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Sensitivity of Rat Cortical Neurons in Distinguishing Taste Qualities by Individual and Correlative Activities

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Abstract

To examine the possibility that different taste qualities are represented by the correlative activity of cortical gustatory neurons, we made simultaneous recordings of neuron pairs during application of four basic tastes into the oral cavity of anesthetized rats and the following observations were made: (i) in 30 of 67 pairs of taste responsive neurons, peaks (troughs in a few cases) were produced in the cross-correlograms (CCs) during stimulation with some tastants; (ii) the correlative discharges occupied 6–8% of the total spikes discharged by individual neurons during taste stimulation and occurred, in a considerable number of cases, even during stimulation with tastants to which one or both of the component neurons of a pair were apparently non-responding (often sucrose and quinine); (iii) the number of tastants to which a neuron pair responded with a significant correlative activity was often greater than the number of tastants to which the component neurons of the same pair responded with significant changes in discharge rate; (iv) there was no significant difference between the correlative (formation of peaks or troughs in the CC) and individual (change in discharge rate of individual neurons) ways of coding in the sensitivity to distinguish between two taste qualities ranked to be adjacent on the basis of the number of spikes composing the response; and (v) the peaks or troughs appearing in two CCs during stimulations with two kinds of tastants were compared with regard to overlapping of their delay ranges and widths. The spikes in the non-overlapping portion of each peak (suppressed spike number in the case of troughs) are supposed to be able to contribute to two-taste discrimination: the correlated discharges occurring with a delay time that corresponds to the overlapping portion can in no way be judged differently, but the spikes falling in the non-overlapping portion may contribute to the differentiation. The ratio of the non-overlapping portion to the entire peak (or trough) was 0.35 on average. It is concluded that temporal coding of taste qualities seems to operate effectively in the gustatory cortex. **Chem. Senses 22: 363–373, 1997.**

Introduction

The conventional criterion applied to the sensory neurons is a significant change in discharge rate following adequate stimulation. However, in the gustatory system, selectivity of individual neurons with regard to taste quality is often very low, because the neurons respond usually to multiple taste qualities. This observation has led to the proposal of various hypotheses: an across-neuron activity pattern hypothesis (Erickson, 1963; Erickson *et al.*, 1965), a matrix-pattern hypothesis (Yamamoto and Kawamura, 1972, 1978) and an across-region response-pattern hypo-

thesis (Yamamoto *et al.*, 1985). What is lacking in these hypotheses is the temporal aspect of the relationship among activated neurons. In the gustatory portions of the solitary tract nucleus (NTS) (Adachi *et al.*, 1989), parabrachial nucleus (PBN) (Yamada *et al.*, 1990) and cerebral cortex (Yokota *et al.*, 1996) of rats, two neurons located close to each other (~0.1 mm or less) in the same relay station often (in ~50% of the randomly sampled neuron pairs) show synchronous discharges in a taste-quality-dependent manner. Thus the possibility emerges that in the ascending gustatory system temporal coding takes some parts, in parallel with the change in the discharge rate of individual neurons, in differential representation of taste qualities. The importance of temporal coding of taste quality may be evaluated quantitatively in a pair of simultaneously recorded neurons by counting the number of spikes occurring with specific temporal correlation during different taste stimulations (correlative index: C-index). The sensitivity of C-index in distinguishing between different taste qualities may be determined by comparing C-index with the taste-quality-dependent change in the number of stimulus-induced spikes of the component neurons of each pair (individual index: I-index). The magnitude of C- and I-indices may be evaluated also by their proportion of the total number of spikes discharged during taste stimulation. However, the magnitude of C-index can change depending on the partner neuron with which the recording happened to be made simultaneously; C-index may have a higher value when the inputs from the neurons commonly innervating the recorded pair are of higher frequency and/or have higher synaptic efficacy. Therefore a variety of C-indices obtained in numerous neuron pairs would show the frequency range within which the correlative activities of the cortical neurons take place.

In the present experiment, we have determined C- and I-indices in the neuron pairs of the gustatory cortex of rats and compared the results with the corresponding measures already obtained in the brainstem neuron pairs to evaluate the importance of correlated discharges in the gustatory system.

Materials and methods

Stimulation and recording procedures

Male Wistar rats of 260–460 g body wt were anesthetized i.p. initially with a mixture of urethane (0.6 g/kg) and pentobarbital sodium (40 mg/kg). A tracheal cannula was inserted and the incised skin was infiltrated with lidocaine HCl. To avoid damaging the chorda tympani, the lateral surface of the temporal bone was directly clamped with the ear bars to mount the animal on the stereotaxic apparatus. The exposed insular cortex was covered with warm saline. The heating pad maintained the rectal temperature at 37°C. Recording was made under urethane (70 mg/kg per hour) and gallamine triethiodide (90 mg/kg per hour). The airway was secured by frequent aspiration. The electrocardiogram was monitored continuously and the artificial ventilation was adjusted to control the level of the expired CO_2 at 4%.

