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Responsiveness of the Cortical Taste Area Neurons to a Mixture of the Four Basic Tastants in Rats

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Abstract

The taste coding mechanism in the cortical taste area was investigated by analyzing the responses of 59 neurons in the cortical taste area of the anesthetized rat to a mixture of the four basic tastants in both absence and presence of bicuculline methiodide, a specific antagonist to the $GABA_A$ receptors. The mixture caused response suppression more frequently than response facilitation, both in the control state and during bicuculline application. Cluster analysis revealed that only a group of the neurons with the best response to both NaCl and HCl (group NH) showed the best response to the mixture in the control state, whereas during bicuculline application, in addition to group NH, two other groups of neurons responding to sucrose, or to HCl and quinine responded vigorously to the mixture. Multidimensional scaling located the mixture outside the space of the four basic tastants facing an NaCl–HCl line in both states. GABAergic inhibition caused the group NH to represent the taste of the mixture in the control state. Thus, the mixture probably tastes salty and sour to rats. No cortical neuron was found which specifically responded to the mixture.

Key words: cortex, GABA, rat, taste interaction, taste mixture

Introduction

Animals, including humans, interact with the world through perception of various sensory stimuli. Investigation of the central nervous system (CNS) involved in such perception sometimes requires the use of the same stimuli as encountered under natural conditions. In particular, a study of the central gustatory system essentially requires investigation of the responsiveness of neurons to mixtures of various taste substances, since foods are often composed of multiple chemicals that individually elicit different tastes and the gustatory system is adaptively evolved to detect such complex taste mixtures. However, most behavioral, psychophysical and physiological experiments on taste so far undertaken have preferred the use of simple, single chemicals as taste stimuli to complex mixtures and have not attempted to study the neural mechanism for complex taste mixtures. Only occasionally, investigators have examined the interactions between the basic tastants that underlie the gustatory perception of foods. Psychophysical studies of the mixtures of basic taste stimuli (Kroeze, 1989) have disclosed various phenomena, such as mixture suppression or facilitation. Mixture suppression is more frequently observed than mixture facilitation.

Previous studies of binary taste mixtures in animals have confirmed that mixture suppression and facilitation occur in a wide range of gustatory systems from the peripheral to the central neurons (Frank, 1989; Vogt and Smith, 1993; Miyaoka and Pritchard, 1996). The fraction of neurons involved in mixture suppression increased from the nucleus of the solitary tract (NTS) to the thalamic taste relay nucleus, or the parvicellular part of the thalamic posteromedial ventral nucleus (VPMpc) (Hasegawa *et al.*, 2002). Investigation of the coding mechanism of taste mixture in the cortical taste area (CTA) would be very helpful in understanding the neural mechanism of the information processing of complex tastes, because the CTA is the site of final decoding of taste information and probably includes mechanisms for detecting taste stimuli. The neural responses to binary, trinary and quadruple mixtures have been reported in the cortex of primates (Miyaoka and Pritchard, 1996; Plata-Salaman *et al.*, 1996), but rarely in rodents except for a mixture of umami substances (Ogawa *et al.*, 1997).

The present study investigated the taste coding mechanism in the CTA of the rat by recording the responses of CTA neurons to the four basic tastants and the mixture of all these four tastants and by analyzing the responses using multivariate analyses to clarify what sort of neuron groups represent the mixture taste in the CTA. The

GABAergic inhibitory system contributes to modification or selection of taste information in the cortex (Ogawa *et al.*, 1998). Therefore, the contribution of GABAergic inhibition to the coding of the mixture in the CTA was also assessed by iontophoretic application of bicuculline methiodide (BMI), a specific antagonist to the $GABA_A$ receptors.

Materials and methods

Animals and surgery

Adult female Sprague–Dawley rats were used. The animals were anesthetized with urethane (1 g/kg body wt, i.p.). After cannulation of the trachea and femoral vein, the animal's head was mounted on a standard stereotaxic frame with a pair of ear bars. The left cheek from the mouth corner to the ramus of the mandible was resected and the frame was rotated ~45° with the left side up. The rat was immobilized with D-tubocurarine (1.5 mg, i.v.) and artificially ventilated. The end-tidal CO_2 was maintained at 3.5–4.5%. Whenever the effect of D-tubocurarine seemed to be wearing off, the level of anesthesia was checked at the corneal reflex and urethane (100 mg/kg) was given if necessary. Body temperature was kept at 37°C with a water heater. The bone covering the left middle cerebral artery was removed and a small opening was made on the dura over the granular and/or dysgranular insular cortices—areas GI or DI (Ogawa *et al.*, 1992). The mouth was opened to ~30–40° and the tongue stretched out anteroventrally. Cut wounds were infiltrated with 1% xylocaine.

Stimulation and recording

The taste stimuli used were the four basic tastants (0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl, 0.02 M quinine–HCl) and a mixture solution containing all of the four basic tastants at the above-mentioned concentrations. Taste stimulation was controlled by a 16-bit microcomputer: distilled water was delivered in a prestimulus period of 15 s, followed by a taste stimulus for 10 s and then by the water for 15 s as a rinse. Taste responses were identified when, during the 10 s of taste stimulation, there was a change in the discharge rate by 2 SD above or below the average prestimulus discharge rate that lasted for at least 1 s (Ogawa *et al.*, 1984). The magnitude of the response was calculated as the number of impulses in the first 5 s following the onset of stimulation minus the number of background impulses in a corresponding prestimulus period. Average net responses of ≥5 impulses/5 s were assumed to indicate significant responses and such responses were used for statistical analysis. The response to the mixture was always compared with the most effective component of the mixture (MEC), the stimulus which, of the four component tastants, evoked the largest response in the neuron under study.

