

# Modulation of the Inspiratory-Related Activity of Hypoglossal Premotor Neurons During Ingestion and Rejection in the Decerebrate Cat

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**Ono, Takashi, Yasuo Ishiwata, Noritaka Inaba, Takayuki Kuroda, and Yoshio Nakamura.** Modulation of the inspiratory-related activity of hypoglossal premotor neurons during ingestion and rejection in the decerebrate cat. *J. Neurophysiol.* 80: 48–58, 1998. Single-unit activities of the bulbar reticular inspiratory neurons directly projecting to hypoglossal motoneurons were studied during fictive ingestion (e.g., swallowing) and rejection elicited by repetitive stimulation of the superior laryngeal nerve and by application of water to the pharynx in immobilized decerebrated cats. The single-unit activity was recorded during 113 episodes of fictive ingestion from 25 inspiratory neurons directly projecting to hypoglossal motoneurons (single projection neurons) and 7 inspiratory neurons directly projecting to both hypoglossal and phrenic motoneurons (dual projection neurons) in the regions ventrolateral to the nucleus tractus solitarius and dorsomedial to the nucleus ambiguus. All of single projection neurons ceased inspiratory-related rhythmical discharges coincidentally with the onset of repetitive stimulation of the superior laryngeal nerve. The majority of them (19/25, 76%, type A) showed a spike burst during ingestion, whereas the minority (6/25, 24%, type B) kept silent until the end of repetitive stimulation of the superior laryngeal nerve. During fictive ingestion elicited by application of water to the pharynx, the type-A neurons showed a spike burst activity, whereas the type-B neurons kept silent. All dual projection neurons (7/7, 100%, type C) ceased inspiratory-related rhythmical discharges at the onset of repetitive stimulation of the superior laryngeal nerve and showed no activity during fictive ingestion. Likewise, the type-C neurons kept silent during fictive ingestion elicited by application of water to the pharynx. A spike burst was induced during 33 episodes of fictive rejection in all of 5 tested type-A, 3 tested type-B, and 6 tested type-C neurons. It is concluded that the premotor neurons involved in the respiratory-related rhythmical activity of hypoglossal motoneurons is responsible for switching from respiration to ingestion and rejection.

## INTRODUCTION

The tongue is a multifunctional organ participating in a variety of functions, such as sucking, licking, mastication, vocalization, respiration, and swallowing (Lowe 1981). Although some of these motor activities can occur simultaneously by utilizing the same motoneurons (Mns) as the output device (Bramble and Carrier 1983; Carter and Smith 1986a,b), others are mutually exclusive and cannot be simultaneously performed (Kaplan and Grill 1989; McFarland and Lund 1993; Weijnen et al. 1984). For instance, respiratory movements cease during swallowing, and the tongue activity coincidentally switches from the respiratory-related pattern to the swallowing-related one. It was proposed that this was

probably because the central pattern generator (CPG) responsible for one motor act suppresses the activity of CPGs involved in the others (McFarland and Lund 1993; Smith and Denny 1990).

Extracellular recording from neurons in the hypoglossal (XII) nucleus in awake animals provided some insight into the neural coordination of licking and swallowing (Travers and Jackson 1992). It was suggested that the CPG for swallowing had effects on a subset of XII Mns involved in swallowing and delayed the generation of a lick cycle. DiNardo and Travers (1994) investigated the XII neural activity during ingestion (licking and swallowing) and rejection (gape) elicited by gustatory stimuli in the awake rat. They demonstrated that subsets of XII Mns were involved in switching of the motor activity from ingestion to rejection by changing their firing patterns, suggesting the overlapping between the XII premotor neurons that produce ingestion and rejection. However, the behavior of premotor neurons projecting to XII Mns (Amri et al. 1990; Manaker et al. 1992; Ono et al. 1994; Sumino and Nakamura 1974) has not been examined to date in relation to interaction of CPGs.

We previously reported that, in the bulbar regions ventrolateral to the nucleus tractus solitarius (vl-NTS) and dorsomedial to the nucleus ambiguus (dm-AMB), there were inspiratory neurons monosynaptically projecting to XII Mns that showed the respiratory-related activity (Ono et al. 1994). Some of these inspiratory neurons had bifurcating axons projecting to both XII and phrenic (PH) Mns. We proposed that the heterogeneity of subpopulations of premotor neurons that project to XII and PH Mns may be involved not only in coordination of the activity of the tongue muscle and the diaphragm during spontaneous breathing but also in their differential responses to various stimuli.