Four basic taste stimuli (0.2 M NaCl, 0.25 M sucrose, 0.03 M HCl and 0.005 M quinine HCl) of 25–26°C were spread over the tongue and oral mucosa through the nozzle placed in the oral cavity. The test solution first touched the soft palate and then filled the oral cavity, to flow out finally along the lingual tip. The flow rate (1.1–1.3 ml/s) was controlled with air pressure as the driving force, which was turned on and off with electromagnetic valves. Each 10 s stimulation period was followed by rinsing with distilled water, which was continued until 1 s before arrival of the next test solution. Different test solutions were applied in random order with 18 s inter-stimulation intervals. Stimulations with longer intervals (≥100 s) did not increase the response magnitude, indicating that adaptive attenuation did not occur at 18 s intervals. Each solution was applied eight times.

Two groups of electrodes were prepared by gluing together four glass micropipettes. The distance between the tips of the micropipettes was between 50 and 100 μM. Two microstep drivers were used to insert the electrode groups into the unilateral gustatory cortex located around the middle cerebral artery. Each micropipette contained 0.5 M Na acetate and 2% pontamine sky blue. The tip diameter was 1.5 μm. The impedance was 4–6 MΩ at 1 kHz. The spike activities were stored on magnetic tape at frequency responses flat between 0 and 5 kHz. The inter-electrode distance was estimated from the iontophoretically produced dye spots in the serial frozen sections of the cerebral cortex stained with cresyl violet.

Data analysis

All the recorded spike-candidates were A/D converted and displayed off-line on the monitor scope. The templates best fitting the wave forms of target units were created to sort out and process separately two or more concurrent units

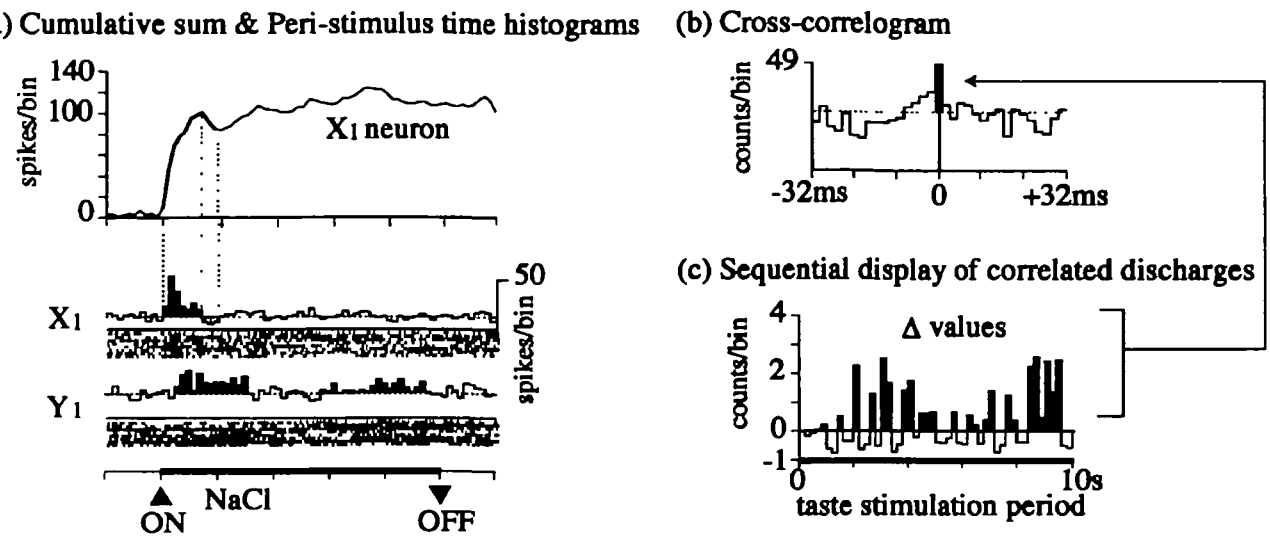

Figure 1 (a) Upper part, cumulative sum histogram of neuron X_1 during stimulation with NaCl for 10 s. The thick line, a sequence of excitatory and inhibitory responses Lower part, PSTHs of X_1 and Y_1 neurons Dark parts, statistically significant responses Horizontal dotted lines, background activity levels during application of distilled water Under each PSTH is the raster display of action potentials during eight trials (b) The CC produced by X_1 and Y_1 neurons The dark bin (2 ms) in the center, statistically significant peak. Horizontal dotted line, background noise level (c) Dark columns, sequential display of the number of statistically significant correlated discharges (Δ values) The sum of these Δ values is approximately proportional to the peak value in (b) pointed to by the arrow

picked up with a single electrode (Forster and Handwerker, 1990).

I-index of every neuron was calculated for each taste quality from the peri-stimulus time histograms (PSTHs) constructed with 0.2-s bins (Figure 1a, neurons X_1 and Y_1).

$$\text{I-index} = \frac{\left(\sum_{i=1}^{N^P}\sum_{j=1}^{P^{Bi}}\left(P^{Sij} - BG\right) + \sum_{i=1}^{N^T}\sum_{j=1}^{T^{Bi}}\left(BG - T^{Sij}\right)\right)}{D}$$

where P^{Sij} is the number of spikes in a jth bin composing an ith statistically significant (see below) peak in the PSTH that appeared during stimulation with a given tastant, BG is the mean number of spikes per bin during water adaptation, P^{Bi} is the number of bins comprising an ith significant PSTH peak, N^P is the number of significant PSTH peaks, T^{Sij} is the number of suppressed spikes in a jth bin comprising an ith significant trough in the PSTH, T^{bi} is the number of bins comprising an ith significant PSTH trough, N^T is the number of significant PSTH troughs and D is the stimulation period (=10 s × 8).