Spikes from the soma of single neurons were recorded with a glass microelectrode (tip diameter <l μm) filled with 2% pontamine sky blue in 0.5 M sodium acetate as described previously (Ogawa *et al.*, 1992). The recording microelectrode was glued to the side of a seven-barreled micropipette used for drug application and protruded by 15–30 μm from the end of the micropipette (the overall tip diameter of the micropipette was 5–10 μm).

BMI application

In several CTA neurons, GABAergic inhibition was blocked by BMI (5 mM, pH 3.2) applied from one of the barrels. Taste responses were recorded in the absence (control state) or presence (BMI state) of BMI. BMI was electrophoretically ejected with 3–10 nA current onto neurons for 30–40 min when three to five series of responses to the four basic tastants and the mixture of those tastants were obtained. After the recordings of the series of responses, the ejection of BMI was discontinued and CTA neurons were allowed to recover. A significant change in the magnitude of taste responses in the BMI state was a mean increase or decrease by 30% in the responses of the neurons compared to those in the control state and a change by not less than eight impulses in the first 5 s following the onset of stimulation (Ogawa *et al.*, 1998). Retaining currents of from –10 to –5 nA were used to prevent BMI from leaking during the control state.

Data analysis

Data analysis was performed with a microcomputer. The responses of 59 taste neurons to the four basic tastants and the mixture were arranged into a matrix of 59 neurons × five stimuli and analyzed with two multivariate statistical methods using SPSS software (SPSS Inc., MI), hierarchical cluster analysis (Bieber and Smith, 1986) and multidimensional scaling (MDS) (Schiffman *et al.*, 1981). The former analysis used the Pearson product-moment correlation coefficients (corr. coefs) calculated for each pair of taste response profiles for all 59 neurons. Hierarchical cluster analysis was used to determine the relative similarity between the neurons, or between the neuron clusters based on responses to the four basic taste stimuli. Cluster amalgamation used the simple average linkage method and the results were plotted as a dendrogram. To determine the number of clusters, each containing functionally similar neurons, a scree test was used (Bieber and Smith, 1986). Cluster similarity was plotted against the number of clusters obtained during amalgamation process. The point (elbow) where a sudden decrease in similarity occurs when the amalgamation process proceeds, indicates the number of cluster to extract. MDS was applied to a set of data in both the control and BMI states to measure the multidimensional distance between taste stimuli in both states. Taste stimuli were mapped in a taste space to reveal the spatial organization.

Histology

Recording sites were histologically identified by extra-

cellular dye marks produced by negative currents of 10 μA passed for 5 min through a recording electrode containing the dye. The marks were made at two or three recording sites along a single electrode track to reconstruct the track. At the termination of the experiments, the animals were deeply anesthetized with urethane and perfused through the heart with 10% formalin in a 0.1 M phosphate buffer. Blocks of the tissue containing the recording sites were frozen, cut into 50 μm thick sections and stained with thionine. Cytoarchitectonic identification of the areas GI and DI forming the CTA was made as reported previously (Ogawa *et al.*, 1992).

Results

A total of 80 taste neurons were studied in the CTA in the control state. Of these, 59 neurons were successfully studied in the BMI state. Since the sample size was small, we collectively analyzed neurons in both areas GI (n = 33) and DI (n = 26) together.

Response of neurons to the tastants and mixture

Responses of the neurons

Most neurons (62.7%) responded to two or more of the four basic tastants. Many neurons (55.9% of the 59 neurons) responded to the mixture. Neurons discharged 11.1 ± 13.5 impulses/5 s in response to NaCl, 6.6 ± 8.8 to sucrose, 9.4 ± 14.9 to HCl, 6.2 ± 10.8 to quinine and 13.4 ± 20.0 to the mixture. Figure 1A shows the representative response of a single neuron to the four tastants and the mixture. The neuron in the figure yielded a tonic response to NaCl and phasic responses to HCl and quinine. The response to the mixture was tonic with the phasic component, the magnitude of which was rather comparable to that for HCl. The MEC was NaCl in this neuron and the response to the mixture was smaller than that to the MEC. In the BMI state the responses to the three tastants and the mixture were enhanced and the response to sucrose emerged (Figure 1B). The response to the mixture was slightly increased, but did not exceed that to the MEC, as in the control state.

BMI significantly affected 44 of the 59 neurons. The responses to both the tastants and the mixture were affected in 26 neurons. Only the responses to some of the tastants, but not to the mixture, were affected in 14 neurons. Only the responses to the mixture were affected in four neurons. Mean magnitudes of the responses in the BMI state relative to those in the control state were 182.1% for NaCl, 190.3% for sucrose, 164.1% for HCl, 201.6% for quinine and 183.5% for the mixture.

