Neural Interaction between Cortical Taste Neurons in Rats: a Cross-correlation Analysis

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Abstract

In the rat cortical taste area (CTA), we recorded 31 pairs of taste neurons and seven pairs of taste and non-taste neurons, with single or double electrodes. By using a cross-correlogram (CCG) in a stationary state, we examined the functional interaction between neurons of the pairs while activating them by taste stimulation. Though only 14.3% of the taste and non-taste neuron pairs were correlated, 54.8% of the taste neuron pairs showed correlated activities, 41.9% of them showing common inputs, including one with an additional excitatory connection. The remainder (12.9%) showed excitatory connections with a time lag of 1–3 ms. When pairs were recorded using single or double electrodes with an intertip distance of <50 μm in a dorsoventral direction, a larger fraction had correlated activities than when the intertip distance was >50 μm. Whereas pairs of neurons showed correlated activities in area DI whatever the vertical intertip distance was, most of the pairs having correlated activities in area GI were found within 50 μm of the vertical intertip distance. The taste profiles of common inputs to the pair were estimated on the basis of peak at time 0 in CCGs for various taste stimuli. The efficacy contribution of the source to target neurons tended to be larger when both had the same best stimulus. This tendency held true for pairs showing excitatory connections. Interlayer excitatory connections were also evident. It is concluded that a functional column with a diameter of 50 μm may present in the CTA in rats, and that information flow is larger between pairs of neurons with the same best stimulus. **Chem. Senses 22: 517–528, 1997.**

Introduction

Anatomical studies have shown that neurons in the cerebral cortex are arranged vertically from the pial surfaces to the white matter, sending processes mainly vertically (Lorente de No, 1933; Jones and Powell, 1973) with few connections tangential to the surface. Physiological studies have revealed the existence of functional columns with a given diameter in sensory cortices (Mountcastle, 1957; Hubel and Wiesel, 1962), in which neurons with similar receptive features are vertically arranged. Afferents from specific thalamic nuclei terminate at the middle of the six cortical layers, i.e. layer IV, and the efferents stem out of layers III, V or VI to other cortical areas or to lower relay nuclei (Toyama *et al.*, 1974; Douglas and Martin, 1990). It is suggested that the sensory information is processed and integrated within functional columns.

The cortical taste area (CTA) receives inputs from the parvicellular part of the ventral posteromedial thalamic nucleus (VPMpc), one of the specific nuclei in the thalamus

(Yamamoto *et al.*, 1980; Kosar *et al.*, 1986; Ogawa *et al.*, 1990). The CTA is at the rostral part of the insular cortex dorsal to the rhinal sulcus (Yamamoto *et al.*, 1980; Kosar *et al.*, 1986; Ogawa *et al.*, 1990). Cytoarchitectonically, the CTA consists of the granular (GI) and dysgranular insular cortices (DI) (Ogawa *et al.*, 1990). It has been reported that when the recording electrode is advanced through the CTA obliquely to the cortical surface, taste neurons are not always recorded sequentially but found among many mechanoreceptive or non-responsive neurons (Ogawa *et al.*, 1990). If the columnar structure exists in the CTA, taste columns are scattered among many non-taste columns. However, no anatomical or physiological report has yet confirmed such a neuronal network in the CTA.

A cross-correlation method was developed for analyzing the functional interaction between pairs of neurons (Perkel *et al.*, 1967), and it has been found useful in revealing the nature of common inputs to the pair, or excitatory or inhibitory connections between the two in which one constituent of the pair (source neuron) sends afferents to the other (the target). This cross-correlation method has been widely used in various areas of the cerebral cortex (Toyama *et al.*, 1974; Ts'o *et al.*, 1986; Gochin *et al.*, 1989; Douglas and Martin, 1990; Hata *et al.*, 1991; Johnson and Alloway, 1996). Though it was also used in the gustatory cortex in rats (Yokota *et al.*, 1996), no study has been performed to reveal functional connections.

In the present study, pairs of neurons were simultaneously recorded in the rat CTA by using single or double electrodes, and their functional connections were studied by means of cross-correlation (Perkel *et al.*, 1967) to clarify the neuronal network.

Materials and methods

Preparation

Thirty-six female SD albino rats (>100 days old; 250–400 g) were anesthetized with an i.p. injection of urethane (1000 mg/kg). After cannulation of the trachea and femoral vein, each animal was mounted on a stereotaxic instrument following the method of Paxinos and Watson (1982). During the experiment, the rats were tilted ~45°, with the left side up. The animals were immobilized by an i.v. infusion of D-tubocurarine and ventilated artificially. Whenever the infusion of D-tubocurarine seemed to wear off, the level of anesthesia was checked by the corneal reflex and urethane (100 mg/kg) was supplemented as necessary. The bone covering the left middle cerebral artery was carefully removed. A small opening was made in the dura to insert an electrode. The left buccal wall was cut from the mouth corner to the anterior edge of the ramus of the mandibula, the mouth was opened ~30–40°, and the tongue was stretched out anteroventrally. All wound margins and pressure points were infiltrated with local anesthetic (1% lidocaine hydrochloride). Body temperature was kept at 37°C with a water heater; the electrocardiogram (ECG) was monitored throughout the experiment. End-tidal CO_2 concentration was monitored with a pCO_2 analyzer during ventilation and adjusted to 3.5–4.5%.

