Gustatory Signal Processing in the Glossopharyngeo-hypoglossal Reflex Arc of the Frog

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Abstract The relationship between the gustatory input and motor output in the glossopharyngeo-hypoglossal reflex was analyzed on the basis of neuronal activities in the solitary tract and hypoglossal motor nuclei of bullfrogs. Concentration-response relations for NaCl, quinine and acetic acid, obtained from the glossopharyngeal (IXth) nerve and simultaneously recorded from the hypoglossal (XIIth) nerve, were expressed relative to the response of each nerve to 1 M NaCl. Compared with a relatively small amount of the afferent input for acid, the reflex motor output was much larger in the relative value. A similarly high output relation was obtained for warmed saline but not for quinine and cooled saline. Although the responsiveness of the nucleus tractus solitarius neurons to 1 M NaCl and 1 mM quinine was not significantly different from that of the hypoglossal motoneurons, responses to 10 mM acetic acid were greater in the latter neurons than in the former by a factor of about 5.2. These phenomena were consistent with those in the peripheral nerves. The solitary tract neurons responsive to NaCl, quinine and acid showed both the phasic and tonic components of discharges. According to classification by a transiency index, the discharge mode became more phasic for the hypoglossal motoneurons responsive to NaCl and quinine, but more tonic for those responsive to acid. The above-mentioned chemoreflex is thus regulated by the intrinsic neural network which sends signals to the XIIth nerve after modifying not only the amount but also the temporal pattern of gustatory nerve signals for a particular taste.

Key words: glossopharyngeo-hypoglossal chemoreflex, bullfrog.

The frog's tongue serves as a manipulative organ in prey-catching behavior; it is propelled from the mouth, to impact on, adhere to, and pull prey into the buccal cavity. The visual nervous system of the animal apparently plays a role in initiating a posture suitable for prey-catching (EWERT, 1973), but the neural mechanism which controls the complex tongue movements has been poorly understood to date.

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Several authors have reported that afferent impulses in the frog glossopharyngeal (IXth) nerve elicited by chemical and mechanical stimulations of the tongue evoke reflex discharges in the hypoglossal (XIIth) nerve that innervates extrinsic and intrinsic lingual muscles (NAKAHARA *et al.*, 1969; KŌZAI, 1974; KUMAI, 1981a, b; NOMURA and KUMAI, 1981, 1982). Effective chemical stimuli capable of eliciting the XIIth nerve discharges are NaCl (3.65%), quinine (0.1%), tartaric acid (1%) (NAKAHARA *et al.*, 1969) and in addition, HCl at pH 2.5, but not water (KUMAI, 1981a). KUMAI (1981b) found that the amount of reflex discharges produced by these chemicals in the three main branches (KŌZAI, 1974) of the XIIth nerve differs with different tongue areas stimulated. He attributed the differential effectiveness of different tongue areas in eliciting reflex discharges in the XIIth nerve to two reflex pathways: one between a medial branch of the IXth nerve (ISHIKO *et al.*, 1979) and the hypoglossal branch of the XIIth, and the other between the lateral branch of the IXth and the genioglossal branch of the XIIth. However, the fundamental question of to what extent a variety of gustatory signals could faithfully contribute to reflex discharges in the motor nerve remains unanswered.

In mammals, the reflex, often referred to as the linguo- or glossopharyngeo-hypoglossal reflex, has long been recognized (TARKHAN, 1936; BLOM and SKOGLUND, 1959; BLOM, 1960; HUNTER and PORTER, 1974) since the report of MILLER and SHERRINGTON (1916). Ample evidence has now been accumulated on the reflex, especially on the activity of mammalian hypoglossal motoneurons in response to various orofacial sensory inputs. On the other hand, reflex neural activity in single XIIth nerve fibers elicited by taste and thermal stimulation of the tongue is not parallel with the magnitude of taste afferent input in the chorda tympani in rats (cf. YAMAMOTO *et al.*, 1982).

Electrophysiological (KUMAI, 1980; HANAMORI and ISHIKO, 1981, 1983a; NAKACHI *et al.*, 1985) and histological (HANAMORI and ISHIKO, 1983b; STUESSE *et al.*, 1984) studies on frogs have revealed that a majority of chemical information in the frog IXth nerve is transmitted to the nucleus tractus solitarius (NTS) which, in turn, sends it to the hypoglossal motor nucleus (HMN). The glossopharyngeo-hypoglossal reflex arc thus should contain at least two neuron pools in the lower brain stem, the NTS and HMN. However, the previous studies on the above-mentioned chemoreflex in amphibia as well as in mammals have not revealed an essential relation between the activity of NTS neurons and that of the HMN neurons.

