Receptive Fields and Gustatory Responsiveness of Frog Glossopharyngeal Nerve

A Single Fiber Analysis

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ABSTRACT   Receptive fields and responsiveness of single fibers of the glossopharyngeal (IXth) nerve were investigated using electrical, gustatory (NaCl, quinine HCl, acetic acid, water, sucrose, and $CaCl_2$), thermal, and mechanical stimulation of the single fungiform papillae distributed on the dorsal tongue surface in frogs. 172 single fibers were isolated. 58% of these fibers (99/172) were responsive to at least one of the gustatory stimuli (taste fibers), and the remaining 42% (73/172) were responsive only to touch (touch fibers). The number of papillae innervated by a single fiber (receptive field) was between 1 and 17 for taste fibers and between 1 and 10 for touch fibers. The mean receptive field of taste fibers ($\overline{X}$ = 6.6, n = 99) was significantly larger than that of touch fibers ($\overline{X}$ = 3.6, n = 73) (two-tailed t test, $P < 0.001$). In experiments with natural stimulation of single fungiform papillae, it was found that every branch of a single fiber has a similar responsiveness. Taste fibers were classified into 14 types (Type N, Q, A, NA, NCa, $NCaA$, $NCaW$, $NCaAW$, $NCaWS$, NQ, NQA, $NQAS$, $NQWarm$, $Multiple$) on the basis of their responses to gustatory and thermal stimuli. The time course of the response in taste fibers was found to be characteristic of their types. For example, the fibers belonging to Type NQA showed phasic responses, those in Type NCa showed tonic responses, etc. These results indicate that there are several groups of fibers in the frog IXth nerve and that every branch of an individual fiber has a similar responsiveness to the parent fiber.

INTRODUCTION

In frogs, sensory information from the tongue is conveyed from only the IXth nerve. The responses of the IXth nerve obtained by whole-nerve recording show that frogs are sensitive to four basic taste stimuli, warming, and mechanical stimulation of the tongue, although the sugar sensitivity is somewhat weak (Kusano and Sato, 1957; Yamashita, 1963; Kashiwagura et al., 1976; Miyake et al., 1976; Ishiko

et al., 1979; Hanamori and Ishiko, 1981; Morimoto and Sato, 1982). The responses of single fibers from the IXth nerve of the frog have been studied by some authors. These studies dealt with temperature effects on the taste response (Kimura, 1961, 1962), dealt with influence of ions and chemical substances on the mechanical response (Yamane, 1978), or dealt with interactions between taste receptors (Taglietti et al., 1969). Kusano (1960) has examined extensively the responses of 105 fibers of the IXth nerve in the Japanese common frog (*Rana nigromaculata*) during stimulation of the tongue with a wide range of stimuli. Unfortunately, these data were rather qualitative. In contrast to the peripheral taste nerves in mammals (e.g., Frank, 1973), a systematic analysis of fibers in the frog IXth nerve is still incomplete.

Anatomical experiments have demonstrated that taste fibers branch before entering fungiform papillae in rats (Miller, 1974) and frogs (Graziadei and Dehan, 1971; Düring and Andres, 1976). In mammals, several electrophysiological studies have mapped the receptive fields of the fibers. In these studies, the receptive fields are described as the number of papillae connected to a given fiber. Pfaffmann (1970) demonstrated that an average of 4.5 fungiform papillae were innervated by a single rat chorda tympani (CT) fiber. The receptive fields of rat CT fibers were also investigated by Miller (1971), who showed that there are excitatory or inhibitory interactions among fungiform papillae in response to taste stimuli and that an average of two papillae were connected to the same fiber. In cats, Oakley (1975) has reported that single taste fibers innervate a mean of 2.7 fungiform papillae. Boudreau and his colleagues (Boudreau et al., 1971, 1982, 1985; Boudreau and Alev, 1973) have reported that the mean number of fungiform papillae innervated by a single geniculate ganglion cell was 3.0 for cats, 4.1 for dogs, 5.0 for goats, and 4.4 for rats. Recently, Mistretta et al. (1987) demonstrated that a single CT fiber in the lamb innervates receptive fields on the tongue that contain an average of 11 fungiform papillae.

In the frog, receptive fields of taste fibers in the IXth nerve was first investigated by Rapuzzi and Casella (1965). They estimated that each fiber gives rise to about six branches. They also showed that the number of branches of the fibers in the IXth nerve responding to tactile stimulation was 2.7 on average. In a similar experiment, Taglietti et al. (1969) showed that the mean number of fungiform papillae connecting to the same fibers in the frog IXth nerve responding to $CaCl_2$ was 2.99. Since these data on the frog were not obtained from single-fiber recording, more direct and systematic experiments are needed to delineate the receptive fields.

In the present study we recorded the orthodromic action potentials from a single fiber or from a few fibers of the frog's IXth nerve after electrical stimulation of single fungiform papillae and determined the receptive fields of each fiber. We then investigated the responsiveness of each fiber to gustatory, thermal, and tactile stimulation of individual fungiform papillae.

METHODS

Preparation

46 American bullfrogs (*Rana catesbeiana*), ranging in weight from 230 to 650 g, were used. The frogs were anesthetized with dorsal lymphatic injection of urethane (1 g/kg) and suxame-

thonium chloride (20 mg/kg). The animal was placed in a supine position in a shallow pool of water to prevent drying of the skin. The caudal tip of the tongue was pulled out from the oral cavity and fixed to an acrylic platform of the chamber with pins. The right IXth nerve was dissected free from surrounding connective tissue from the junction of its two peripheral branches to a point 4 cm more centrally located, transected at the point where it passed throught the hyoid bone,and then it was desheathed and teased into fine strands for single-fiber recording. The hypoglossal nerves were transected bilaterally to prevent tongue movements.

Recording

Action potentials were recorded from single fibers by placing a fine strand of the IXth nerve on a pair of platinum wire electrodes. Neural activity was amplified (AVB-9; Nihon Kohden, Tokyo, Japan), displayed on an oscilloscope (VC-9; Nihon Kohden), and stored on magnetic tape (UN-61430; Sony, Tokyo, Japan). When single papillae were stimulated to determine the receptive field of a single fiber, the action potentials elicited were passed through a signal processor (7T07; NEC-San-ei, Tokyo, Japan), and displayed on an inkwriter (WS441; Watanabe, Tokyo, Japan). Therefore, we could check the exact shape of the action potentials of a fiber induced by electrical stimulation of a single fungiform papillae (Fig. 1 C).

Stimulation

To locate the approximate receptive field of a single fiber, a small amount of taste solution was first applied to a small area of the tongue at random by using a pipette. Mechanical stimulation was applied with a cotton wick, a small brush, and a glass rod. After the receptive field of a fiber was roughly determined, then every fungiform papilla within and around the assumed receptive field was drawn inside a small suction electrode (inner-tip diameter, 200 μm) (Fig. 1 A, SE) under a binocular microscope. Each papilla was stimulated by a brief rectangular pulse (0.1 ms, 0.2–10 V, usually 5 V was used as the maximal stimulation; Hanamori and Ishiko, 1981, 1983) through a pair of thin silver wires, one placed inside of the glass pipette (+) and the other on the tissue near the papilla (−).

Taste stimulation was delivered using a V-shaped glass pipette (Fig. 1 A). One side of the pipette was connected to a peristaltic pump (PP-V; Furue, Tokyo, Japan) via polyethylene tubing, which applies negative pressure. The other side of the pipette was connected to a bottle of taste solution via polyethylene tubing. The bottom of the pipette was open so that a single papilla could be attached (600 μm diameter of inner tip) via negative pressure. The flow rate of the taste solution was 0.03 ml/s and the duration of the stimulation was ~10 s. Before and after each stimulation, the papilla was constantly rinsed with 0.01 M NaCl (pH 5.6). The taste stimuli were: 0.5 M NaCl (pH 5.1), 0.001 M quinine HCl (QHCl, pH 5.9), 0.01 M acetic acid (pH 3.4), 1.0 M sucrose (pH 5.3), 0.001 M $CaCl_2$ (pH 5.5) and deionized water (water, pH 6.3). All of the stimuli except water and 0.5 M NaCl were dissolved in 0.01 M NaCl, which was also used as a rinse between stimuli, since the frog's tongue is known to respond to water and a rinse of 0.01 M NaCl produces a minimal response (Yamashita, 1963). These taste stimuli were chosen as "usual" taste stimuli although the foodstuffs have been used by some authors (e.g., Boudreau et al., 1971). All stimuli were delivered at room temperature (~20°C). The concentrations of these stimuli are those that produce >60% of the maximum integrated responses in the frog's IXth nerve (Morimoto and Sato, 1982; Sato, 1972). For thermal stimulation, 0.01 M NaCl at 35°C was applied after adaptation of the tongue to 0.01 M NaCl at 20°C. The tongue was mechanically stimulated with a small brush, a glass rod, or a cotton wick. Usually a cotton wick was used, since it was the most effective in eliciting mechanical responses in the frog tongue. The order of presentation of the eight stimuli (NaCl, QHCl, acetic acid, sucrose, $CaCl_2$, water, warming, or touch) was random.

Data Analysis

Recorded action potentials were passed through a window discriminator (EN-601J; Nihon Kohden). The pulsed outputs of the window discriminator were accumulated in 100-ms bins over 12.8 s, including pre- and poststimulus periods, using a signal processor (7T07; NEC-San-ei). Peristimulus time histograms were displayed on an inkwriter.