Recording

Single and double glass micropipettes were filled with 2% pontamine sky blue in 0.5 M Na acetate (5–7 MΩ) to record pairs of cortical taste neurons extracellularly. In the double electrode, the two electrodes were glued with the tips separated by ~50–500 μm. Electrodes were inserted from a dorsolateral to a ventromedial direction, parallel to the coronal plane, towards the CTA through the opening of the dura. Unitary activities were isolated extracellularly from the somas of the cortical neurons using the criteria of Bishop *et al.* (1962).

Identification of two different neurons recorded with single electrodes was made using the following criteria: (i) two kinds of spikes displayed on an oscilloscope were totally different in form and size, and triggering the trace of the oscilloscope with one of the two spikes showed that the other was not due to the IS/SD block; (ii) with movement of the electrode, the two spikes changed in size independently; and (iii) after the termination of recording, a further advance of the electrode injured one of the two spikes, with the other remaining intact. Unless all three criteria were met, the pair of neurons recorded were not used for analysis.

Impulse discharges recorded from two cells were fed to window discriminators after amplification. The spike was converted to a pulse (5 V, 1 ms) by the window discriminator and led to a personal computer (NEC PC9801RX) through an I/O board. We measured two trains of inter-spike intervals by a counter with a time resolution of 10 μs, and stored them on the hard disk for later analysis.

Stimulation

The taste stimuli used were 0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl, 0.02 M quinine–HCl, and a 'search' stimulus

containing all four chemicals at the above-mentioned concentrations. Taste solutions were delivered to the whole oral cavity, including the tongue and soft palate, from a system of overhead funnels via gravity flow at a rate of 3 ml/s. We used two modes for finding taste neurons and examining their characteristics. The first mode (searching) was to apply distilled water for 5 s, and then to give a taste solution for 5 s. When taste responses seemed doubtful, taste solutions were delivered in the second (data collection) mode, which delivered rinse water for 15 s, a given taste stimulus for 10 s and then rinse water for 15 s to characterize the neurons (Ogawa *et al.*, 1984). During the interstimulus period, the oral cavity was repeatedly rinsed with distilled water until it was confirmed with an audio monitor that the rate of background discharges had returned to the level of the prestimulus period. Taste responses were identified when, during 10 s of taste stimulation, there was a change in the discharge rate of at least 1.0 s and 2 SD above or below the prestimulus mean. 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 control period. However, in this study, responses to taste stimuli and distilled water were used to construct a taste profile of neurons without subtraction of the number of impulses in a corresponding prestimulus period. When one or both neurons comprising the pair showed responses for taste stimuli, continuous stimulation with the given taste stimulus or water for 50 s was delivered following rinse water for 10 s for cross-correlation analysis in the stationary state. One and the same stimulation was repeated three times to collect as many spikes as possible.

Data analysis

To obtain trains of impulses in the stationary state in response to a given taste stimulus, impulses which in the first 5 s after the onset of stimulation contained phasic responses were discarded from each trial, with the impulses from the remaining 45 s in the three trials being collected. Then the side test (Griffith and Horn, 1966) was applied to the latter series of impulses to test whether or not they were in a stationary state. In this test, series of impulses were divided into several blocks of a certain time period, the length of which was chosen to contain an average number of 25 impulses, instead of the 100 in the original version. This was done because the number of impulses discharged was small in the cortical taste neurons but the number 25 was considered large enough to meet the assumption that the Poisson distribution could be approximated by the Gaussian distribution. Most of the records passed the side test at $P = 0.01$ by excluding the phasic responses in the 5 s after taste stimulation. Several records did not have a sufficient number of impulses to divide them into blocks. The rest of the records passed the side test only when discharges at 10–20 s after stimulation were excluded. As a control, similarly paired spike trains during three repeated applications of distilled water, except during the first 5 s each trial, were subject to the side test when the number of impulses discharged was sufficiently large. Thus, spike trains in pairs of neurons during taste or water application were utilized for stochastic analysis, i.e. cross-correlation and auto-correlation analysis.

Cross-correlation (CCG)

To examine correlated firings of paired neurons, the cross-correlation between two trains of spikes was calculated by a method established previously (Perkel *et al.*, 1967; Gochin *et al.*, 1989). Raw CCGs were constructed that displayed changes in the activity of one neuron (neuron 2) as a function of discharges of the reference neuron (neuron1) occurring at time 0. Stimulus coordination effects were removed by subtracting a shift predictor from the raw CCG to produce neural CCG (Gochin *et al.*, 1989). The shift predictor was used to calculate 95% confidence limits, and peaks or troughs that exceeded the 95% confidence limits were regarded as statistically significant (Gochin *et al.*, 1989). A peak or trough judged significant was confirmed by the synchronization index method of Wienger and Wierzbicka (1987).

A significant peak at time 0 indicates that both neurons in the pair under consideration may have a common input or share a source neuron, while a peak at other times shows that the neurons may have excitatory interaction (Perkel *et al.*, 1967). In the latter case, peaks to the right of time 0 may represent interaction in the direction from neuron 1 (source neuron) to neuron 2 (target), whereas peaks to the left of time 0 may represent interaction in the opposite direction. Similarly, a significant trough may indicate an inhibitory interaction between the two neurons studied.