The present study aims to clarify the degree of parallelism in the stimulus intensity-response functions between the IXth and XIIth nerves, while examining gustatory activities of neurons in the NTS and HMN, the two centers in the glossopharyngeo-hypoglossal reflex arc of the frog.

MATERIALS AND METHODS

The experiment was performed on bullfrogs (*Rana catesbeiana*), weighing

between 200 and 400 g. Anesthesia was introduced by immersing the animal into MS-222 solution (tricaine methanesulfonate, 1 g/l) and they were kept in the solution until the end of surgical procedures. When performing the experiments, the animals were removed from the MS-222 solution and immobilized with suxamethonium chloride (20 mg/kg body weight, i.p.), and the immobilization was maintained by subsequent injections of the drug. In an early series of experiments on 18 frogs, the animals were placed in a supine position to expose the IXth nerves on either side and the XIIth nerves on both sides. A length of the IXth nerve from the junction of its medial and lateral branches (ISHIKO *et al.*, 1979) to a point 2–3 cm proximal to the junction was dissected free from the surrounding tissue and kept intact, while the three main branches of the XIIth nerve, each innervating the hyoglossal, genioglossal and intrinsic muscles (KŌZAI, 1974; STUESSE *et al.*, 1983), were isolated and cut peripherally for recordings. Some frogs were further decerebrated by removing the telencephalon above the optic tecta, using a small cotton ball. Unless otherwise mentioned, all the experiments were performed on non-decerebrated animals. The free end of the tongue resting in the mouth cavity was pulled, turned inside out on the axis of the mandibular symphysis, and fixed in a lucite chamber.

In a later series of experiments on 35 frogs, the animals were placed in a ventral position and fixed firmly with the upper jaw clamp. The dorsal part of the cranium was removed to expose the medulla oblongata and the upper portion of the spinal cord. The posterior choroid plexus and the dura overlying the exposed area were then carefully removed. The IXth and XIIth nerves on the ipsilateral side were exposed at the caudoventral region of the tympanic membrane, freed from surrounding tissue, and kept intact. The tongue was pulled out of the oral cavity similar to the earlier experiment.

Recordings. For recording electrical activities from the IXth and XIIth nerves simultaneously, a pair of Ag-AgCl electrodes was placed underneath the exposed IXth nerve, while another pair was placed on the XIIth nerve trunk at a point proximal to the cut end. When simultaneous recording was made from the peripheral three branches of the XIIth nerve, a pair of Ag-AgCl electrodes was attached to the central cut end of each branch. Electrical activities led from these nerves were amplified by conventional amplifiers, summed by integrators (0.3 s in the time constant) and displayed on a pen recorder. The amplified signals were continuously monitored on an oscilloscope while they were stored on FM tape. The data stored on the tape were later displayed and photographed by a kymographic camera, if necessary.

For extracellular recording of single-unit discharges from the NTS and HMN neurons, a glass microelectrode filled with 0.5 M sodium citrate solution containing 2% pontamine-skyblue (resistance, about 20 M Ω) was used. The electrode, mounted on a three-dimensional manipulator, was penetrated into the NTS or HMN. Electrical activities were amplified, displayed, stored and recorded in a way similar to that employed in the above experiment. As an indifferent electrode, an Ag-AgCl

plate (area: 1 cm²) was buried into the subcutaneous tissue in the submandibular or neck region.

To establish the recording sites of a single unit in the NTS and HMN, the dye was deposited electrophoretically from the electrode by applying a cathodal current to the electrode. At the end of each experiment, the animal's brain was perfused with a mixture of 0.5% paraformaldehyde and 1% glutaraldehyde in a phosphate buffer at pH 7.2 through the heart with hydrolic pressure. The brain stem was removed, cut every 50 μ m in thickness and mounted on slide glass for later microscopic examination.