The number of impulses occurring during the first 3-s of stimulation were used as a measure of a fiber's responsiveness to each papilla. This 3 s count was further quantified by subtracting the preceding 3-s count during the prestimulus period, even though the spontaneous activity of fibers in the IXth nerve was very low (below 0.03 impulses/s). To evaluate the responsiveness of a fiber, we adopted the mean response to stimulation of every papilla connected to the fiber.

Sampling

In the present study we used the number of papillae connected to a single fiber as a measure of the size of the receptive field. One reason for this decision is that the frog's tongue is so elastic that it is difficult to determine the actual physical size of the receptive fields. Secondly, fungiform papillae in the frog are distributed across almost the entire dorsal tongue surface with relatively uniform density.

We measured the longitudinal tongue length (L) in the experimental condition from the rostral edge of the tongue to the base where the caudal portion of the tongue starts to divide into two parts. The mean value of L was 2.9 cm ($n = 46$, SEM = 0.1), with a range of 2.2–3.5 cm. The position of the center-point of each receptive field (l) was also measured from the rostral edge of the tongue in respective samples. The relative locations of the receptive fields of individual fibers was obtained from l/L. The mean l/L was 0.50 ($n = 99$, SEM = 0.03) for taste fibers (responding to taste stimuli) and 0.47 ($n = 73$, SEM = 0.03) for touch fibers (responding to only touch). The IXth nerve branches into two (medial and lateral) branches just before entering the tongue. The medial and lateral branches innervate the caudal two-thirds and the rostral one-third of the tongue, respectively (Ishiko et al., 1979). In the present study, 65 of 99 (66%) taste fibers had receptive fields in nearly two-thirds of the caudal area $(l/L > 0.35)$ and the remaining 34 (34%) fibers innervated about one-third $(l/L < 0.35)$ of the rostral area of the tongue. For touch fibers, 27 of 73 (37%) fibers had receptive fields in the rostral area of the tongue $(l/L < 0.35)$, and the rest (46/73; 63%) innervated the caudal area $(l/L > 0.35)$.

RESULTS

Receptive Fields of Fibers in the IXth Nerve

Receptive fields of 172 single fibers were investigated by recording the orthodromically evoked action potentials of single fibers after electrical stimulation of individual papillae. One sample of the receptive field of a fiber is shown in Fig. 1 *B*. This map of the papillae shows that the four papillae numbered *1, 2, 3,* and *4* were connected to the same single fiber (fiber 97), since action potentials similar in size and shape were recorded after electrical stimulation of these respective papillae (Fig. 1 *C, 1, 2, 3,* and *4*). Electrical stimulation of the rest of the papillae tested (Fig. 1 *C, 5–27*) did not elicit any action potentials except for two papillae (Fig. 1 *C, 22* and *24*). The size, shape, and latency of the action potentials of papilla *22* and *24* were quite different from those of the papilla *1–4*. Therefore, it is clear that these two

papillae are not innervated by fiber 97. In this way, even if we isolated more than one fiber, the receptive field of a single fiber could be accurately determined.

Of 172 fibers, 99 responded to at least one of the gustatory stimuli (taste fibers) (see Figs. 2 and 3). The remaining fibers (73 fibers) responded to only mechanical stimulation (touch fibers) (see Fig. 4). Individual taste fibers branched and innervated between 1 and 17 papillae with a mean of 6.6 (n = 99, SEM = 0.4) (Fig. 5,
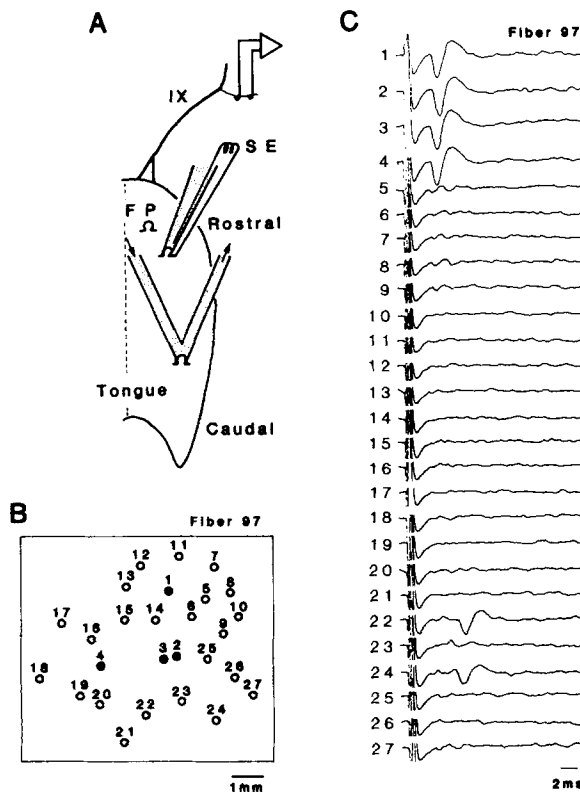

FIGURE 1. (A) Schematic drawing of the system for recording action potentials from the single fibers of the IXth nerve and for stimulation of a single fungiform papilla of the frog tongue. IX, glossopharyngeal nerve; FP, fungiform papillae; SE, suction electrode. Electrical stimulation of single fungiform papillae was made using a suction electrode. Chemical stimulation of single fungiform papillae was delivered using a V-shaped glass pipette. (B) A map of fungiform papillae of the frog tongue innervated by fiber 97. Each empty or filled circle represents a fungiform papilla. The four filled circles (1–4) were connected to a single fiber, whereas the empty circles were not. The numbers above the circles show the conventional numbers for the papillae. The disposition of the tongue is shown in A. Namely, in this map upward indicates the rostral side, and the left side of the map indicates close to the midline of the tongue. (C) Action potentials of a few fibers of the IXth nerve elicited after electrical stimulation of individual papillae (1–27). The map of the papillae were shown in B. Four papillae (1–4) are connected to the same single fiber (fiber 97). Action potentials from another fiber with a smaller amplitude and a later latency were also recorded after electrical stimulation of papillae 22 and 24. The complex deflections at the start of each record show the stimulus artifact of the electrical stimulation.

Taste). Taste fibers most frequently branched 4 papillae (Fig. 5, *Taste*). On the other hand, the number of the papillae innervated by individual touch fibers was between 1 and 10 with a mean of 3.6 (n = 73, SEM = 0.2, Fig. 5, *Touch*). Most touch fibers innervated two or three papillae (Fig. 5, *Touch*). Thus, taste fibers innervate more papillae than touch fibers (two-tailed t test, P < 0.001).

The relationships between the receptive fields (number of papillae connecting to
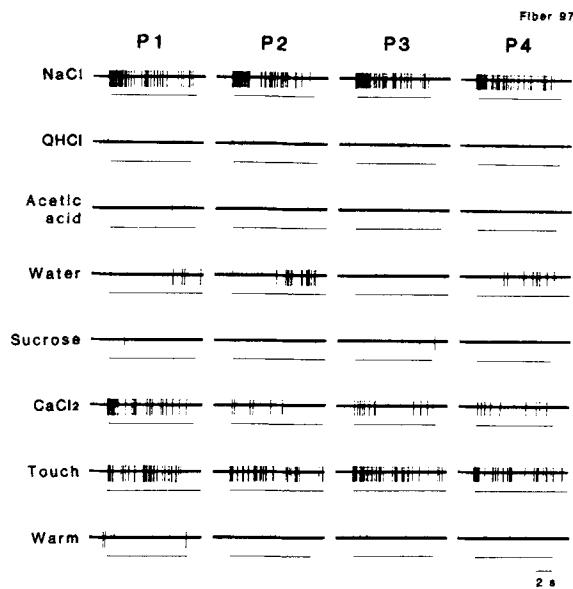
FIGURE 2.   Response of a single fiber (fiber 97) to chemical, tactile, and thermal stimulation of four individual papillae (P1, P2, P3, and P4). This fiber responded tonically to NaCl, water, $CaCl_2$, and touch stimulation. This fiber was classified as Type $NCaW$ (details in the text). Two spikes elicited at just before stimulation of warming for P1 are evoked mechanically by the air bubbles contained in the stimulus tube. Note that all four papillae connected to the same fiber responded very similarly. In P3, although there is no response to water in the record shown, a response to water was observed after a longer delay.

the same fiber) and taste stimuli were examined (Table I). The mean receptive field of the fibers responding to QHCl ($\overline{X}$ = 8.4, n = 46) was significantly larger than the receptive fields of those responding to other stimuli (two-tailed t test, $P < 0.02$, Table I). The receptive fields of fibers responding to $CaCl_2$ and water were found to be relatively small (two-tailed t test, $P < 0.05$, Table I).
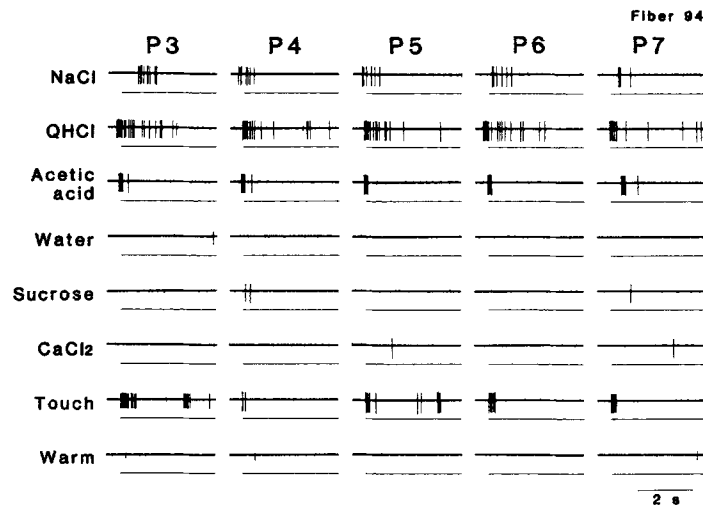