Response profiles

The taste response profiles of the neurons to the four tastants and the mixture are illustrated in Figure 2A. The

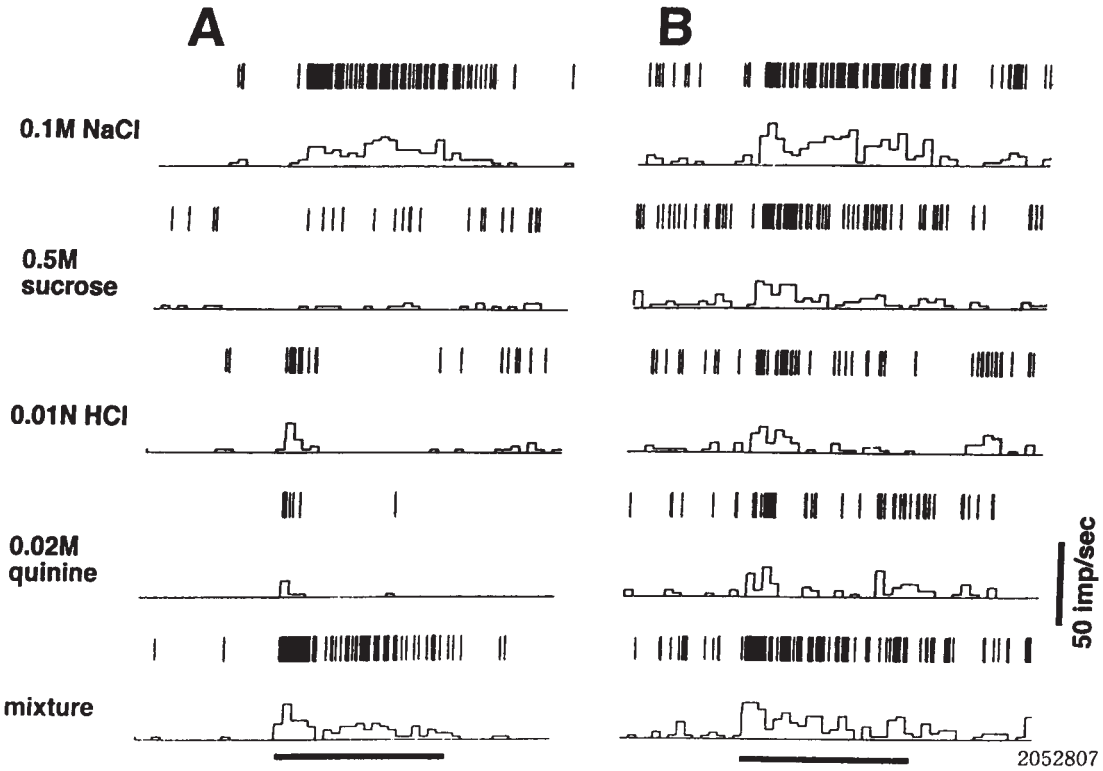

Figure 1 Responses of a single CTA neuron to the four basic tastants and to the mixture. Taste responses are shown as spike images and peristimulus time histograms. Stimulus is indicated at the left. **(A)** Control state. **(B)** During application of BMI (BMI state). Horizontal bars under the responses indicate the period of taste stimulation (10 s). The neuron showed maximum response to NaCl in both states. Layer V, area DI. Unit 2052807.