Stimulation. For chemical stimulation of the tongue, NaCl, quinine-HCl and acetic acid of varying concentrations were employed. Of these three kinds of taste substances, NaCl was dissolved in deionized water, but quinine and acetic acid were dissolved in 10 mM NaCl to eliminate the IXth nerve response to water (KUSANO and SATO, 1957). For thermal stimulation, warmed or cooled 10 mM NaCl solution which had temperature differences of 10°C and 20°C from the room temperature to which the tongue had been adapted was used. After rinsing the tongue with 10 mM NaCl solution for 30 s, each test solution was poured over the tongue for 10 s, with a flow rate of about 4 ml/s, followed by another rinse for 30 s. The tongue was then allowed to rest for 10 min to avoid influence from the preceding stimulus (YAMASHITA, 1964; KASHIWAGURA *et al.*, 1976). Taste stimulation was controlled by the use of a microcomputer. All the taste and rinsing solutions were adapted to room temperature ranging from 24 to 26°C.

In the second series of experiments, a brief electric pulse (0.1 ms in width) was applied to the IXth nerve at a rate of 1/s through a pair of Ag-AgCl electrodes to evoke a single neuron discharge accompanied by the negative field potentials in the NTS (HANAMORI and ISHIKO, 1983a). The electric pulse was also used to antidromically stimulate the XIIth nerve to elicit the action potential in the HMN neurons. To examine the mechanical sensitivity of these neurons, the tongue surface was stroked with a Japanese writing brush.

Measurements. To express the amount of electrical activities in the IXth nerve, the area enveloped by an integrated IXth nerve response from the onset of the stimulus to 10 s after was measured with the use of a digital planimeter. The area thus obtained was expressed relative to the area for the response to 1 M NaCl. The integrated XIIth nerve responses representing the efferent electrical activities of the HMN neurons were also measured in the same way as above and expressed relative to the area for the XIIth nerve response to 1 M NaCl.

The magnitude of responses of the NTS and HMN neurons to chemical as well as thermal stimuli was expressed by the number of impulses discharged during the 10 s of each stimulus, while the spontaneous activity of each neuron was expressed by the number of impulses in the 10 s before stimulation. The response of a neuron to a given stimulus was judged to be excitatory when the number of impulses discharged in any second of the stimulus period was greater than the mean + 2 S.D. of the number of spontaneous discharges/s calculated from those obtained during

the 10 s before stimulation. On the other hand, when the discharge rate was smaller than the mean -2 S.D. of the spontaneous discharge rate, the response was judged to be inhibitory.

For quantitative expression of the initial transient versus later tonic discharge of a neuron, we used a transiency index (Tr. I. in Fig. 9) which is expressed by the following equation (SCHILLER and MALPELI, 1977):

$$\text{Tr. I.} = [I/(I + T)] \times 100$$

where I stands for initial and T for terminal response. We employed I , as the large response magnitude evoked either in the 1st or 1st-2nd s after the onset of stimulus and T , as the mean response magnitude during the terminal 5 s (OGAWA *et al.*, 1985). The range of this index in the present study was 0–100, with higher values indicating a greater transiency.

Statistical analyses. For statistical comparison of the mean response magnitudes between two samples, Student's t -test was employed throughout the present study.

RESULTS

Relationship between sensory input and motor output

Figure 1 demonstrates afferent responses in a lingual branch of the IXth nerve

Fig. 1. Records demonstrate the IXth nerve responses (upper row) of a frog to taste and thermal stimulations of the tongue and resulting reflex motor activities in the XIIth nerve (lower row) taken simultaneously. In each record, the lower and upper trace show impulse discharges and their summed record, respectively. Horizontal bar marks period of application of stimulus here and in Fig. 3. -20°C and $+20^{\circ}\text{C}$: the temperature of saline solution applied was 20°C lower and higher than tongue temperature, respectively, here and in Figs. 3, 5, 6 and 8.

(upper records) to taste and thermal stimuli, and efferent motor activities in a proximal branch of the XIIth (lower ones), taken simultaneously. In each record, the lower trace shows a train of nerve impulses elicited during stimulation (horizontal bar) or, on some occasions, at the end of a stimulus, whereas the upper trace exhibits the corresponding integrated record. As seen in the figure, integrated neural activity comprised a large transient response followed by a tonic one of varying amplitudes depending on the stimuli applied. Furthermore, there existed a certain degree of dissimilarity between the time course of integrated afferent activity in the IXth nerve and that of the corresponding efferent activity in the XIIth. Particular attention should be paid to the response of the XIIth nerve to 10 mM acetic acid at pH 3.4 (3rd record from left in the lower row), which produced a large tonic response characteristic of its recruiting activity, reached a greater amplitude than the transient response, and lasted for more than 30 s even after cessation of the stimulus. On the other hand, the enhancement seen in the XIIth nerve response to warmed saline at a temperature 20°C higher than the tongue temperature (+20°C)

Fig. 2. Stimulus intensity-response relationships for three kinds of taste stimuli (quinine, acetic acid, and NaCl) and temperature changes in six frogs. In each animal, simultaneous recordings were made from the IXth and XIIth nerves. The magnitudes of response of each nerve to all stimuli were expressed relative to the response to 1 M NaCl. Vertical bar at each point represents mean $\pm$ S.E. Numerals on temperature scale: temperature of saline applied was lower (minus) or higher (plus) than tongue temperature (0), here and in Fig. 4.

lacked the late recruiting activity seen in the response to acid.