Neuronal mechanisms subserving behavioral switching between respiratory and nonrespiratory function have been reported (DiNardo and Travers 1994; Gestreau et al. 1996; Grélot et al. 1992; Hooper and Moulins 1989; Huang and Satterlie 1990; Larson et al. 1994; McFarland and Lund 1993; Travers and Jackson 1992). In this study, single-unit activities of subpopulations of these XII premotor neurons with inspiratory-related activity were recorded during fictive ingestion and rejection as well as during spontaneous breathing. This was done to elucidate their roles in switching from one function to another within neuronal networks controlling the multiple functions of the tongue.

## METHODS

Experiments were performed in 12 adult cats of either sex weighing 2.7–4.7 kg. The animal use protocol was reviewed and approved by the Screening Committee for Animal Research of Tokyo Medical and Dental University. From two days before the surgical procedures, animals were given a prophylactic dose of antibiotics. Chlorpromazine hydrochloride (2.0 mg/kg im) and atropine sulfate (0.1 mg/kg im) were initially administered; 30 min later, ketamine hydrochloride was given (40 mg/kg im). After the trachea and right superficial radial vein were cannulated, halothane (1.5–2.0%) insufflation was started and continued throughout the surgical procedures.

A midline skin incision was made from the symphysis mentalis to the top of the sternum. The medial and lateral branches of the XII nerve were dissected free and sectioned distally on the left side. A bipolar silver-wire collar electrode (interpolated distance: ~5 mm) was fixed to the central cut end of the medial branch for recording, which innervates tongue protruding muscles including the genioglossus (GG) muscle. The C<sub>5</sub> and/or C<sub>6</sub> portion of PH nerve on the left side was dissected free near the diverging point of the jugular and subclavian veins and sectioned distally. A pair of steel needle electrodes (diameter: 0.2 mm, enamel-coated except for 0.2 mm at the top; interpolar distance: ~5 mm) was inserted into the GG, the geniohyoid, the hyoglossus and the styloglossus muscles and the costal diaphragm on the right side, respectively, for recording the electromyogram (EMG). The superior laryngeal nerve (SLN) was dissected free and sectioned bilaterally near the larynx. A bipolar silver-wire electrode (interpolated distance: ~5 mm) was fixed to the central stump of the nerve on each side for electrical stimulation. Repetitive stimulation was applied to the SLN to elicit ingestion (30 Hz, 0.5–1.0 V) and rejection (30 Hz, 3.0–5.0 V). In some experiments, a flexible plastic tube (diameter: ~1.5 mm) was inserted to the pharynx transnasally for water application (volume: ~0.5 ml for each trial). An occipital, parietal, and frontal craniotomy was performed, and the cerebellum was aspirated to expose the floor of the fourth ventricle. Decerebration was made at the precollicular level. A laminectomy at the C<sub>5</sub> and C<sub>6</sub> segments was performed, and the dura was removed. After separation of the splenius muscle from the platysma, a bipolar silver-wire collar electrode (interpolated distance: ~3 mm) was fixed to the central cut end of the left PH nerve. In some animals, a bilateral cervical vagotomy was performed. When surgical procedures were completed, halothane anesthesia was terminated. The animal was paralyzed with pancuronium bromide (0.1 mg · kg<sup>-1</sup> · h<sup>-1</sup>) and artificially ventilated throughout the recording sessions. A bilateral pneumothorax was made to improve recording stability.

An enamel-coated electropolished tungsten microelectrode was inserted into the left PH nucleus at the C<sub>5</sub>–C<sub>6</sub> segments for monopolar stimulation and fixed to the point where the largest negative antidromic field potential was evoked by stimulation of the left PH nerve. To minimize stimulus artifacts, a pair of enamel-coated electropolished tungsten microelectrodes was inserted into the left XII nucleus for bipolar stimulation. Each electrode of the pair was inserted one after the other, separately. First, one of the pair was inserted into the XII nucleus at the middle level of its rostrocaudal extent, then the other into its close vicinity. The two electrodes were fixed to the points in the XII nucleus where the largest negative antidromic field potentials were evoked along each insertion track by stimulation of the medial branch of the left XII nerve (interpolated distance, ≤1.0 mm).