When two paired neurons showed a common input, characteristics of the source neurons were estimated on the basis of the magnitude of the CCG's peak. The magnitudes of the peak at time 0 for four basic stimuli were assumed to represent the taste profile of the source neuron. When the

CCG could not be obtained for a certain taste stimulus because the impulse trains of either neuron failed the side test, the common input for the stimulus was assumed to be nothing. The efficacy contribution of the source neuron to the target neuron for a certain taste stimulus was calculated as

$$\text{efficacy contribution (for neuron } I) = CE/N_i \qquad (i = 1,2)$$

where CE represents the maximum number of coincident events occurring at time 0 in the neural CCG and N_i (i = 1,2) stands for the total number of discharges of neuron 1 or neuron 2 (Levick *et al.*, 1972). However, when the CCG could not be calculated for a stimulus, the efficacy contribution of the common input to the pair was assumed to be zero for the stimulus. The total efficacy contribution of all four basic taste stimuli was calculated by $\Sigma CE_j/\Sigma N_{ij}$ (i = 1,2 representing neuron 1 or 2; j = 1–4 for taste stimuli), where each numerator represents the total number of significantly correlated discharges in response to all four basic stimuli in each neuron, and each denominator the total number of discharges during administration of the four taste stimuli.

When either or both of the neurons in a pair exhibited mutual excitatory interactions, the efficacy contribution of the source neuron to the target neuron for a single stimulus, and that for all four taste stimuli, were calculated similarly: CE represents the maximum number of coincident events at the bin of the significant peaks and N stands for the total number of spike discharges of the target neuron.

Histology

The dye was deposited iontophoretically from the recording electrode at three points, including the recording site(s) and the end of the electrode track. When double electrodes were used, dye was deposited from the protruding tip. At the end of the experiment, the animal was deeply anesthetized and perfused with 10% formalin. The brain was frozen, serially sectioned at 50 μm in parallel to the coronal plane and stained with thionin, and the taste neurons were reconstructed histologically. Cytoarchitectonic identification of areas GI and DI was based on the description by Cechetto and Sapar (1987). When double electrodes were used, the angle of the electrode track to the cortical surface was determined, and the distances vertical or tangential (dorsoventral) to the surface between the two electrode tips were measured.

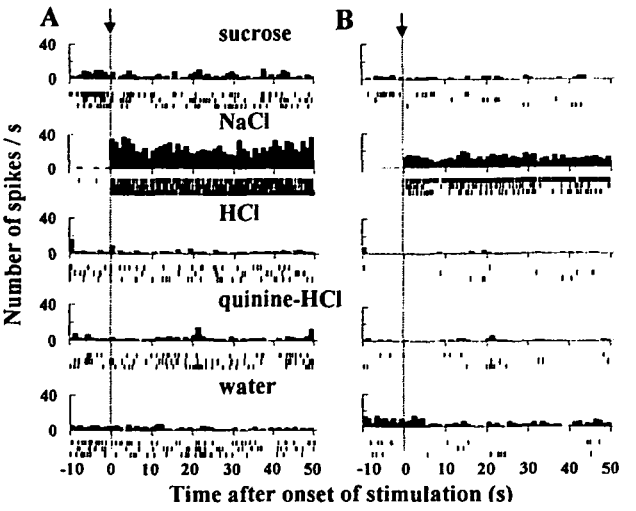

Figure 1 Responses of a pair of NaCl-best neurons to three trials of sustained taste or water stimulation for 50 s after a water rinse of 10 s. **(A, B)** Discharge patterns and peri-stimulus time histogram of two neurons (neurons 1 and 2), simultaneously recorded with a single electrode.

Results

A total of 856 neurons were encountered. Thirty-one pairs of taste neurons were recorded, and in 29 (15 in area GI, 14 in area DI) the recording sites were identified. Seven pairs (five in area GI, two in area DI) in which only one of the constituents was a taste neuron were obtained, and all of the recording sites were identified.

Figure 1 shows the discharges of a pair of neurons in response to three trials of stimulation with the four basic stimuli and water. Both constituents of the pair responded best to NaCl among the four stimuli. Since the neuron in Figure 1B did not produce a sufficiently large number of impulses in response to taste stimuli and water other than NaCl, CCGs were obtained only for the NaCl response (see Figure 2A). In 29 pairs of neurons, 161 of the 232 series of impulses discharged in response to the four basic tastes contained a sufficiently larger number of impulses for the side test of the stationary state. Of the 161, 154 series passed the test after excluding the discharges in the first 5 s after stimulation, the remaining seven passing it only after excluding the discharges in the first 10–20 s after stimulation. Thus, 76 of the 116 paired impulse trains during taste stimulation were utilized for CCG calculation. Of the series of impulses (n = 71) to which the side test was not applicable, almost all (n = 64) were found not to be taste-responsive, only seven being taste-responsive. Out of the impulse trains of 58 neurons during water application, those of 33 neurons showing a sufficient number of

Table 1 Relation between best stimulus of constituents in pairs and cross correlation in areas GI and DI