In Fig. 2, the relation between the relative magnitude of response and the intensity of stimulus was obtained from both the IXth and XIIth nerves. It illustrates that, unlike the intensity-response relationship for NaCl, quinine and cold saline, the relationship for acetic acid and warmed saline had a much steeper slope in the XIIth nerve than in the IXth. The question of whether such the reflex activity in the XIIth nerve differs among the three peripheral branches (KÖZAI, 1974) was studied. Figure 3 demonstrates sample records obtained simultaneously from all the branches in a non-decerebrated frog, and the intensity-response relation for each branch is presented in Fig. 4 (solid symbols). Figures 3 and 4 show that the responses of the intrinsic branch (int br.) to all stimuli did not differ substantially from those of the genioglossal branch (gen br.), while the response of the hyoglossal branch (hyo br.) to each stimulus was in general less prominent than the corresponding response in the other two branches. The result in Fig. 4 thus confirms that the reflex motor activity in the XIIth nerve trunk seen in Fig. 2 distributes over the three peripheral branches, though to different degrees. To determine whether the relation in non-decerebrated frogs differs from that in decerebrated ones, the intensity-response functions for taste and thermal stimuli were also studied in six decerebrated frogs and presented in Fig. 4 (empty symbols with dotted lines). It was found that there was no significant difference ($p > 0.02$) in the intensity-response relation in each branch between the non-decerebrated and decerebrated frogs. This is consistent with the results reported by NAKAHARA *et al.* (1969).

Fig. 3. Integrated reflex discharges in a frog, recorded simultaneously from the three XIIth nerve branches innervating hyoglossal (hyo br.), intrinsic (int br.) and genioglossal (gen br.) muscles. Note that responses of the hyoglossal branch to taste and thermal stimuli are less pronounced than those of the other two branches. For minus and plus 20°C, see legend in Fig. 1.

Fig. 4. Stimulus intensity-response relationships obtained from the three main branches of the XIIth nerve in six non-decerebrated (solid symbols and lines) and six decerebrated (empty symbols and dotted lines) frogs. Graphs obtained from the hyoglossal (hyo br.), intrinsic (int br.) and genioglossal (gen br.) muscle branches are arranged from top to bottom. The magnitudes of response of each branch to taste and thermal stimuli are expressed relative to the response of each branch to 1 M NaCl. Vertical bar at each point: $\pm$ S.E. For temperature scale, see legend in Fig. 2.

Response characteristics of the NTS and HMN neurons

Response type. *NTS.* When the glass capillary electrode was advanced to the NTS, there usually appeared a single unit spike having negative or positive-negative polarity on the top or on the skirt of a negative field potential (HANAMORI and ISHIKO, 1983a) in response to electrical stimulation of the IXth nerve (*s*, in the diagram in Fig. 5). Not all the units isolated followed the stimulation rate greater than 50 Hz (SESSLE, 1973) accompanying a deterioration of the field potential (HANAMORI and ISHIKO, 1983a). The mean latency for the spike initiation in 32 units was found to be 7.2 ± 0.61 ($\pm$ S.E.) ms with a range from 3 to 13.5 ms. Estimated from the conduction distance (3.5 cm) in the peripheral nerve and the conduction velocity of the IXth nerve fibers (HANAMORI and ISHIKO, 1981), units with a spike latency of more than 3 ms can be considered to be NTS neurons rather than primary afferents. Individual NTS neurons were then identified by their sensitivity of 1 M

Table 1. Response type and taste sensitivity of NTS and HMN neurons.