The onset and offset times of peaks and troughs in the PSTH were determined on the cumulative sum histogram (Ellaway, 1978) as obvious flexion points demarcating the rising and falling phases (Figure 1a, top). The statistical significance of each peak and trough was examined with the t-test ($P < 0.01$) in reference to the spike density during application of distilled water (degree of freedom = 14).

Correlation coefficients of the activity of each neuron pair were calculated to depict CCs. The CCs constructed with bin width giving most salient peaks and/or troughs were used to determine C-index. C-index of individual neurons was calculated for each taste quality by the following equation (Figure 1b).

$$\text{C-index} = \frac{\left(\sum_{i=1}^{N^P}\sum_{j=1}^{P^{Bi}}\left(P^{Sij} - BG\right) + \sum_{i=1}^{N^T}\sum_{j=1}^{T^{Bi}}\left(BG - T^{Sij}\right)\right)}{D}$$

Here P^{sij} is the number of spikes in a jth bin composing an ith statistically significant (see below) peak in the CC that appeared during stimulation with a given tastant, BG is the mean number of spikes per bin within the total lag time used to construct CCs (in the case of Figure 1b, ± 32 ms) (the bins adopted as peaks and troughs were excluded), P^{Bi} is the number of bins comprising an ith significant CC peak, N^P is the number of significant CC peaks, T^{Sij} is the number of suppressed spikes in a jth bin comprising an ith significant CC trough, T^{Bi} is the number of bins composing an ith significant CC trough, N_T is the number of significant CC troughs and D is the stimulation period (= 10 s × 8).

The statistical significance of the peaks and troughs produced during taste stimulation was examined by the use

of a synchronization index (Wiegner and Wierzbicka, 1987; $P < 0.01$). Furthermore, a train of inter-spike intervals of the component neurons were shuffled in temporal order with the frequency of inter-spike intervals preserved and the disappearance of the peak in the shuffled CC was confirmed. Auto-correlograms were constructed for each component neuron to exclude the peaks due to bursts of spikes with short and regular intra-burst intervals. In the cases where significant peaks or troughs also appeared during application of distilled water, the magnitude of the water-induced change was subtracted from the corresponding bins.

To see at what moments and with what frequency the correlated discharges occurred in each neuron pair, a time window was settled for each pair of PSTHs. The spikes that fell within the time window corresponding to the width of the peak in the CC (troughs excluded) were counted at 0.2 s intervals during the stimulated periods. The number of net correlative spikes at an ith epoch (Δi) was calculated for each taste quality in the following way and displayed in parallel with PSTHs (Figure 1c):

$$\Delta_i = Ob_i - Ex_i \text{ where } Ex_i = R_i M_i Pw/De$$

where Ob_i is the number of observed correlative spikes that fell in the bins forming a significant peak in the CC during an ith 0.2 s stimulated epoch (= De), Ex_i is the number of correlative spikes expected to occur by chance during the period corresponding to Pw at an ith 0.2 s epoch, R_i and M_i are the number of spikes observed in the paired neurons R and M at an ith 0.2 s epoch, Pw is the time-width in seconds of a significant CC peak and De is the duration of each epoch (=0.2 s in this study).

Results

The test solutions were applied repetitively and continuously during advancement of the electrodes. The taste responsive neurons were encountered in the area 0.8–2.1 mm anterior to the bregma. The frequency to encounter taste neurons was in the following order; dysgranular > granular > agranular insular area in the cortex (Ogawa *et al.*, 1992) and V > IV > III > II in the cortical layer. Among 67 pairs of taste responsive neurons simultaneously recorded in 31 animals, 30 pairs exhibited significant peaks and/or troughs in the CCs during stimulation with one or more tastants (cross-correlation-positive pairs). Peak formation in the CCs, however, was not limited to the taste-sensitive pairs (Table 1). It occurred relatively frequently in the pairs composed of a taste-sensitive neuron and a neuron which did not show any significant response in the PSTH. Only a small number of touch-sensitive neurons were involved in the correlative activity. In the present study, we defined the neurons as touch-sensitive ones, when they generated similar PSTHs in response to all stimuli including water and four basic tastants. Furthermore, those responses to water and tastants were always much smaller in magnitude as compared with the typical taste responses. Very small peaks were also observed, in a small number of pairs, during spontaneous activity without liquid application or during application of distilled water. In the latter case, the peak was enhanced under stimulation with certain taste solutions, but disappeared under stimulation with other tastants.

