of motile sperm) was highest in group 1 and 2 males. These results, at first sight, suggest a positive influence of female odor on the hypothalamus–pituitary gonad circuit, which in turn could be thought to have raised sperm motility. But the testosterone level of males was remarkably high only when they were able to interact with females and when they were dominants. It was not high when only the odor of females was provided to the males. Some factor other than testosterone was considered to have worked on raising sperm motility.

## P12. Which molecular features determine odor qualities of odoriferous molecules?

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The purpose of this work is to find out important molecular features that determine the odor quality of odoriferous molecules. We took molecules having the odor of 'lily of the valley' into consideration. They include lilial®, bourgeonal® and mugetanol®. Conformational analyses, structure optimizations and electrostatic potential (ESP) charge calculations were performed using the SYBYL QSAR module (Ver. 6.2 and 6.3). The size of the conformers and the octanol/water partition coefficient (log *P*) were also estimated. As a result, a good accordance of molecular size and the position of the oxygen atoms are seen in the conformers. We assumed these conformers to be odor-active conformers. Their ESP charge values and calculated log *P* values were also met. We tried to propose a novel compound based on these molecular features. However, the proposed compound was not a novel one. The odor is not likely to be 'lily of the valley' judging by descriptions in the literature. Other important molecular features should be considered in future studies.

## P13. Brewing process control utilizing a surface plasmon resonance chemical sensor

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We studied a chemical sensor utilizing surface plasmon resonance (SPR) in order to control the brewing processes. The SPR sensor detects the change of ingredients in the fermentation processes as that of a refractive index. Using the SPR sensor, we could get specific response curves depending on each brewing process.

## P14. Functional design of a polymer-film-coated quartz resonator gas sensor for harmful gas detection

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The copolymerized propylene-butyl, of which the 'solubility parameter' almost coincides with that of toxic gases such as toluene, xylene, octane, diethylether, chloroform and acetone, was chosen as the material for a sensing membrane coated onto a quartz resonator. It was found that the copolymerized propylene-butyl-film-coated quartz resonator gas sensor exhibits high sensitivity and excellent selectivity for these toxic gases, especially for toluene and xylene gas.

## P15. Measurement of the aroma of soup using potentiometric gas sensors

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Involving such ambient and personal factors as temperature, humidity or physical conditions, objective evaluation of odor duly entails appropriate devices for various odor substances. We have studied methods of measuring the aroma of soup using gas electrodes, and the possibility of their use as sensors. The system presented is easily controllable at room temperatures but has been little studied, though similar prospects have been checked elsewhere. Here discussed are the qualitative variations of soup and, using the results, applications of the present system to the related quality control.

Gas electrodes used are of ammonia-, hydrogen sulfide- and newly designed oxidation–reduction types. Notably the last type has not been applied to gaseous substances. Also, five known ingredients of the aroma of soup—2-methylpyrazine, 2-pentylfuran, capronaldehyde, dimethyldisulfide and acetone—produced characteristic responses to the above three electrode types.

Further, the aromas of two common types of soup on the

market are compared under two conditions. After their unpacking, one is immediately tested dissolved in water, with the other left intact to be tested five days after. The comparison has shown a discernible difference in their electric potential.

Thus, the foregoing point out some possibilities for the effective use of the introduced plural gas electrodes as multisensors, enabling them to be instrumental in food quality control.

## P16. Application of a gas sensor to the quality control of heat-stored coffee

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Although coffee tastes best when freshly brewed, it is generally brewed in large quantities at once and then stored on a heater. However, coffee deteriorates over time, and becomes unfit for consumption. Therefore, establishing a method of measuring coffee quality simply and quantitatively would greatly facilitate quality control.

An aroma measurement system using a gas sensor (Figaro) was fabricated to measure the aroma of coffee stored on a heater in real time. The aroma of heat-stored coffee was then measured using this system for two cases, with 600 and 1200 ml in the coffee pot respectively. The results showed that the output voltage of the gas sensor decreased gradually according to the storage time, and that the ratio of this decrease was greater when the amount of coffee was smaller. After 1 h of heated storage, the coffee quality was measured by a conducting polymer odor sensor (Aromascan) and by sensory evaluation (taste tests).

As a result, it was found that the quality of heat-stored coffee can be measured by a gas sensor, and that this system can be applied to the quality control of coffee.

## P17. The application of the Olfacto-Senser odor sensor system to the quality control of wine

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The application of the Olfacto-Senser, made of a semiconductor, to the quality control of wine was studied. Ethanol effected a response of the sensor. However, dilution of wine with water to 8% v/v of alcohol cut off the effect of ethanol.

Examination of the lingering flavor of wine in a glass is important for the quality control of wine. So, a new method to measure the strength of the lingering flavor of wine with the Olfacto-Senser was made. 0.2 ml of wine was added to a 1 cm dia. cell made of stainless steel. The cell was covered with a 200 ml beaker, and after 15 min the wine was discarded from the cell. The cell was then put into the chamber of the Olfacto-Senser, and the voltage response of six channels was measured. Each channel's voltage value of response of a model wine, containing ethanol, organic acid and flavored components, was regarded as a standard of value 100.0, and the value of sample wine was converted into a number relative to that standard. Relative radar-charts were made.

This method was very useful for the quality control of wine.

## P18. The odor stained on the evaporator of the air conditioner

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In a car's cabin, the odor from the air conditioner is an important factor for comfortable driving. Having recognized the importance of the odor of the air conditioner, we studied the odor from various angles. We were able to identify a rotten odor as caused by a microorganism that propagates on the evaporator of the air conditioner and a dusty odor caused by the corrosion product (aluminum hydroxide) of the evaporator material.

In this report, the odor that stains on the evaporator of the air conditioner was studied by sensory evaluation and instrumental gas analysis. With the multivariate analysis method, it is obvious that the staining odor was made up of the components of the car's exhaust gas and the volatile components from the interior material. The components of tobacco smoke were believed to stain the interior material in the car's cabin rather than evaporator. Also, it was found that the staining odor was influenced by humidity, as was the dusty odor. Under high humidity, the odor became stronger and more unpleasant.

## P19. Human responses to the odor of building materials for a house

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Hyperairtight and hyperinsulated buildings are built to save energy and to improve comfort. We live in the airtight space. The influence of the odor of the building materials on humans has not been fully examined. We examined the psychological and physiological responses of the odor of building materials in a climate control chamber. The materials were concrete, lauan plywood, cryptomeria, *Hiba arborvitae*, American pine, Hinoki of Yoshino district and rush. A highly efficient activated charcoal deodorization filter was installed in the control chamber. Material was exposed to the subject for 150 s at the position of the floor top 1.2 m high and 1 m backward. At this time, an electroencephalogram, eardrum and rectum temperature, and blood quantity were measured. Decision of working efficiency is in key touch number and waiting time of the personal computer. Dry bulb temperature, wet bulb temperature, glove temperature and air velocity were measured. Due to the existence of odors from the building material, working efficiency and thermal sensation were examined.

When there was an exposure to a smell, skin temperature was slightly raised. The blood quantity of the forehead was increased when *Hiba arborvitae* was smelled, whereas the temperature of the sole of the foot decreased. Rectum temperature fell at a rate of 0.05°/h when the subject was sitting on the chair under the rest condition. Rectal temperature rose at ~0.02°/h during the time of the addition work.

## P20. Familiar odors for Japanese people: a cross-generation comparison

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It is important to measure the decline of olfaction in elderly people for their safety in daily life. The purpose of this study was to investigate familiar odors for Japanese elderly people, compared with other generations. One hundred and seventy-eight subjects, separated into three generations, were asked about their experience and knowledge of 119 odors using written descriptions, and familiar odors for each generation were compared. The familiar odors for the young generation (20–39 years) were characterized by mint and caramel, and the ones for elderly people (60–89 years) were characterized by traditional foods and things in Japanese life like salted rice-bran paste for pickling, seaweed, lily, shoe polish, dust, Japanese incense stick, naphthaline and india ink. More than 50 odors were selected as common familiar odors for all generations. The appropriate odors for an identification test in different generations were then discussed.

## P21. A smell test for Japanese people: a study using a new stick-type tool for odor presentation.

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A new tool for olfaction testing was developed and its efficiency and stability were examined. The tool was made by mixing a microencapsulated odor substance in a solid base shaped like a lipstick. Twenty-two odors were selected from the cross-generation comparison on familiar odors for the odor identification test given to young, middle-aged and elderly Japanese. The odor quality and intensity of this tool were verified by asking for an identification of odor quality and perceived intensity from 33 olfactory normal persons, and the stability of the quality and intensity over a period of 6–9 months was also confirmed. The olfaction test using this tool was applied to elderly people and the results were compared with that of young and middle-aged people. The ability to identify odors was measured by choosing one of four odor descriptors, and the perceived intensity of odor was measured on a six-point scale from no odor to very strong. The interstimulus interval was 4 min. The elderly people showed lower performance in both odor identification and perceived intensity.

## P23. Effect of the threshold on the evaluation of odor quality for five odorants of a T&T olfactometer using concrete adjectives

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In a previous paper, odor quality was evaluated for five odorants of a T&T olfactometer by a semantic differential method using twenty-eight concrete adjectives. As a result, the expressions of the odor were extracted as 'rotten smell', 'burnt smell'and 'floral odor'. In the present paper, 44 naive subjects evaluated the odor quality of the five odorants using the same adjectives to ascertain the effect of variation of threshold. The threshold of the subject was measured twice to implement evaluation experiments of two concentration levels. Because it changed by the day, it was measured twice. A variation of the threshold between the two measurements occurred with most subjects. When the difference of the threshold is large, it influences used concentration levels of the odor, and the effect of concentration levels might not be estimated precisely. Some of naive subjects showed large variations in the threshold, and attention is above all necessary for the measurement of the threshold.

## P24. The influence of odors upon the autonomic nervous system

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It is known that the physiological measurements of the influence of odors upon the autonomic nervous system are cerebral blood flow, an electrocardiogram, blood pressure, the beam reflection of the pupil, skin surface temperature and so on. Using Peltier's element temperature control unit, which may be a good method of simple physiological measurement, we studied the influence of odors upon the autonomic nervous system by the mean of recovery rate of skin surface temperature, blood pressure and pulse rate.

## P25. Effect on humans of inhalation of essential oils: sensory test and physiological measurement using fingertip temperature

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The effect on humans of inhalation of essential oils was examined in terms of sensory evaluation, in which the perception of fragrance was assessed by 13 contrasting pairs of adjectives and fingertip temperature measurements. The essential oils examined in this study were those of basil and peppermint. For evaluating a change in the perception of a given aroma, scores were recorded after inhaling a fragrance before and after each type of work, and the statistical significance of the change of score for the 13 impression descriptors was examined by Student's *t*-test for mental work, physical work and hearing environmental sounds. It was found that the inhalation of each caused a different subjective perception of the fragrance depending on the type of work, and the feature in the sensory test was the reverse when mental work was assigned to subjects: inhalation of basil after mental work produced a much more favorable impression than that before work, in contrast to the case for peppermint with mental work, which produced an unfavorable impression when compared with that before work. These features of basil and peppermint in relation to mental work were then evaluated by fingertip temperature measurements. As a result, the following trends were

apparent: (i) inhalation of basil after mental work was accompanied by a considerable increase in fingertip temperature, while (ii) that of peppermint resulted in a decrease in fingertip temperature. Therefore, it may be suggested that an increase of fingertip temperature causes favorable impressions of basil after mental work, while a decrease causes agitated inclination of peppermint after mental work.

## P26. Kansei evaluation from a finger plethysmogram by odor stimuli

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A finger plethysmogram was measured on 20 subjects who sniffed five ordorants (rose odor, peach odor, burnt odor, sweaty odor, and moldy odor) of a T&T olfactometer, using scentless as a control. The finger plethysmograms were standardized such that the mean was zero and the variance was unity. The standardized finger plethysmograms were analyzed by fast Fourier transform (FFT) and FFT parameters were used for discriminant analysis. The five odorants and scentless were separated by discriminant analysis. From a group scatterplot of discriminant analysis, it was found that the pleasant odor group (peach odor, rose odor and burnt odor), the unpleasant odor group (sweaty odor and moldy odor) and scentless were discriminated respectively. It was suggested that it is possible to estimate the different emotions between pleasantness and unpleasantness using the plethysmogram.

## P27. Improvement score on olfactory tests

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Patients with an olfactory disorder often reveal a difference between their improvement on olfactory tests and their subjective symptoms. Accordingly we need an inclusive and simple index of the change in olfactory function that reflects a change in subjective symptoms.

We performed olfactory testing (T&T olfactometry and Alinamin injection test) on 366 patients twice at the Olfaction Clinic of the Osaka City University Medical School from 1981 to 1997. We scored four items of olfactory tests ('detection threshold' and 'recognition threshold' of T&T olfactometry, 'latent time' and 'duration time' of the Alinamin injection test) as follows: we assigned +1 points to 'improvement', 0 points to 'no change', and –1 points to worsening', then summed the score of the four items and defined the total score as 'improvement score'.