Single-unit activities were recorded from respiratory neurons by enamel-coated electropolished tungsten microelectrodes (impedance: 10–20 MΩ at 1 kHz) in the lower brain stem on the right side (from 3.0 mm rostral to 3.0 mm caudal to the obex in the rostrocaudal direction; from 1.5 mm to 4.5 mm lateral to the mid-

line). Respiratory neurons projecting to the XII nucleus or both the XII and PH nuclei were identified by 1) a rhythmical firing activity corresponding with the respiratory cycle and 2) spike responses after a fixed short latency to microstimulation of the XII nucleus (<50 μA; duration, 0.1 ms) or both the XII and the PH (<100 μA; duration, 0.1 ms) nuclei, which showed collision with spontaneous spikes. During recording, the tracheal pressure and discharges in both the XII and PH nerves were simultaneously displayed on oscilloscopes after amplification and recorded on a tape recorder. Instantaneous firing frequency was obtained by inverting the duration between a spontaneous spike discharge and a succeeding one recorded from the respiratory neuron (i.e., 1,000 ms divided by an interspike interval in ms). To detect monosynaptic connection between the respiratory neuron and either the XII Mns or the XII Mns and PH Mns, spike-triggered averaging method was employed (Fedorko et al. 1989; Kirkwood and Sears 1973; Lipski et al. 1983). Spontaneous spike discharges of a respiratory neuron were fed to a window discriminator; the discriminated pulses were used to trigger the averager (Signal processor 7T07, San-ei, Tokyo, Japan) of simultaneously recorded XII and PH nerve discharges, which were full-wave rectified and integrated (time constant: 10 ms). Usually >2,000 sweeps were averaged with respect to trigger pulses.

Throughout the experiment, the end-tidal CO<sub>2</sub> was maintained at 3.5–4.0%. The tracheal pressure and electrocardiogram were monitored. The rectal temperature was maintained at ~37°C by a radiating heating lamp from above and a heating pad under the abdomen. At the end of each experiment, an overdose of pentobarbital sodium was given intravenously to the animal. Small electrolytic lesions were made in the stimulation and recording points by applying DC cathodal current (20 μA for 20 s) through electrodes. The animal was perfused transcardially with 0.9% saline followed by 10% Formalin. Serial transverse frozen sections (thickness: 75 μm) were made of the brain stem, and stained by the Klüver-Barrera method to verify the sites of stimulation and recording.

An analysis of variance (ANOVA) was used for statistical comparison in the number of spikes during ingestion and rejection among the type-A, type-B, and type-C neurons. Statistical significance was established at  $P < 0.05$ .

## RESULTS

*EMG activity of the tongue muscles and the diaphragm during ingestion and rejection*

A typical record of EMG activity of the extrinsic tongue muscles (i.e., the hyoglossus, the styloglossus, the geniohyoid, and the GG muscles) and the diaphragm during ingestion and rejection before immobilization is illustrated in Fig. 1. Repetitive SLN stimulation was applied to elicit swallowing (Fig. 1, downward arrows). Shortly after the cessation of repetitive SLN stimulation (Fig. 1, upward arrows), occurrence of swallowing was observed (Fig. 1, i). During swallowing, a burst activity was coincidentally observed in the styloglossus, the geniohyoid, and the GG muscles during expiration. No change was observed in the EMG activity of the diaphragm. When a volume of water was applied into the pharynx (Fig. 1, arrowhead), expulsive behavior was elicited. The most visible component of this rejection response was a large jaw opening (gape). During rejection (Fig. 1, r), a burst activity was coincidentally found in the hyoglossus, the styloglossus, the geniohyoid, and the GG muscles as well as the diaphragm. The amplitude of EMG activities of these muscles during rejection was significantly larger than those recorded during ingestion (swallowing).