Table 1 The neuron pairs recorded in the gustatory cortex

Neuron pair as classified by the response type	Number of pairs with peak or trough in the CC	Number of pairs without peak or trough in the CC	Total
Taste versus taste	30	37	67
Taste versus touch	0	11	11
Taste versus none	14	52	66
Touch versus touch	3	4	7
Touch versus none	1	25	26
None versus none	6	31	37
Total	54	160	214

On the basis of the responses in the PSTHs, the neurons were divided into three categories taste-sensitive (taste), touch-sensitive (touch) and no detectable response (none)

The 30 pairs, in which both neurons were taste sensitive, were composed of 51 neurons. The distance between a pair of neurons as estimated from the inter-electrode distance was significantly ($P < 0.001$, Mann–Whitney U-test) shorter in the cross-correlation-positive pairs (80 ± 17 μm; mean ± SEM) than in the pairs without statistically significant cross-correlation (225 ± 36 μm).

Magnitude of I- and C-indices at single neurons during different taste stimulations

Sample PSTHs and CCs are shown in Figure 2. Two neurons, X_2 and Y_2, were responsive to HCl (I-index = 2.79 for X_2 and 2.59 for Y_2) and NaCl (1.31 and 4.74), but not to sucrose and quinine (Figure 2a). Significant peaks were produced at the origin of the time base of the CCs for all

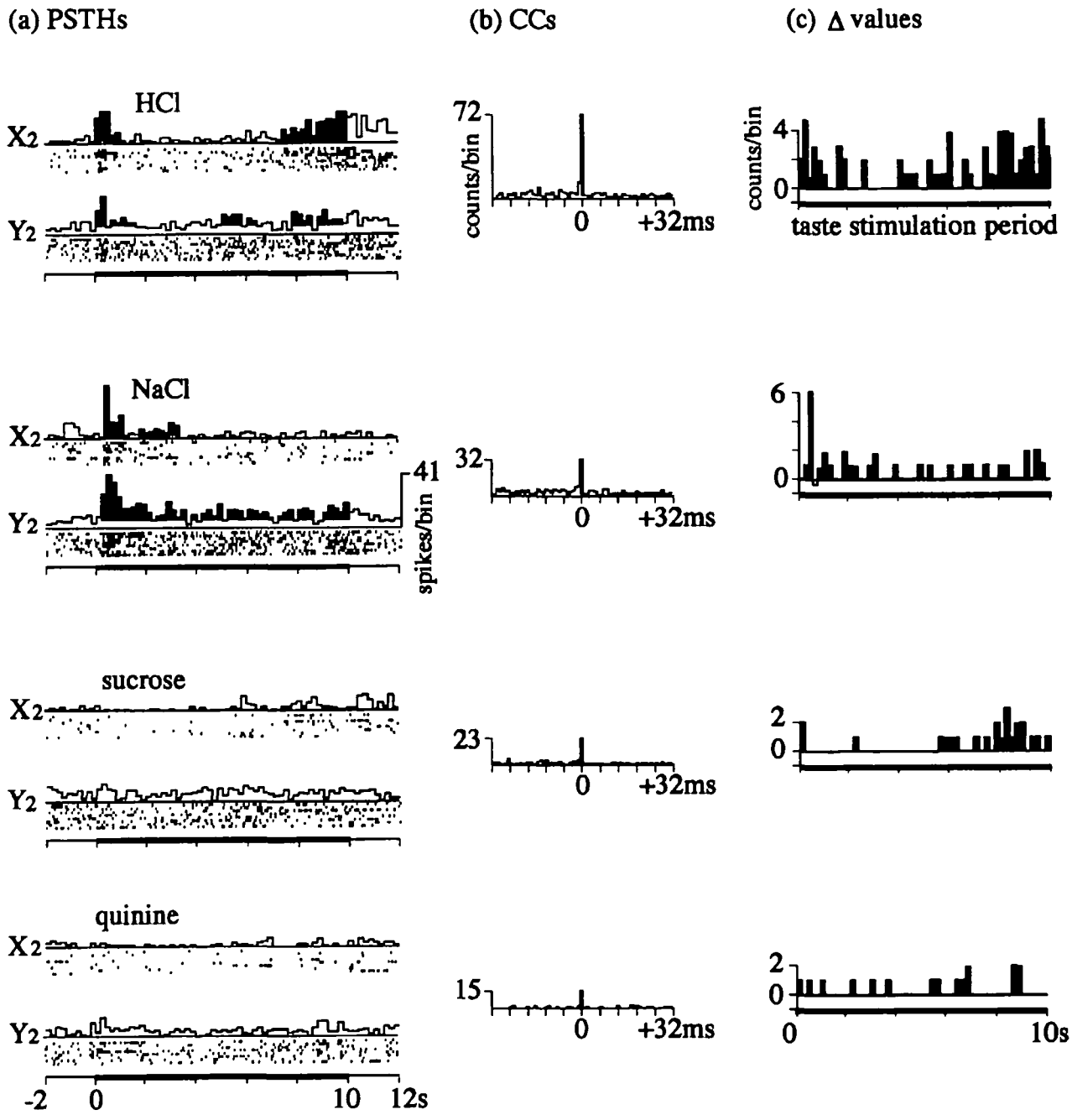

Figure 2 (a) PSTHs of a pair of neurons (X_2 and Y_2) responding to HCl and NaCl, but not to sucrose and quinine. (b) CCs with significant peaks at the center bin (c) Sequential display of Δ values

kinds of test solutions (Figure 2b). C-indices of X_2 and Y_2 neurons were 0.86 for HCl, 0.36 for NaCl, 0.28 for sucrose and 0.18 for quinine. The temporal course of the occurrence of correlated discharges is shown in Figure 2c.