A. NTS neurons

Type	No. of effective stimuli	No. of responsive neurons	Effective basic taste
E	3	18	N, Q, A
	2	9	N, A
		3	N, Q
	1	14	N
I	3	1	N, Q, A
	2	1	N, A
	1	1	N
E & I	3	4	N, Q, A
	2	2	N, A
Total		53	

B. HMN neurons

Type	No. of effective stimuli	No. of responsive neurons	Effective basic taste
E	3	47	N, Q, A
	2	3	N, A
		1	Q, A
	1	2	A
Total		53	

E, I and E & I: neurons exhibited excitatory, inhibitory and a combination of both excitatory and inhibitory responses, respectively. N, Q, and A: 1 M NaCl, 1 mM quinine, and 10 mM acetic acid solutions, respectively.

NaCl, 1 mM quinine, 10 mM acetic acid, warmed saline solutions at a temperature of +20°C and mechanical stimulation of the tongue. Of the 53 single neurons studied, 38 neurons responded to more than two of the three kinds of taste substances, 43 (81%) showed resting discharges without application of stimuli, 8 (15%) responded to the warmed saline, and 26 (48%) responded to tactile stimulation of the tongue. The mean rate of the "spontaneous discharges" in the NTS neurons was 21.5 ± 2.8 ($\pm$ S.E.) impulses/10 s (Fig. 9A).

Taste responses of all the NTS neurons fell into one of three distinct types as given in Table 1A: 44 (83%) out of the 53 neurons responded only with the excitatory response (E-type) to any stimulus, 3 (6%) only with the inhibitory

Fig. 5. Diagram at left: experimental set-up for recording unitary neuron discharges from the NTS. Right: records demonstrating excitatory response (E-type, in Table 1) of NTS neuron (No. 17, in left graph in Fig. 8) to taste and thermal but not to tactile stimulation of the tongue. Arrow marks onset of stimulus here and in Figs. 6 and 7. Note that each trace does not include end of stimulus. TEC, optic tectum; CBL, cerebellum; NTS, nucleus tractus solitarius; IX, glossopharyngeal nerve; R and S, recording and stimulating electrodes, respectively. For $+20^{\circ}\text{C}$, see legend in Fig. 1. Voltage calibration: 2 mV.

response (I-type), and the remaining 6 (11%) with varying combinations of the excitatory and inhibitory responses (E- and I-type) depending on the kinds of stimuli applied. Figure 5 demonstrates an example of the E-type showing the excitatory responses of a neuron to all but mechanical stimulation of the tongue. The I-type is presented in Fig. 6 in which the spontaneous discharges of the neuron were inhibited markedly by application of NaCl and acetic acid, weakly by quinine and tactile stimuli, and unaffected by warmed saline. In Table 1A, the neurons belonging to either one of the three response types were further divided according to their sensitivity to the number of taste stimuli. As seen in the table, NaCl was the most effective among the three taste in evoking either the E-type or the I-type response in the NTS neurons.

HMN. While penetrating the recording electrode into the region caudomedial to the NTS, we identified the HMN neurons which generated a large positive-negative spike in response to antidromic stimulation (s, in the diagram in Fig. 7) of the XIIth nerve. The neurons isolated were then tested to see whether they could discharge efferent impulses following taste stimulation of the tongue. Fifty-three taste-sensitive neurons thus obtained had a mean latency of 1.5 ± 0.04 ($\pm$ S.E.) ms, with a range from 0.87 to 1.98 ms, for initiating the antidromic spike. They showed

Fig. 6. Records demonstrating inhibitory response (I-type) of a NTS neuron (No. 53, in Fig. 8) to all but thermal stimulation of the tongue. For $+20^{\circ}\text{C}$, see legend in Fig. 1. Voltage calibration: 2 mV.

only the E-type response to any taste substance. As shown in Table 1B, all the neurons were further subdivided into three groups in a way similar to the NTS neurons. From Table 1B, the HMN neurons sensitive to more than two taste qualities were found to number 51, 96.2% of all 53 neurons, whereas the number of NTS neurons having the same property was 38 (71.7%) out of the same number of neurons as studied in the NTS. Therefore, HMN neurons are more dominant in multiple taste sensitivity than the NTS neurons. The responsiveness of the HMN neurons to tongue warming was not studied.

Responsiveness to taste substances. The response profile of all the 53 NTS neurons to the four kinds of stimuli is presented in Fig. 8A with their spontaneous discharges shown at the bottom. In the figure, the neurons are arranged in the order of the responsiveness or the number of impulses during 10 s of stimulation by 1 M NaCl. The response profile of all the HMN neurons to all but thermal stimuli is shown in Fig. 8B, in a manner similar to Fig. 8A. The figure illustrates that the responsiveness of the HMN neurons to acetic acid is obviously greater than that of the NTS neurons, although the spontaneously firing neurons in the HMN were very few, only 5 (9%), which contrasts with the number (81%) in the NTS. Such an observation has also been reported in mammals (YAMAMOTO *et al.*, 1982).