The coincidence rate of the change in subjective symptoms and improvement score was 66.1%. This was higher than the rate of change in subjective symptoms and recognition threshold, which revealed the best rate among each olfactory test item. This result was not affected by the cause and severity of the olfactory disorder.

Accordingly, 'improvement score' is expected to serve as a good index to express the degree of improvement of the olfactory function.

## P28. Psychological and physiological human responses to odor

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The physiological responses of the autonomic nervous system are often used for the evaluation of the stress and mental work load. In particular, the measure of HRV (heart rate variability) is very simple and often used as the parameter of the activity of both the sympathetic and parasympathetic nerves. Shimono *et al*. pointed out that the component part of the Mayer wave increased in high blood pressure and irregular breath as the worker became irritated or tired by the simple work stress. HRV using lemon odor during a simple additional task was measured. As the result, a power spectral deviation was observed, but it is not clear whether this was the effect of the odor or mathematical treatment. Furthermore, the effect of irregular breath should be considered.

## P29. Investigation about 'Soko' (first report)—from the viewpoint of the behavior of coping with stress

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'Soko' is the system for the study of creating fragrances by self-expression. The system of 'Soko' was established 20 years ago in Japan, in order to separate it from a vocational fragrance designer. There have been many reports on the fragrances about the effects of coping with stress by an experienced person. There have been few reports about the neurophysiological and psychological investigation of 'Soko', so we have attempted to investigate this using the galvanic skin reflex (GSR).

Subjects were 15 female (29.1 ± 3.9 years old) for the group of 'Soko' and 13 female (33.6 ± 5.5 years old) for the controls. GSR was determined by Biofeedback Trainer (OG Giken Co. Ltd, Tokyo, Japan). Measurement was started at 14.00 h, because subjects had a little stress by daily work after that time. For the 'Soko' group, GSR was determined for 5 min before and after 'Soko' for 30 min. The difference of the width of GSR was determined. There was a significant difference between the 'Soko' group and the controls ($P < 0.01$); nevertheless, there was no significant difference before 'Soko'.

We conclude that 'Soko' had an effect on coping with stress via the autonomic nervous system.

## P30. Analysis of MEG cognitive responses in an olfactory oddball paradigm

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To draw recognitory responses of human, we tried an experiment using two kinds of odorant stimuli in an oddball paradigm technique by MEG. The two kinds of odor were quite different—one sweet and one unpleasant—so that the subject could distinguish between them. The sweet odor was amylacetate, and the bad- smelling odor was isovaleric acid. Pulsed odors were given to the other nasal cavity directly and were synchronized with breathing. The ratio of presentation of the two kinds of odor was rare 1:frequent 3 and each was given at random. We imposed tasks as a subject gained only an odor on rare stimulation. From the results of three men (21–23 years old, all right-handed), there were some responses of late latency (>450 ms) besides current odor responses. These MEG responses showed a tendency to appear on both sides of the head, and were regarded as a response of recognition. When we looked at these activity areas within the cerebral hemisphere, a response to amylacetate was found in the contralateral area when either nasal cavity was stimulated, but in case of isovarelic acid, the right side was dominant, and produced a response to the side ipsilateral to the nasal cavity stimulated.

## P31. Effects of musk-like odors on P300

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Musk has been valued as an aphrodisiac since ancient times, but its use has been prohibited for the preservation of nature. Therefore, synthesized compounds which show a musk-like odor were developed. The mental effects of the three following compounds, galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-benzopyran), pentalide (cyclopentade canolide) and TM-II (5-cyclohexadecen-1-one) were studied using the event-related potential, P300. Twenty-four female subjects aged 19–21 years, cooperated in this study. The odors were inhaled for 5 min by natural respiration, and changes in P300 were studied. Pentalide and TM-II augmented or inhibited the amplitude of P300, while galaxolide showed a tendency to inhibit. Changes in P300 were well correlated with the questionnaire of preference to these odors, that is, subjects who like an odor augmented the P300, while dislike inhibited it. This suggests that galaxolide had a greater stimulatory effect than the other two compounds.

## P32. α-wave transition under odors of coffee and whisky—comparison by the additional mean spectrum

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It has previously been reported that not only the subjective evaluation of an odor but also the daily liking and disliking of its taste take part in the effect of the transition of α-wave power concerning the taste of drinks. This study tried to visually mark the effect of odors of coffee and whisky using the additional mean spectrum.

Subjects were 21 healthy adult females whose ages ranged from 18 to 59 years (mean 30.7 ± 12.8). Their EEGs were recorded in a resting state and under the odors of coffee and whisky, and then the power of $\alpha_1$ and $\alpha_2$ waves were analyzed using fast Fourier transformation. The power of each of the groups which were separated by subjective evaluation of the presented odor and their daily taste for that drink were examined using Wilcoxon's Signed Rank Test. And the additional mean spectrum was recorded using groups which showed characteristic transition.

The major results were as follows:

### 1. Effect by subjective daily taste for coffee

The additional mean spectrum of the subjects who disliked the presented odor of coffee but like coffee itself increased the predominant α-band and those lower than that. The subjects who disliked the odor and dislike coffee itself showed three patterns. First, the α-wave power decreased entirely. Secondly, the predominant α-wave increased but the bands higher than that were restrained. Lasty, the predominant α-wave shifted to a lower band.

### 2. Effects by subjective odor evaluation of whisky

The subjects who disliked the presented odor of whisky and dislike whisky itself showed that the predominant α-wave shifted to the higher, α-wave power decreased entirely and lower bands were restrained. But the subjects who disliked the odor but like whisky did not show such a transition.

## P33. Neuromagnetic responses during sniffing behavior

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MEG activities were measured with Neuromag-122 in three healthy subjects while they sniffed air or odor (lavender, lemon or soy sauce) within ~0.5 s and with rates faster than in their normal respiratory inhalations. The responses were averaged with respect to the onsets of the sniffing. Olfactory responses were observed above frontal cortices, similar to our previous studies (Tonoike *et al.*, 1986), where odor pulses were injected with the 'blast' method. However, the responses in this study were stronger on the right hemisphere than on the left, which is in accordance with Zatorre *et al.* (1992). Interestingly, some MEG channels showed activity before the onset of sniffing.

## P34. The estimation of olfactory activity in the human brain by EEG and MEG

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Kettenmann *et al*. (1996, 1997), by magnetoencephalographic (MEG) recordings, found that odorants activated the area between the superior temporal plane and the parainsular cortex at a latency corresponding to the P1 component of olfactory evoked potentials (OEP), the anterior-central parts of insula cortex to the N1, and the areas around the superior temporal sulci (STS) to the P2 of both hemispheres. They revealed a neocortical area involved in olfactory processing. In this experiment, we aimed to trace the activated cortical areas by olfactory stimulation using a different SQUID system from that of Kettenmann *et al*. We recorded magnetic fields (MFs) after stimulation of an odor using Kobal's olfactometer, and simultaneously recorded OEP. For recording MFs, we employed a 64-channel whole-head SQUID system. Four subjects participated in the experiment. As results, we localized equivalent current dipoles (ECDs) in insula cortex, lateral sulci, STS and temporal inferior gyrus ~300–1000 ms after stimulus onset. Although these areas were activated at the latencies of the components of the OEP responses, we did not find the clear correspondence between the activated areas and each OEP component that Kettenmann *et al*. had reported. A certain component of OEP responses seemed to have arisen from different cortical areas by each subject.

## P35. Analysis of gustatory related neural responses detected by the brain magnetic field

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To understand the mechanism of gustatory related brain neural responses, we have tried to make a new gustatory stimulation system for measuring the brain's magnetic field. A taste solution is supplied via a Teflon tube directly into the subject's mouth and flows over the surface of the tongue. We used color solutions to detect, using a light sensor as a trigger, the onset of the taste stimulant. The brain's magnetic field is measured by Neuromag-122, and is averaged over 38 measurements by the stimulation trigger. 500 mM sucrose and 50 mM citric acid were used for the taste stimulants. We also examined the effect of miracle fruits (*Synsepalum dulcificum*) as a taste-modifying substance.

The positions of signal sources on the subject's MRI were almost identical for sucrose and citric acid. The area was near the insula and operculum. However, the latencies of the signals were different between sucrose and citric acid. Citric acid was activated earlier than sucrose. This would suggest that a different transduction mechanism lies in peripheral taste receptors. Miracle fruits had the same latency as sucrose. It is suggested that active components of miracle fruits activated the sweet taste receptor and blocked the ion channel for sour taste transduction.

## P36. The effects of ethanol on the taste of beer

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We previously investigated the effects on taste intensity when ethanol was added to solutions of isohumulones, which are main bitter components of beer, and found that bitterness intensity increased as ethanol concentration was increased and the average rating for sweetness intensity increased slightly, though not all subjects perceived sweetness. In the present study, to understand the effects of ethanol on the taste of a more complicated solution, beer, we examined the changes of taste intensity when ethanol was added to non-alcoholic beer. Two brands of non-alcoholic beers with added water or ethanol (2, 4, 6 or 8% after addition) were prepared. Samples were served to subjects at 8°C. In a completely randomized design, bitterness, sweetness and sourness intensity were rated by 30 subjects using a 13-point category scale. Each subject rated the samples twice. In the evaluations of both brands of non-alcoholic beers, increasing the ethanol concentration from 0 to 6 or 8% produced significant increases in bitterness ratings (ANOVA, $P < 0.05$). Sweetness intensity also increased with the addition of ethanol. On the other hand, sourness intensity decreased as ethanol concentration was increased. These data suggest that ethanol may contribute to the taste of beer.

## P37. Comparison between young and elderly people in quality discrimination and identification of mixed odors and tastes (Part II)

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The threshold of tastes [sucrose (SU) and tartaric acid (TA)] and odors [β-phenylethyl alcohol (PA) and γ-undecalactone (UL)] in young and elderly people were compared using the triangular test. The elderly showed significantly higher thresholds of TA, PA and UL compared with the young. However, there was no difference in threshold of SU between the elderly and the young. The threshold of TA, PA or UL showed a correlation with the percent correct in a discrimination or identification test of a mixture of SU and TA or of PA and UL. The thresholds of TA, PA and UL showed a correlation with the percent correct in the short-term memory test. Therefore, these results support the hypothesis that the decrease in discrimination and identification abilities for odors and tastes with aging is due to decreases not only in the peripheral but also in the central nervous system.

## P38. Functionality of taste localization in humans

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We have previously demonstrated that people can localize a

punctate gustatory stimulus on the lingual epithelium in the absence of discriminative tactile cues. The localization of tastes could be based upon gustatory intensity or quality cues, or both. The purpose of this study was to determine whether humans can localize two compounds that differ substantially in taste quality, NaCl versus citric acid, or Na-saccharin (Sac) versus Na-glutamate (MSG), using only their qualitative cues. In the first experiment, subjects always received 100 mM NaCl on either the left or right tip of the tongue while various concentrations (0.1–32 mM) of citric acid were placed simultaneously on the other side and subjects were requested to determine on which side they perceived a salty taste. In the second experiment, 100 mM MSG and various concentrations (0.1–10 mM) of Sac were used in the same procedure, except the subjects were requested to answer on which side they perceived an umami taste. When NaCl and citric acid were presented, subjects could discriminate two tastes with some difficulty. In the absence of intensity cues localization was very difficult by qualitative cues alone, but possible nonetheless. When MSG and Sac were presented, subjects were able to locate the position of the umami stimulus almost perfectly across all Sac concentrations. These results strongly suggest that taste localization can occur strictly as a function of gustatory quality information in the absence of intensity cues. Thcse data have implications for the existence of a gustatory map in the CNS.

## P39. Effect of physical exercise on preference of various sour taste solutions

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The effect of physical exercise on preference of various sour taste solutions was examined. After 30 min of exercise using a bicycle ergometer, at intensity 50% $\dot{V}O_{2max}$ (maximal oxygen uptake), a taste preference test was performed in 41 healthy university students aged between 19 and 24 years. Test solutions were citric acid, α-ketoglutaric acid, L-malic acid, acetic acid, L-tartaric acid and ascorbic acid.

Preference scale values of citric acid and ascorbic acid increased after exercise. In particular, the degree of change was large in citric acid solutions. However, the preference scale values of α-ketoglutaric acid, L-malic acid, acetic acid and L-tartaric acid were not changed between pre-exercise and post-exercise in all concentrations.

These results suggest that the effect of preference of sour taste by physical exercise was different among sour taste substances.

## P40. Study of evoked potentials induced by stimuli of four basic tastes under the influence of taste modifiers

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We investigated the influence of miracle fruit, which can change the taste of a sour solution, on four basic taste solutions. The solutions used were NaCl (0.1 M) for salt taste, tartaric acid (0.12 M) for sour taste, sucrose (0.1 M) for sweet taste and quinine-HCl (0.003 M) for bitter taste. Results of the evoked potentials before and after treatment with the taste modifier (miracle fruit) showed that the latency of the *sour* solution after treatment was significantly shorter than that before the treatment, and the amplitude was also significantly smaller as compared with that before treatment. It was also found that the latency of the *sweet* solution after treatment was longer than that before the treatment, the amplitude also being larger than that before treatment. Few changes could be found from the stimulus of salty and bitter solution.