FIGURE 3.   Responses of a single fiber (fiber 94). This fiber innervated 10 papillae. In the figure, the responses for five papillae (P3–P7) are shown. Each branch shows a phasic response to NaCl, QHCl, acetic acid, and touch (Type NQA). Note that the time scale is different from that in Fig. 2.
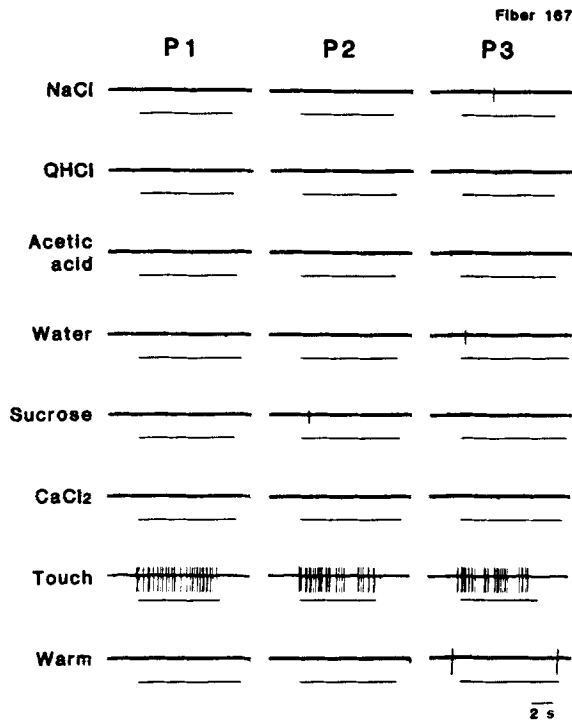
Fiber 167

P1   P2   P3

NaCl

QHCl

Acetic
acid

Water

Sucrose

FIGURE 4. Responses of a single fiber (fiber 167), which responded only to mechanical stimulation in three individual papillae (P1, P2, and P3).

CaCl2

Touch

Warm

2 s

Taste
n = 99

No. of fibers
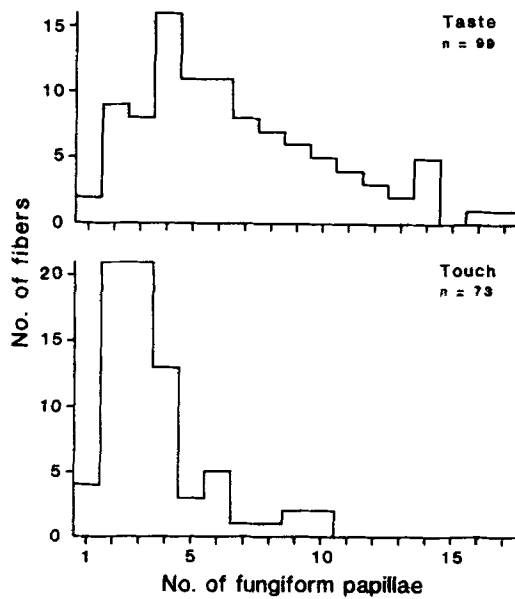
Touch
n = 73

No. of fungiform papillae

FIGURE 5. Frequency histogram of the number of taste or touch fibers innervating different numbers of papillae.

Classification of Receptive Fields

The papillae connected to the same single fibers of the IXth nerve were found to be lined up or clustered in a square or elongated area and were never found in two or more separated areas on the tongue. The receptive fields of the 172 fibers were classified into five types (Fig. 6 B) according to the following procedures. First, all of the papillae connected to the same fiber were mapped (cf. Fig. 1 B) and enclosed by the most closely fitting rectangle (Fig. 6 A, Receptive field). If the rectangles were close to a square, i.e., the ratio of the long side (l) to the short side (s) of the rectangle was smaller than 1.5 ($l/s < 1.5$), their receptive fields were named Type e (Fig. 6 B, Type e; fiber 66). If the receptive fields were more elongated in shape ($l/s > 1.5$, e.g., Fig. 6 A; $l/s = 4.3$ for fiber 17), they were further classified into four groups (Fig. 6 A, Orientation; Types a, b, c, and d) according to their orientation. The rectangles with orientation close to the horizontal axis (i.e., with angles between $-22.5°$ and $22.5°$) were named Type c (Fig. 6 A, Orientation; Fig. 6 B, Type c). The

TABLE I

Size of the Receptive Fields of Fibers Responding to Each Taste
and Warming Stimuli

Stimuli	No. of the papillae	No. of fibers
	mean ± SEM	*n*
NaCl	6.7 ± 0.4	92 (93)
QHCl	8.4 ± 0.6	46 (46)
Acetic acid	7.8 ± 0.6	45 (45)
Water	5.2 ± 0.6	14 (14)
Sucrose	9.5*	4 (4)
CaCl$_2$	5.2 ± 0.4	35 (35)
Warm	7.5*	2 (2)

Numbers in parentheses are the percent of the total fibers.
*SEMs from a small number of fibers (below $n = 5$) are not shown.

rectangles that were close to the vertical axis (the angle of the rectangle was between 67.5° and 112.5°) were named Type a (Fig. 6 A, Orientation; Fig. 6 B, Type a). The orientation of the rectangles in Type d was between that in Type c and Type a (Fig. 6 A, Orientation; the angle of the rectangles was between 22.5° and 67.5°). In Type b, the orientation of the rectangles was between that in Type a and c and orthogonal to that of Type d; the angle of the rectangles was between 112.5° and 157.5° (Fig. 6 A, Orientation; Fig. 6 B, Type b).

The number of fibers showing these five types of receptive field is shown in Fig. 6 C. In touch fibers (Fig. 6 C, Touch), fibers showing a receptive field of Type a were more common (20 of 73 fibers). The next most frequent type was Type e (15/73), followed by Type b (14/73), Type d (12/73), and Type c (12/73). A somewhat similar distribution was also seen for taste fibers (Fig. 6 C, Taste; Type a, 26/99; Type e, 22/99; Type c, 16/99), although the number of fibers for Type b was decreased (8/99) and that for Type d increased (27/99) in comparison to those in touch fibers.

Response Characteristics of Fibers in the IXth Nerve

Responses to the entire array of stimuli were recorded from 99 taste fibers. These fibers were classified into 14 types on the basis of their patterns of response to seven stimuli (NaCl, QHCl, acetic acid, water, sucrose, $CaCl_2$, and warming), as shown in
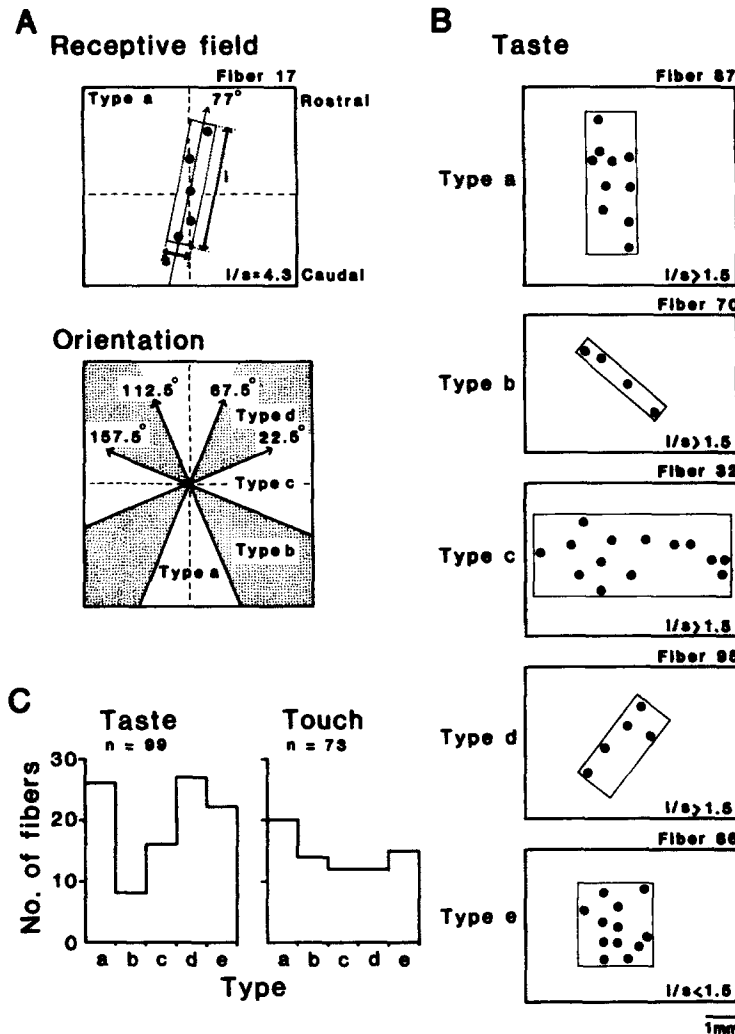

FIGURE 6. (A) Classification of the receptive fields of the IXth nerve fibers. Five papillae (*Receptive field; filled circles*) connected to the same single fiber (fiber 17) were enclosed by a rectangle. The value of long side (*l*) to short side (*s*) was 4.3. This fiber belonged to Type a (see below). We named the receptive fields Type e if the value of *l/s* was <1.5 (close to the square). If the value was >1.5, like fiber 17, we subclassified them into four types (Types a, b, c, and d) according to the orientation of the rectangles (*Orientation*, showing the range of the angles of the rectangles in each type). (B) Five receptive field types in taste fibers are shown. In these maps, the disposition of the tongue is the same as in Fig. 1, A or B. (C) Frequency histogram of the number of taste or touch fibers as a function of the receptive field type.