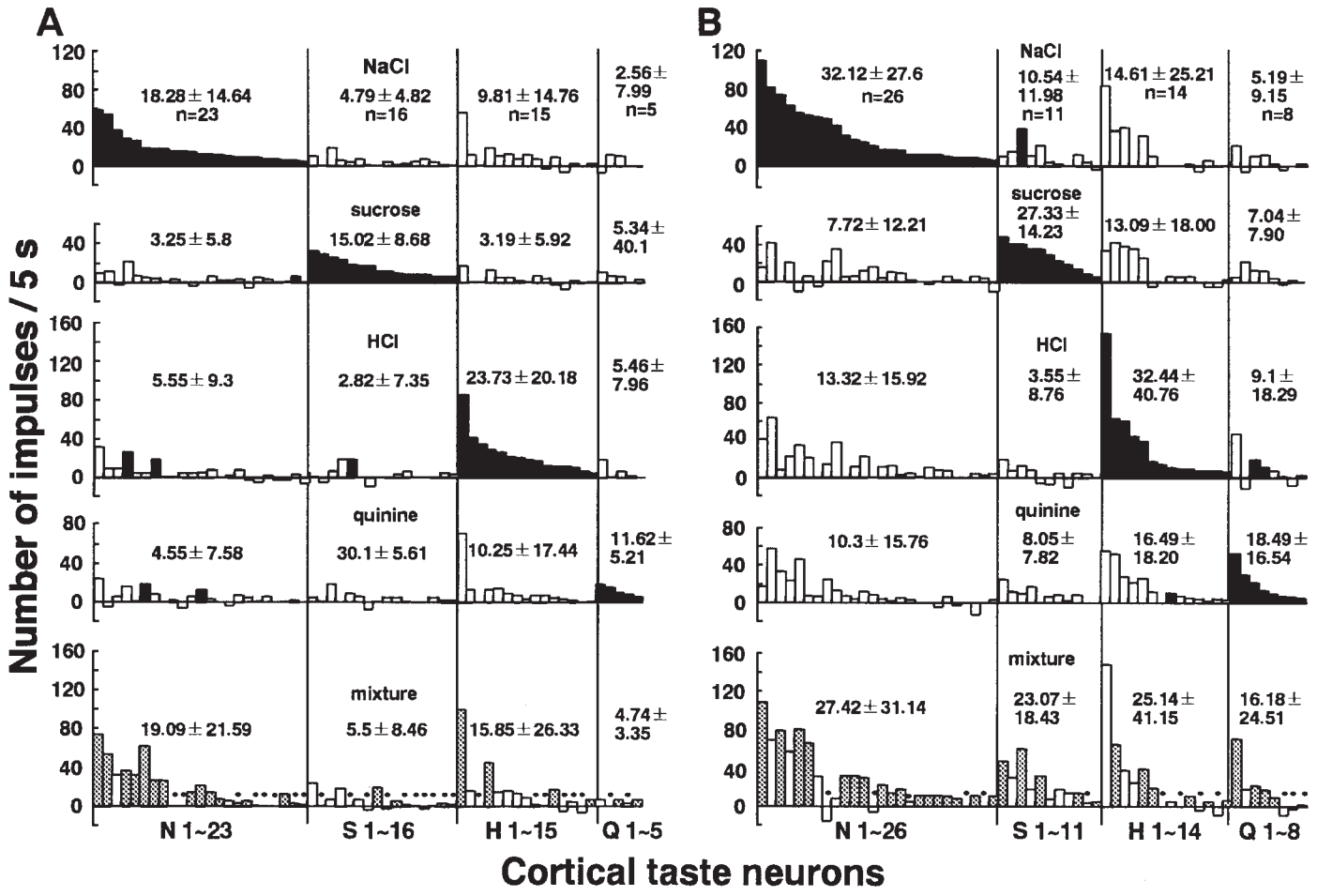

Figure 2 Response profiles of 59 CTA neurons to the four tastants and to the mixture. **(A)** Control state. **(B)** BMI state. Neurons were first categorized into four classes according to the most effective component of the mixture (MEC) and then plotted in descending order of response magnitude to the MEC within each class. Solid bars indicate the maximum or the second maximum responses (>90% of the maximum response). Shaded bars indicate the response to the mixture that was greater than or equal to the response to the MEC. Solid circles indicate the responses to the mixture <5 impulses/5 s.

neurons were categorized according to the MEC. In each category, neurons are arranged from the left to right according to the response to the MEC in decreasing order. In both the NaCl-best and HCl-best categories, mixture responses tended to decrease from the left to right in a manner in which the responses to the MEC in the corresponding category decreased. Mean response to the mixture was 104% of the mean MEC response in the NaCl-best neurons (n = 23) and 67% of the mean MEC response in the HCl-best neurons (n = 15). No significant differences were noted between the mean responses to the mixture and to the MEC in both categories ($P > 0.05$, Student's t-test). However, the sucrose-best (n = 16) or quinine-best (n = 5) neurons showed smaller responses to the mixture than to the MEC. Mean response to the mixture was 37% of the mean MEC response in the sucrose-best neurons and 41% in the quinine-best neurons, and was significantly smaller than the mean MEC response in the these two categories of neurons ($P < 0.005$ and $P < 0.05$, respectively, Student's t-test).

The taste response profiles of the neurons in the BMI state are illustrated in Figure 2B. The neurons are also categorized according to the MEC. In all categories, mixture responses tended to decrease from left to right in a manner in which the responses to the MEC in the corresponding category decreased. Most of the NaCl-best (n = 26) or HCl-best (n = 14) neurons showed similar magnitudes of responses to both the mixture and the MEC. Mean responses to the mixture were 85 and 77% of the mean response to the MEC, respectively, in these categories, although no difference was noted between the mean response to the mixture and the MEC in both categories ($P > 0.05$, Student's t-test), as seen in the control state. In contrast, the sucrose-best neurons (n = 11) showed different response profiles from those in the control state. Namely, the response to the mixture did not differ from that of the MEC. This was also the case in the quinine-best neurons. ($P > 0.05$, Student's t-test).

The number of neurons responding to the mixture in the

total population increased from 33/59 (55.9%) in the control state to 46/59 (78.0%) in the BMI state.

Mixture suppression and facilitation

The mixture might cause a response as large as the sum of the responses to the four tastants, if the tastants in the mixture do not interact in a transduction at either receptor or neuronal level. In previous studies (Vogt and Smith, 1993), the responses to binary mixtures have been compared to the response to each component in the mixture, especially the MEC. We also compared the response to the mixture with that to the MEC, as well as with the sum of the responses to the four tastants.

Figure 3A illustrates the analysis of the neural responses in the control state, in which the across-neuron patterns (ANPs) of the responses to the mixture and the MEC are compared (Figure 3A-a) and those to the mixture are also compared with the sum of the responses to the four tastants (Figure 3A-b). The response to the mixture was greater by at least 5 impulses/5 s than the response to the MEC in only 12 of 59 neurons and greater than the sum of the responses to the four tastants in five neurons (Table 1). The response to the mixture was smaller than the response to the MEC in 33 neurons and smaller than the sum of the responses to the four tastants in 50 neurons. Response to the mixture greater or smaller than the sum of the responses to the four tastants indicates mixture facilitation or suppression, respectively.

Figure 3B shows the analysis of the neural responses in the BMI state, as in the control state. The response to the mixture was greater than the response to the MEC in seven neurons and greater than the sum of the responses to the four tastants in two neurons (Table 1). The response to the mixture was smaller than the response to the MEC in 25 neurons and smaller than the sum of the responses to the four tastants in 52 neurons. The response to the mixture was similar to the response to the MEC in 27 neurons, almost double the number (14) in the control state. BMI increased the difference between the response to the mixture and the sum of the responses to the four tastants (Figure 3B-b).