## P41. Influences of gustatory information on swallowing in normal humans

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Gustatory information from the oral and pharyngolaryngeal regions of humans has been presumed to play a role in swallowing, but no study has evaluated the role. Eight healthy subjects were used for the evaluation in both perceptual and motor aspects of swallowing. A food for dysphagic patients was dissolved in distilled water (DW) and five taste solutions of sucrose (Suc), sodium chloride, acetic acid (AA), quinine hydrochloride (QHCl) and monosodium glutamate. High and lower concentrations were prepared for the five taste solutions. The perceptual aspect was estimated by subjective judgement of the difficulty of swallowing. The difficulty tended to be lower if the food was dissolved in Suc solutions than in DW, while it tended to be higher if the foods were dissolved in the AA and QHCl solutions. The motor aspect was evaluated by recording the laryngeal movement and electromyographic activities accompanied with swallowing. The duration of the 'oral phase' of swallowing was measured. No clear differences were found in the duration among the six tested foods. These results indicate that gustatory information from the oral and pharyngolaryngeal regions mainly affect the perceptual aspect of swallowing in normal humans.

## P42. Labeled magnitude scale in Japanese

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The Labeled Magnitude Scale (LMS; B. Green *et al*., 1993, Chem. Senses, 18: 683–702), which was translated into Japanese, was examined to measure taste intensities of five basic taste stimuli and compared with Magnitude Estimation (ME) and the seven-point Category Scale (CS). Thirteen Japanese subjects participated in four sessions for each method. Stimulus solutions presented in a session were as follows: 0, 0.032, 0.1, 0.32 and 1 M sucrose (S; sweetness); 0, 0.032, 0.1, 0.32 and 1 M NaCl (N; saltiness); 0, 0.00032, 0.001, 0.0032 and 0.01 M citric acid (CA; sourness); 0, 0.001, 0.0032, 0.01 and 0.032 M caffeine (C; bitterness); and 0, 0.0032, 0.01, 0.032 and 0.1 M MSG (M; umami).

Stevens' law was applied to the data from LMS and ME, and Weber–Fechner's law to those from CS. The slopes were S > C > N > M > CA in all methods, but the 'bend' was near 'Strong' on LMS. In cases of C, CA and M, the rank order of individual

subject means from LMS was correlated to those from CS. The N–S relation from LMS was consistent with that from the Gust scale. The finding that slopes derived from ME were steeper than those from LMS for all stimuli was not compatible with results from the USA. The results concerning LMS in Japanese indicate that the order of descriptors is suitable but the location of descriptors might be slightly different from that of the original scale. Further investigation is needed to confirm these findings.

## P43. A taste-recognition sensor system using ISFET and a quartz resonator with membranes of mixtures of sweetness and salty taste

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The importance of human sensibility is increasing in the field of the sensory sensor. In this paper, we attempted to construct a taste-recognition sensor system for supporting the manufacture and management of food.

We examined the taste sense of taste substances by presenting subjects with a questionnaire. In the test, the 22 subjects tasted mixtures of substances, and evaluated their impressions of taste of those substances. They were classified into three groups from evaluation of their taste preference. The impressions of taste were extracted from the mean value of evaluation of the taste of each group by factor analysis. Each extracted factor was related with quality, continuation and depth of taste.

A sensor for the detection of taste substances was constructed by coating several kinds of lipid/polymer membranes onto the surface of an ion-sensitive field effect transistor (ISFET) and quartz resonator device. The sensor system is composed of one quartz resonator sensor and three ISFET sensors, and is capable of expressing taste sensory quantity using a neural network. The network receives the output pattern from the sensors and learns from the result of the human sensory test.

We have confirmed that a sensor system is capable of estimating the sense of taste.

## P44. Images of the taste and smell of foods: cognitive psychophysical approaches

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Wooley and Wooley (1973) reported that increases in salivation occurred regularly when subjects looked at or thought of palatable food. As the subjects did not eat or were sham fed the foods, this salivation was suggested to be a conditioned response to the visual stimuli or imagery of that food. Images of foods are developed by the association of the flavor of foods and words which signal for those foods. We are investigating how the images of the foods develop, and what roles they play. In this experiment, we asked the subjects to evaluate the food with several scores (sweetness, saltiness, bitterness, strength of odor, etc.) in one of the following conditions: (1) Eat: the subjects eat a food and evaluate that food. (2) Odor: the subjects smell a food, imagine the taste of that food, and evaluate the food with their images. (3) Flavor: the subjects smell an artificial flavor (Takasago Co.), imagine the taste and smell of that food, and evaluate the food with their images. (4) Word: the subjects read a food-related word, imagine the taste and smell of that food, and rate scores with the imagery of food. The results showed that imagery of foods under the Word conditions was similar to the perceptual experience of the taste and smell of those foods.

## P45. Ethnic comparisons of sweet preference between Japanese and Korean students

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Ethnic differences in sweet preference for sweet foods were investigated between groups of Japanese and Korean students. A questionnaire survey and sensory tests using sweet food were conducted. For the sensory tests, two kinds of chocolate tablets popular in both countries having different intensities of sweetness were used as samples. Each subject ate a total of 15 tablets of one kind of chocolate and evaluated the palatability, liking the flavor and liking the sweetness intensity successively. The results of the questionnaire survey showed that the Japanese, especially the female students, liked sweets in general and ate them more often than the Koreans. Successive evaluation of chocolate tablets showed that the Koreans felt the sweetness of both samples more strongly than the Japanese. The more they ate the samples, the stronger the sweetness and liking score decreased. The pattern of evaluation changes showed that the Japanese liked the chocolate samples significantly more than the Koreans.

## P46. Taste perception of 6-*n*-propylthiouracil in Japanese university students

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The perception of the bitter taste of 6-*n*-propylthiouracil (PROP) was studied in 145 Japanese university students (94 females and 51 males). The perception of the bitterness of PROP ($3.2 \times 10^5$ M, $1.6 \times 10^4$ M) and saltiness of NaCl (0.3 M) was measured with a 100 mm visual analogue scale (0 for no taste, 100 for extremely strong). The results revealed that the perception of taste showed a unimodal distribution for the saltiness of NaCl but a bimodal distribution for the bitterness of $3.2 \times 10^5$ M PROP. A sex difference in the ability to taste PROP was found: females rated the bitterness of PROP higher than males ($t = 2.24$, $P < 0.05$). According to the criterion of Bartoshuk and colleagues (1994, Physiol. Behav., 56: 1165–1171), the subjects were classified as nontasters and tasters. Nontasters could not detect the bitterness of $1.6 \times 10^{-4}$ M PROP. The percentage of nontasters in 145 Japanese university students was 6.2%. The sex difference was also seen in the rate of nontasters: the rates of nontasters were 11.8%

for males and 3.2% for females. The relationships between the ratings of the bitter taste of PROP and the preferences for the stimulative foods were discussed.

## P47. Mechanism of heat-induced sweet taste disappearance of the sweet protein thaumatin

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The heat-induced sweet taste disappearance of the sweet protein thaumatin was examined. On heating at pH 7.0 to >70°C, thaumatin lost its sweetness and an insoluble aggregate was observed. The aggregate could be solubilized by heating in the presence of both a thiol reducing reagent and SDS. The molecular aggregation was repressed by the addition of either *N*-ethylmaleimide or iodoacetamide. This suggests that the disulfide bond between heat-denatured thaumatin molecules plays an important role in the formation of aggregates, and suggests that disulfide bonds were formed through the thiol-catalyzed disulfide interchange reaction. From the amino acid analysis of the aggregates, the reduction of the cystine and lysine residues in a molecule was observed and the formation of cysteine and lysinoalanine residues was confirmed. The reduction and formation of these residues stoichiometrically satisfied the sequential reaction of the degradation of cystine residue through a β-elimination reaction. This study shows that both the degradation of cystine residues and the formation of intermolecular disulfide bonds are the main reactions in the thermal aggregation of thaumatin. These observations indicate that the sweet taste of thaumatin is stable in the heating-condition if no aggregate is formed.

## P48. Mice perceiving the taste-sensation from sweet proteins

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Protein is generally tasteless and induces no taste sensation, while thaumatin and some other proteins elicit strong sweetness. It is reported that the sweetness of thaumatin and other sweet proteins can be recognized only by monkeys and humans. However, as the sensitivity of micee against various sweeteners depends on the strain of mouse, we tried to discover a mouse strain which can perceive the taste-sensation of sweet proteins, using the two-bottle preference (TBP) test and the conditioned taste aversion (CTA) test. One of the strains tested selected thaumatin solution rather than water by the TBP test, and the perception of the sweetness of thaumatin has been confirmed by the CTA test. Some other strains also perceived the taste of sweetness of thaumatin by the CTA test. Lysozyme elicits the sweetness taste in humans. Mice also perceived the taste of lysozyme; however, they dislike the taste, as observed by the TBP and CTA tests.

## P49. Behavioral analysis of gurmarin inhibition of the sweet taste response in mice

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Our previous electrophysiological studies in C57BL mice have demonstrated that gurmarin inhibited responses of the chorda tympani nerve to various sweeteners to ~50% of control and the inhibited responses recovered immediately after rinsing the tongue with β-CD. In the present study, such effects of gurmarin and β-CD on sweetener responses were behaviorally examined in C57BL mice by use of a single bottle test (counting the number of licks per 10 s to each test solution). Test solutions were prepared as mixtures with 3 mM quinine. The numbers of licks per 10 s for mixtures of sucrose with quinine decreased to ~60% of control after oral administration with 10 μg/ml of gurmarin, and recovered to ~80% of control by the subsequent administration of 15 mM β-CD. The numbers of licks to other various sweeteners mixed with quinine also significantly decreased after gurmarin. These results suggest that gurmarin inhibited behavioral responses to all tested sweeteners and β-CD facilitated the recovery of sucrose responses inhibited by gurmarin, which are comparable with those observed in previous electrophysiological studies.

## P50. Biochemical characteristics of gurmarin-binding proteins in rat saliva

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Our previous study demonstrated that preference for 0.1 M sucrose in rats temporally decreased until 3 days after beginning to feed on a gurmarin-containing diet and recovered to the control level on day 14. The subsequent restoration of the preference was possibly attributed to the induction of salivary gurmarin-binding proteins. In the present study, the salivary gurmarin-binding proteins were analyzed for some biochemical properties. Male Wistar rats were fed a diet containing gurmarin (3% in the form of dry *Gymnema sylvestre* leaves) for 2 weeks. Changes in preference for a sucrose solution were ascertained. Submandibular saliva inhibited immunoreaction with gurmarin and mouse anti-gurmarin antiserum, indicating the presence of salivary gurmarin-binding proteins. Such inhibition was strong in the gurmarin-containing diet groups compared with the ordinary chow group (control). Affinity chromatography of saliva was performed on a gurmarin-coupled Sepharose gel. Several salivary proteins selectively absorbed on the matrix in a pH range where gurmarin has been reported to suppress responses of the chorda tympani to sweet taste stimuli. The absorbed proteins were effectively eluted by adding β-cyclodextrin solution, a candidate for the inhibitor of gurmarin binding to the sweet receptor. The molecular weights of the salivary gurmarin-binding proteins were estimated at 15.0, 16.0, 46.5, 60 and 66 kDa by SDS–PAGE. These

results suggest that the binding sites of the proteins for gurmarin are structurally similar to those of a gurmarin-sensitive sweet receptor.

## P51. Release of P-endorphin to taste stimulation in the rat

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Several studies have suggested that opioids are involved in the generation of palatability and the facilitation of consumption of food and fluid. We measured the level of an endogenous opioid, β-endorphin, in the blood serum and cerebrospinal fluid (CSF) after the drinking of water and taste solutions in Wistar rats. When the water-deprived animals were allowed to drink 10 ml of water, the level of β-endorphin increased significantly 60 and 90 min after the start of drinking in both samples. β-Endorphin in the CSF increased most after ingestion of 0.5 M sucrose and 0.005 M saccharin followed by 0.1 M NaCl, 0.1 mM quinine and water. An intragastric infusion of 7 ml of water did not change the β-endorphin level. Although essentially the same results were obtained for the serum sample, the NaCl and quinine solutions did not increase the level of serum β-endorphin. The sweeteners became ineffective in releasing β-endorphin in both samples after the establishment of conditioned taste aversions to these taste stimuli. These results suggest that the release of β-endorphin is positively correlated with the palatability of taste stimuli, and that CSF β-endorphin also reflects the reinforcement of fluid intake in thirsty animals.