Table II. For example, fiber 97 (Fig. 2), responding to NaCl, water, CaCl$_2$, and touch, was classified Type *NCaW*, and fiber 94 (Fig. 3), responding to NaCl, QHCl, acetic acid, and touch, was named Type *NQA*, etc. If we consider every possible combination of the seven stimuli, 127 combinations could occur ($_7C_1 + {_7C_2} + {_7C_3} + {_7C_4} + {_7C_5} + {_7C_6} + {_7C_7} = 127$). However, in the present study only 14 combinations or types were found. It is assumed that the probability of responses in each fiber will not be the same among the seven stimuli and, further-

TABLE II

Response Types of Fibers of the IXth Nerve

Type	Stimuli								No. of fibers
	N	Q	A	W	S	Ca	T	Warm	
N	+	0	0	0	0	0	0	0	7
	+	0	0	0	0	0	+	0	9
Q	0	+	0	0	0	0	0	0	6
A	0	0	+	0	0	0	+	0	1
NA	+	0	+	0	0	0	+	0	2
NCa	+	0	0	0	0	+	+	0	12
NCaA	+	0	+	0	0	+	0	0	1
	+	0	+	0	0	+	+	0	8
NCaW	+	0	0	+	0	+	+	0	9
NCaAW	+	0	+	+	0	+	+	0	3
NCaWS	+	0	0	+	+	+	+	0	1
NQ	+	+	0	0	0	0	0	0	5
	+	+	0	0	0	0	+	0	4
NQA	+	+	+	0	0	0	0	0	2
	+	+	+	0	0	0	+	0	25
NQAS	+	+	+	0	+	0	+	0	2
NQWarm	+	+	0	0	0	0	0	+	1
Multiple	+	+	+	+	+	+	0	+	1
Total									99

+, responded; 0, no response; *N*, 0.5 M NaCl; *Q*, 0.001 M quinine HCl; *A*, 0.01 M acetic acid; *W*, deionized water; *S*, 1 M sucrose; *Ca*, 0.001 M CaCl$_2$; *T*, touch; *Warm*, 0.01 M NaCl at 35°C.

more, that the combinations among seven stimuli will not occur at random. In the present study, 46% of all taste fibers responded to QHCl and 35% responded to CaCl$_2$, but there was only one fiber responding to both QHCl and CaCl$_2$.

These 14 fiber types can be classified as major, medium, and minor groups according to the number of fibers belonging to each type (Table II). The major groups were Type *NQA* (27/99 fibers), Type *N* (16/99), and Type *NCa* (12/99). The medium groups were Type *NCaA* (9/99), Type *NCaW* (9/99), Type *NQ* (9/99), and

Type Q (6/99). The minor groups were Type *NCaAW* (3/99), Type *NA* (2/99), Type *NQAS* (2/99), Type *A* (1/99), Type *NCaWS* (1/99), Type *NQWarm* (1/99), and Type *Multiple* (1/99).

The responses of 99 taste fibers (impulses/3 s) for eight stimuli are shown in Fig. 7. The fibers are arranged along the abscissa according to the order of the 14 types in Table II, and within each type the fibers are ordered by the magnitude of their responses to NaCl or QHCl (Fig. 7). These response profiles show that the IXth nerve fibers respond best to NaCl and touch, somewhat less to QHCl, $CaCl_2$, and acetic acid, and only weakly to water and sucrose. Only two taste fibers showed a response to warming but these responses were relatively high in comparison with those to sucrose or water (Fig. 7). These response profiles in Fig. 7 also show that most of the fibers responding to $CaCl_2$ also respond well to NaCl and touch, but never to QHCl. All of the fibers responding to water were among those responding to $CaCl_2$. Most of the fibers responding to QHCl showed better responses to QHCl than to NaCl and showed relatively low responses to touch in comparison with the fibers responding to $CaCl_2$.

The distributions of responses to the eight stimuli across the 14 fiber types are shown as average profiles in Fig. 8. In the figure it seems that the response histogram of Type *NCaA* is similar to that of Type *NCaW*. The similarity (correlation coefficient; r) of the response histogram between Type *NCa* and the other types was calculated. The correlation coefficient for Type *NA*, Type *NCa*, Type *NCaA*, Type *NCaW*, Type *NCaAW*, and Type *NCaWS* was 0.96, 1.0, 0.96, 0.92, 0.99, and 0.97, respectively. The remaining types showed low values of r (e.g., 0.21 for Type *NQA*) except for Type *N* which showed a high correlation of 0.98. On the other hand, when the correlation coefficients were obtained between Type *NQA* and the other types, high values of r were obtained for Type *NQ* (0.91), Type *NQA* (1.0), and Type *NQAS* (0.96), and the remaining types showed low values of r. Considering these results the 14 types can be rearranged into five groups. Group 1 is sensitive to specific stimuli: Type *N*, Type Q, and Type *A*. Group 2 is highly sensitive to NaCl and $CaCl_2$: Type *NA*, Type *NCa*, Type *NCaA*, Type *NCaW*, Type *NCaAW* and Type *NCaWS*. Group 3 is highly sensitive to QHCl: Type *NQ*, Type *NQA*, and Type *NQAS*. Group 4 is sensitive to warming: Type *NQWarm*. Group 5 is sensitive to variable stimuli: Type *Multiple*. Most of the IXth nerve fibers (98%, 97/99) examined in this study were included in three groups: those sensitive to specific stimuli (Group 1), to NaCl and $CaCl_2$ (Group 2), and to QHCl (Group 3).

Of 99 taste fibers, 92 (93%) responded to NaCl, 46 (47%) to QHCl, 45 (46%) to acetic acid, 35 (35%) to $CaCl_2$, 14 (14%) to water, 4 (4%) to sucrose, and 2 (2%) to warming (see Fig. 11 *A*, below). The mean responses of fibers responding to NaCl was 15.6 impulses/3 s ($n = 92$), 14.4 to QHCl ($n = 46$), 5.9 to acetic acid ($n = 45$), 7.9 to $CaCl_2$ ($n = 35$), 2.4 to water ($n = 14$), 2.8 to sucrose ($n = 4$), and 16.5 to warming ($n = 2$).