Correlation of the taste mixture with four tastants

The relationships of responses to the mixture and those to the four tastants were analyzed using Pearson product-moment corr. coefs between the responses to the mixture and those to the four tastants (Figure 4A). This analysis quantitatively evaluated the similarities of the ANPs to the mixture and the four tastants. The responses to the mixture correlated significantly with those to NaCl, HCl, or quinine

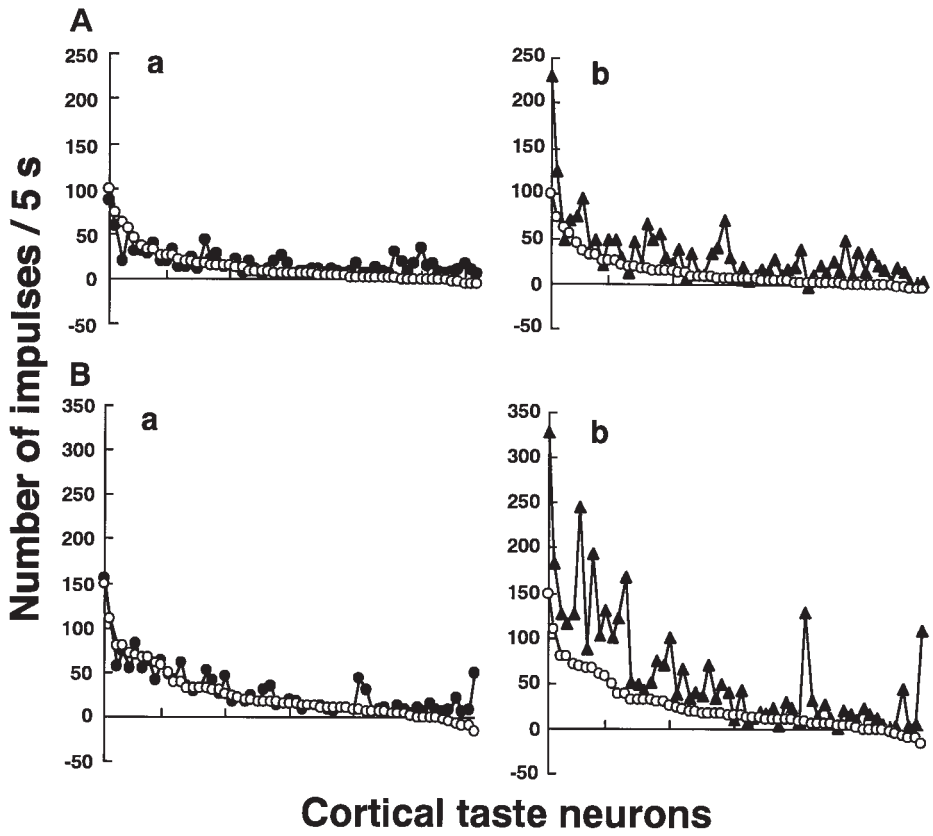

Figure 3 Across neuron pattern (ANP) of the cortical taste neurons in the control state **(A)** and in the BMI state **(B)**. ANP of the responses to the mixture (open circles) is compared with that to the MEC (filled circles) in **(a)** and with that constructed by the sum of the responses to the four tastants (filled triangles) in **(b)**. The neurons in each plot are arranged according to their responses to the mixture.

Table 1 Relationship between the responses to the mixture and to the MEC, or the sum of the responses to the four basic tastants*

(A) Responses to the mixture versus to the MEC

State	Mixture > MEC	Mixture = MEC	MEC > mixture	Total
Control	12 (20.3%)	14 (23.7%)	33 (55.9%)	59 (100.0%)
BMI	7 (11.9%)	27 (45.8%)	25 (42.4%)	59 (100.0%)

(B) Responses to the mixture versus the sum of the responses to the four basic tastants

State	Mixture > sum	Mixture = sum	Sum > mixture	Total
Control	5 (8.5%)	4 (6.8%)	50 (84.7%)	59 (100.0%)
BMI	2 (3.4%)	5 (8.5%)	52 (88.1%)	59 (100.0%)

[a]CTA neurons were classified on the basis of response to the mixture greater or less than (by at least 5 impulses/5 s), or equal to the response to the MEC or the sum of the responses to the four basic tastants.

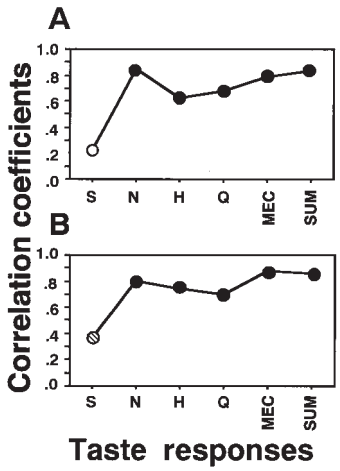

Figure 4 Correlation profiles of the responses to the mixture across responses to the four basic tastants and the MEC, and the sum of the responses to the four basic tastants in the control state **(A)** and in the BMI state **(B)**. Across neuron correlation coefficients between pairs of responses are plotted. S, response to sucrose; N, to NaCl; H, to HCl; Q, to quinine; MEC, to the MEC; SUM, sum of the responses to the four tastants. Solid and hatched circles indicate statistically significant correlation at the levels of $P < 0.001$ and $P < 0.01$, respectively.

(corr. coef. = 0.849, 0.625 and 0.680, respectively; $P < 0.0001$, Student's *t*-test), but not correlated with sucrose (corr. coef. = 0.217; $P > 0.05$, Student's *t*-test). The corr. coef. was also calculated between the responses to the mixture and those to the MEC, and between the responses to the mixture and the sum of the responses to the four tastants. The response to the mixture was significantly correlated with the response to the MEC and the sum of the responses to the four tastants (corr. coef. = 0.804 and 0.835, respectively; $P < 0.0001$, Student's *t*-test).