## P52. The effect of intracranial microinfusions of antisense oligodeoxynucleotide against *c-fos* mRNA on conditioned taste aversion in the rat

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The functional involvement of the *c-fos* gene product, Fos protein, induced by the gustatory (conditioned stimulus, CS) and visceral stimuli (unconditioned stimulus, US) have not been much reported for the formation of conditioned taste aversion (CTA). The effects of suppression of Fos production by intracranial microinfusions of the anti-sense oligodeoxynucleotide (A-ODN) against *c-fos* mRNA on OTA formation were investigated in the rat. Bilateral infusions of A-ODN 8–9 h before a pairing of the ingestion of saccharin (CS) with an i.p. injection of LiCl (US) into the parabrachial nucleus (PBN), but not into the amygdala (AMY) or gustatory cortex (GC), significantly disrupted the acquisition of CTA. However, the retention of CTA was impaired by the infusions of A-ODN into the PBN, AMY or GC. In contrast, infusions of randomized oligodeoxynucleotide (R-ODN) with the same deoxynucleotide content into each of these sites had no effect on both the acquisition and retention of CTA. Simultaneous infusions of A-ODN, but not R-ODN, into both the AMY and GC of each rat significantly weakened the acquisition and retention of CTA. These results suggest that the Fos proteins in the PBN, AMY and GC each contribute differently for the acquisition of CTA and th eretention of a long-term gustatory memory.

## P53. Hyperglycemia suppresses taste responses to glucose selectively in a sugar-sensitive subgroup of NTS cells in the rat

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Multiunit recordings indicate that hyperglycemia causes suppression of gustatory responsiveness to glucose in the nucleus tractus solitarius (NTS). We repeated this study, but at the level of single NTS neurons, to determine which taste cells were responsible. We used micropipettes to isolate the activity of NTS cells, and monitored their responses to oral application of 1.0 M glucose, 0.1 M NaCl, 0.01 M HCl and 0.01 M quinine-HCl, each applied 3–4 times over a 30 min period. We then infused 0.5 g/kg glucose over 3 min through a jugular cannula and continued the recordings for 60 min post-infusion. The total taste response to glucose declined by 19%. Cells were divided into three types based on their response profiles before glucose infusion. Responses of the 10 salt, 20 acid and two outlying cells were not significantly affected by hyperglycemia. However, glucose-induced responses in the five glucose cells were suppressed from 20.5 to 5.5 spikes/s (73%). Accordingly, the proportion of the total response to glucose that was carried by glucose cells declined from 25% to 8%. Thus, nearly the entire effect of hyperglycemia on taste was attributable to the impact on one neuron type. This provides evidence for a sweet coding channel.

## P54. Effects of electrical stimulation of the amygdala on taste responsive units in the parabrachial nucleus of rats

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It is well documented that there are reciprocal connections between the taste responsive region of the parabrachial nucleus and the central nucleus of the amygdala. Compared with the parabrachio-amygdaloid projection, the projection from the central nucleus of the amygdala to taste responsive neurons in the parabrachial nucleus has not been electrophysiologically demonstrated in detail. In the present experiment, we examined the effects of electrical stimulation of the central nucleus of the amygdala on taste responsive neurons of the parabrachial nucleus in urethane-anesthetized rats. Nineteen units (21%) of 92 taste responsive units were antidromically and 17 (18%) were orthodromically activated by the amygdala stimulation. Antidromic response latencies were <3 ms in nine (90%) of 10 units recorded from the region dorsal to the brachium conjunctivum. On the other hand, five (56%) of nine units recorded from the region ventral to the brachium conjunctivum had longer antidromic response latencies (4–6 ms). The recording sites of orthodromically activated units were dorsal to (10; 59%), ventral to (5; 29%) or within (2; 12%) the brachium conjunctivum. The relative numbers of units that responded to only one taste stimulus or all four stimuli were higher in units activated orthodromically than in other units. These

results suggest that the projection from the central nucleus of the amygdala to the parabrachial nucleus is actually related to taste function.

## P55. Cortico-cortical connections of the cortical taste area in rats

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With a tract tracing method using WGA-HRP, we examined cortical connections of the cortical taste area (CTA) in two kinds of cortical preparations, tangential sections of flattened cortices and conventional coronal sections of the cortex. After the tracer was injected in a portion of the CTA, we saw ipsilaterally labeled cell bodies and/or terminals in a rostrocaudally elongated portion including the injection site. Denser labels were recognized in six regions rostrally to the injection site: (1) a rostrolateral portion of the agranular lateral field [frontal areas 1 and 3 of Paxinos and Watson (1986)], (2) a rostrolateral portion of the agranular medial field (frontal area 2 or agranular insular area) and caudally to the injection site, (3) an insular area, (4) SII, (5) PV (parietal ventral area) and (6) PR (parietal rhinal area) [terms after Fabri and Burton (1991)]. In the contralateral cortex dense retrograde and anterograde labels were found in the site homotopical to the injection site while sparse labels were seen in other sites. The results indicate that ipsilateral cortical connections of the CTA were quite similar to those of the SI (Fabri and Burton, 1991) but that its callosal connections were different from those of the SI except for SI jaw regions (Hayama and Ogawa, 1997).

## P57. Differences in developmental changes of taste buds innervated by the facial and glossopharyngeal nerves of the postnatal rat

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The development of taste buds on the soft palate (SP), fungiform (FF), foliate (FL) and circumvallate papillae (CV) was examined histologically in the postnatal rat. After paraffin embedding, complete serial sections (10 μm) were made and stained by H&E. Digitized images for each section were examined, and the existence of a taste pore was determined. Both the height × width (i.e. size) and height/width (i.e. shape) for each taste bud were calculated. At birth, 53% of taste buds on the SP contained taste pores, while only 12% within FP contained them. After the first week, 90% of the SP and 80% of the CT taste buds contained taste pores. In contrast, no taste bud with a pore was observed at birth within the FL and CV. An increase in the number of mature taste buds occurred later in these two latter types of papillae. The height × width of taste buds at four different areas increased similarly until 4 weeks of age. However, the ratio of height/width in the CV was ~2 from 1 to 9 weeks of age, and that in the FL was 2.4 at 1 week and decreased to 1.6 at 4 weeks. In contrast, the ratios for SP and FF were ~1 from 1 to 9 weeks of age. These results indicate that developmental changes of taste buds may depend on the innervating nerves, either facial or glossopharyngeal.

## P58. Properties of taste responses to sweet stimuli applied to the soft palate in the greater superficial petrosal nerve of the rat

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To examine properties of taste responses to sweet substances in the greater superficial petrosal nerve of the rat, the inhibitory effects of gurmarin (10 μg/ml), a protein extracted from *Gymnema sylvestre*, were examined in integrated responses to gustatory stimuli applied to the soft palate. Stimuli included 0.1 M NaCl, 0.01 M HCl, 0.01 M quinine-HCl, 0.01 M saccharin, six sugars at 0.5 M, 0.1 M L-Arg-HCl and L-Lys-HCl, 0.1 M L- and L-forms of His, Asn, Phe, Gln and Ala, and 0.05 M Trp. The phasic responses to the six sugars and saccharin were significantly depressed to 40–50% after gurmarin treatment for 10 min, whereas little inhibitory effect was observed for responses to NaCl, HCl, QHCl and two L-basic amino acid-HCl salts. With the single exceptions of D-Phe, responses to the D-amino acids were significantly inhibited by the gurmarin treatment. Phasic responses to L-Phe and L-Ala were also depressed, but gurmarin treatment was without significant effect on the remaining L-amino acids. These response characteristics of the GSP reflect well the sweetness to these substances in humans. Considering that D-amino acids produced smaller responses than each D-amino acid in the chorda tympani, the results from this experiment suggest that the receptor molecule and/or transduction mechanisms for sweet taste in the GSP are different from those for the CT in the rat.

## P59. Electrophysiological study of the umami receptor using agonists and an antagonist of the glutamate receptor in the rat

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A recent study has suggested that mGluR4, a metabotropic glutamate receptor, is a chemosensory receptor for the umami taste of monosodium glutamate in rats (Chaudhari *et al*., 1996). To investigate the characteristics of the umami receptor, we recorded integrated neural responses from the chorda tympani nerve using three agonists and one antagonist of glutamate receptor. As agonists, L-2-amino-4-phosphonobutyrate (L-AP4; 5 mM), *N*-methyl-D-aspartate (NMDA; 5 mM), kainic acid (KA; 5 mM) and α-amino-3-hydroxy-5-methylisoxazole-4-propionionic acid (AMPA; 5 mM) were used. *S*-2-Amino-2-methyl-4-phosphono-butanoic acid (MAP4) was used as an antagonist. Results were as follows: (i) on mixing with 0.01 M 5′-inositol monophosphate (IMP), L-AP4 showed synergistic effects like monopotassium glutamate (MPG), but other agonists did not. (ii) 40 mM MAP4, an antagonist of mGluR4, did not suppress any of the responses to four agonists and MPG. (iii) Gurmarin, an anti-sweet peptide, suppressed the responses to the mixtures of both MPG and IMP, and L-AP4 and IMP. It is possible that

umami receptors may not be simply understood by established glutamate receptors in the nervous system.

## P60. Aversive behavior and taste nerve responses to various acid solutions with identical pH in rats

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Although acid taste is essentially dependent on $H^+$, rats show distinct aversive behaviors to various acid solutions with identical pH. For instance, acetic acid adjusted at pH 3 induces strong aversive behavior, as assessed by the two-bottle preference test, compared with hydrochloric acid (HCl) with identical pH. In the present study, we examined the factor(s) which is responsible for the behaovioral differences. Anosmia induced by $ZnSO_4$ did not influence behavioral responses to both acetic acid and HCl, indicating that olfaction does not play a pivotal role in the differential behavior between these two acids. In contrast, responses of two major taste nerves, the chorda tympani and glossopharyngeal nerves, to the acid solutions were closely coincident with the behavior. Responses of these nerves to acetic acid were larger that those to HCl with identical pH. Since the $CH_3COO^-$ was less effective than $Cl^-$ as a taste stimulant, anions coexisting in the acid solutions would not be the factor accounting for the mechanism by which acetic acid and HCl produce notable differences in taste nerve responses. Further study is needed to evaluate the mechanism for the acid avoiding behavior.

## P61. Possible mechanism for the enhancement of chorda tympani nerve resopnses to NaCl after cold exposure in rats

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The mechanism by which a cold (4°C) ambient temperature increases the taste responses of the chorda tympani nerve to NaCl was examined in rats. The nerve responses to NaCl were time-dependently enhanced when the animals were exposed to cold for >3 days, and reached maximal levels at 7–14 days. A similar time course was observed when the enhanced response by cold exposure returned to the control level in a warm environment (22°C). It has been known that taste cells are continuously renewed over an average of 10 days. It is thus probable that the sensitivity of taste receptor cells to sodium salts was substantially improved during the process of cell replacement in the cold environment. We also examined the effects of amiloride on the cold-induced hypersensitivity to NaCl. The residual NaCl responses after amiloride in cold-exposed rats were similar to those in control rats. In addition, responses to Na-acetate were elevated similarly to NaCl, and those to KCl were unchanged in the cold environment. These results indicate that cold exposure potentiates the chorda tympani nerve responses to $Na^+$ but not to $Cl^-$.

## P62. Water response of the superior laryngeal nerve in humans

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When the mucous membrane of the larynx is stimulated with water and taste solutions, the superior laryngeal nerve responds most successfully to water. This phenomenon is called water response. To confirm the existence of water response in humans, we examined the responses of the superior laryngeal nerve during a surgical operation on the human. The laryngectomy is performed on patients with laryngeal or pharyngeal cancer. Informed consent was obtained from the patients before the operation. The laryngeal aspect of the epiglottis is stimulated with water and some taste solutions using eyedroppers. The exposed nerve is fixed onto a bipolar electrode. The responses guided from the electrode are electrically integrated and recorded. We evaluated these integrated responses. The maximum response is to distilled water. The minimum is to physiological salt solution. These results are compatible with the reports concerning water responses in other species of animals.

## P63. Effects of various ion transport inhibitors on the water response in the superior laryngeal nerve in rats

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The effects of various ion transport inhibitors on the water response in the superior laryngeal nerve were investigated in anesthetized and paralyzed rats. The taste stimuli used were deionized water, 0.15 M NaCl, various concentrations (0.001, 0.01, 0.1, 1, 5 and 10 mM) of amiloride, an inhibitor of Na transport, 5 mM furosemide, an inhibitor of Na-K-2Cl cotransport, 2 mM acetazolamide, an inhibitor of carbonic anhydrase, and 2 mM DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid), an inhibitor of Cl transport. All chemicals were dissolved in deionized water. The magnitude of the response to each stimulus was measured as the height of the integrated response, and was expressed relative to that of deionized water. The responses to amiloride at low concentrations (0.001, 0.01 and 0.1 mM) were slightly smaller than those to deionized water ($0.92 \pm 0.04$, $0.92 \pm 0.07$ and $0.88 \pm 0.06$ respectively), but these suppressions were not statistically significant (one sample $t$-test, $P > 0.05$). On the other hand, the responses to amiloride at high concentrations (1, 5 and 10 mM) were significantly small ($P < 0.05$); the responses to these stimuli were $0.76 \pm 0.05$, $0.63 \pm 0.02$ and $0.59 \pm 0.06$ respectively. Acetazolamide, DIDS and furosemide also suppressed the water response significantly ($0.91 \pm 0.03$, $0.68 \pm 0.04$ and $0.63 \pm 0.03$ respectively). These results suggest that several ion transport systems are directly or indirectly involved in the water response in the rat larynx.