Most of the taste fibers (76/99, 77%) responded also to mechanical stimulation of the single papillae (Table II, Figs. 2, 3, 7, and 8). There were some differences in the characteristics of the mechanical sensitivity among the 14 types of fibers. That is, none of the fibers of Type Q (0/6) responded to mechanical stimulation, and only the 44% (4/9) of Type *NQ* fibers and 56% (9/16) of Type *N* fibers responded to
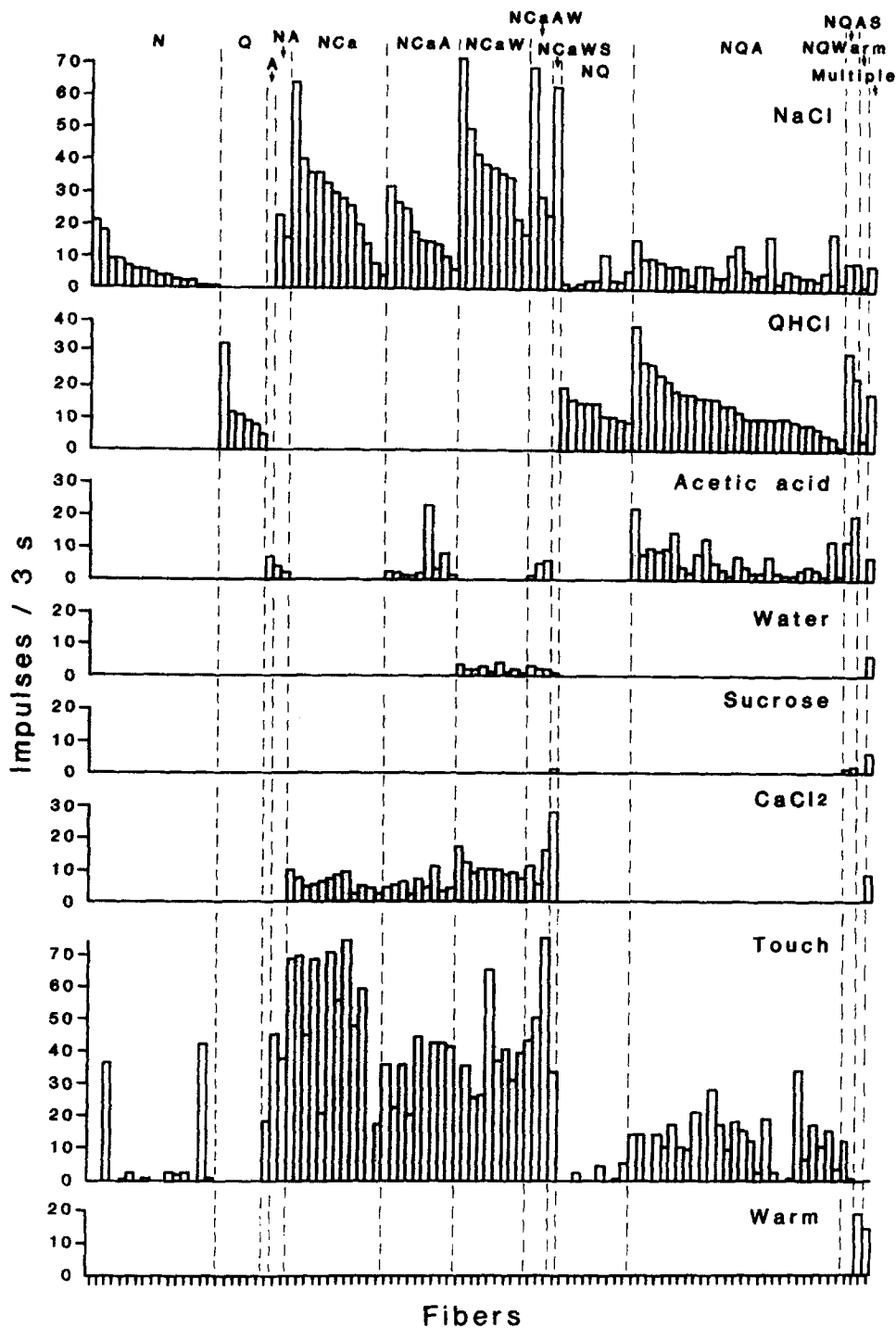
FIGURE 7. Across-fiber patterns evoked by eight stimuli (NaCl, QHCl, acetic acid, water, sucrose, CaCl₂, touch, and warm). Abscissa indicates 99 taste fibers in order of 14 response types (shown at the top of the figure) and within the types the fibers are arranged according to the magnitude of the response to NaCl or QHCl. The ordinate shows the number of impulses in 3 s. N, NaCl; Q, QHCL; A, acetic acid; Ca, CaCl₂; W, water; S, sucrose; T, touch; Warm, warming; Multiple, a fiber responding to all taste stimuli used in the present study.

mechanical stimulation (Table II). On the other hand, all fibers of Type *NCa* (12/12) and Type *NCaW* (9/9) responded to mechanical stimulation, and 93% (25/27) of Type *NQA* fibers and 89% (8/9) of Type *NCaA* fibers responded to mechanical stimulation (Table II).

Time Course of Taste Fibers

Time course of the mean responses in the 92 fibers responding to NaCl is shown as a phasic response followed by a tonic response (Fig. 9 *A*, *NaCl*). A similar time
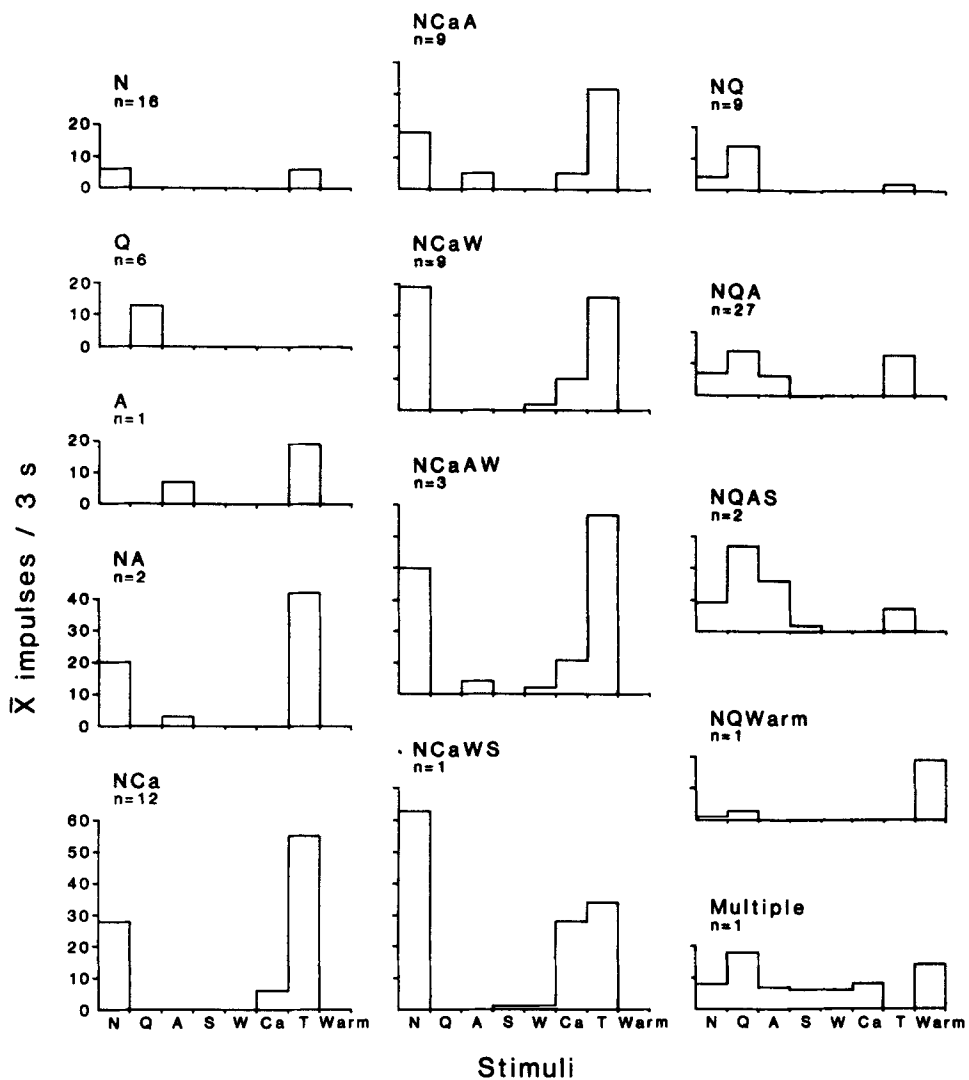

FIGURE 8. Mean responses (impulses/3 s) of the taste fibers in 14 response types across eight stimuli.
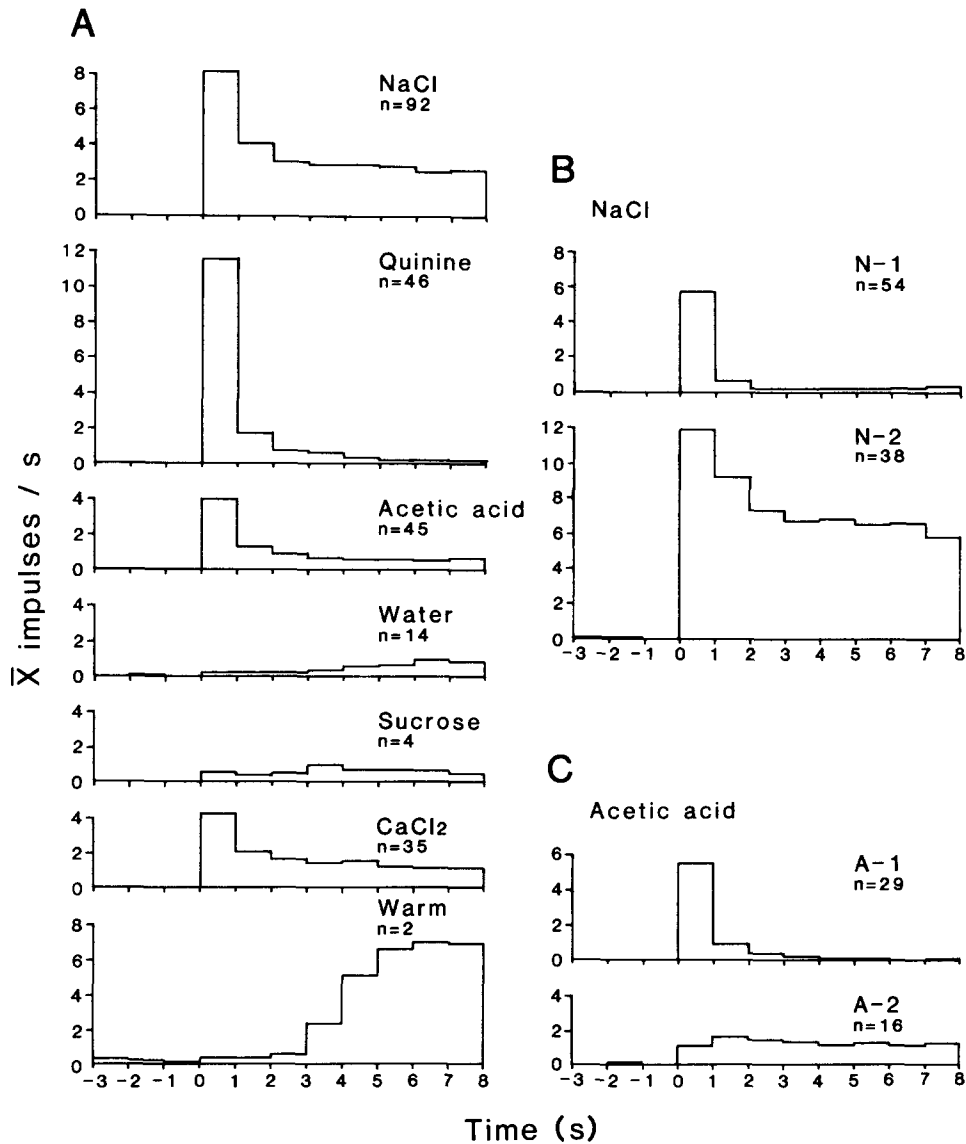
FIGURE 9. (A) Time course (mean impulses/s) of the responses in the taste fibers across seven stimuli. (B) Time course of the responses of fibers for NaCl subclassified (N-1 and N-2). The fibers of N-1 are constituted from the fibers in Type N, NQ, NQA, and NQAS, and those of N-2 are from the fibers in Type NA, NCa, NCaA, NCaW, NCaAW, NCaWS, NQWarm, and Multiple. (C) Time course of the responses of the fibers for acetic acid subclassified (A-1 and A-2). The fibers of A-1 are constituted from the fibers in Type NQ, NQA, and NQAS, while those of A-2 are from the fibers in Type A, NA, nCaA, NCaAw, and Multiple. The abscissa indicates the time before and during stimulation. The zero on the abscissa indicates the start of the taste stimulation.