Area	Details of CCG	Best stimulus of paired neurons							
		Pairs of neurons with same best stimulus					Pairs of neurons with different best stimuli		Gross total
		Sum	Specifics				Sum	Specifics	
			S–S	N–N	H–H	Q–Q			
GI	Total	9(1)	1	3	2(1)	3	6(2)	S–N, S–Q(×3), H–Q,,N–H	15(3)
	CCG(+)	7(1)	0	3	2(1)	2	2	S–Q, N–H	9(1)
	Common input	4	0	2	1	1	2	S–Q, N–H	6
	Excitatory connection	2(1)	0	0	1(1)	1	0		2(1)
	Com. input + exc. connection	1	0	1	0	0	0		1
DI	Total	9(5)	2(2)	5(3)	2	0	5(1)	S–N(×2), S–H(×2), N–H	14(6)
	CCG(+)	5(1)	1	2	2	0	3	S–N, S–H, N–H	8(1)
	Common input	4(1)	1	1	2	0	2	S–N, N–H	6(1)
	Excitatory connection	1	0	1	0	0	1	S–H	2

S, N, H and Q indicate sucrose, NaCl, HCl and quinine–HCl. S–S, H–H and so forth represent types of combination of best stimuli of paired neurons. Numerals in parentheses indicate the number of cases in which the tangential distance between electrode tips of double electrodes was >50 μm. (×2) and (×3) stand for observation of two and three pairs of the same type.

discharges passed the side test; water discharges in only 15 pairs were utilized for CCG calculation.

We classified pairs of taste neurons by the stimulus which gave rise to the maximum responses (best stimulus) (Table 1). In 18 pairs, both constituents showed the same best stimulus, whereas in the remaining 11 pairs (six in area GI, five in area DI) the consistuents had different best stimuli. A majority of pairs consisted of NaCl-best neurons in both GI and DI areas. Among the possible combinations of neurons with different taste stimuli, pairs of NaCl- and HCl-best neurons or pairs of NaCl- and sucrose-best neurons were found in both areas. However, fractions of pairs of taste neurons with different best stimuli differed with area.

Pairs consisting of taste neurons only

Common input and excitatory connection

Seventeen pairs (nine in area GI, eight in area DI) had significant cross-correlations. Of these, 13 (seven in area GI, six in area DI) had cross-correlations (CCGs) with peaks at time 0 showing common inputs (Perkel *et al.*, 1967). Figure 2A and B shows two examples: a pair of NaCl-best neurons and a pair of HCl-best neurons. Each pair had a single peak at time 0 and a width of 1 ms (Figure 2Ab and Bb). The width of the peak in 13 pairs was <10 ms except in one pair where it was 30 ms. It was 1 ms in half of the narrow peaks (six cases).

Four pairs of neurons (two in area GI, two in area DI) had peaks other than at time 0, showing excitatory connections between two constituents in pairs (Perkel *et al.*, 1967), and a peak delay of 1–3 ms. Figure 3 shows the CCG of a pair of both NaCl-best neurons in area DI which also gave good responses to quinine–HCl (see bar graphs in Figure 3Ca and b); the peak was at the bin of 1 ms right to time 0 (Figure 3B). An excitatory connection is suggested from one constituent of the pair (source) to the other (target). Three pairs showed a CCG peak for distilled water as well as for all four taste stimuli. One pair in area GI showed a peak at the bin of 2 ms delay for all the taste stimuli, in addition to the peak at time 0; that is, the pair had a common input to both constituents and an excitatory connection between them.

Pairs showing common inputs were more numerous than those with excitatory connections, as previously reported (Dickson and Gerstein, 1974; Toyama *et al.*, 1981a; Ts'o *et al.*, 1986; Kruger and Aiple, 1989; Hata *et al.*, 1991).

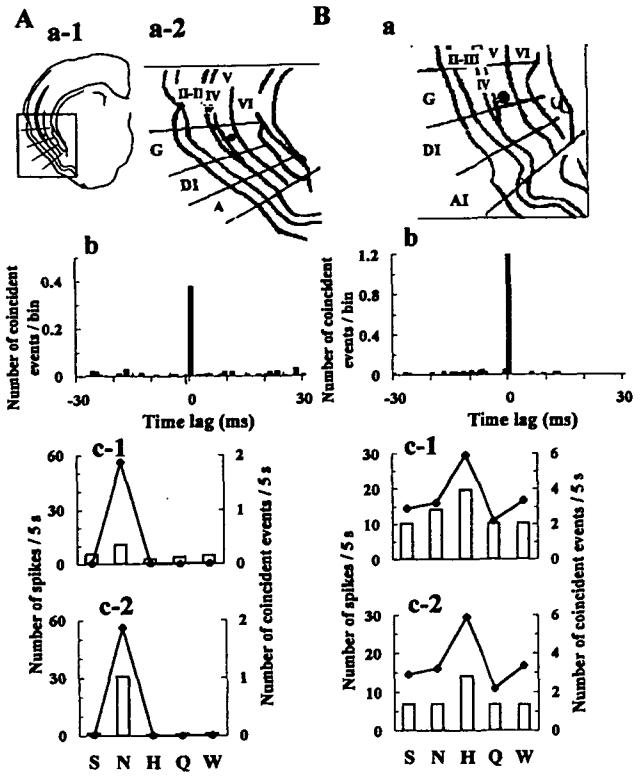

Figure 2 Two pairs of neurons which showed correlated activities at time 0, indicating the reception of common inputs. Both were recorded with single electrodes. **(A)** A pair of NaCl-best neurons in layer V in area GI. **(B)** A pair of HCl-best neurons in layer V in area GI. **(a)** Recording sites marked by solid circles. **a-1** in A shows the section of the hemisphere containing the recording site magnified in **a-2**. a in B shows a magnified section. **(b)** Cross-correlogram (CCG) with a peak at time 0, due to concurrent excitation by the best stimulus, 0.1 M NaCl (A) or 0.01N HCl (B). **(c-1, c-2)** Taste profile of neurons 1 and 2 shown by blank bars. Dots connected with continuous lines show the taste profile of the common input estimated on the basis of the peak heights in concurrent excitation by the four basic tastes. The dimension at the left ordinate applies to the taste profile of paired neurons and that at the right to that of the common input. The efficacy contribution of the common input was assumed to be zero for a certain stimulus when the CCG could not be calculated for the paired impulse trains in response to the stimulus. The total efficacy contribution of the common input for all four basic stimuli to neurons 1 and 2 was 0.171 and 0.061 in A, respectively, and 0.262 and 0.410 in B.