## P64. Taste responses of the pharyngeal branch of the glossopharyngeal nerve in rats

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Pharyngeal afferent signals are important in the perception of food taste in the pharynx. However, very few reports are available about the neural activity of afferent nerves from the pharynx. This study was therefore designed to examine the responsiveness of the pharyngeal branch of the glossopharyngeal nerve to water, beer and several taste solitions. The pharynx and larynx of urethane-anesthetized rats were surgically opened and test solutions were applied to the internal surface of the pharynx. Fresh beer, 5% ethanol and water elicited marked responses. Soda water produced a marked transient response. A solution of 1 M NaCl elicited a remarkable response. Amiloride failed to inhibit the response to NaCl. Red pepper dissolved in water facilitated water response whereas no response was elicited when it was dissolved in 0.15 M NaCl solution. The properties of taste receptors in the pharynx were quite similar to those in the larynx except for the response to 1 M NaCl, because the solution of 1 M NaCl did not elicit any excitatory responses but depressed the spontaneous activity of the laryngeal nerve.

## P65. Characterization of ion channels involved in three types of MSG responses in mouse taste cells

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We obtained three types of MSG responses (transient inward current, sustained inward current and outward current) in the isolated taste cells from C57BL/6J mouse by using a whole cell patch clamp technique. The transient inward current response appeared to be due to $Ca^{2+}$ ion influx through the NMDA-sensitive ionotropic glutamate receptor rather than through the voltage-dependent Ca channel, though both channels exhibited $Ca^{2+}$ current 'run-down'. In the presence of verapamil (30 μM, a voltage-dependent Ca channel blocker), MSG (10 mM) elicited a transient inward current. NMDA (1 mM) stimulation also elicited a transient inward current. The sustained inward current response was related to cation ($Na^+$, $K^+$, $Ca^{2+}$) influx through the non-selective cation channel. The outward current response by MSG was seen to be similar to that by L-AP4 (1 mM; a potent agonist of mGluR4) stimulation. It seems that MSG and L-AP4 have similar perceptive mechanisms including G-protein-coupled receptors in the taste cells. These results suggest that MSG responses involve more than three types of transduction mechanisms in mouse taste cells. MSG binds directly to the NMDA-sensitive glutamate receptor to activate ionotropically, and it also opens the nonselective cation channel. Further, MSG closes the nonselective cation channel metabotropically by binding to an alternate receptor, possibly mGluR4.

## P66. Effects of G protein modulator on responses to quinine stimulation in mouse taste cells

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Various studies have been made on the signal transduction of quinine in taste tissue, and it has been suggested that quinine blocks ion channels or activates G proteins via receptor. In the knockout mouse lacking gustducin, the sensitivity to quinine was impaired, so it was suggested that the gustdusin and cyclic nucleotide are involved in quinine signal transduction. In the present work, the effects of cGMP on responses to quinine in isolated mouse taste cells were examined.

Taste cells were isolated from 8-week-old female mice (C57BL/6J) by enzyme treatment. A whole-cell patch clamp technique was used for the electrical recording of taste cells. Ten millimolar quinine dissolved in Ringer solution was applied to the taste cells by pressure ejection from a capillary glass. In the voltage clamp mode (holding potential –80 mV) using pseudo-intracellular solution, quinine induced an inward current response. From the observation of current–voltage relationships by ramp voltage commands, it was demonstrated that quinine blocked the $K^+$ channel, and then activated membrane conductance independently of the blockade of that channel. Using a $Cs^+$ pipette, in which $K^+$ was substituted by $Cs^+$ in the pseudo-intracellular solution, the response from the$K^+$ channel blockade was eliminated. When 1 mM cGMP was applied to the $Cs^+$ pipette solution, the extent of the activation of membrane conductance was higher than that observed with a $Cs^+$ pipette not containing cGMP. This suggests that the quinine response in the taste cells involves an alternate transduction pathway stimulated by an increase in cGMP level.

## P67. Measurements in cAMP and inositol-1,4,5-trisphosphate levels of the fungiform papilla in response to umami substances in C57BL and BALB/c mice

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Our previous electrophysiological studies have shown that strain differences of mice were found in the chorda tympani responses to umami substances and in the magnitudes of inhibition of umami substance responses by the lingual treatment of the proteolytic enzyme pronase E. We measured the cAMP and inositol-1,4,5-trisphosphate ($IP_3$) levels of the fungiform papilla when the tongues in C57BL and BALB/c mice were treated with pronase E followed by stimulations with 0.5 mM inosinate (IMP), 0.03 M monosodium glutamate (MSG) and 0.5 mM IMP + 0.03 M MSG. Within ~10 s after the onset of taste stimulation, each fungiform papilla was removed with a pair of fine forceps to pool 60 fungiform papillae in each sample. Mass levels of both cAMP and $IP_3$ in each tissue pool were measured by radiobinding assay kits. In C57BL mice, the levels of both cAMP and $IP_3$ in the fungiform papilla synergistically increased by stimulation with 0.5 mM IMP + 0.03 M MSG. Similarly to the case in sucrose stimulation, pronase treatment suppressed the increase in cAMP level but not in the $IP_3$ level of the fungiform papilla stimulated with 0.5 mM

IMP + 0.03 M MSG. In BALB/c mice, which are poorly sensitive to umami substances, stimulation with 0.5 mM IMP + 0.03 M MSG did not elicit an increase in the $IP_3$ level of the fungiform papilla. These results suggest that in the transduction for umami a pronase-insensitive receptor coupled with the $IP_3$ pathway may be more important than a pronase-sensitive receptor coupled with the cAMP pathway in the fungiform papilla in mice.

## P68. A quinine-activated cationic channel in the excised patch membrane of the bullfrog taste receptor cell

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We have reported that quinine increases a cationic conductance in bullfrog taste receptor cells recorded in the whole-cell configuration. In the present study we record membrane currents in outside-out patches excised from isolated taste receptor cells. The quinine-induced channel openings were seen without adding any cytoplasmic factors (cyclic nucleotides, ATP or GTP) to either side of the membrane. Quinine was effective only when it was applied to the outside. The reversal potential was +20.5 ± 2.3 mV (mean ± SD, $n$ = 3) in a Na/Cs bi-ionic condition ($P_{Cs}/P_{Na}$ = 0.39), and the single channel conductance was 10.2 ± 1.4 pS. The reversal potential was independent of $[Cl^-]_o$, indicating that the channel is cation-selective. Removal of extracellular $Ca^{2+}$ increased the single channel current amplitude: –0.38 ± 0.10 pA ($n$ = 4) in 1.8 mM $Ca^{2+}$ (holding voltage, –64 mV) and –0.78 ± 0.09 pA ($n$ = 3) in nominally $Ca^{2+}$-free saline. The properties of the quinine-activated channel were almost identical to those obtained in the whole-cell recording by noise analysis. Since it is very unlikely that small diffusible cytoplasmic second messengers are involved in the events seen in an excised patch membrane, it is strongly suggested that quinine activates the channel directly rather than via second messengers.

## P69. KCl stimulation to mouse taste cells induces an inwardly rectifying current

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We examined KCl- and $NH_4Cl$-induced responses in non-dissociated taste cells of mouse fungiform papillae using a whole-cell clamp technique and a localized taste stimulation method. Ninety-four percent of cells responded to 0.5 M KCl with depolarization under current clamp or with inward currents at –80 mV under voltage clamp. KCl-induced responses were not suppressed by amiloride. Mouse taste cells displayed an inwardly rectifying current ($I_{ir}$) which showed the reversal potential between –60 and –80 mV and was strongly suppressed by 1 mM $Cs^+$. The reversal potential of $I_{ir}$ shifted to the potential near the equilibrium potential of $Cl^-$ (–42.4 mV) at the basolateral membrane during KCl responses. The responsivity, the magnitude of responses and the reversal potential were not affected by removal of $Na^+$ from the bathing solution. KCl-induced current responses were suppressed not only by the inwardly rectifying $K^+$ channel blockers, $Cs^+$ and TEA, but also by the $Cl^-$ channel blockers, NPPB and niflumic acid. Similar properties were observed in $NH_4Cl$-induced responses. The results suggest that the generation mechanism for KCl- and $NH_4Cl$-induced responses is different from that for NaCl-induced responses and is mainly mediated by the activation of $Cs^+$-sensitive inwardly rectifying $Cl^-$ channels.

## P70. The space distribution of $Cl^-$ and the function of the $Cl^-$-pump in newt olfactory cells

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We recorded the fluorescence intensity of the $Cl^-$-sensitive fluorescent dye probe, *N*-(6-methoxyquinolyl)acetoethyl ester bromide (MQAE), loaded into isolated olfactory cells from newts in normal Ringer's solution, then in solutions with different $Cl^-$ concentrations containing a $Cl^-$-ionophore cocktail to estimate the intrinsic concentration of $Cl^-$ in the cell ($[Cl^-]_i$). Under the assumption that $Cl^-$ was uniformly distributed in the cell, $[Cl^-]_i$ was estimated to be ~40mM, while the $[Cl^-]_i$ is kept lower at the knob area. This $[Cl^-]_i$ gradient along a longitudinal axis of the cell became obscure within 5 min after 1.0 mM ethacrynic acid, the $Cl^-$-pump inhibitor, was applied to the cell. These results suggest that the $Cl^-$ is constantly pumped out to the outside of the cell at the olfactory knob and/or cilia in normal conditions. It has been proposed and widely accepted that the $[Cl^-]_i$ in olfactory receptor cells is rather high (~100 mM), and that the receptor current is amplified by the $Cl^-$-efflux to the outside of the cell. This proposal, as well as our present result, may require more careful examination.

## P71. Saccharin-induced responses in glossopharyngeal nerves and taste cells of the bullfrog

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Sweet stimuli interact with receptor proteins located in the apical taste membrane, triggering a biochemical cascade via G protein activation. We examined the glossopharyngeal nerve activity and taste cell membrane current in response to saccharin in bullfrogs. The gustatory nerve displayed the transient impulse discharges elicited by saccharin. The magnitude of the neural activity increased in proportion to the saccharin concentration and the $EC_{50}$ was ~0.4 mM. Cross-adaptation experiments between 3 mM saccharin and 0.3 mM quinine were performed. The magnitude of saccharin response after quinine adaptation was approximately equal to that after normal saline adaptation. The membrane currents of rod cells were measured using the conventional whole-cell patch clamp technique. In 4/13 cells dialyzed with 10 mM $Cl^-$, 30 mM saccharin elicited an inward current of –10 to ~–60 pA at the membrane potential of –50 mV. The $EC_{50}$ of the current response was also ~0.4 mM. Intracellular dialysis of 1 mM 8-Br-cAMP did not change the magnitude of the saccharin-induced current significantly. Regardless of intracellular 8-Br-cAMP, saccharin inhibited the voltage-gated $Na^+$ and $K^+$ currents in 5/29 cells, but the effects were irreversible. The results

suggest that frog taste cells generate a cation current in response to saccharin.

## P72. Effect of amiloride on the taste responses of the frog glossopharyngeal nerve to cations

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In the frog tongue, amiloride-blockable sodium ion channels are found in isolated taste cells (Avenet and Lindemann, 1988). However, we have reported that amiloride failed to reduce the response of the frog glossopharyngeal nerve to 100–500 mM NaCl (Kitada and Mitoh, 1997). In the present study, we examined whether amiloride affects the responses to KCl, $NH_4Cl$, $MgCl_2$, $CaCl_2$ and $CaSO_4$. Unitary discharges were recorded from single fibers in the glossopharyngeal nerve in anesthetized frogs. Amiloride at 0.5 mM did not affect the responses to 500 mM KCl, 500 mM $NH_4Cl$ or 10–100 mM $MgCl_2$, whereas it strongly reduced the response to $CaCl_2$ and $CaSO_4$ (the Ca response) at 0.1–1 mM. It has been suggested that the affinity of the calcium receptor responsible for the Ca response might be charge-specific. The inhibition of the Ca response by amiloride was due to competitive antagonism between Ca and amiloride. Since amiloride has a positive charge in the solution, it appears that amiloride serves as a cation. Therefore, amiloride does not have a specific action on the cation response of the frog glossopharyngeal nerve. Since amiloride, a large molecule, is unable to pass through tight junctions, the calcium receptor may reside in the apical membrane of the taste cells.

## P73. Response properties of the facial and glossopharyngeal taste systems in the clawed toad, *Xenopus laevis*

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The purpose of the present experiment is to characterize the relative responsiveness of the facial (VII) and glossopharyngeal (IX) nerves of the clawed toad, *Xenopus laevis*.

The L-proline data obtained from both the VII and IX nerve recordings showed no differences in the threshold and the concentration–response curve. Similar results were obtained for L-tyrosine and L-arginine. The IX nerve recordings showed L-valine, L-cysteine, L-tryptophan and L-isoleucine to be relatively less stimulatory as compared with the results obtained for the VII nerve, although the threshold did not change between the two nerves.

Thresholds for the quinine-HCl, strychnine-HCl and brucine-HCl did not differ for the two nerves, whereas the response magnitude for the IX nerve was significantly larger than that for the VII nerve at higher concentrations. These differences in responses between the VII and IX taste systems to bitter substances may be a result of a lower total number of sensitive units in the VII system as compared with the IX system.