Positive Δ values, i.e. statistically significant correlated discharges, tended to be larger and to be obtained more frequently during the periods when the component neurons responded vigorously (HCl and NaCl), but they were also observed sporadically over the 10 s stimulation periods, with a loose relationship with the responding moments of individual neurons. Furthermore, it should be noted that the peaks could be produced even during stimulation with tastants to which both of the component neurons of a pair were apparently non-responding (I-index = 0; see sucrose and quinine in Figure 2 and also Table 4).

The mean value of I-index of cross-correlation-positive

Table 2 The mean values (spikes/s) ± SE of I- and C-indices during stimulations with NaCl (N), HCl (H), sucrose (S) and quinine–HCl (Q)

	Individual index					Correlative index				
	N	H	S	Q	Total	N	H	S	Q	Total
n	46	38	16	22	122	24	21	16	17	78
Mean	2.93	2.37	1.15	1.46	2.26	0.40	0.40	0.33	0 28	0 36
±SE	0.50	0.41	0.18	0 16	0.24	0.08	0.12	0 06	0 04	0.04

n, the number of neurons with I-index > 0 and of pairs with C-index > 0 The data are based on 51 taste neurons and 30 pairs

Table 3 The magnitude of C- and I-indices relative to the mean discharge rate and the ratio of C-index to I-index of the sampled neurons

	C-index/mean discharge rate				I-index/mean discharge rate				C-index/I-index			
	N	H	S	Q	N	H	S	Q	N	H	S	Q
Mean	0.08	0.06	0 07	0.08	0 41	0.38	0 20	0 23	0 25	0.24	0 58	0.44
±SE	0.01	0.02	0 02	0.02	0 05	0 04	0.06	0 04	0 04	0.04	0 21	0 14
n	46	36	30	32	37	32	10	19	35	24	8	13

The data are based on 51 taste neurons and 30 pairs

neurons with significant taste responses was 2.26, whereas the mean C-index with significant peaks and/or troughs was 0.36 (Table 2). In order to find out the weight of C- and I-indices in the mean discharge rate of individual neurons, C- and I-indices were divided by the mean discharge rate of each neuron during different taste stimulations. The ratio of C-index to the mean discharge rate was between 0.06 and 0.08, whereas that of I-index was between 0.20 and 0.41 (Table 3). The magnitude of C-index relative to I-index was between 0.24 and 0.58.

Sensitivity of C- and I-indices to encode different taste qualities

Table 4 shows the overall frequency of the cross-correlation-positive neurons to detect different tastants with different combinations of C- and I-index values. The detectability of C-index, i.e. C-index >0, was slightly higher (59 + 97 = 156 test sessions) than that of the I-index (I-index > 0; 37 + 97 = 134). In the great majority of cases (n = 97) both I- and C-indices were >0, indicating that both methods of taste quality coding that are represented by C- and I-indices are operating simultaneously at numerous neurons. Such a two-way coding occurred more frequently during stimulation with NaCl (42/97) and HCl (31/97). However, in many cases (n = 59) where there was no significant response in the PSTH, i.e. I-index = 0, C-index was useful in detecting different tastants, especially sucrose (21/59) and quinine (21/59). These 59 responses included 19 occasions in which I-index of a tastant was zero in both of the component neurons. In contrast, the opposite case, i.e. I-index > 0 and C-index = 0, was much less frequent (n = 37) as compared with the combination of I-index = 0 and C-index > 0.

Figure 3 is the matrix showing the frequency of occurrence of different combinations of the number of tastants to which single neurons responded with both C- and I-indices > 0. The number of tastants detected in a single neuron with I-index was most often two or three [(32 + 17)/60 = 82%], whereas the number of tastants detected in a single neuron with C-index tended to be greater; the total number of observations falling in the squares to the right of the equivalent diagonal of the matrix was greater (28/60 = 47%) than that falling in the squares to the left of the equivalent diagonal (18/60 = 30%).

To evaluate the sensitivity of a single neuron in distinguishing between two taste qualities by C- and I-indices, the taste quality giving the maximum C- or I-index in a neuron was named the first taste and the rest were named the second, third and fourth tastes in decreasing order and each index of lower rank was divided by the index of the adjacent higher rank (Table 5). The ranks of four taste qualities as assessed from I- and C-indices at each

Table 4 Frequency of occurrence of different combinations of I- and C-indices in sampled neurons during four kinds of taste stimulations

	N	H	S	Q	Total	(%)
C-index > 0 and I-index = 0	6 [1]	11 [3]	21 [8]	21 [7]	59 [19]	(24.6)
C-index = 0 and I-index > 0	10	10	6	11	37	(15 4)
C-index = 0 and I-index = 0	2	8	22	15	47	(19.6)
C-index > 0 and I-index > 0	42	31	11	13	97	(40.4)
Total					240	

In square brackets is the number of pairs with C-index > 0 and I-index = 0 in both component neurons The data are based on 51 taste neurons and 30 pairs

neuron were different in most cases. There was no statistically significant difference between I- and C-indices in distinguishing between two qualities of adjacent rank.