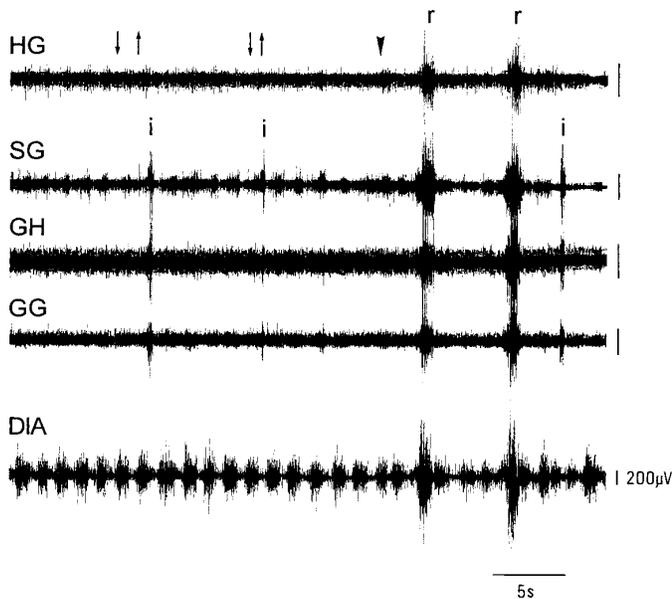


FIG. 1. Electromyographic activity of the hyoglossus, the styloglossus, the geniohyoid, the genioglossus muscles, and the diaphragm during ingestion (swallowing) and rejection (gape). Downward and upward arrows show the onset and offset of repetitive superior laryngeal nerve (SLN) stimulation, respectively. Arrowhead indicates the onset of water application. Three episodes labeled *i* and 2 episodes labeled *r* denote ingestion and rejection, respectively. HG, hyoglossus muscle; SG, styloglossus muscle; GH, geniohyoid muscle; GG, genioglossus muscle; DIA, diaphragm.

In addition, the duration of EMG activities of these muscles during rejection was significantly longer than that recorded during ingestion. The second episode of rejection was followed by swallowing characterized by simultaneous activation of the styloglossus, the geniohyoid, and the GG muscles.

#### Activity of bulbar inspiratory neurons during fictive ingestion

Single-unit activity was recorded from 25 respiratory neurons directly projecting to XII Mns (single projection neurons) and 7 neurons directly projecting to both XII and PH Mns via bifurcating axons (dual projection neurons) during fictive ingestion elicited by both SLN stimulation and water application to the pharynx after the animal was immobilized. All these neurons were inspiratory neurons.

Figure 2 illustrates the single-unit activity recorded from a respiratory neuron in the right vl-NTS region. It fired continuously during the inspiratory phase, indicating that this neuron was an inspiratory neuron. It responded to stimulation of the left XII nucleus with a spike potential after a constant latency of 0.5 ms (Fig. 2*Ca*), which collided with spontaneous spike potentials (Fig. 2*Cb*). Averaging the rectified discharge of both the XII and PH nerves by spontaneous spikes of this neuron revealed a facilitation in the XII nerve discharge after 1.6 ms (Fig. 2*D*, arrow), but not in the PH nerve discharge (Fig. 2*D*). Thus this neuron was a single projection neuron (Ono et al. 1994). In an early inspiratory phase, repetitive SLN stimulation was applied to elicit ingestion (Fig. 2, *A* and *B*, downward arrows). The respiratory-related rhythmical activity in the PH nerve disappeared half way to the peak (Fig. 2, *A* and *B*). Coincidentally

the inspiratory neuron completely ceased firing, while the XII nerve discharge frequency exceeded its preceding inspiratory-related level. Shortly after the cessation of repetitive SLN stimulation (Fig. 2, *A* and *B*, upward arrows), a transient burst activity was observed in this inspiratory neuron. The burst consisted of spikes firing at a higher rate than the normal inspiratory discharge and was accompanied by a similar burst in the XII nerve discharge but not in the PH nerve discharge (Fig. 2, *A* and *B*, *i*). Because this pattern of discharge in the XII and PH nerves was the same as that seen in association with actual swallowing (ingestion) before paralysis (Fig. 1), the bursts were considered to represent a fictive ingestion (DiNardo and Travers 1994; Gestreau et al. 1996; Grélot et al. 1992; Jiang et al. 1991; Withington-Wray et al. 1988). The moment when the transient burst activity was present in the XII nerve, no activity was seen in the PH nerve discharge. In a total of 25 single projection neurons, 19 neurons showed this type of activity (type-A neurons), whereas the remaining 6 neurons showed no activity during fictive ingestion (type-B neurons).