In the BMI state, the responses to the mixture correlated significantly with the responses to NaCl, HCl and quinine (corr. coef. = 0.809, 0.739 and 0.703, respectively; $P < 0.0001$, Student's *t*-test; Figure 4B), as seen in the control state. The responses to sucrose became significantly correlated with those to the mixture (corr. coef. = 0.361; $P < 0.005$, Student's *t*-test), although the coefficient was small. The response to the mixture was significantly correlated with the response to the MEC and the sum of the responses to the four tastants (corr. coef. = 0.901 and 0.846, respectively; $P < 0.0001$, Student's *t*-test).

Multivariate statistical analysis

Cluster analysis

Cluster analysis of the similarity between neurons was performed to identify which group of neurons carries the response to the mixture. Since the scree test indicated an elbow at the number of clusters = 5, five groups were identified in the dendrogram (Figure 5A-a): groups of neurons with the best responses to HCl (i.e. MEC: HCl, group H), to quinine (i.e. MEC: quinine, group Q), to NaCl–quinine (i.e. MEC: NaCl and quinine, group NQ), to NaCl–HCl (i.e. MEC: NaCl and HCl, group NH) and to sucrose (i.e. MEC: sucrose, group S). Group NH contained almost all neurons yielding the largest responses to the mixture, as marked with stars at the left column in Figure 5A-a (see mean response profiles in Figure 5A-b). This indicates that group NH carries the information of the mixture.

In the BMI state, the scree test identified seven groups of neurons categorized according to the best stimuli among the four basic tastants: i.e. groups NH, N, Q, S, HQ-1, HQ-2 and H in the dendrogram (Figure 5B-a). Groups NH and HQ-1 contained neurons most strongly responding to the mixture, marked with stars at the right column in Figure 5B-a (see the mean response profiles in Figure 5B-b). A fraction of group S also contained neurons most strongly