Two-taste discrimination affected by the difference in the timing of correlative discharges

In the case where the formation of peaks and/or troughs occurs in a neuron pair under stimulation with various tastants and their C-indices are not very different, distinguishing between taste qualities by temporal coding would be difficult. However, if the timing, i.e. the delay, of the correlative discharges of a neuron pair during stimulation with a given taste quality differs from that during stimulation with another quality, the ability to distinguish between the two tastes would become higher. For example, consider the case in which two peaks or two troughs produced by two kinds of tastants have totally different delays, that is, they appear at completely different positions in the CCs [Figure 4 (1)]. In such a case, the ability to distinguish between the two tastants would be higher than in the opposite case in which both the delay and width of the two peaks or of the two troughs are identical [Figure 4 (2)]. The intermediate cases would be a partial overlapping of two peaks or two troughs [Figure 4 (3) and (4)]. The ability to distinguish would be reduced in proportion to the extent of overlap, because the portions of peaks or troughs that are equivalent in timing are not considered effective in distinguishing between two tastes. We have evaluated the ability to distinguish from such an aspect and expressed it by the fraction of the correlative discharges (suppressed spikes in the case of troughs) that did not overlap with the peaks or troughs produced by another test solution (effective fraction). The procedure for calculating effective fractions was as follows: in the case where the peak

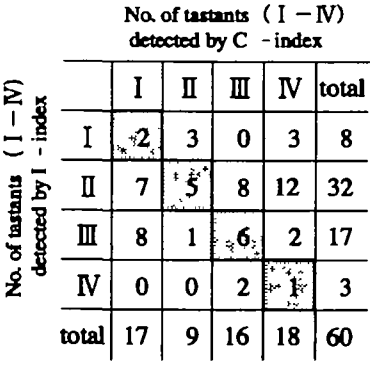

Figure 3 The matrix illustrating different combinations of the number of taste qualities detected (index > 0) by single neurons with C- and I-indices The number of taste qualities detected is in Roman numerals The number of observations is in Arabic numerals The cells composing the equivalent diagonal of the matrix, i e both C- and I-indices detecting the same number of tastants, are shaded and encircled by thick lines The Arabic numerals in the squares on the right of the equivalent diagonal indicate the frequency of observation that single neurons detected a greater number of taste qualities with C-index than with I-index.

produced by a given tastant, say tastant A, appeared in the CC at a position completely different from the peak position of another tastant B without any overlapping portion, all the correlative discharges during stimulations with A and B were regarded to be effective in distinguishing taste A from B and thus the effective fraction was 1.0 for both A and B [Figure 4 (1)]. If two peaks produced by A and B overlapped completely, the effective fraction scored zero for both tastants [Figure 4 (2)]. When the peak produced by tastant A was narrower than peak B and the delay range of A was totally covered by peak B, peak A scored zero and the score for peak B was reduced by the amount corresponding to the spike number in the bins overlapping with peak A [Figure 4 b_2 in (3)]. When two peaks overlapped each other partially, the effective fractions were proportional to the spike number in the non-overlapping portions [Figure 4 a_1 and b_2 in (4)]. In the

Table 5 Sensitivity of I- and C-indices in distinguishing between two taste qualities of adjacent rank

	Individual index			Correlative index		
	2nd/1st	3rd/2nd	4th/3rd	2nd/1st	3rd/2nd	4th/3rd
n	46	20	3	25	14	9
Mean	0.64	0.63	0.62	0 65	0 72	0 73
±SE	0 05	0.06	0.15	0 04	0 05	0 05

n is the number of neurons (I-index) and pairs (C-index) The data are based on 46 taste neurons and 25 pairs