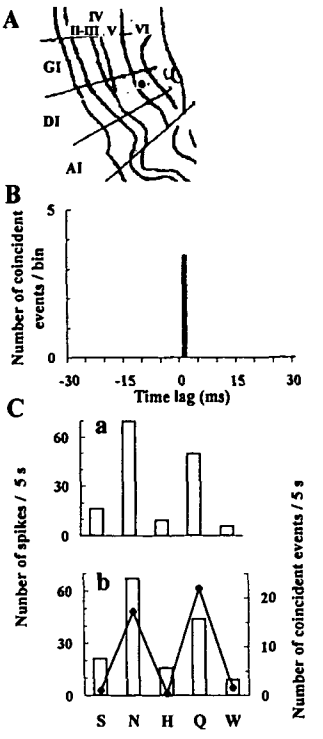

Figure 3 A pair of neurons with excitatory connection recorded with a single electrode. **(A)** Recording site. Both neurons were located in layer VI in area DI. **(B)** CCG with a peak at 1 ms due to delayed excitation (by 1 ms) of neuron 2 in reference to the discharge of neuron 1. Stimulation with 0.1 M NaCl. **(C-a, C-b)** Taste profile of both neurons 1 and 2. Both were NaCl-best. Taste profile of the source neuron estimated from the peak height in the CCG, which was quinine–HCl-best. The total efficacy contribution of the source neuron to the target neurons for all four basic stimuli was 0.307.

Cross-correlation for neuron pairs recorded with single electrodes vs. double electrodes

In eleven pairs (nine in area GI, two in area DI) recorded with single electrodes the distance of paired neurons could be assumed to be short enough. Six pairs (five in area GI, one in area DI; see Figure 2) had common inputs, two pairs (one in each area; Figure 3) showed excitatory connections and one pair (area GI) showed both a common input and an excitatory connection.

Eighteen pairs (six in area GI, 12 in area DI) were recorded with double electrodes. Among them six pairs (five in area GI, one in area DI) showed common inputs and two pairs (one in each area) showed excitatory connections.

Neuron pairs recorded with single electrodes tended to show correlated activity more frequently than those recorded with double electrodes, as reported by Dickson and Gerstein (1974).

Relations between electrode distance and correlated activities

Assuming that the distance between the electrode tips represented the distance between the neurons, we examined the correlated activities of neuron pairs recorded with double electrodes against the tangential or vertical distance between the electrode tips (Figure 4A and B). We defined the tangential and vertical distance between the electrode tips in a single electrode recording 0 μm (Figure 4A and B).

Of the 20 pairs (12 in area GI, eight in area DI) recorded within a distance of <50 μm in the dorsoventral, tangential direction, 15 (75%; eight in area GI, seven in area DI) showed correlated activities (Figure 4A). On the other hand,

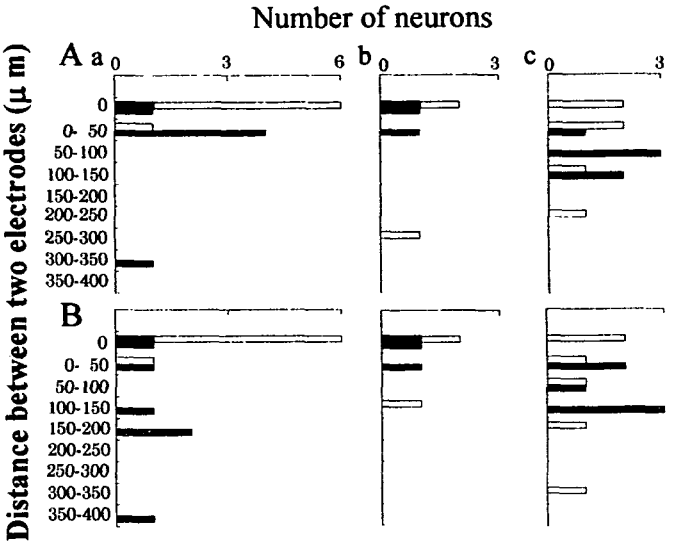

Figure 4 Relation of correlated activities of paired neurons to distance between electrode tips in areas GI and DI. **(A)** Relation between various correlated activities and tangential distance between electrode tips. **(B)** Relation between various correlated activities and vertical distance between electrode tips. **(a)** Common input; **(b)** excitatory connection; **(c)** non-correlated activity. Numerals in ordinate indicate distance in μm. The distance between electrode tips for single electrode recording was defined as 0 μm. Blank bars represent neurons in area GI, and hatched bars neurons in area DI. A solid bar stands for the pair in area GI with both common input and excitatory connection.

of the nine pairs (three in area GI, six in area DI) in which neurons were separated by >50 μm, only two pairs (22%; one in area GI, one in area DI) were correlated (Figure 4A). As for correlation patterns, 11 pairs (six in area GI, five in area DI) had common inputs (Figure 4Aa), three pairs (one in area GI, two in area DI) had an excitatory connection and one (area GI) had both a common input and excitatory connection among the pairs obtained within <50 μm tangential distance (Figure 4Ab). Among the nine pairs of neurons separated by >50 μm, however, only one pair (area DI) had a common input (Figure 4Aa) and one (area GI) had an excitatory connection (Figure 4Ab). Only a limited fraction of neuron pairs had common inputs or excitatory connections at >50 μm.