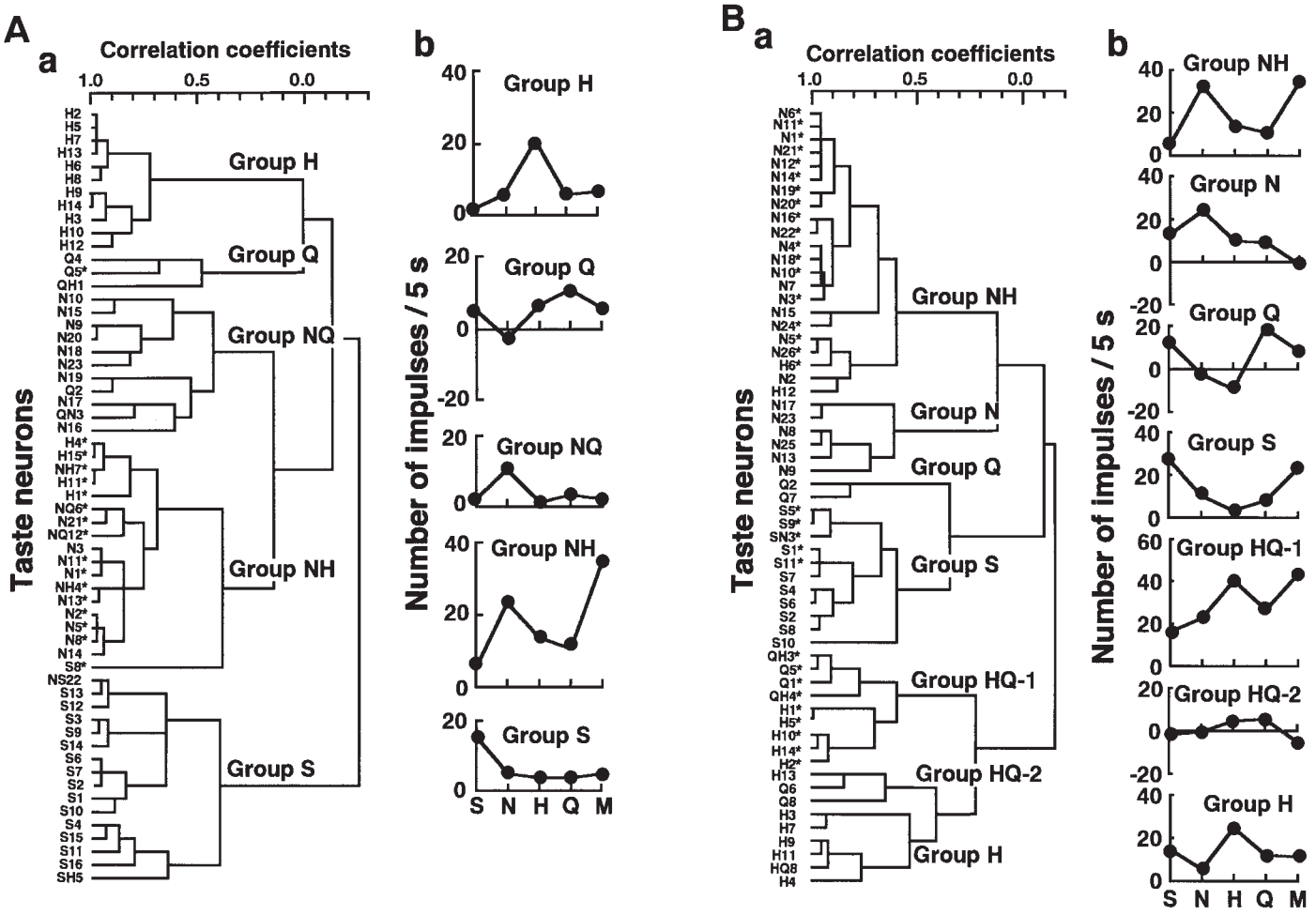

Figure 5 Cluster analysis of cortical taste neurons in the control state **(A)** and in the BMI state **(B)**. Cluster dendrogram of neurons **(a)** and profiles of mean taste responses across the four tastants and the mixture **(b)** are shown. (a) Each neuron shown along the left of the dendrogram is labeled according to the MEC and the order of arrangement from the left within the MEC category in Figure 2. A secondary effective stimulus is indicated as further letters. Abscissa shows the degree of similarity between neurons or neuron clusters, expressed by correlation coefficients. Neurons or neuron clusters linked on the left of the dendrogram are more similar than those linked further to the right. Asterisks indicate neurons with responses to the mixture greater than or equal to the response to the MEC. (b) The profile of each group of neurons is shown according to cluster analysis in (a). Sucrose (S), NaCl (N), HCl (H), quinine (Q) and the mixture (M) are arranged from left to right along the abscissa and the mean response of neurons in the cluster is plotted against the tastant.

responding to the mixture. This indicates that both groups NH and HQ-1 together with a fraction of group S carry the information of the mixture in the BMI state.

MDS analysis

Distances between the four tastants and the mixture were assessed by the MDS method. Figure 6 shows the two-dimensional map of the taste stimuli in the control and BMI states. In both states, the mixture was located outside the area defined by the four basic stimuli facing a line connecting NaCl and HCl, and sitting closer to NaCl. The spatial relationship of the relative positions of the mixture and four tastants did not change in the BMI state, but the distances among the five tastants increased.

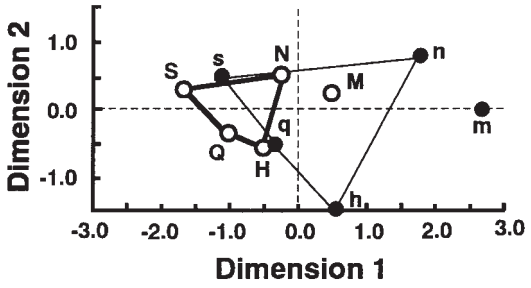

Figure 6 Two-dimensional taste space, showing the relative locations of the four tastants and the mixture in the control and BMI states. The distances indicate stimulus proximity. Open circles represent relative locations of the four tastants and the mixture in the control state; solid circles represent those in the BMI state. N or n, NaCl; S or s, sucrose; H or h, HCl; Q or q, quinine; M or m, mixture. Upper-case letters, control state; lower-case letters, BMI state.

Discussion

Mechanisms of mixture suppression and facilitation

The phenomenon of mixture suppression has been primarily identified for heterogeneous mixtures of tastants in psychophysical studies (Pangborn, 1960). The interactions of heterogeneous tastants may involve central or both peripheral and central mechanisms (Kroeze, 1989). Neurophysiological studies in rodents have shown that mixture suppression (20–50%) is more frequently observed than mixture facilitation (5–15%) for binary mixtures from the peripheral to the CNS (Frank, 1989; Vogt and Smith, 1993; Miyaoka and Pritchard, 1996). In the present study, mixture suppression was observed in many neurons in the CTA. The fraction of mixture suppression for the quadruple mixture (84.8%) was much larger than that for the binary mixture (20–50%) (Frank, 1989; Vogt and Smith, 1993; Miyaoka and Pritchard, 1996).

Previously, we studied responses of neurons in NTS and VPMpc to the mixture of the four basic tastes (Hasegawa *et al.*, 2002). The fraction of mixture suppression in the CTA was significantly larger than that in the NTS (63.4%; $P < 0.025$, χ^2 test), but did not differ from that in the VPMpc (77.5%; $P > 0.05$, χ^2 test). The finding suggests that additional mixture suppression is not generated by a synaptic transfer from the VPMpc to the CTA, but might be generated somewhere in the gustatory pathway from the NTS to the VPMpc.

Human psychophysical studies indicate that enhancement of the intensity of one taste quality in a tastant by the addition of another tastant usually occurs only when the added tastant is of weak stimulation (Kroeze, 1989) and homogeneous in quality (Pangborn, 1962). Mixture facilitation is found occasionally for a heterogeneous taste solution, particularly when the tastes of two components in the solution overlap in quality (Bartoshuk, 1975). In the present study, mixture facilitation was observed in a small fraction of taste neurons (8.5%), probably because individual taste qualities of the components in the mixture do not overlap. Mixture facilitation occurs also in the chorda tympani fibers (15%) (Hyman and Frank, 1980), NTS (14.5%) (Hasegawa *et al.*, 2002) and VPMpc (10.0%) (Hasegawa *et al.*, 2002). There was no significant difference in these fractions of mixture facilitation ($P > 0.1$, χ^2 test).

Taste quality of mixture

The cluster analysis in the present study identified five separate groups of taste neurons in the CTA, but only the group NH contained neurons strongly responding to the mixture. The NTS, parabrachial nucleus (PBN) and VPMpc contain three to four groups of neurons responding to one of the four basic tastants and each group contains neurons with a high sensitivity to the mixture (Hasegawa *et al.*, 2002). The findings indicate that mixture information may be carried in the lower CNS by multiple groups of neurons, each sensitive to one of the four tastants, but in the CTA only by a single group of neurons, i.e. group NH. Therefore, the mixture may be represented as a taste similar to that of NaCl and HCl in the CTA.