course is seen for acetic acid (Fig. 9 *A*, *Acetic acid*) and for CaCl$_2$ (Fig. 9 *A*, *CaCl$_2$*). On the other hand, the time course for QHCl shows only a phasic response which declines to baseline. The time course for sucrose (Fig. 9 *A*, *Sucrose*) shows only a tonic response. The responses to both warming and water increased gradually with time (graded response) (Fig. 9 *A*, *Water and Warm*).

The mean time course of 92 fibers responding to NaCl showed both a phasic and tonic component. However, this mean response could be divided into two components, one showed a phasic response and the other a tonic response (Fig. 9 *B*). In the figure, the time course of the upper histogram (*N-1*) shows only a phasic response, in which the data were obtained from the fibers of Type *N*, Type *NQ*, Type *NQA*, and Type *NQAS*. The time course of lower histogram (Fig. 9 *B*, *N-2*) shows mainly a tonic response, in which the data were obtained from the fibers of Type *NA*, Type *NCa*, Type *NCaA*, Type *NCaW*, Type *NCaAW*, Type *NCaWS*, Type *NQWarm*, and Type *Multiple*. Also, the time course for acetic acid could be divided into two components, a phasic and a tonic response type (Fig. 9 *C*). The phasic response curve for acetic acid (Fig. 9 *C*, *A-1*) was obtained from the fibers of Type *NQA* and Type *NQAS*, and the tonic response (Fig. 9 *C*, *A-2*) was constructed from the fibers of Type *A*, Type *NA*, Type *NCaA*, Type *NCaAW*, and Type *Multiple*. It is noted that most types in *N-1* or *A-1* are belonging to Group 3 (see above, highly sensitive to QHCl) and those in *N-2* or *A-2* are from Group 2 (highly sensitive to NaCl and CaCl$_2$).

Responses and Receptive Fields of the Fibers in Each Type

The mean responses and the mean size of the receptive fields across five receptive field types were examined for touch fibers and for taste fibers. In touch fibers, the mean response of fibers of Type e was the largest among the five types ($\overline{X}$ = 32.4 impulses/3 s, SEM = 8.1, *n* = 15), but there was no significant difference in the responsiveness among these types (two-tailed *t* test, *P* > 0.05). Also, the size of the receptive fields was not different among these types (two-tailed *t* test, *P* > 0.05). In taste fibers, as well as in touch fibers, there was no significant difference across five receptive field types in both the responsiveness to taste stimuli and the size of the receptive fields (two-tailed *t* test, *P* > 0.05). There was a significant difference in the responsiveness for touch in taste fibers between Type d ($\overline{X}$ = 28.0 impulses/3 s, SEM = 4.9, *n* = 27) and Type b ($\overline{X}$ = 8.9 impulses/3 s, SEM = 4.0, *n* = 8) (two-tailed *t* test, *P* < 0.01). However, it is noted that since we applied the tactile stimuli to the single papillae by hand using a cotton wick in the present study, the data for touch are expected to be rather qualitative.

In taste fibers, there was some relationship between response type and the size of the receptive field (Table III). The receptive fields of fibers in Type *NQA* (the mean number of papillae was 9.3) were significantly larger than those in other types (Type *N*, Type *Q*, Type *NCa*, Type *NCaA*, Type *NCaW*; Table III, two-tailed *t* test, *P* < 0.02). No significant difference between types was seen in other case (two-tailed *t* test, *P* > 0.1).

Responses of Branches Connecting to the Same Single Fiber

The taste-responsive IXth nerve fibers of the frog (*n* = 99) branched and innervated an average of 6.6 papillae. It is interesting to examine whether each branch of the

same single taste fiber conveys the same information. In Fig. 2, fiber 97 innervated four papillae and every papilla responded to NaCl, water, $CaCl_2$, and touch (a delayed response to water that was observed in P3 is not shown in the figure). It seems that every branch of fiber 97 shows the same response pattern. Similar results are shown in Fig. 3. In Figure 10 A, fiber 39 innervates 11 papillae, whose response patterns looks similar to each other. The similarity of these response patterns to that of the averaged response pattern from these 11 papillae (Fig. 10 A, Av) were calculated. They all showed high correlation coefficients; the values of r were 0.93 for P1 (papilla 1), 0.99 for P2, 0.93 for P3, 0.98 for P4, 0.88 for P5, 0.97 for P6, 0.97 for P7, 0.96 for P8, 0.97 for P9, 0.99 for P10, and 0.93 for P11.

In the present study we tested 654 papillae that were innervated by 99 taste

TABLE III

Size of the Receptive Fields in Each Response Type

Type	No. of papillae	No. of fibers
	mean ± SEM	
N	5.8 ± 0.7	16
Q	5.2 ± 1.5	6
A	4*	1
NA	1.5*	2
NCa	5.2 ± 0.7	12
NCaA	5.1 ± 0.7	9
NCaW	4.7 ± 0.5	9
NCaAW	5.0*	3
NCaWS	5*	1
NQ	7.2 ± 1.6	9
NQA	9.3 ± 0.8	27
NQAS	11*	2
NQWarm	4*	1
Multiple	11*	1
Total		99

*SEMs from a small number of fibers (below $n - 5$) are not shown.

fibers. Of 654 papillae, 56 (9%) did not show any response to the taste stimuli used in this work; 62% (31/56) of these nonresponsive papillae responded only to touch. The remaining 598 papillae showed taste responses. In 4 of 598 papillae, the responses of a single fiber depended on one branch; in two cases the single fiber innervated only one papilla and in two cases, although the single fiber innervated more than two papillae, only one of them showed a taste response. Therefore, for the 594 papillae innervated by 95 taste fibers, correlation coefficients were obtained in the same way as in Fig. 10 A (Fig. 10 B). The correlation of the response pattern among papillae connecting to the same single fiber was found to be very high; the mean value was 0.93 ($n = 594$, SEM $= 0.01$) (Fig. 10 B). In most of the papillae (92%, 546/594), the correlation coefficient was >0.8 (Fig. 10 B).
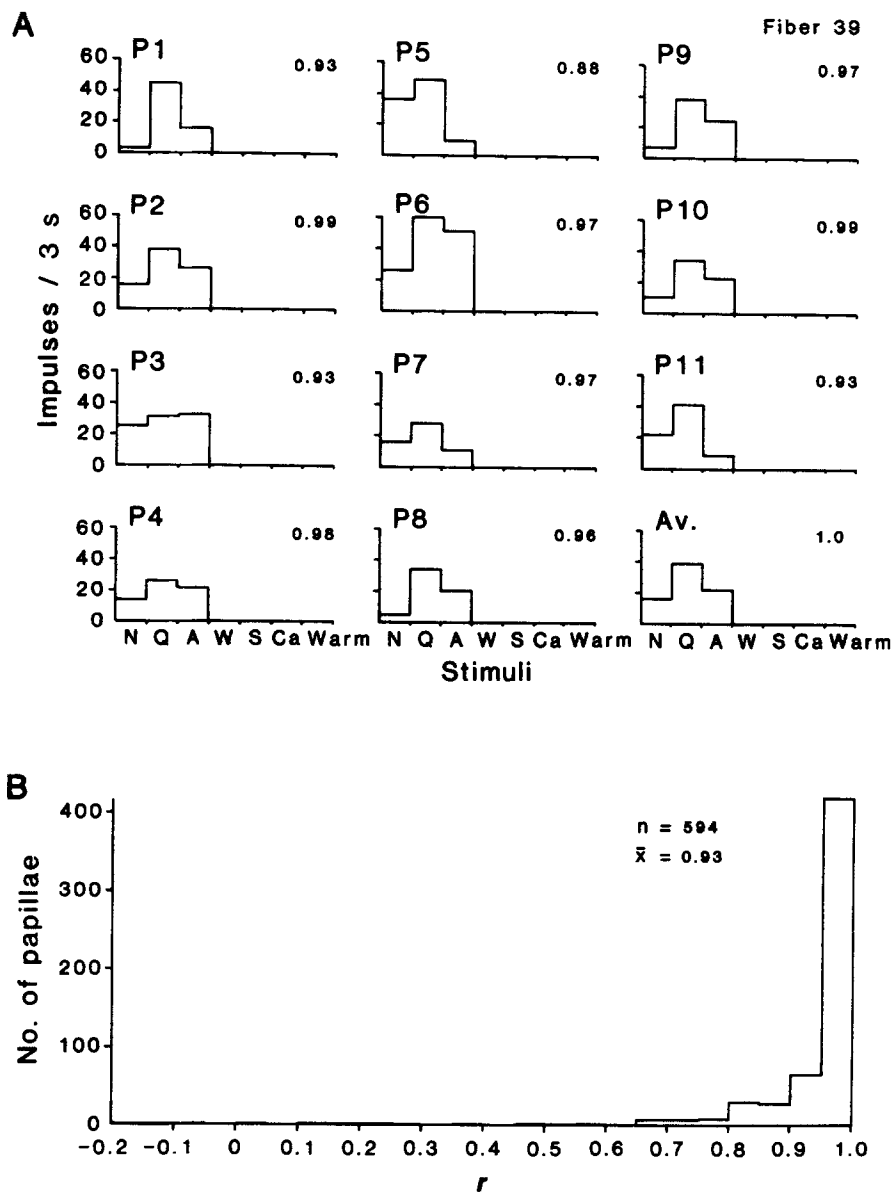
FIGURE 10. (A) Response histograms of a single taste fiber (fiber 39) branching and inner-vating 11 papillae. *P1–P11:* fungiform papillae innervated by fiber 39. *Av:* averaged response from all papillae (P1–P11). Ordinate indicates the number of impulses (impulses/3 s) and abscissa shows the stimuli. The numerals indicated at the top right of each histograms are the similarity (r) of each histogram to the averaged histogram (*Av.*). (B) Frequency histogram of papillae as a function of similarity between an averaged response profile and that to each papillae as in Fig. 10 A. This histogram was obtained from 594 fungiform papillae in 95 taste fibers.