On the other hand, 12 (71%; eight in area GI, four in area DI) among the 17 pairs (11 in area GI, six in area DI) recorded at a vertical distance of <50 μm showed correlative activities (Figure 4B); eight pairs (six in area GI, two in area DI) showed common inputs (Figure 4Ba), three pairs (one in area GI, two in area DI) an excitatory connection (Figure 4Bb) and one (area GI) both a common input and an excitatory connection. Five (42%; one in area GI, four in area DI) of the 12 pairs (four in area GI, eight in area DI) recorded at a distance of >50 μm were correlated (Figure 4B): four pairs (all in area DI) showed common inputs (Figure 4Ba) and one pair (area GI) showed an excitatory connection (Figure 4Bb).We found that many pairs of neurons in area GI within 50 μm vertically showed a strong tendency for correlated activities, whereas those in area DI had a strong tendency for common inputs irrespective of the vertical distance.

Table 2 Relation between cross correlation of neuron pairs and cortical layer recorded

Layers	Details of CCG			
	Common input	Excitatory connection	No interaction	Total
A. Single electrode				
II–III(II–IV)	1(0, 1)	0	0	1(0, 1)
IV	1[a](1, –)	2[a](2, –)	2(2, –)	4(4, –)
V	5(5, 0)	0	0	5(5, 0)
VI	0	1(0, 1)	0	1(0, 1)
Total	7[a](6, 1)	3[a](2, 1)	2(2, 0)	11(9, 2)
B. Double electrodes				
Same layers				
II–III(II–IV)	0	0	1(0, 1)	1(0, 1)
IV	0(0, –)	0(0, –)	1(1, –)	1(1, –)
V	5(1, 4)	0	5(2, 3)	10(3, 7)
VI	0	0	0	0
Total	5(1, 4)	0	7(3, 4)	12(4, 8)
Different layers[b]	1(0, 1)	2(1, 1)	3(1, 2)	6(2, 4)
Total	6(1, 5)	2(1, 1)	10(4, 6)	18(6, 12)

Numerals in parentheses indicate the number of cases in areas GI and DI. In area GI, neurons in layers II–III and IV are treated separately, but in area DI they are done collectively.

[a]One pair had both common input and excited connection, and so was put in both cells.

[b]Almost all pairs consisted of neurons located in layers II–III (or II–IV) and V, except for one pair of layers V and VI in area DI without interaction.

Cortical layer and correlated pairs

In Table 2, types of functional interactions are related to whether pairs of neurons were recorded at the same or different layers. Types of electrodes used are also shown.

Among the nine pairs recorded with single electrodes in area GI, four were obtained from layer IV and five from layer V. All five pairs showing common inputs were found in layer V, whereas one pair with an excitatory connection and one with both a common input and an excitatory connection were in layer IV. On the other hand, among the two pairs recorded with single electrodes in area DI, one pair with a common input was in layers II–IV and one with an excitatory connection was in layer VI.

The tips of the double electrodes were separated vertically. Thus, both tips might be in the same or in different layers. In 12 of the 18 pairs recorded with double electrodes, both electrode tips were located in the same layer (four in area GI, eight in area DI). Among them, five pairs (one in area GI, four in area DI) showed common inputs and were located in layer V. None showed excitatory connections.

In six pairs (two in area GI, four in area DI), each electrode tip was located in a different layer. Among them, three pairs (one in area GI, two in area DI) showed correlated activities: one pair (consisting of neurons in layers II–IV and V in area DI) had common inputs, and two pairs with one pair in area GI (each neuron in layers II–III and V) and one in area DI (each neuron in layers II–IV and V) showed excitatory connections. In one of the pairs showing a common input in area DI, the neurons were tangentially separated by >50 μm.

In the pairs with excitatory connections, we examined the possible dependency of the time lag of correlated activities upon the location of the constituents. Among the five pairs of excitatory connections, two pairs consisted of constituent neurons in the same layer (one in area GI in layer IV, one in area DI in layer VI) (a single electrode recording); their time lag was 1 ms. Two other pairs consisted of constituents in different layers; one pair (source neuron in layers II–III, target neuron in layer V in area GI) had a time lag of 1 ms, but the other (source neuron in layers II–IV in area DI, target in layer V in area GI) had a lag of 3 ms. The remaining pair (both constituents in layer IV in area GI) showed an excitatory connection with a time lag of 2 ms in addition to a common input. No difference in time lag was evident between pairs from the same layer and from different layers.