Fig. 7. Diagram at left: experimental set-up for recording single neuron discharges from the HMN. Right: records exhibiting different modes of impulse discharges resulting from application of the three kinds of taste stimuli. Records in A and B show responses of the HMN neuron (No. 14 and No. 5 in Fig. 8, respectively). HMN, hypoglossal motor nucleus; TEC, CBL, R and S, same abbreviations as in Fig. 5. Voltage calibration: 2 mV.

To compare the taste responsiveness of the NTS neurons with that of the HMN, the mean number ($\pm$ S.E.) of impulses/10 s discharged by all the neurons in the respective nuclei during taste stimulation was calculated and is presented in Fig. 8. All the values in Fig. 8 include the spontaneous discharges. It reveals that the responsivenesses of the NTS neurons to NaCl and quinine does not significantly differ ($p > 0.02$) from that of the HMN neurons, whereas a difference in the responsiveness to acetic acid is highly significant ($p < 0.001$) between the NTS and HMN neurons.

Temporal pattern of discharges. Figure 7 demonstrates discharge patterns of two HMN neurons (A and B) responding to application of three kinds of taste substances to the tongue. In the records, it can be seen that HMN neuron discharges can roughly be divided into three classes according to their temporal patterns during and after taste stimulation of the tongue: (i) the initial phasic discharges (henceforth referred to as *P* mode) often accompanied by off-discharge at the end of stimulus, as in the second trace in A; (ii) the initial phasic discharges followed by a tonic impulse train (*P* + *T* mode), as in the top and third traces in A; and (iii) tonic discharge alone (*T* mode) which includes a mode of delayed discharges with a long

Fig. 8. Response profiles of 53 NTS (A) and 53 HMN (B) neurons for 1 M NaCl, 1 mM quinine, 10 mM acetic acid and warming (saline 20°C higher than the tongue temperature). Each block represents the number of impulses elicited during the initial 10 s of stimulation in an individual neuron. The neurons in the NTS and HMN were, respectively, arranged in the order of responsiveness to NaCl. Numerals in each graph present the mean $\pm$ S.E. of impulses/10 s with the number of neurons in parenthesis. **: difference is highly significant ($p < 0.001$) compared with corresponding value in the NTS. Bottom: spontaneous discharge. For +20°C, see legend in Fig. 1.

latency of more than 3 s from the onset of stimulus, as in the bottom record (B). These three modes of discharge patterns were also recognized in the NTS neurons responding only with the E-type discharge. For quantitative expression of the three discharge patterns, the index of transiency (SCHILLER and MALPELI, 1977) was calculated for individual neuron responses to the three taste stimuli. The indices obtained here scattered over the range from 0 to 100, in which 50 means a state of discharge just between the purely phasic discharge (100) and the extremely tonic one (0). Therefore, the transiency indices within the range between 25 above and 25 below the midpoint (50) were considered to be *P* + *T* mode discharges, whereas the indices within 100–75 and 25–0 could be classified as *P* and *T* modes, respectively. In Fig. 9, the NTS and HMN neurons responding to each of the three tastes are

Fig. 9. Distribution of transiency indices for the NTS (white bars) and HMN (dotted bars) neurons responsive to 1 M NaCl, 1 mM quinine and 10 mM acetic acid. Individual neuron discharges responding to each stimulus were classified into three categories according to their transiency indices: phasic (*P*), phasic with tonic (*P* + *T*), and tonic (*T*) mode corresponding, respectively, to the indices ranging from 100–75, 75–25 and 25–0. The number of neurons belonging to either one of the three discharge modes is expressed relative to the total number of neurons responding to each stimulus. Numerals in each histogram indicate the mean ± S.E. of the transiency index with the number of neurons in parenthesis. **: difference is highly significant ($p < 0.001$) compared with corresponding value in the NTS. *P*, *P* + *T*, and *T* in the upper right histogram apply to the corresponding bar in the remaining histograms.