DISCUSSION

Receptive Fields

To determine the receptive field of a single taste fiber, different methods of stimulation and recording have been used by several authors. Both electrical (Rapuzzi and Casella, 1965; Pfaffmann, 1970; Boudreau et al., 1971, 1982, 1985; Boudreau and Alev, 1973; Mistretta et al., 1987; Nagai et al., 1988) and taste stimulation (Taglietti et al., 1969; Miller, 1971; Oakley, 1975) have been used. For recording, single-fiber (Pfaffmann, 1970; Miller, 1971; Oakley, 1975), single-unit (in the geniculate ganglion; Boudreau et al., 1971, 1982, 1985; Boudreau and Alev, 1973), and single-papilla recording (Rapuzzi and Casella, 1965; Taglietti et al., 1969) have been done. To investigate the receptive fields of the single fibers, it might be better to use electrical stimulation than taste stimulation, since in the present study a small number of papillae (9% of total papillae) or a few branches of the single fibers did not respond to taste stimulation; although they did respond to electrical stimulation of the single papillae. In this work we determined the receptive fields of single fibers using single-fiber recording and electrical stimulation of individual papillae.

In the taste fibers of the frog's IXth nerve there was some relation between the responsiveness of the fibers and the size of the receptive fields. The mean number of the branches of the IXth nerve fibers responding to QHCl ($\overline{X}$ = 8.4, Table I) was significantly larger than those of fibers responding to other stimuli. This largeness of the receptive fields for QHCl is mainly contributed by the fibers of the Type NQA ($\overline{X}$ = 9.3, Table III). On the other hand, the fibers responding to $CaCl_2$ had relatively small receptive fields ($\overline{X}$ = 5.2, Table I). Taglietti et al. (1969) also reported small receptive fields, where the number of papillae of a fiber responding to $CaCl_2$ was about three on average. In mammals there are a few studies concerning the relationship between receptive field size and the taste responsiveness of the fibers. Boudreau et al. (1985) summarized the relations between the receptive fields and classified groups of fibers (acid units, salt units, amino acid units, X units) in cats, dogs, goats, and rats. According to their data there seems to be no difference in the size of the receptive fields of the fibers in these four groups. Recently, Nagai et al. (1988) have reported that in lambs there is negative correlation between receptive field size and $NaCl/NH_4Cl$ response ratio. That is, in lambs the receptive fields most responsive to NaCl had fewer papillae than those most responsive to NH_4Cl.

In touch fibers of frogs, the mean number of papillae connected to a single fiber was 3.6 with a range from 1 to 10 (Fig. 5). Most of the fibers (42/73) innervated two or three papillae (Fig. 5). This is almost the same as the results obtained by Rapuzzi and Casella (1965). They reported that the number of papillae connected to the same tactile fiber ranged between two and four (mean 2.7). In comparison with the receptive fields of taste fibers, it is concluded that touch fibers have smaller receptive fields than the taste fibers (two-tailed t test, $P < 0.001$). Thus, touch fibers may play an important role in the localization of the area stimulated.

Time Course of Responses

From the whole-nerve recording in the frog it has been demonstrated that the integrated responses of the frog IXth nerve to QHCl show a phasic response, while

those to NaCl show both phasic and tonic responses during stimulation (Kashiwa-gura et al., 1976; Morimoto and Sato, 1982). These tendencies of the adaptation of the response in the IXth nerve were also seen in the results obtained from single-fiber recording in the frog (Kimura, 1961). In the present study we showed that for each of these stimuli there is a similar adaptation of the response (Fig. 9 *A*), although, we could subdivide the fibers responding to NaCl or acetic acid into two groups (N-1, N-2 for NaCl and A-1, A-2 for acetic acid). Namely, N-1 fibers showed a phasic response and N-2 fibers showed mainly tonic responses. The fibers in these subgroups (N-1, N-2, A-1, A-2) are subdivided into several components or several types of response. For instance, N-1 is constituted from Type *N*, Type *NQ*, Type *NQA*, and Type *NQAS*. Therefore, most types showed either phasic or tonic responses. It was also interesting that responses of fibers to QHCl were phasic and those to $CaCl_2$ were mainly tonic (Fig. 9 *A*). In addition to these difference in the time course of the responses to QHCl and $CaCl_2$, there was a clear separation of fibers responding to these two stimuli, that is, there were no fibers responding to both QHCl and $CaCl_2$ except one (Type *Multiple*).

In the frog IXth nerve, the responses of single fibers to water increased gradually (graded response) (Fig. 9) as have been demonstrated from integrated responses of the whole nerve (Akaike and Sato, 1976; Morimoto and Sato, 1982). The responses to warming were also graded as water, but the rate of the response for warming was higher than that for water (Fig. 9). It can be concluded that there are three groups (phasic, tonic, and graded) in the fibers of the IXth nerve concerning the time course of the response. Considering specificity of the fibers and similarity of the response histogram between types (Fig. 8), 14 response types could be grouped into five groups. It is interesting that there is a relationship between the group and the time course of the response. That is, Group 2 showed a tonic response and Group 3 showed a phasic response.

Responses of Fibers of the IXth Nerve Compared with the Nucleus Tractus Solitarius (NTS)

We have previously described the responses of 115 NTS taste neurons (111 excitatory neurons, 4 inhibitory neurons) using the same stimuli as in the present study except for $CaCl_2$, which was not used in the frog's NTS study (Hanamori et al., 1987). In Fig. 11 *B*, we reclassified the IXth nerve fibers on the basis of the responses to six stimuli (NaCl, QHCl, acetic acid, water, sucrose, warming) without $CaCl_2$ (cf., Table II). In this case, 12 types were found (Fig. 11 *B*, IX). Also, we could classify the NTS cells into 14 types in the same way (Fig. 11 *B*, NTS).