Behavioral responses of animals to binary mixtures should be consistent with the neural recordings in the CTA if neural responses are adequately analyzed. Hamsters usually generalize an aversion conditioned to a binary mixture to both components of stimuli when tested individually, except for the mixture of NaCl and quinine (Nowlis and Frank, 1977). The animals strongly generalize an aversion conditioned to the latter mixture to NaCl, but not to quinine at all. The neural mechanism underlying this phenomenon was suggested when responses of best-stimulus categories of PBN neurons in hamsters were analyzed (Travers and Smith, 1984): the mean response of the NaCl-best neurons to a mixture of NaCl and quinine was comparable to that for NaCl alone, but the mean response of the quinine-best neurons to the mixture was only 75% as large as that to quinine alone. A similar mechanism may be present in the brainstem or cortical gustatory neurons of the rat, as well. On top of that, the present study clarified the role of the GABAergic inhibition in the CTA that selects a certain group of neurons to represent the mixture.

The MDS analysis located the mixture outside the taste space defined by the four basic stimuli, though facing a line connecting NaCl and HCl. This indicates that the mixture is represented in the CTA as a taste different from each taste component of the mixture, which is not compatible with both behavioral and physiological experiments on taste. Therefore, the mixture information is not coded by a large number of neuron groups, but by a certain group of neurons.

GABAergic inhibition in the coding of taste mixture

Most CTA neurons contain $GABA_A$ receptors and the GABAergic inhibitory system apparently contributes to modifying or selecting taste information in the CTA (Ogawa *et al.*, 1998). Therefore, GABAergic inhibition is likely to contribute to the coding of taste mixtures. The suppression of GABAergic inhibition caused responses to the mixture in neurons in group HQ-1 and a fraction of group S, in addition to those in group NH that responded to the mixture in the control state (Figure 5B). Results in the BMI state are quite similar to those found in the NTS, PBN and VPMpc (Hasegawa *et al.*, 2002). Therefore, because of GABAergic inhibitory action which suppresses several groups of neurons otherwise responsive to the mixture, only group NH neurons seem to represent the taste mixture in the CTA in the control state. Presumably, the group NH neurons suppressed the activities of the other groups of neurons through GABAergic interneurons in the CTA.

Two different multivariate analyses (cluster analysis and MDS) used in the present study showed different findings concerning the effects of GABAergic inhibition on coding

of the taste mixture. The cluster analysis showed that GABAergic inhibition increased discrimination of tastants by filtering out a single group of cortical neurons responding to the mixture. On the other hand, however, MDS showed that GABAergic inhibition decreased distances, i.e. caused hard discriminations, between tastants in a two-dimensional taste space. The present findings indicate that cluster analysis is more suitable for data analysis of CTA neurons than MDS.

Tastant detection and mixture as a taste stimulus

Taste stimuli are received at the taste receptor cells in the oral tissue, coded into a series of nerve impulses in the peripheral taste fibers, such as the chorda tympani fibers and transmitted to the NTS and then to the PBN. The afferents from the NTS to the PBN converge without receiving much inhibitory action, so the response magnitude for taste stimuli reaches the maximum in the PBN (Ogawa *et al.*, 1984). Receptive fields are enlarged in the VPMpc and CTA, probably because of the afferent convergence from the bilateral PBN in the thalamus (Nomura and Ogawa, 1985; Ogawa and Nomura, 1988; Ogawa *et al.*, 1992) or callosal afferents from the other hemisphere in the cortex (Kadohisa *et al.*, 2000). However, various forms of neural modification, e.g. descending inhibition from the cortex (Ogawa and Nomura, 1988) or intracortical inhibition (Ogawa *et al.*, 1998), probably decrease the response magnitude to the basic tastants in the VPMpc and thus in the CTA. The decreased response magnitude together with the enlarged receptive fields, a manifestation of neural integration, suggests changes in the adequate stimuli from basic stimuli to complex stimuli as seen in other sensory cortices, e.g. the somatosensory (Iwamura *et al.*, 1985), visual (Gross *et al.*, 1972) and auditory cortices (Maruyama *et al.*, 1979). Thus, it is assumed that neurons in the CTA and probably also in the VPMpc could be tuned to some natural food consisting of several taste qualities, but not to simple basic stimuli.

The mixture evoked significant responses (see Materials and methods) in ~90% of the taste neurons in the NTS, the PBN and the VPMpc (Hasegawa *et al.*, 2002), but in only 56% of the CTA neurons in the present study. Changes in the adequate stimulus to excite most neurons are observed along the ascending pathway in other sensory systems. For example, in the visual system in cats, a concentric circle of light is an adequate stimulus for the retina ganglion cells, whereas a slit of light with adequate slant is needed for neurons in the primary visual cortex. Light covering most of the visual field of the cat evokes little or no response in the retina ganglion cells to the visual cortex neurons (Kuffler and Nicholls, 1977).

In the present study, group NH neurons carried the information of the mixture, but only a few neurons produced greater responses to the mixture than to the sum of the responses to the four tastants, and the magnitude of the response of all cortical neurons to the mixture was small. Therefore, no neuron was specially tuned to the present mixture, although the CTA probably has mechanisms for detecting mixtures of several basic tastants as found in natural products. It is possible that the present species of the taste mixture was not suitable to activate neurons involved in such a mechanism. Further studies are necessary to search for such complex taste stimuli that specifically activate the mechanism for detecting the taste mixture in the CTA.

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