Figure 3 shows the single-unit activity recorded from one of seven inspiratory neurons that projected to both the XII and PH Mns in the right dm-AMB region. This dual projection neuron was activated by stimulation of both the left XII and PH nuclei after fixed latencies of 0.8 and 1.6 ms, respectively (Fig. 3, *Ca* and *Cb*). When stimulation in the left XII nucleus preceded that of the left PH nucleus by 3.0 ms (Fig. 3*Da*) or 2.0 ms (Fig. 3*Db*), two spike potentials evoked by stimulation in respective nucleus persisted. When the interval was further shortened to 1.0 ms, the spike potential could not be evoked by stimulation of the left PH nucleus (Fig. 3*Dc*). Thus the inspiratory neuron had a bifurcating axon terminating in both the XII and PH nuclei. Averaging the rectified XII and PH nerve discharges by spontaneous spike potentials of this neuron revealed a facilitation in discharges of both nerves after 1.9 and 2.6 ms, respectively (Fig. 3*E*, arrows). This inspiratory neuron ceased its rhythmical discharge at the onset of repetitive SLN stimulation (Fig. 3, *A* and *B*, downward arrows), and the PH nerve discharge also disappeared (Fig. 3, *A* and *B*). During repetitive SLN stimulation, fictive ingestion occurred, which was indicated by the transient burst activity in the XII nerve discharge (Fig. 3, *A* and *B*, *i*). During this fictive ingestion, this inspiratory neuron showed no activity. At the end of SLN stimulation (Fig. 3, *A* and *B*, upward arrows), the inspiratory neuron resumed its respiratory-related rhythmical activity. All of seven neurons showed this type of activity: inactive during fictive ingestion and active during fictive rejection (type-C neurons).

#### Activity of bulbar inspiratory neurons during fictive rejection

Single-unit activities recorded from a type-A neuron and a type-B neuron during fictive rejection are illustrated in Fig. 4. Shortly after application of water (Fig. 4, *A* and *B*, arrows), a sporadic burst activity occurred in the XII and PH nerve discharges (Fig. 4, *A* and *B*, *r*). These episodes were regarded as fictive rejection and were distinguished from other behaviors, because 1) the same pattern of discharge in both the XII and PH nerves were demonstrated in