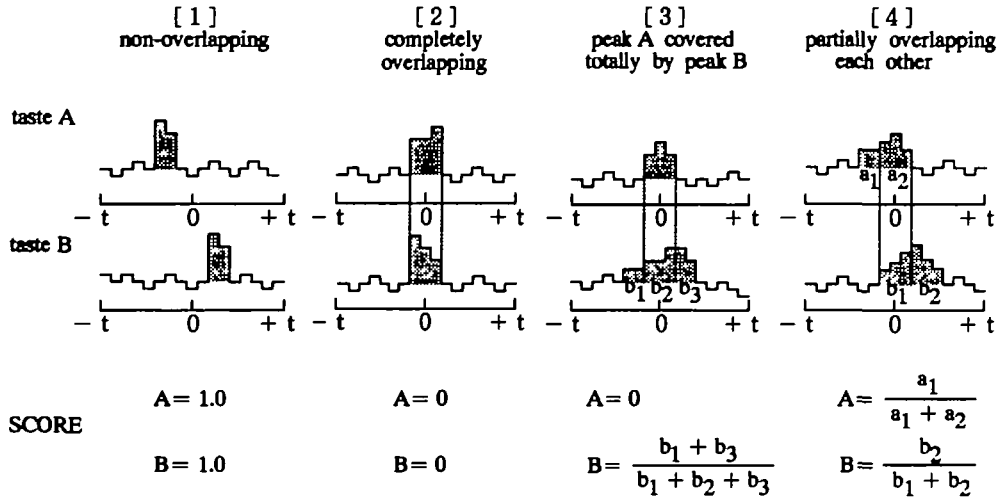

Figure 4 The method to calculate the score of effective fraction from the significant peaks (shaded parts) produced in four pairs of model CCs during stimulation with two kinds of tastant A and B. **(1)** The two peaks produced during stimulation with A and B had no overlapping portion Both A and B scored 1 0 **(2)** The two peaks overlapped completely Therefore, the score was zero for both A and B **(3)** Peak A was totally covered by peak B Therefore, the score for A was zero The score for B was calculated by dividing the spike number in the non-overlapping portion (b1 + b3) by the total spike number forming peak B. **(4)** The scores were calculated in the same way as in peak B in (3)

case where changing of the test solution was associated with inversion of response polarity, i.e. a peak becoming a trough or a trough becoming a peak, the score was 1.0 irrespective of the extent of overlap.

In 22 neuron pairs, significant peaks and/or troughs appeared in the CCs in response to two or more kinds of taste qualities. The probability of a taste quality being differentiated from another taste was scored by the method described above. Among 85 two-taste comparisons a total of 91 tastants (54%) scored zero, i.e. the delay range of their correlative discharges was totally covered by that of the paired tastant (Table 6). In the remaining 79, the score was 1.0 (n = 32) or there was some non-overlapping effective portion in their peaks and troughs and their average score was 0.76. The overall effective fraction was, therefore, $0.76 \times 79/(91 + 79) = 0.35$.

Discussion

Preferential synchronization of taste-sensitive neurons

Almost all neuron pairs that exhibited correlative discharges were taste-sensitive (Table 1). The neurons apparently sensitive to tactile stimulus showed correlative discharges only in a small number of cases. These results seem to indicate strongly the importance of correlative activities in the processing of the gustatory information in the insular cortex. Processing of tactile information seems to be less important in this cortical area. Very weak correlative activities were sometimes observed during spontaneous activity without liquid application into the oral cavity. These correlative activities of unknown origin might mean that synchronization is a basic property of cortical neurons and

Table 6 Number of taste responses in the CCs without (score = 0) or with (1.0 ≥ score >0) effective fraction and the mean of the score

	N	H	S	Q	Total
Score = 0	27	22	18	24	91
1.0 ≥ score > 0	24	23	18	14	79
Mean	0 39	0 36	0 37	0.27	0.35

The data are based on 38 taste neurons and 22 pairs

that enhancement or attenuation of the synchronization is a significant signal. The weak synchronization sometimes found during water application was also enhanced or abolished under stimulation with tastants.

Synchronization of closely located neurons

Most of the cross-correlation-positive pairs were recorded with the inter-electrode distance at ~0.1 mm or less, and the peaks and troughs in the CCs had no distinct trailing skirts or oscillatory side peaks. This would suggest that the mechanism producing the peaks and troughs observed in the present experiment is essentially different from that reported recently in the visual cortex (cf. Singer and Gray, 1995), because in the visual cortex oscillation can occur in the form of generalized synchronization of numerous neurons with a much looser restriction on the inter-neuronal distance. In the gustatory cortex, in contrast, synchronization occurs with a smaller jitter in a population of neurons located close to each other. This observation is rather similar to 'the precisely correlated firing' found in the lateral geniculate nucleus (Alonso *et al.*, 1996). Correlation with a small jitter is expected to increase the spike density in local neuronal circuits momentarily but repetitively. Synchronous spike discharges of nearby neurons would enhance the probability of post-synaptic potentials to summate at their common target neurons, resulting in sequential activation of a set of neurons in a short period of time. The troughs in the CCs might indicate that a lack of activity at specific moments of particular neurons in a local network plays also some role in signaling taste qualities.

Two modes of taste quality coding: salt and sour versus sweet and bitter

Timing of the occurrence of correlative discharges, the frequency of which was evaluated by the Δ values, was not necessarily concomitant with the high frequency discharge of the component neurons of a pair. This would mean that the temporal relationship between the two kinds of activities at a single neuron, which were represented by I- and C-indices, is rather random. The random relationship might be favorable for minimizing a discontinuous flow of information, that is, the individual and correlative activities may often be complementary and thereby can reduce silent periods in monitoring gustatory information.

In the responses to NaCl and HCl, both C- and I-indices of individual neurons were often >0 (42/60 = 70% and 31/60 = 52% respectively), whereas such cases were quite infrequent in the responses to sucrose and quinine (18 and 22%). In contrast, the type of combination of C- and I-indices most often observed during stimulation with sucrose and quinine was C-index > 0 and I-index = 0 (35% each). Furthermore, there was a substantial number of cases where the response type of both of the component neurons was C-index > 0 and I-index = 0 to the same taste quality and those cases were found much more frequently during stimulation with sucrose and quinine (15 pairs in total) than during stimulation with NaCl and HCl (four pairs). In the brainstem relay stations the most frequently observed combination was both C- and I-indices > 0 [78% in NTS (Adachi *et al.*, 1989) and 86% in PBN (Yamada *et al.*, 1990)] and a small number of another type of combination, C-index > 0 and I-index = 0, was found only during stimulation with sucrose or quinine. It seems that in the cortex the correlative processing is increasingly important, especially for sweet and bitter tastes.