Pairs of taste and non-taste neurons and cross-correlation

We examined the CCGs of seven pairs consisting of taste and non-taste neurons by coactivating them with applications of taste stimuli or distilled water. Among them, four pairs were recorded within 50 μm tangentially, and the rest at >50 μm. Only one of the seven pairs (layers II–IV in area DI) was recorded with single electrodes; it showed a correlated activity with a peak at time 0 for all four basic tastes and water. Since the highest peak was to distilled water, the source of the common input was probably mechanoreceptive.

Taste response characteristics and cross-correlation

Relation between best stimulus and correlation

Table 1 shows the relation between the best stimulus of the constituents in each pair and the CCGs. In area GI, the pairs of neurons with the same best stimulus amounted to 60% of the total sample (n = 15). Such pairs were found in 67% of the six pairs with common inputs, and in both of the two pairs with excitatory connections. The pair with both common input and excitatory connection had the same best stimulus.

By contrast, in area DI, the pairs of neurons with the same best stimulus were in 64% of the total (n = 14), accounting for 67% of the pairs with common inputs (n = 6) and half of those with excitatory connections (n = 2). Findings in areas GI and DI were similar except for those with excitatory connections. However, the fraction of pairs with correlated activities did not differ between samples of neurons with the same best stimulus and those with a different best stimulus.

Characteristics of common inputs

In seven (four in area GI, three in area DI) of the nine pairs (five in area GI, four in area DI) consisting of neurons with the same best stimulus, the stimulus which concurrently activated the pair to produce the largest peak at time 0 was the same as the best stimulus for paired neurons (Figure 2Ac and Bc). It seems that the common input strongly affects the response of the target neuron to the best stimulus. We therefore examined the effects of the taste responses of the source neurons to the responses of the target neurons by means of efficacy contribution (Levick *et al.*, 1972). Among the 18 neurons constituting the nine pairs (five in area GI with one showing additional excitatory connection, four in area DI), the total efficacy contribution to the response for all four basic taste stimuli was very small (i.e. <0.2) in eight neurons (seven in area GI, one in area DI), and it varied from 0.2 to 1 in the remaining 10 neurons (three in area GI, seven in area DI) (Figure 5Aa).

In four pairs (two in each area), the best stimulus of the common input differed from the best stimulus of the target neurons. The total efficacy contribution to the responses of the seven constituent neurons (three in area GI, four in area DI) was <0.2 (Figure 5Ba). In the remaining neuron (area GI) it was 0.2–0.4 (Figure 5Ba).

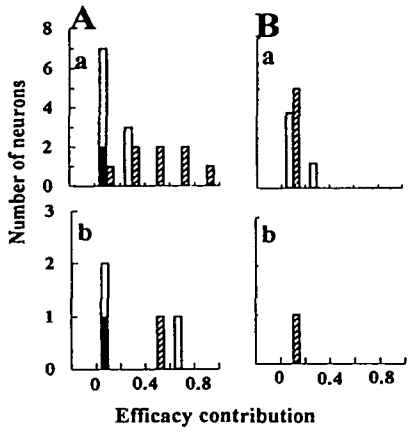

Figure 5 Difference in efficacy contribution of the source neurons between pairs of neurons with the same best stimuli **(A)** and those with different best stimuli **(B)** in the rat cerebral cortex. **(a)** Total efficacy contribution of the common input to constituents of the pairs. **(b)** Total efficacy contribution of source neuron to the target neuron of the pairs with excitatory connections. Abscissa: total efficacy contribution of the source neuron for the four stimuli. For types of bars, see legend for Figure 4.

Characteristics of taste excitatory connection

Excitatory connections were found in the four pairs of neurons with the same best stimulus (one pair of NaCl-best and two pairs of HCl-best in area GI; one pair of NaCl-best in area DI). The total efficacy contribution for two pairs in area GI was <0.2, for one pair in area DI it was 0.4–0.6 and for one pair in area GI it was 0.6–0.8 (Figure 5Ab). Thus, in excitatory connection between neurons with the same best stimulus, the efficacy contribution of the source to the target neurons in the pair showed a large variation. When the best stimulus was different between the two constituents (a pair of NaCl-best and HCl-best neurons in area DI), the efficacy contribution was <0.2 (Figure 5Bb).

In the pair with both common input and excitatory connection (NaCl-best neuron pair in area GI), the contribution of the source neurons was <0.2 in either connection.

Among the three pairs which showed excitatory connections with a time lag of 1 ms, two pairs showed an efficacy contribution of >0.4. The rest, as well as the pair with a time lag of 3 ms and the pair with a time lag of 2 ms and a common input, showed an efficacy contribution of <0.2.

Discussion

We recorded pairs of taste neurons in the cortical taste area in rats. However, since taste neurons occupy only a small percentage of the total population in the CTA (Ogawa *et al.*, 1990), we could not record a large number of pairs. We applied maintained taste stimulation three times for each of the four basic taste stimuli, though we were able to record only a few hundred spikes at best. This could be the reason why a smaller fraction of taste neuron pairs passed the side test for stationarity (Griffin and Horn, 1966). Many scientists have used a series of impulse trains in phasic responses obtained by repeated stimulations, such as 50 times for CCG calculation, and have obtained correlated activities in a relatively larger number of neurons in other sensory areas (Dickson and Gerstein, 1974; Toyama *et al.*, 1981b; Michalski *et al.*, 1983; Johnson and Alloway, 1996; Hata *et al.*, 1991).