The responses to sugars of 1 M concentration were significantly larger for the VII nerve than for the IX nerve in all sugars tested.

HCl and other salts tested did not show any differences in the threshold and the concentration–response relationship.

## P74. The primary taste center in the goatfish

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This study was carried out to reveal general morphological features of the primary taste center in the goatfish. The primary taste center was found as a pair of columns in the medulla. Its anterior portion is extraordinarily developed as the facial lobe (FL) to occupy the dorsal regions of the medulla. Tracing experiments with DiI show the topological projections of the facial, glossopharyngeal and vagal nerves; the facial fibers end at the anterior portion of the column (FL), glossopharyngeal fibers at the intermediate part of the column and vagal fibers at the posterior part of the column.

The facial lobe has a highly convoluted surface. Histological examination reveals fold-like and laminated structures in the FL. Each fold consists of four layers: (i) an outer marginal layer, (ii) a layer of neuropile, (iii) a layer of cell-clusters and (iv) an ventral layer of fibers.

Sublaminar organization was also found in the layer of cell-clusters: densely packed round cells are located in the upper portion of the cluster, followed by medium-sized cells. In the bottom of the cluster, large cells are lined. The layer of cell-clusters is located in the central part of the fold and each end of this layer connects to the layer of cell-clusters in the adjacent fold. No laminar organization was found in the other regions of the primary taste center.

## P75. Distribution of the taste buds and nerve fibers in the barbels of the catfish and goatfish

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We examined the distribution of the taste buds and nerve fibers in the barbels of the catfish and goatfish and revealed considerable differences between the two species.

In the catfish, taste buds are concentrated in the rostral and caudal surfaces of the barbel, with relatively few in the intermediate surface. The barbel nerve fibers are distributed in a loop-like fashion to make neural networks under the epithelium. Small fiber strands frequently originate from the networks perpendicular to the surface and enter the ventral portions of taste buds to make a plexus.

In the goatfish, on the other hand, taste buds are much larger than in the catfish and are found throughout the whole surface of the barbel at high density.

Barbel nerve fibers are much greater in number in the goatfish than in the catfish. The large barbel nerve is located around the cartilage in the center of the barbel. Some bundles originate from the nerve and run longitudinally in the barbel. Each bundle gives off strands running toward the surface of the barbel at regular intervals. This strand ramifies into 10–12 substrands under the epidermis to make 'bunch of grapes'-like structures. Each substrand ends as a swelling in the ventral portion of the taste bud. The differences in the distribution of taste buds and nerve fibers

are thought to reflect the different organizations of the facial lobe between the catfish and goatfish.

## P76. Taste receptor sites of the largemouth bass, *Micropterus salmoides*

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Receptor sites for different amino acids, nucleotides and nucleosides in the facial taste system of the largemouth bass *Micropterus salmoides* were determined by *in vivo* electrophysiological cross-adaptation experiments. Forty largemouth bass ranging from 156.7 to 604.8 g in body weight were used for the experiments. The tested amino acids were L-Arg, D-Arg and L-Lys, the potent amino acids for this species. These amino acids were tested at equally stimulatory concentrations ranging from $6 \times 10^{-4}$ to $10^{-2}$ M. The tested nucleotides were ATP, ADP, AMP, GMP and IMP, and the nucleosides were inosine and adenosine. These substances were also stimulatory to the taste receptors of this species. The stimuli were adjusted in concentration from $6 \times 10^{-5}$ to $3 \times 10^{-3}$ M to provide equal response magnitudes. The cross-adaptation experiments were performed for combinations of (i) three amino acids, (ii) L-Arg with nucleotides or nucleosides and (iii) nucleotide-related substances. The results of the cross-adaptation experiments indicated that (i) L-Arg, D-Arg and L-Lys shared the same receptor sites; (ii) the receptor site for L-Arg and those for nucleotides and nucleosides were completely independent of each other; and (iii) the existence of at least four different receptor sites for nucleotides and nucleosides, an ATP-ADP-AMP site, an IMP-GMP site, an inosine site and an adenosine site, were estimated.

## P77. The effect of amino acids on the feeding behavior of the cuttlefish *Sepia esculenta*

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The effect of chemical stimulants on the feeding behavior of cuttlefish was behaviorally determined in a tank for the development of squid jigs baited with chemical stimulants. The chemical stimulants were nine amino acids (L-glutamine, L-glutamate, glysine, L-proline, L-serine, L-α-alanine, L-arginine, taurine and betain), extract of mysids and quinine. These were mixed with starch binder, plasticized as baits and presented to the cuttlefish. The feeding process of the cuttlefish in respect to a bait with mysids extract was as follows: recognition, approach, capture with arms, firm holding in arms and ingestion. The starch was neither held nor ingested. Baits with amino acids, except betain, were completely ingested, indicating an accelerating effect of these amino acids on the feeding behavior. Baits with betain and quinine were neither held nor ingested but repelled, indicating that these were aversive to the cuttlefish.

## P78. Computer-assisted analyses of glutathione-induced hydra response and mechanical frequency-dependent induction of the behavioral response

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We have developed a computer-assisted behavioral response analysis system, using the NIH-Image software, the free image analysis software published by the NIH, into which we incorporated a specific code to extract hydra images from the frame and to analyze the image as a user-p code. After the stimulation, the hydra images were captured every 2 s for 10 min. The 150 images from 5 to 10 min were analyzed to calculate various image quantities and the response was determined from these values.

When the glutathione-induced response of hydra was examined with the computer-assisted analysis system, we found the efficiency resulting in a strong response (images >90 among 150 images were that of 'response') was dependent on the frequency of vibration which was given for the initial 30 s of the chemical stimulation (10 μM *S*-methylglutathione) via a speaker fed with a square wave current from a wave generator. Some 26–36 responses were analyzed at each frequency from 13 to 32 Hz (total 609 responses; ~90,000 images were analyzed). An average ratio of the strong response over all the frequencies examined was 26.8%, with 52.9% at 29 Hz (significantly different, $P < 0.01$, by the chi-square test).

## P78. Mutational analysis of the methyltransferase-binding sequence of the bacterial chemoreceptor, which is critical for chemotactic adaptation

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Adaptation to a persisting stimulus plays a crucial role in many sensory systems. In the chemotaxis of *Escherichia coli*, adaptation requires methylation and demethylation of transmembrane receptors, which are catalyzed by methyltransferase CheR and methylesterase CheB respectively. CheR binds to major chemoreceptors (Tsr and Tar) through their highly conserved C-terminal motif NWETF, which is distinct from the sites of methylation. In this study, we carried out systematic mutagenesis, including alanine-scanning, on the NWETF sequence of Tar. Receptor methylation and adaptation were severely impaired by the alanine substitution of W550 and, to a lesser extent, by that of F553. Those of N549 and T552 had little effect. The alanine substitution of E551 enhanced slightly the receptor's ability to be methylated and to support swarming. The defects of Tar-W550A and -F553A were suppressed by high level and low level overproduction of CheR respectively. Preliminary results suggest that CheR is localized predominantly to the membrane fraction of cells expressing wild-type Tar, but to the cytoplasmic fraction of cells expressing Tar-W550A. These results and further mutagenesis suggest that the hydrophobicity and the size of W550 and F553 are

involved in the interaction of the NWETF sequence with CheR, whereas the charge of E551 seems to be rather inhibitory to the receptor–CheR interaction. Therefore, the pentapeptide sequence may interact with CheR via hydrophobic interactions.

## P80. The enhancement effectiveness of phosphoric acid on the gustatory response of the fleshfly

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Phosphoric acid remarkably enhances the stimulatory effectiveness of ADP on the sugar receptor cell of the fleshfly *Boettcherisca peregrina*. Hill plot analysis of dose–response curves showed that the addition of 100 mM phosphoric acid in ADP solution lowered the concentration required to produce the half maximum response ~8-fold (from 14.9 ± 6.0 to 2.4 ± 1.8 mM), although it did not affect the maximum response. The Hill coefficient value was also lowered from 1.29 ± 0.41 to 0.92 ± 0.25 ($n$ = 8). The addition of $2^{-9}$ M phosphoric acid in 5 mM ADP solution significantly enhanced the stimulatory effectiveness. The enhancement effectiveness increased as the concentration of phosphoric acid increased. The stimulating effectiveness of sugars and amino acids on the sugar receptor cell were not remarkably affected by phosphoric acid. The responses of the salt receptor cell to NaCl and GMP were inhibited by it.

We tested the enhancement effectiveness of phosphoric acid on the stimulating effectiveness of ADP analogues on the sugar receptor cell, and found that the responses to GDP and IDP were not enhanced.

## P81. Amiloride inhibits the sugar taste response of the fleshfly

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Amiloride is known to inhibit the salt taste response in various vertebrate species by blocking the amiloride-sensitive $Na^+$ channel. In this study, we investigated the effect of amiloride on the taste response of an invertebrate, the fleshfly. When 0.5 mM amiloride was mixed with taste solutions, the response of the salt receptor cell to NaCl was not depressed but those of the sugar receptor cell to sucrose, glucose, fructose, L-Phe and L-Val were strongly inhibited. After treatment of a taste hair with 0.15 mM amiloride for 10 min, the response of the salt receptor cell to NaCl was not affected compared with that before the treatment. On the other hand, the responses of the sugar receptor cell to sugars and amino acids were depressed after the treatment. These results suggest that amiloride-sensitive conductance was involved in the taste transduction mechanism of the sugar receptor cell of the fleshfly.

## P82. Recall of olfactory memory and its effect on feeding behavior of the blowfly, *Phormia regina*

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It has been generally accepted that olfactory memory accompanied by a daily meal influences preference or appetite. Using blowflies fed with sucrose with a smell of dried fish or cheese, we examined the influences of such a memorized smell on the appetite or the feeding sensitivity of the fly.

First, the flies fed with sucrose with a certain smell showed reduced feeding sensitivity to plain sucrose (without any smells) compared with the flies fed with sucrose without any smells. It is considered that the olfactory memory which had been experientially constructed was recalled by taste stimulation and referred to the tested taste of plain sucrose. The absence of smell in the plain sucrose solution resulted in reduced feeding sensitivity. Thus, the reduced feeding sensitivity was recovered when the flies were tested with regard to their feeding behavior to sucrose with the smell. Moreover, it was also experimentally shown that such flies could recall the olfactory memory accompanied by the daily meal of sucrose, discriminating it from a background smell.

We are now commencing a study on the recall process of memory with this behavioral experiment.

This study was supported by HFSP (M.O.).

## P83. The taste enhancement effect of umami substances in the sugar receptor of the blowfly, *Phormia regina*

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Umami is regarded as the fifth fundamental taste independent of sweet, salty, bitter or sour. Monosodium glutamate (MSG) shows remarkable taste enhancement in humans. Using the blowfly, we investigated the taste-enhancing effect of MSG and its relative umami substances. In the presence of 50 mM MSG, the feeding sensitivity of the fly to sucrose was increased threefold, as was the electrophysiological response of the sugar receptor cell. In case of the fly, glutamate does not act as a taste substance but enhanced the sweet taste of sucrose. As we reported previously, the sucrose affinity of the P site type of putative sugar receptor protein, which is specifically inhibited by starch, was also increased in the presence of MSG. Considering the results of the behavioral, eleetrophysiological and molecular experiments together, it is suggested that glutamate or other umami amino acids act on a sugar receptor protein for the P site and increases its affinity for stimulants, resulting in taste enhancement and the promotion of appetite to sucrose.

This study was supported by Association of Umami Research (M.O.).

## P84. Changes in the gustatory sugar sensitivity induced by P-element excision mutations of the gene *Tre* in *Drosophila*

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P-element insertions near the gustatory gene *Tre* (trehalose sensitivity, 1–13..6) on the X chromosome of *Drosophila melanogaster* were used to produce small deletions that partially or fully uncover *Tre*. They were crossed to a strain that carries a transposase gene, and the F2 progenies where the P element had been excised from the X chromosome were tested by a feeding test that detects

changes in gustatory sugar sensitivity. Among four P-insertion lines at the 5A and 5B regions on the salivary X chromosome only a line with the insertion at 5A produced viable deletions that change the gustatory sensitivity in the feeding test to trehalose. Quantitative comparisons of the amount of intake for different sugars showed that the deletion causes a specific and severe reduction of intake for trehalose without significantly reducing the intake for other sugars. Electrophysiological investigation of the labellar sugar-sensitive taste neurons confirmed that the deletion causes a specific reduction in the spike frequency of the neuron to trehalose as in the spontaneous mutation that has been described in wild populations of *D. melanogaster*.

## P85. *gekoB*, *C*, *D*, *E:* novel olfactory mutants of *Drosophila melanogaster*

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For fruit flies the olfactory senses as well as the visual senses are vital in the search for food in nature. Although *Drosophila* is very useful for behavioral, evolutionary and physiological studies of olfactory senses, the olfactory receptor itself has not been cloned.