divided into the three classes (P , $P+T$, and T , as in the upper right histogram) according to their transiency indices and presented against the number of neurons expressed relative to the total neurons encountered. This illustrates clearly that the distribution of transiency indices was different between the NTS (white bar) and the HMN (dotted bar) neurons, although some degree of overlap was recognized between the two. As indicated in Fig. 9, the mean transiency index for NaCl-sensitive NTS neurons, i.e., 66.7 ± 2.8 ($\pm$ S.E.), was significantly smaller than the corresponding index of the HMN neurons (81.9 ± 2.6 , $p < 0.001$), indicating that the transiency is augmented in the HMN compared with the NTS. A similar tendency was noted between the mean transiency index for quinine-sensitive neurons in the NTS and HMN. In contrast, the mean transiency index (52.2 ± 3.9) for acetic acid-sensitive neurons in the NTS was large in comparison with the index (31.2 ± 3.1) for the corresponding HMN neurons, showing that an apparent shift in the transiency took place from a sustained to more tonic discharge pattern.

Localization of taste-sensitive neurons

Locations of the medullary neurons responding to chemical stimulation of the tongue were reconstructed histologically and presented in Fig. 10, in which a series of transverse sections of the lower brain stem are arranged from the obex to 2.2 mm

Fig. 10. Cross-sections of the lower brain stem showing location of taste-sensitive NTS (○) and HMN neurons (●). TS and HMN: tractus solitarius and hypoglossal motor nucleus, respectively. NTS locates around TS and extends ventromedially. Numeral indicates rostral distance from the obex.

rostral to the obex. All the 53 neurons (empty circles) depicted were found to distribute at 0.6–2.2 mm rostral to the obex, 0.3–1.2 mm from the midline, and 0.25 to 1.8 mm in depth from the dorsal surface of the medulla. As seen in the figure, all the neurons distribute approximately within the NTS, the region surrounding the tractus solitarius (TS) and its ventromedial portion (OPDAM *et al.*, 1976; HANAMORI and ISHIKO, 1983b; STUESSE *et al.*, 1984).

Neurons which responded to both antidromic stimulation of the XIIth nerve and taste stimulation of the tongue existed within the region caudomedial to the NTS. All the 53 neurons studied (solid circles) were distributed 0.15–0.35 mm lateral to the midline, 0–0.9 mm rostral to the obex, and within a depth of between 0.3 and 0.6 mm from the dorsal surface of the medulla, as seen in Fig. 10. The area circumscribed by the above three dimensions approximately coincides with that for the HMN in *Bufo bufo* L. (WEERASURIYA and EWERT, 1981) and in *Rana pipiens* (STUESSE *et al.*, 1983).

DISCUSSION

Unlike the results reported by previous authors (NAKAHARA *et al.*, 1969; KŌZAI, 1974; YAMAMOTO, 1975; KUMAI, 1981a, b; NOMURA and KUMAI, 1982; YAMAMOTO *et al.*, 1982), our results revealed a degree of parallelism between gustatory nerve input and reflex motor output, by showing the IXth and XIIth nerve responses to varying intensities of taste and thermal stimuli (Fig. 2). In their study on the frog, *Rana nigromaculata*, NAKAHARA *et al.* (1969) reported that the threshold concentration for initiating reflex discharges in the XIIth nerve is 3.65% (0.63 M) for NaCl, 0.1% (2.5 mM) for quinine and 1% for tartaric acid. Although we did not use the last stimulant, the concentrations of the former two are apparently higher than those expected from the present results (Fig. 2), i.e., less than 0.05 M for NaCl and less than 0.01 mM for quinine-HCl. The reason for such a discrepancy may be ascribed in part to the species difference between the two sets of experimental animals, but largely to a difference in the concentration of saline solution to which the tongue had been adapted; NAKAHARA *et al.* (1969) used Ringer solution (111.2 mM NaCl) instead of 10 mM NaCl solution as employed by the present authors. Similar consideration should be paid to the threshold concentration for reflex discharges in the three branches of the XIIth nerve, when comparing the present results (Fig. 4) with the results reported by KŌZAI (1974).

In interpreting the results in Figs. 1 and 2, two questions may arise: (i) whether acetic acid could stimulate other sensory receptors such as those in the olfactory mucosa; and (ii) whether warmed saline at a temperature of +20°C could stimulate nociceptive fiber endings in the lingual mucosa. The first possibility is less probable, since we found that all the reflex activities in the XIIth nerve were completely abolished in the presence of gustatory and thermal stimulations of the tongue in three frogs whose IXth nerves had been cut bilaterally. Therefore, there may exist no specific sensory pathway except for the IXth nerve for signalling information

from the tongue to the central neurons. For the second question, one should note that magnitude of the IXth nerve response to warmed saline increased at $+10^{\circ}\text{C}$, i.e., at a solution temperature of about 35°C (Fig. 2). This agrees with the previous finding that warm fibers in the frog IXth nerve respond to solutions at a temperature above 25°C (KIMURA, 1962; YAMASHITA, 1964). However, the question of whether warming the tongue to about 45°C could excite a sufficient number of nociceptive fibers remains obscure, because there exists no appreciable difference between the magnitudes of the IXth nerve responses to saline at $+10^{\circ}\text{C}$ and $+20^{\circ}\text{C}$ (Fig. 2).