Basic neuronal network in cortical taste area in rats

When the distance between the electrode tips was <50 μm in the dorsoventral direction tangential to the cortical surface, a high fraction of pairs showed correlated activities. When it was larger than that, the fraction declined sharply. However, the fraction of neuron pairs with correlated activities in area DI was unaffected by the vertical distance between the recording sites, whereas it was larger in area GI when the vertical distance was <50 μm.

In other sensory areas, e.g. the visual cortex, it is known that the fraction of neuron pairs with correlated activities changes beyond 400 μm in a horizontal direction (Michalski *et al.*, 1983; Ts'o *et al.*, 1986; Hata *et al.*, 1991). If functional columns are present in the CTA in rats, 50 μm may correspond to the diameter of the functional column in which neurons have some response features in common.

Among the nine pairs of neurons whose tangential distance was >50 μm, only two pairs showed correlated activities: one with common input, one with excitatory connection. If the diameter of the columnar structure is as small as 50 μm, neurons of these pairs might have a connection or a common input beyond the size of the columns: in other words, intercolumnar interaction may present in the CTA. It is reported that thalamic afferents bifurcate into several clusters and terminate at several columns in other sensory cortices (Gilbert and Kelly, 1975; Jones and Friedmann, 1982). The existence of the intercolumnar connections are also shown (Gilbert and Wiesel, 1989). But the probability of finding the latter is less by one degree of magnitude than that of finding the intracolumnar connection (Michalski *et al.*, 1983; Ts'o *et al.*, 1986).

In the present study, we examined the correlated activities of pairs of neurons separated tangentially to the cortical surface in the dorsoventral direction, but we did not study the functional connections or basic neural network between the neurons separated in the rostrocaudal direction. Such connections remain to be studied.

Source of common inputs

It is reported that there are two types of peaks at time 0; a narrow type and a broad type (Kruger and Aiple, 1989). In the present study, all peaks except one were of the narrow type. A narrow peak indicates the possibility that source neurons as common input were near the pair studied, and that few interneurons were involved. They are possibly thalamocortical neurons in specific nuclei of the thalamus (Humphrey *et al.*, 1985) or neighboring neurons in the cortex separated by, say, 1500 μm (Dickson and Gerstein, 1974). In the visual cortex, layer IV is the place where afferents from the lateral geniculate body mainly terminate, though thalamic axons provide <20% of the excitatory synapses even in this layer, the remainder coming from intracortical sources (Douglas and Martin, 1990). Thus it is plausible that source neurons for common inputs in the present study are in a specific sensory relay nucleus of the thalamus, i.e. the VPMpc, or in the cortex near by, except in one case. Broad peaks suggest that the pairs received common inputs from source neurons at a more peripheral part of the sensory pathway (Kruger and Aiple, 1989). Since correlated activities between taste neurons in the taste relay nuclei of the brain stem have been reported (Adachi *et al.*, 1989; Yamada *et al.*, 1990), the broad peak may be the result of common inputs in these regions. Recently, some authors (Yokota *et al.*, 1996) have more frequently recognized CCGs with a broad peak in the CTA.

The present study suggests that afferents from the VPMpc or intracortical afferents come as common inputs to neurons in layer V in the CTA. In many cortical areas, thalamic afferents make direct synapses with the neuron in layer III or V as well as in layer IV (Douglas and Martin, 1990; Johnson and Alloway, 1996). In the present study, we found only a single pair of neurons in layer IV with common inputs. This may be due to the small sample, since the neurons in the layer to be recorded were small in size.

Excitatory connection across layers

Within a tangential distance of 50 μm, all pairs in layer V had common inputs, pairs in layer IV in area GI or in layers II–IV in area DI had common inputs or excitatory connections between them, and neurons in layers II–III (GI) or layers II–IV (DI) projected to neurons in layer V as sources for excitatory connections. These findings show neural information flow across layers, in agreement with the finding obtained within the functional column of the visual cortex (Toyama *et al.*, 1981b). It is suggested that information processing is also done along a vertical structure of small diameter across layers in the CTA.

Possible taste information process in basic cortical neural network

Many pairs of neurons within a tangential distance of 50 μm had the same best stimulus and had common inputs and/or correlated activities with a time lag of 1–3 ms, indicating mono- or disynaptic connections (Toyama *et al.*, 1981a; Ts'o *et al.*, 1986). These findings suggest that in the CTA functional columns present in which neurons with a common best stimulus have common inputs and make excitatory connections with each other.

Concerning the peak at time 0, common inputs tended to have the same best stimulus as that of target neurons with the same best stimulus in common (7/9). But even though they had the same best stimulus, the efficacy contribution of the common input to the pairs varied widely in the range 0–1. This is probably because common inputs did not derive from a single source detected by CCGs but rather from several sources which may not contribute to the discharges of both constituents. The efficacy contribution of common inputs may depend on how many afferents the two constituent neurons share. When pairs consisted of neurons with a different best stimulus, or when the best stimulus of common inputs was not the same as that of the pairs, the efficacy contribution was <0.4. It is suggested that afferents proper to each constituent of the pair had contributed to its firing more than did common afferents to both constituents.

In almost all cases (4/5), excitatory connections were found between neurons with the same best stimulus. The time lag was in the range of 1–3 ms. The efficacy contribution of the source to target neurons in a long time lag (3 ms, one case) was smaller than that in a short time lag (1 ms), which suggests a larger number of interneurons involved and the lack of a unique contribution from the source.

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