The *Drosophila* olfactory organs are the antenna and maxillary palpus in the adult, and the antennal-maxillary complex (AMC) in larvae. 6144 GAL4 enhancer trap lines were screened for GAL4 expression at the antenna to achieve olfactory mutants. By using UAS-GFP as a reporter, the load for screening mutants was relieved very much compared with other vectors or reporters. Lines that showed ubiquitous expressions were screened out despite having expression at the antenna. The number of lines that had localized (or partial localized) expression at the antenna was 190. Four mutants that had reduced olfactory responses to some attractants were isolated from these lines. These mutants were named *gekoB*, *C*, *D* and *E*. *gekoB* had a very poor response to ethanol, although it retains a normal response to other attractants. *gekoC* had a reduced level of response to acetic acid but a normal level of response to ethanol. *gekoD* and *gekoE* seem to have reduced levels of response to all attractants.

## P86. Chemical communication in scarab beetles with one pheromone binding protein, two olfactory receptor neurons and two enantiomeric pheromones

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Two enantiomers of japonilure, (*R*) and (*S*), play an interesting role in the chemical communication of two species of scarab beetles, the Osaka beetle (*Anomala osakana*) and the Japanese beetle (*Popillia laponica*). Each species produces only one of the two stereoisomers as its own sex pheromone and utilizes the other species' pheromone as a behavioral antagonist (stop signal). Using a single sensillum recording technique, we found that the antennae of each species possess olfactory receptor neurons extremely specific to (*R*)- and (*S*)-japonilure. In both species, different neurons detecting the two enantiomers co-occur within the same olfactory plate and are surrounded by a sensillum lymph. This lymph contains only a single pheromone binding protein, which binds both enantiomers to a similar extent. Therefore, filtering of chiral pheromone molecules by perireceptor soluble proteins alone, as a means of achieving specificity, seems unlikely. Enantiomeric discrimination must be achieved by the interaction of the pheromone or the appropriate ligand–PBP complex with membrane receptors.

## P87. Spatio-temporal activities in the antennal lobe analyzed by an optical recording method in an insect brain

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Optical recordings with a voltage-sensitive dye showed that the spatio-temporal pattern of depolarizing responses evoked by electrical stimulation of the antennal nerve was nonhomologously distributed in the antennal lobe of a male silkwonn moth, *Bombyx mori*. Time courses of postsynaptic activities and GABAergic inhibitory potentials of antennal lobe neurons were individually demonstrated by pharmacological experiments, i.e. $Ca^{2+}$-free and bicuculline conditions. GABAergic inhibitory potentials began with an ~3 ms delay from the beginning of the postsynaptic activities. Relatively strong postsynaptic activities and GABAergic inhibitory potentials were consistently observed in some parts of the macroglomerular complex and/or in some ordinary glomeruli in the medial and ventral parts of the antenna lobe.

The antennal nerve bifurcates into the medial and lateral nerves in the antenna. Our results using both optical recording and histological methods demonstrate that the differences in the projection area in the antennal lobe by the medial and lateral nerves reflect the topology of the distribution pattern of the receptor cells on the antenna.

We also applied the method to recording olfactory responses of the antennal lobe of a bumble-bee. We obtained some specific oscillatory responses in the glomerular region during stimulation (citral, isoamyl acetate). The frequency of power peak was ~26 Hz.

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## P88. Odour-evoked, spatial neural activity patterns in the deutocerebrum of *Periplaneta americana*

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Odour receptor neurons in the antennae of insects synapse onto neurons in the antennal lobes of the deutocerebrum where the first processing of information takes place. Synaptic contacts are made in glomeruli: distinct morphological entities which contain dense, synaptically connected neural arborizations. There is mounting evidence for an odotopy, i.e. that olfactory receptor neurons with similar odour-specificities project to the same subset of glomeruli. Here we show how six food-related odours and the female sex-pheromone component periplanone B map onto the antennal

lobes of male American cockroaches ($n$ = 10) by visualizing neural activity (synaptic activity) through optical recording of the [$Ca^{2+}$]-sensitive dye calcium green I [method adapted from Galizia *et al*. (1997, *Neurosci. Methods*, 76: 61–69)]. Different odours indeed evoke different spatial patterns of neural activity in the antennal lobes, as was reported earlier by Friedrich and Korsching (1997, Neuron, 18: 737–752) for the zebrafish olfactory bulb and by Joerges *et al.* (1997, *Nature*, 387: 285–288) for the antenna lobes of the honey bee. With the exception of the female sex-pheromone, which elicits a sharp focus of activity in the macroglomerular region, all other odours tested in the cockroach give broad and overlapping activity patterns. This indicates that either (i) the antennal afferents from receptors responsive to each odour project to a large array of glomeruli in the lobes or (ii) that the subsequent processing is widely distributed. We are now using pharmacological antagonists for acetytcholine and GABA receptors to understand how the spatial patterns are generated. Future experiments will focus on the spatial coding of odour mixtures.

## P89. Physiological properties of Kenyon cells in the cockroach *Periplaneta americana*

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The mushroom bodies of insect brains are thought to be crucial in learning and memory. They are composed of many intrinsic neurons, called Kenyon cells. Although the morphological organization of the insect mushroom bodies has been known for a very long time, the physiological properties of the Kenyon cells are still unknown. We focus here on the electrophysiology of Kenyon cells in the cockroach *Periplaneta americana*. Responses of Kenyon cells to intracellular injections of current were recorded *in vivo* by the patch-clamp whole-cell method ($n$ = 44); spike activation was voltage-dependent. The amplitudes of spikes were decreased by application of 10 mM TTX and recovered after washing ($n$ = 8). We have examined the relation between odor and spike activation in Kenyon cells: three of eight Kenyon cells showed spike activation during stimulation with complex food odor for 200 ms. This suggests that the Kenyon cells receive input from the antennal lobes and participate in olfactory discrimination.

## P91. The functional analyses of neuromedin B receptor in the olfactory region of rodents

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Neuromedin B receptor (NMB-R), which is one of the mammalian bombesin-like peptide receptors, is known to be expressed in the olfactory region. In the present study we conducted a series of experiments in order to investigate the functional properties of NMB-R in the olfactory systems. In *in vitro* analysis, we utilized cultured cells from the anterior olfactory nucleus (AON) of SD rats. AON is a region which expresses NMB-R abundantly. Application of neuromedin B (NMB) elicited transient oscillatory responses in some cells of the AON cultures in a dose-dependent fashion. Next, we evaluated *in vivo* the function of NMB-R using NMB-R KO mice, which we generated in our laboratory. Two-bottle odor discrimination tests revealed no functional deficiency of olfaction in NMB-R-deficient mice. We next evaluated the pharmacological effect of NMB using C57BL/6 mice. An i.p. administration of NMB (10 nmol/kg) inhibited the performance of olfactory avoidance tasks. These results suggest that NMB-R in the AON is be directly related to odor detection, olfactory learning and memory but may play some role in the modulation of olfactory systems.

## P92. Involvement of 5-$HT_2$ receptors in olfactory learning in young rats

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After odor exposure paired with foot shock, young rats show an aversion to that odor. Somatosensory stimulation disinhibits mitral cells via noradrenergic activation. From the raphe nucleus, centrifugal 5-hydroxytryptamine (5-HT) fibers project to mitral cells in the olfactory bulb. In order to clarify their function, we infused ritanserin, a 5-$HT_2$ antagonist, and methylserotonin, a 5-$HT_2$ agonist, directly into the olfactory bulb during odor exposure training on postnatal day (PND) 11 via cannulae implanted bilaterally prior to training. Animals exposed to odor and foot shock that were also infused with 1.0 nM ritanserin did not show aversion to the odor on testing on PND 12. However, at high (10 nM) or low (0.5 mM) concentrations ritanserin fails to prevent olfactory learning. Thiothixene HCl, a dopamine antagonist, infused simultaneously with 10 nM ritanserin restores the capability of 10 nM ritanserin to prevent olfactory learning. Since ritanserin has a low affinity to dopamine receptors, it is thought that a high dose of ritanserin activates mitral cells through dopaminergic excitation. Methylserotonin infusion during odor exposure only training mimics foot shock-induced aversive olfactory learning. These results suggest that 5-$HT_2$ receptor activation of mitral cells may be involved in aversive olfactory learning in young rats.

## P93. Regionalization of Fos immunostaining after exposure to urine in the rat accessory olfactory bulb

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We examined Fos-immunoreactive (Fos-ir) structures in the accessory olfactory bulb (AOB) of the rat when the vomeronasal organ was exposed to urine. Following exposure to male and female Wistar rat urine, Fos-ir cells were found in the mitral, granule and periglomerular cell layers of the AOB of the female Wistar rat, with the highest number in granule cell layer. Exposure to water or removal of the vomeronasal organ suppressed the expression of Fos-ir cells. These results suggest that female Wistar

rats specifically detect urinary pheromones derived from male or female Wistar rats via the vomeronasal organ.

As for the mitral cell layer, the density of Fos-ir cells in the anterior part ($G_{i2\alpha}$-positive) of all regions of the AOB was about two tinies higher than that in the posterior part ($G_{o\alpha}$-positive) when male urine was given. Following exposure to female urine, the density of Fos-ir cells in the posterior part was, however, slightly larger than that in the anterior part in the lateral region, while in other regions the density in the anterior part was higher than that in the posterior part. These results suggest that information of different pheromones is transmitted to the higher brain through the different regions of the AOB.

## P94. Study of the distribution and the function of nerve growth factor in the olfactory tract of mice

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The purpose of this research is to examine the distribution and function of nerve growth factor (NGF) in the olfactory tract of mice. In our first study we showed that expression of trk A immunoreactivity in the mouse olfactory epithelium and olfactory bulb increased during the regenerative period following olfactory nerve section. In the second study $^{125}$I-labeled NGF injected into the olfactory bulb was taken up and transported to the olfactory epithelium 18 h after injection. In the last study we found the following. In mice continuously infused with anti-NGF, degeneration of the olfactory cells and trk expression were observed at day 7, and the olfactory cells were regenerated by day 28. Trk expression was still recognized and the function of olfaction was not restored by day 28. From these examinations, it is suggested that NGF produced in the olfactory bulb was transported retrogradely to olfactory cells through nerves and were related to sustaining the existence of those cells and to regenerating the olfactory tract after injury.

## P95. Gene expression of neuropeptide during the regeneration of rat olfactory receptor neurons after axotomy

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In mammals olfactory receptor neurons have a recognized ability to regenerate following axotomy. In this study we observed the process of regeneration in rat olfactory receptor neurons after transection of the fila olfactoria using *in situ* hybridization histochemistry. Unilateral olfactory nerves of male Sprague–Dawley rats (6 weeks old) were transected. The survival times were 3, 14, 30 and 60 days after transection, after which the animals were killed under deep anesthesia and their olfactory mucosae were dissected out to examine the histological changes. We used neuropeptide for *in situ* hybridization histochemistry; α-CGRP, β-CGRP and PPT.

Expression of β-CGRP mRNAs was seen in the olfactory epithelium 30 and 60 days after axotomy. β-CGRP may play a role in the regeneration of the olfactory nerve.

## P96. Comparison of signal propagation between accessory and main olfactory bulb slices revealed by optical recording

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In the present study, we compared the information flow following orthodromic electrical stimulation between the main (MOB) and the accessory (AOB) olfactory bulb slices of the guinea-pig by optical recording with a voltage-sensitive dye. To activate afferent fibers, a stimulating electrode was inserted into the olfactory nerve layer (ONL) of the MOB, or into the posterior vomeronasal nerve layer (VNL) of the AOB. In the MOB, after ONL stimulation, excitation spread into the glomerular layer (GLL). Large optical responses lasting ~100 ms occurred in several glomeruli. The optical responses in the external plexiform layer (EPL) and mitral cell layer (MCL) were small in amplitude and duration compared with those in the GLL. In the AOB, after posterior VNL stimulation, excitation encompassed the GLL and spread into the EPL/MCL. The excitation in the EPL/MCL showed several alternative phases of increasing and decreasing (oscillation).

From these results, it was concluded that the optically potent neural activities were largely confined to the EPL/MCL of the AOB, whereas these were restricted to the GLL of the MOB, indicating that the manners of information processing in the AOB and the MOB are virtually different, despite their similar layer structures.

## P97. Effects of dithizone on the electro-olfactogram of the frog

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Zinc is thought to be an important element for olfaction, but the effects of chelation of zinc ions are unclear. When the ciliated surface of the isolated olfactory mucosa of the frog was covered with Ringer's solution containing dithizone, a heavy metal ion chelator, electro-olfactograms (EOGs) were attenuated; odorants were *n*-amylacetate and menthone. These EOGs were recovered when the dithizone was washed out. Forskolin increases cAMP concentration in olfactory cells and produces EOG-like responses, but these were not attenuated by dithizone. The olfactory transduction mechanism comprises five steps: (1) binding the odorant to the olfactory receptor molecules. (2) Activating G-binding protein (Golf). (3) Stimulating adenylate cyclase. (4) Increasing cAMP. (5) Opening cAMP-dependent channels. Therefore, the chelation of heavy metal ions affects the third process in olfactory transduction.