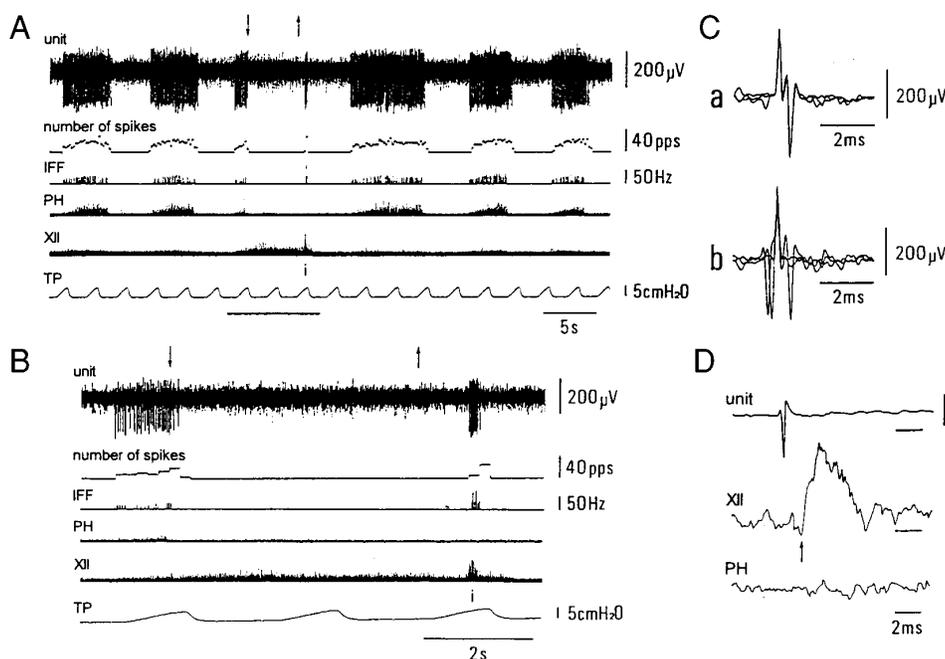


FIG. 2. *A–D*: activity of a respiratory neuron directly projecting to hypoglossal (XII) motoneurons (Mns) during fictive ingestion. *A*: simultaneous record of extracellular spikes of a respiratory neuron, number of spike potentials per second (200 ms/bin), instantaneous firing frequency of spike potentials, rectified phrenic (PH) nerve discharge, rectified XII nerve discharge, and tracheal pressure (from top to bottom). Downward and upward arrows show the onset and offset of repetitive SLN stimulation, respectively. The episode labeled *i* denotes ingestion. *B*: record of the part underlined in *A* on an expanded time base. The episode labeled *i* denotes ingestion. *Ca*: fixed short-latency response of the respiratory neuron shown in *A* to stimulation of the contralateral XII nucleus (0.1 ms; 45 mA). *Cb*: collision of spikes with spontaneous spikes. Three sweeps (1 sweep with a fixed short-latency response and 2 sweeps with collision with spontaneous spikes) are superimposed in *Ca* and *Cb*. Spontaneous spike discharges within 0.5 ms before the onset of stimulation of the XII nucleus were collided with the antidromic spike discharge. The polarity of stimulus artifact was positive, whereas the polarity of spike discharge was negative-positive. *D*: averaging of rectified XII and PH nerve discharges by spontaneous spikes of the neuron shown in *A–C*. One thousand sweeps were averaged. Note a facilitation in the XII nerve discharge after a short latency, but not in the PH nerve discharge. IFF, instantaneous firing frequency; PH, rectified PH nerve discharge; XII, rectified XII nerve discharge; TP, tracheal pressure.

association with a large mouth opening, or gape before the animal was immobilized (DiNardo and Travers 1994; Grill and Norgren 1978; Travers and Norgren 1986) and 2) the magnitude of the XII nerve activity was larger than foregoing inspiratory-related activity. Although the magnitude of rejection seen in both the XII and PH nerve discharges was relatively less in Fig. 4*A* than that in Fig. 4*B*, these responses were qualitatively similar. In Fig. 4*A*, the type-A neuron showed an augmented burst activity during rejection. Likewise, a transient burst activity was observed in the type-B neuron coincidentally with the burst activity in both the XII and PH nerve discharges (Fig. 4*B*).

Figure 5 shows the pattern of activity of a dual projection neuron shown in Fig. 3 in association with fictive rejection. Shortly after application of water (Fig. 5, arrow), a transient burst activity occurred in the XII and PH nerve discharges (Fig. 5, *r*). This episode was considered as a fictive rejection by the two reasons above mentioned. Moreover, the burst activity was followed by swallowing, which was a typical behavior sequential to rejection when the stimulus was aversive (DiNardo and Travers 1994). Coincidentally with the characteristic activities in XII and PH nerve discharges, this inspiratory neuron fired at a higher frequency than its normal rhythmical bursting frequency. Slightly after the fictive rejection, smaller bursts were seen twice in the XII nerve

discharge, but not in the PH nerve discharge (Fig. 5, *i*). These two episodes were considered to be fictive ingestion, because 1) the same patterns were observed in EMG activities of both the tongue muscles and the diaphragm during actual ingestion (swallowing) elicited by repetitive SLN stimulation before the animal was immobilized (Fig. 1), 2) the magnitude of the XII nerve activity was smaller than that recorded during rejection, and 3) it was reported that swallowing usually coincided with the expiratory phase of the breathing cycle in decerebrated cats (Dick et al. 1993). During fictive ingestion, this inspiratory neuron showed no activity.