In their study on single-unit activity of the chorda tympani and hypoglossal motor nerve fibers, YAMAMOTO *et al.* (1982) reported that, in non-decerebrated rats, the magnitude of XIIth nerve responses to four basic taste stimuli was 1.4 (for HCl) and -8.4 (for sucrose) times larger than that of the chorda tympani responses, although, in decerebrated animals, the magnitude of motor output decreased to that of taste input. Such a direct comparison between input and output signals cannot be made in the present study in which the whole IXth and XIIth nerve responses to taste and thermal stimuli were expressed relative to the response of each nerve to 1 M NaCl. Nevertheless, our results show that, compared with a relatively slow rate of rise in the intensity-response function for acetic acid and for warmed saline in the IXth nerve, the corresponding function in the XIIth is much steeper. This contrasts with an approximately parallel increase in the functions for other stimuli (Figs. 1 and 2). To analyze these phenomena, it was essential to know the responsiveness of both the NTS neurons and the HMN neurons to taste stimuli. The results (numerals in Fig. 8) revealed that the mean response magnitudes of HMN neurons to 1 M NaCl and 1 mM quinine were not significantly different ($p > 0.02$) from those of the NTS neurons, whereas response magnitudes of the HMN neurons to 10 mM acetic acid were significantly larger ($p < 0.001$) than those of the NTS neurons, by a factor of about 5.2. All the NTS neurons studied did not necessarily project to the HMN neurons analyzed, since both groups of neurons were recorded independently of each other. Nevertheless, the results obtained were consistent with the findings in the two peripheral nerves (Figs. 1 and 2). Therefore, we may state that the central neuron networks along the NTS-HMN pathway are capable of augmenting the amount of gustatory signals for acid, and that they are to a large extent responsible for the difference in the relative magnitudes of response of the IXth and XIIth nerves to acetic acid. The existence of a similar central mechanism for sucrose but not for acid has been suggested in rats by YAMAMOTO *et al.* (1982).

In the results (Figs. 1 and 2), the relative magnitude of reflex motor responses to warming, but not to cooling of the tongue is obviously greater than that of afferent responses in the IXth nerve. Although we did not study the responsiveness of HMN neurons to thermal stimuli, a neural enhancing mechanism for efferent impulses might be similar to that for acid signals.

According to SCHILLER and MALPELI (1971) the transiency indices for visual neurons vary from 50 to 100. On the other hand, the indices for the present

chemosensitive neurons distributed over the range of 0–100. Such a wide variation could be ascribed to the fact that, in some taste neurons of the NTS and HMN, the tonic component of discharges was larger than the phasic one, as was clearly seen in the responses of the HMN neurons to acetic acid (Figs. 1, 3, 7 and 8). Studies on the phasic versus tonic response of the rat taste neurons at different brain levels have revealed that the phasic component becomes smaller in magnitude as the level of neurons varies from the medulla, pontine and thalamus, to the cortical taste area (SCOTT, 1982; YAMAMOTO *et al.*, 1984). OGAWA *et al.* (1985) examined the same problem along the taste pathway from the chorda tympani to the thalamic level by means of the transiency index (SCHILLER and MALPELI, 1977), and found that the index becomes smaller for the response to NaCl, larger for the responses to sucrose and HCl, and is unchanged, or always phasic, for the quinine response. The present results (Fig. 9) agree with the latter authors in that the change in the level of taste neurons from the NTS to HMN brings about a decrease in the transiency index for acid but not always for other taste stimuli.

In summary, the present results reveal that gustatory signals in the IXth nerve are subject to two kinds of central processing, namely, amplification of the magnitude and modification of the temporal pattern while they are travelling from the NTS to HMN. The question of whether similar changes also occur at the junction between the IXth nerve afferents and the NTS neurons cannot be answered at the moment, because of differences in the method of data sampling between the pre- and post-junctional neurons.

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