## P98. Simultaneous measurements of current responses and changes in $Ca^{2+}$ concentration in response to odorants in turtle olfactory sensory neurons

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Transduction components such as the cAMP-dependent channel and phosphodiesterase in olfactory neurons are regulated by $Ca^{2+}$. In the present study, we simultaneously measured membrane currents and $Ca^{2+}$ concentrations under voltage-clamp conditions (holding potential: –70 mV). The $Ca^{2+}$ concentration in the soma after the breakthrough varied among neurons. Application of odorant cocktails (cAMP-dependent odorant cocktail I: citralva, hedione, eugenol, L-carvone and cineole; cAMP-dependent odorant cocktail II: L-citronellal, geraniol and menthone; and $IP_3$-dependent odorant cocktail: lilial, lyral and ethylvanillin) induced inward currents in neurons accompanied by an increase in $Ca^{2+}$ concentration. There was, however, no correlation between the magnitude of changes in $Ca^{2+}$ concentration and the magnitude of inward currents. There was also no correlation between $Ca^{2+}$ concentration before the odorant stimulation and the magnitude of the inward current in response to the three odorant cocktails.

## P99. Inward current responses induced by dialysis of $IP_3$ in turtle olfactory sensory neurons

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Turtle (*Geoclemys reevesii*) olfactory sensory neurons were isolated with an enzyme-free procedure. Using the whole-cell mode of the patch-clamp technique, we recorded inward currents in response to inositol-1,4,5-trisphosphate ($IP_3$) after the breakthrough and those in response to $Ca^{2+}$-free Ringer solution after dialysis of $IP_3$ in the olfactory neurons. Dialysis of $IP_3$ induced inward currents with an increase in membrane conductance. The magnitude of inward currents induced by $IP_3$ increased with increasing $IP_3$ concentration from 2 μM and reached a plateau at 20 μM. Application of $Ca^{2+}$-free Ringer solution to olfactory neurons which were previously dialyzed with $IP_3$ after being irritated with $Na^+$,$Ca^{2+}$-free Ringer solution induced inward currents. The magnitude of the inward current in response to $Ca^{2+}$-free Ringer solution increased with an increase in $IP_3$ concentration from 2 to 20 μM and decreased with a further increase in $IP_3$ concentration. Inward currents induced by $Ca^{2+}$-free Ringer solution were reversibly blocked by normal Ringer solution, suggesting that the inward current induced by $IP_3$ is desensitized by $Ca^{2+}$. Inward currents induced by $Ca^{2+}$ were also blocked by 50 μM ruthenium red. The present results suggested that $IP_3$-mediated transduction pathways exist in turtle olfactory sensory neurons.

## P100. Responses of the water nose of *Xenopus laevis* to multiple odorants

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In *Xenopus laevis* olfactory neurons, the lateral diverticulum of the olfactory epithelium (the water nose) is considered to receive water-soluble odorants (Altner, 1962) and the medial diverticulum receives volatile odorants. The former selectively expresses 'fish-like' receptors, whereas the latter expresses 'mammalian-like' receptors (Freitag *et al.*, 1995). Until recently, however, differences in physiological functions in these two diverticula have remained unclear. In the present study, we recorded odor responses to water-soluble odorants as well as to volatile odorants from water nose receptor neurons under whole-cell voltage-clamp conditions. The results indicated that the water nose receptor neurons responded to both water-soluble odorants and volatile odorants. Many olfactory neurons (>60%) responded to plural amino acids, including acidic, basic and neutral ones. It is likely that single olfactory neurons in the water nose have multiple receptors for amino acids and volatile odorants.

Dialysis of $IP_3$ into the olfactory neurons in the water nose evoked transient inward currents after membrane rupture, but dialysis of cAMP did not induce any response. In most neurons (12/14 cells), the slopes of the *I–V* curve measured during the odorant-induced response were identical to those measured before the response, indicating that the cAMP- and $IP_3$-independent pathway plays an important role in olfactory transduction.

## P101. The contribution of calcium-activated chloride conductance to amino acid-induced responses of isolated olfactory receptor neurons from the rainbow trout

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To clarify whether or not amino acid-induced inward receptor currents contain a component of $Ca^{2+}$-activated $Cl^-$-conductance, we first studied reversal potential changes in *I–V* relations of receptor responses in different extra- and intracellular $Na^+$ and $Cl^-$ ion concentrations, and then the effects of $Ca^{2+}$-activated $Cl^-$-channel blockers on receptor currents in ciliated receptor neurons from the rainbow trout using the conventional whole-cell clamp technique. A mixture of four amino acids (10 mM L-glutamate, L-arginine, L-alanine and L-norvaline) and one of six different $Ca^{2+}$-activated $Cl^-$-channel blockers were applied separately to cilia of receptor neurons with a two-barrel micropipette using the pressure ejection system. The expected reversal potential shifts to indicate the contribution of $Ca^{2+}$-activated $Cl^-$-conductance occurred in either a positive or a negative direction. Niflumic acid, flufenamic acid, 5-nitro-2-(3-phenylpropylamino)-benzoate and 3′,5-dichlorodiphenylamine-2-carboxylate at 500 μM blocked reversibly not only amino acid-induced receptor currents but also the background activities of receptor neurons perfused with normal Ringer's solution. The blocking effectiveness of these drugs varied from 100 to 50% between the receptor neurons. Both 4-acetamido-4′-isothiocyanatostilbene-2,

2′-disulfonate at 2 mM and 4,4′-diisothiocyanatostilbene-2,2′-disulfonate at 5 mM had little blocking effect. The results suggest that the effective blockers are not definitively specific for $Ca^{2+}$-activated $Cl^{-}$-channels and that the channel distribution density on the receptor neurons varies from one to another.

## P102. The olfactory organs of representative large pelagic and demersal fish

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The paired olfactory organ of the teleost species examined—bigeye tuna (*Thunnus obesus*), yellowfin tuna (*Thunnus albacares*), albacore tuna (*Thunnus alalunga*), striped marlin (*Tetrapturus audax*), dolphin fish (*Coryphaena hippurus*), opah (*Lampris guttatus*) and red sea bream (*Pagrus major*)—are situated on the dorsolateral side of the head and not connected to the oropharyngeal cavity. The sensory epithelium is composed of ciliated receptor cells, microvillar receptor cells, mucous cells and sustentacular cells in all the species. Ciliated non-sensory cells are absent in most of the large pelagic fish.

## P103. An attempt to imprint hime (landlocked sockeye) salmon to phenylethyl alcohol

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Salmonid fishes are well known for their ability to return to their natal stream to spawn. The olfactory imprinting hypothesis of Hasler and Wisby (1951) is generally accepted for salmonid homing. However, the imprinting mechanisms are still unknown. In this study, we tried to imprint hime (landlocked sockeye) salmon (*Oncorhynchus nerka*) to a synthetic chemical, phenylethyl alcohol (PEA). Hatchery-reared hime salmon fry were exposed to $10^{-7}$ M PEA in a flow-through tank for 20 days. Two years later, when the fish matured, we tested whether they remembered the odor of PEA by examining their upstream behavioral preference for PEA in a two-choice Y-maze. Subsequently, their olfactory sensitivities to PEA were recorded electrophysiologically and compared with those of PEA-naive fish. PEA-exposed fish showed no preference for PEA-scented water. No difference was found in the EOG (electro-olfactogram) magnitudes to PEA between PEA-exposed and PEA-naive fish. Thus, we considered that imprinting hime salmon to PEA was unsuccesful in this experiment. Juvenile coho salmon (*Onchorhynchus kistch*), of the same genus as hime salmon, have been successfully imprinted to PEA (Scholtz *et al*., 1976; Nevitt *et al*., 1994; Dillman *et al*., 1996). Furthermore, cells from imprinted coho salmon showed increased sensitivity to the chemical compared with cells from naive fish (Nevitt *et al*., 1994). There may be a species difference in the manner of olfactory imprinting even within the same genus.

## P104. Metals in stream water and the olfactory responses of rainbow trout

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In the homing migration of salmon, it is well established that olfactory systems play an important role for both the identification of natal streams and spawning behavior. Generally, natural stream water contains many species of metals in various concentrations. In order to examine whether the metals in stream water act as the stream odor or an odor response inhibitor, we determined the concentrations of the main metals—Fe, Zn, Mn, Cu and Al—in natural stream water and measured the olfactory responses of rainbow trout to these metals. The analyses showed that the five streams examined showed various concentrations of metals. Artificial stream water containing the same concentrations of the metals as the natural stream water elicited either an extremely small or no response. Fe, Cu and Al ions inhibited olfactory reponses, but the concentrations required to inhibit responses to $10^{-5}$ M L-alanine (>3–~40 μM) were higher than the concentrations in stream water. The responses to $10^{-6}$ M L-alanine dissolved in the artificial stream water containing the same concentrations of metals as natural water were slightly depressed. The present results suggest that metals in stream water do not contribute to the natal stream odor, and that there is a possibility that the mixture of metals depresses the olfactory reponses of salmonids, even though the concentration of each metal is lower than the minimum value that inhibits the olfactory response.

## P105. Relationships between electrophysiological properties and expression of gustducin or ultrastructural features of the taste bud cells in the rat

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We investigated the relationships between the electrophysiological properties and immunocytochemical or ultrastructural features in single rat taste cells. The taste bud cells were dissociated from vallate and foliate papillae by treatment with enzymes and EDTA. The voltage-dependent currents were recorded using a whole-cell patch-clamp method and an on-cell patch-clamp method for further immunocytochemical and electron microscopic studies respectively.

The electrophysiologically characterized cells were subjected to binding with anti-gustducin antibody to detect the expression of gustducin ($G_{gust}$), which is the taste-cell-specific G-protein mediating bitter and/or sweet taste transduction. Three cells out of nine which displayed voltage-dependent $Na^{+}$- and $K^{+}$-currents were $G_{gust}$-positive and only one cell was positive out of eight cells which had only voltage-dependent $K^{+}$-currents. This result suggests the possibility that a voltage-dependent $Na^{+}$-channel might be involved in the intracellular cascade through $G_{gust}$.

The ultrastructural characteristics of taste bud cells which

generated action currents in the cell-attached configuration were examined with electron microscopy. The cells possessed both clear vesicles and dense-cored vesicles, which is characteristic to type III cells. This result indicates that the action currents in the cell-attached configuration may be a good criterion for delineating type III taste cells.

## P106. Decreased carbonic anhydrase activity in the taste buds of zinc-deficient rats

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We have reported that the reception of carbonated water through the lingual trigeminal and the chorda tympani nerves needs the participation of carbonic anhydrase (CA), a zinc-containing enzyme. Moreover, the sensitivities of these nerves to carbonated water are lower in zinc-deficient rats than in those that are zinc-sufficient. Brown *et al*. have shown that CA is densely localized in the taste buds. Furthermore, clinical studies have also shown that the chronic use of a CA inhibitor (for glaucoma) p.o. not only causes carbonated water to taste flat but also causes a basic taste dysfunction (dysgeusia). Taste preference studies have also shown that the sense of taste in zinc-deficient rats is abnormal. Since it appears that CA plays a key role in taste sensation, we investigated the effect of zinc deficiency on the CA activity in the taste buds of the rat. Male SD rats, 4 weeks old, were divided into four groups (Zn-Deficient, Low-Zn, Zn-Sufficient, Pair-fed control). After feeding the experimental diet for 42 days, the rats were sacrificed and their tongues, including the circumvallate papillae, were excised and immediately frozen in liquid nitrogen. Frozen sections were cut (20 μm) in a cryostat and reacted for carbonic anhydrase activity using a slightly modified Hansson's method. The CA activity in the taste buds was significantly lower in Zn-Def and Low-Zn rats than in Zn-Suf and Pair-fed control rats. There was much lower activity in Zn-Def rats than in Low-Zn rats, which agrees well with our previous observation obtained by a biochemical method for the measurement of enzyme activity. These results indicate that CA activity, considered to be an indispensable factor for the maintenance of normal taste sensation, is affected by the dietary zinc content.

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## P107. Effect of ethanol on the lingual trigeminal nerve responses to cold water or carbonated water in SD rats

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It is well known that the irritation sensation caused by the liquid phase of carbon dioxide ($CO_2$) in drinks is mainly transduced through the lingual trigeminal nerve. The effect of ethanol on the lingual trigeminal nerve responses to cold water or carbonated water (liquid-phase $CO_2$) was studied in SD rats. Female adult SD rats were used throughout the present study. In the first experiment we stimulated the tongue surface with cold and cool (5, 15 and 23°C) water and 5% ethanol solutions at the same temperature while keeping the tongue temperature constant at 28°C. In the second experiment, the responses to carbonated water with or without 5% ethanol were measured at the tongue (28°C), cool (23 and 15°C) and cold (5°C) temperatures. It was found that ethanol significantly decreased the lingual trigeminal nerve responses to 5°C cold stimulation irrespective of whether the tongue surface temperature was controlled exactly at the same temperature between the two stimulations. In the second experiment, it was shown that ethanol significantly decreased the firing of the $CO_2$ component of carbonated water at 28 and 23°C. However, 5% ethanol significantly increased it at 5°C. Therefore it is considered that ethanol might have protective role for firing of the $CO_2$ component of very cold carbonated water, whereas it has a lesser effect at 28 and 23°C.

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