Relative magnitude of C- and I-indices

The magnitude of the mean C-index was only one-sixth of the mean I-index. The mean C-indices reported in the brainstem nuclei were usually much greater (0.3–0.9 in NTS; Adachi *et al.*, 1989 and 1.6–7.6 in PBN; Yamada *et al.*, 1990). However, the mean ratio of C-index to I-index in the cortex was not greatly different from that in the brainstem, because the response intensity (comparable with I-index) of brainstem neurons is often very high (Perrotto and Scott, 1976; Hill *et al.*, 1983) as compared with the cortex (Ganchrow and Erickson, 1970; Yamamoto, 1984). If temporal coding of gustatory information actually takes place in a manner which can be detected effectively by C-index, its importance must be reflected in the magnitude of the mean C-index and/or C/I ratio. If such logic is acceptable, temporal coding may be regarded as much less powerful when compared with the conventional way of coding that is considered to be represented by I-index. However, an alternative interpretation is also possible: if the correlative

discharge occurs in a substantial number of neurons quasi-synchronously and/or in succession with a specific spatio-temporal pattern, the total number of correlative discharges could become large. There are several observations supporting this assumption: (i) the probability of encountering cross-correlation-positive neuron pairs was rather high (30/67 = 45%); (ii) in the majority of cases, single neurons detected a greater number of tastants with C-index than with I-index (28/60 = 47%), and the opposite case was less frequent (18/60 = 30%) (Figure 3); (iii) in a considerable number of responses (59/240) C-index was >0 with I-index = 0 (Table 4). Apparently taste-insensitive neurons often showed correlated activity with taste-sensitive neurons (Table 1); (iv) in a small number of cases (n = 5) where three or more neurons were recorded simultaneously, single neurons showed correlative activity with plural neurons during stimulation with certain tastants (data not shown).

Involvement of a single neuron in the processing of multiple taste qualities might be advantageous, because the number of neurons recruited in response to a given tastant can be greater than in the case where single neurons respond to a smaller number of taste qualities. However, multi-taste responsiveness appears to be disadvantageous to differential representation of different taste qualities by single neurons. In this sense, taste-quality discrimination based on C-index might be less effective than in the case with I-index, because C-index tended to detect a greater number of tastes. To quantify the distinguishing ability, we used the ratio of the response magnitudes of two taste qualities of adjacent rank. As assessed by this method, there was no significant difference in distinguishing ability between I- and C-indices (Table 5). The same was true also in the brainstem nuclei (Adachi *et al.*, 1989; Yamada *et al.*, 1990).

Possible improvement in discriminative sensitivity by the difference in the timing of synchronization

It seems possible that a part of the information for differential representation of taste qualities by a neuron pair is in the difference in timing of correlative discharges. We evaluated this possibility by the effective fraction of the peaks and troughs. The average effective fraction was 0.35. Therefore, two-taste discrimination based on the difference in the magnitude of C-index may be improved by a factor of 0.35. In the brainstem nuclei, the mean effective fractions were smaller [0.24 in NTS (Adachi *et al.*, 1989) and 0.14 in PBN (Yamada *et al.*, 1990)], probably because of a smaller number of interneurons intervening between the recorded neurons and their commonly innervating neurons and/or because of a smaller difference in the length of bifurcated terminal axons of the commonly innervating neurons.

In the gustatory cortex where the activity of individual neurons is rather poorly time-locked to the stimulus onset and offset, the temporal coding seems to be a more important mechanism than in the brainstem nuclei and to operate more effectively for coding of sweet and bitter tastes.

In conclusion, the present results suggest that correlated discharges can bind into groups the taste-sensitive neurons which have been analysed as functionally independent units in various hypotheses on taste-quality coding so far proposed. It has also been suggested that the grouping can change in both the composing members and the frequency of synchronization in a taste-quality-dependent manner. The classical hypotheses, however, are based on the assumption that only neurons that can exhibit a change in discharge rate upon changing the quality of test solutions can participate in taste quality coding. Our observations have highlighted the possibility that an unexpectedly large number of neurons can participate in the coding of the taste quality to which they are apparently non-responding. This might mean that the number of neurons concerned is greater than that commonly assumed and detection of the presence of taste signals in the local neuronal pool can be facilitated by the correlative activities.

It has been proposed that the visual cortical neurons can act as both integrator and coincidence detectors (König *et al.*, 1996). This concept is consistent with our finding that, at single neurons in the insular cortex, both C- and I-indices can be expressed at different intensities. However, in view of the complex structural interconnections of the cortical neurons, some additional mechanisms seem to be required to functionally bind numerous neurons distributed over a wide insular area.

ACKNOWLEDGEMENTS

Thanks are due to Miss Tomoe Sato for her technical contributions. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan to T.Y. and T.S.

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Received April 2, 1995, accepted April 1, 1997