A comparison of the responses between two levels (IXth nerve and NTS) is shown in Fig. 11. The distribution of the number of NTS cells responding to eight stimuli was similar to that of fibers in the IXth nerve, although the cells responding to acetic acid were increased in the NTS compared with those in the IXth nerve (Fig. 11 *A*). Note that $CaCl_2$ was not used in the NTS study. In the NTS cells, the magnitude of the responses to acetic acid were also increased (Fig. 11 *C*). The distribution of the cells (NTS) or fibers (IXth) for 18 response types is shown in Fig. 11 *B*. The distribution of types in the IXth nerve was similar to that in NTS, although there were some differences in that Type *NA* was seen more often in NTS than in the IXth
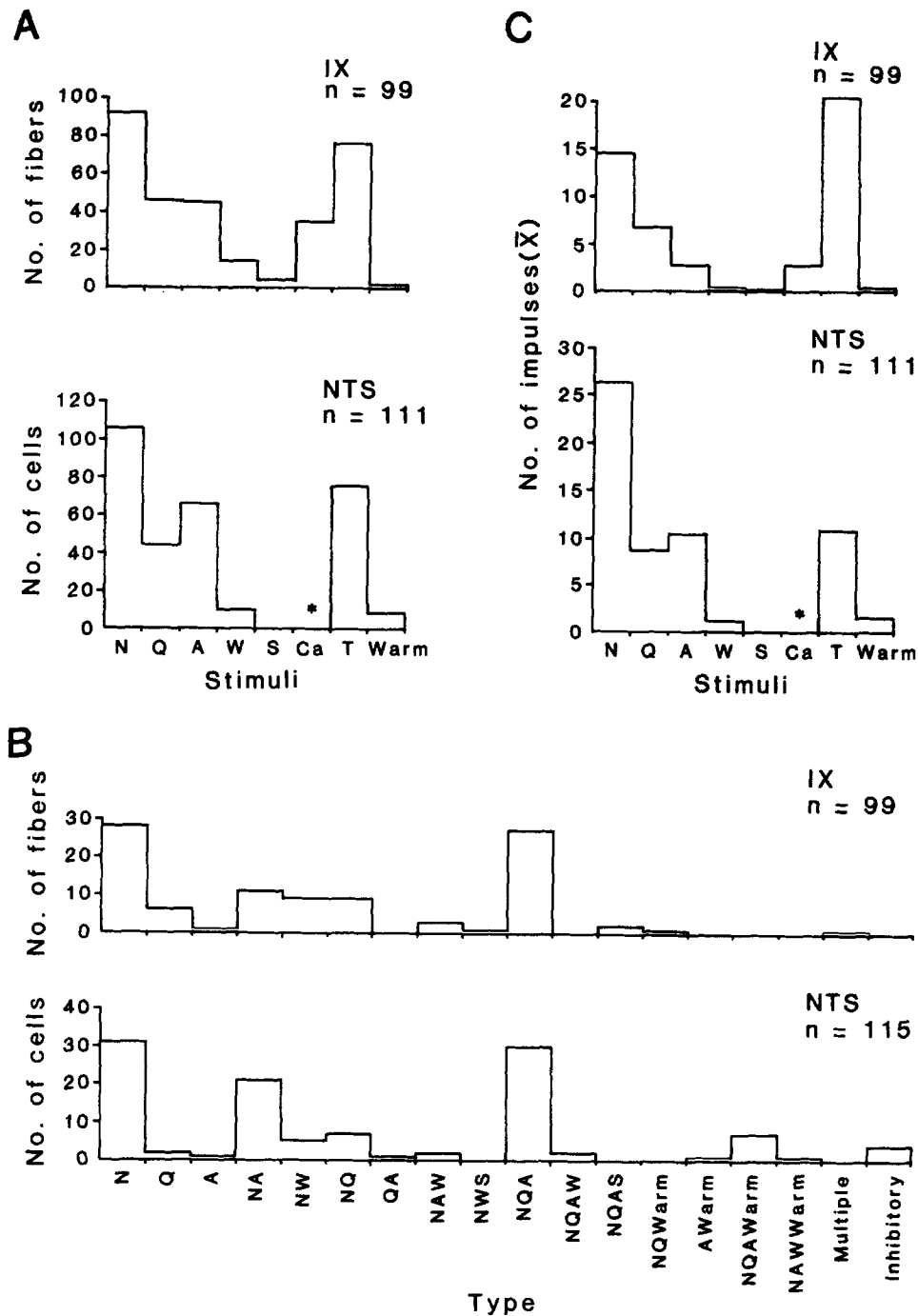
FIGURE 11. Comparison of the data from the IXth nerve with those from the NTS. (A) Frequency histogram of the number of the fibers (IX, n = 99) or cells (NTS, n = 111) as a function of eight stimuli. (B) Frequency histogram of the number of the fibers (IX, n = 99) or cells (NTS, n = 115) as a function of 18 response types. Since $CaCl_2$ was not used in the NTS study, we reclassified the 99 IXth nerve fibers on the basis of their response patterns of response to six stimuli (NaCl, QHCl, acetic acid, water, sucrose, and warming) without $CaCl_2$. Of 115 NTS cells, 111 were classified in the same way as in the IXth nerve. The remaining cells (4) showed inhibitory response to some of the stimuli applied. These cells were named Type Inhibitory. In the present figure, 12 and 14 types are seen for the IXth nerve and the NTS, respectively. (C) Mean number of impulses (impulses/2.5 s for NTS and impulses/3 s for IXth) of the IXth nerve (IX, n = 99) and the NTS cells (NTS, n = 111) to eight stimuli. *, $CaCl_2$ was not used in the NTS study. NTS data from Hanamori et al. (1987).

nerve and that a few types responding to warming (Type *A Warm,* Type *NQA Warm,* and Type *NA W Warm*) and a type showing inhibitory responses to some of the stimuli (Type *Inhibitory*) were found only in NTS (Fig. 11 *C*).

It is concluded that in frogs the responses of the IXth nerve afferents are roughly similar to those of second-order afferent neurons (NTS), although the responses to acetic acid and warming are enhanced in the NTS. Similar results have been reported in mammals; the relationships found among the neural patterns in the CT appear to be fairly well preserved in the NTS and pontine taste neurons since the relative degrees of similarity among the neural patterns were very much alike among these levels, although there are some differences in discharge rates and breadth of responsiveness (Doetsch and Erickson, 1970; Ganchrow and Erickson, 1970; Scott and Erickson, 1971; Travers and Smith, 1979; Smith et al., 1983; Van Buskirk and Smith, 1981).

Multimodal Sensitivity

In the frog's NTS, many cells (67% of all taste NTS neurons studied) responded to both taste and touch stimulation (Hanamori et al., 1987). The results of this investigation demonstrate that the multimodal sensitivity of NTS cells largely depends on the multimodal sensitivity of the IXth nerve fibers. The bimodal responses of the taste nerves (responding to both taste and touch) have been reported in the mudpuppy (Samanen and Bernard, 1981), in the frog (Yamane, 1978), and in mammals (Biedenbach and Chan, 1971; Kasahara and Kawamura, 1975; Stedman et al., 1980; Bradley et al., 1983). On the other hand, the IXth nerve fibers in the carp (Konishi and Zotterman, 1961) and palatine nerve fibers in the puffer (Kiyohara et al., 1985) are sensitive to either taste or touch, but never to both.

The mean response for touch in the touch fibers was 26.5 impulses/3 s ($n = 73$, SEM = 2.4) and that for touch in the taste fibers was 26.9 impulses/3 s ($n = 74$, SEM = 2.5). Although our data for the responses to mechanical stimulation of the single papillae were not quantitative, there seems to be no difference between touch fibers and taste fibers in the rate of response to mechanical stimulation. On the other hand, the receptive fields of the touch fibers ($\overline{X} = 3.6$, SEM = 0.2, $n = 73$) were significantly (two-tailed t test, $P < 0.001$) smaller than those of taste fibers responding to touch ($\overline{X} = 6.8$, SEM = 0.4, $n = 76$).

Using scanning and transmission electron microscopy, Düring and Andres (1976) have demonstrated Merkel cells, located in a ring-like arrangement at the base of the taste disc. This may explain the responsiveness of the fibers to tactile stimulation of the single papillae. However, there is no electrophysiological demonstration of how these Merkel cells might contribute to the mechanical response in taste fibers and touch fibers. In the present study, most touch fibers could easily respond to tactile stimulation using a glass rod as well as a cotton wick. On the other hand, it was relatively difficult to elicit mechanical responses in the taste fibers using a glass rod, whereas stimulation by the hair-like cotton wick was much more effective. Further works are needed to explain the mechanical responses in the taste fibers.

Responses of the Branches of a Single Fiber

The ultrastructure of the taste disc of the frog has been studied by several authors (e.g., Düring and Andres, 1976; Graziadei and Dehan, 1971). Düring and Andres

(1976) have reported that the taste discs of the frog contain an average of ~200 taste cells. Although one-quarter of these taste cells are considered premature cells, there are a lot of receptor cells in a single papilla. Rapuzzi and Casella (1965) have reported that about nine afferent fibers innervate a single papilla. This means that a single fiber could synapse with ~20 taste cells in a single papilla. Also, it has been reported that in rats the life span of the average taste cell was ~250 h (Beidler and Smallman, 1965). Electrophysiologically, it has been reported that single taste cells respond to a variety of taste stimuli in the frog (Sato, 1972; Akaike et al., 1976). From these results, it is easily assumed that if a single taste fiber branches, innervates several papillae, and makes synapses with a number of taste cells at random in each papilla, the information conveyed by a single taste fiber from taste receptors to the brain stem will be nonspecific.

Oakley (1975) has tried to solve this problem by using single CT fiber recordings after taste stimulation of single papillae in the cat. His conclusion was that branches of a taste fiber are not connected at random to the various kinds of taste receptor cells because taste sensitivity between two papillae connected to the same single fiber were similar to each other. In the present study we demonstrated the same result in the frog (Fig. 10). Every branch that was connected to the same single fiber showed similar sensitivity in response to a variety of stimuli (Figs. 2, 3, and 10).

These results suggest the possibility that afferent nerve fibers have some way of detecting taste cells that show a specific responsiveness, or that the fibers provide some substances to taste cells that determine their responsiveness. However, from cross-reinnervation studies Oakley (1967) concluded that the taste nerve itself does not determine the responsiveness of the taste cells and that the responsiveness which taste cells have originally is important in determining the responsiveness of a taste fiber. Recently, Kinnamon et al. (1988) showed anatomically using high voltage electron micrographs that afferent fibers synapse with taste cells of similar morphological type in the circumvallate taste buds of the mouse. However, the question of how every branch of a fiber can select taste cells of similar responsiveness is still unknown.

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