#### Activity of bulbar inspiratory neurons during fictive swallow-breath

Weak activation in the PH nerve discharge occurs in association with swallowing, which is called swallow-breath (Dick et al. 1993; Menon et al. 1984; Wilson et al. 1981). Figure 6 shows the pattern of activity of a type-B neuron shown in Fig. 4*A* during fictive swallow-breath. Shortly after application of water to the pharynx during inspiration (Fig. 6, arrow), a fictive rejection characterized by a transient burst activity in the XII and PH nerve discharges occurred (Fig. 6, *r*). In the following expiratory phase, a transient

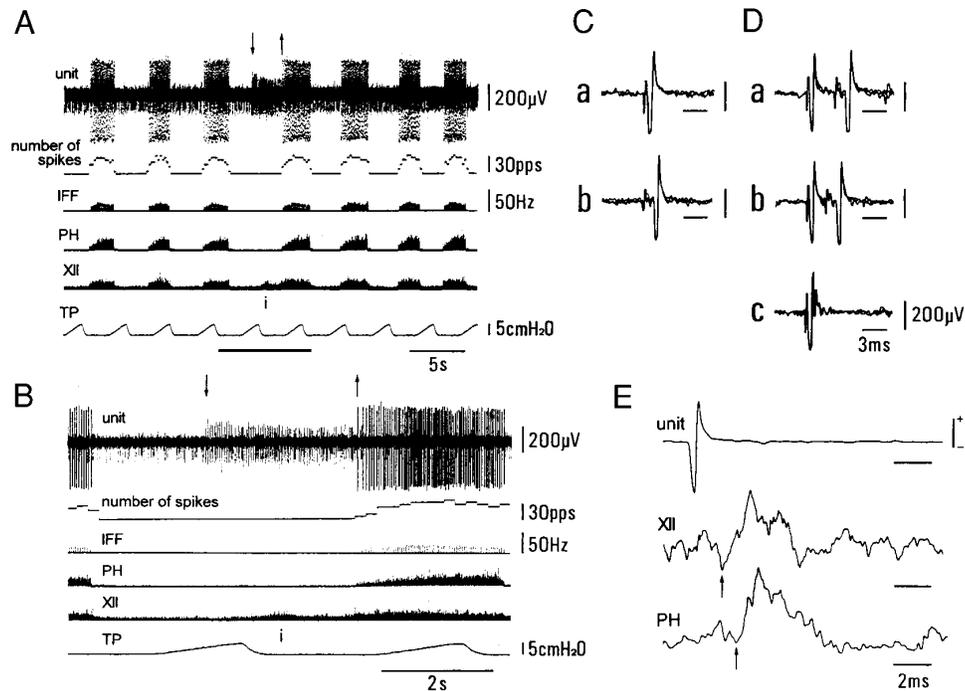


FIG. 3. *A–E*: activity of a respiratory neuron directly projecting to both the XII and PH Mns during fictive ingestion. *A*: simultaneous record of extracellular spikes of a respiratory neuron, number of spike potentials per second (200 ms/bin), instantaneous firing frequency of spike potentials, rectified PH nerve discharge, rectified XII nerve discharge, and tracheal pressure (from *top* to *bottom*). The episode labeled *i* denotes ingestion. *B*: record of the part underlined in *A* on an expanded time base. The episode labeled *i* denotes ingestion. *Ca*: fixed short-latency response of the respiratory neuron shown in *A* to stimulation of the contralateral XII nucleus (0.1 ms; 30 mA). *Cb*: fixed short-latency response of the same respiratory neuron to stimulation of the contralateral PH nucleus (0.1 ms; 12 mA). Three sweeps are superimposed in *Ca* and *Cb*. *D*: collision of spikes shown in *Cb* evoked by stimulation of the PH nucleus with spikes evoked by stimulation of the XII nucleus. Antidromic spike potentials evoked by stimulation of the PH nucleus persisted when stimulation of the XII nucleus preceded 3 ms (*Da*) and 2 ms (*Db*). When the interval was shortened to 1 ms (*Dc*), the antidromic spike potential evoked by stimulation of the PH nucleus was collided with that evoked by stimulation of the XII nucleus. The polarity of stimulus artifact was negative-positive, whereas the polarity of spike discharge was negative-positive. Three sweeps are superimposed. *E*: averaging of the rectified XII and PH nerve discharges by spontaneous spikes of the neuron shown in *A–D*. One thousand two hundred sweeps were averaged. Note the facilitation in both the XII and PH nerve discharges after short latencies.

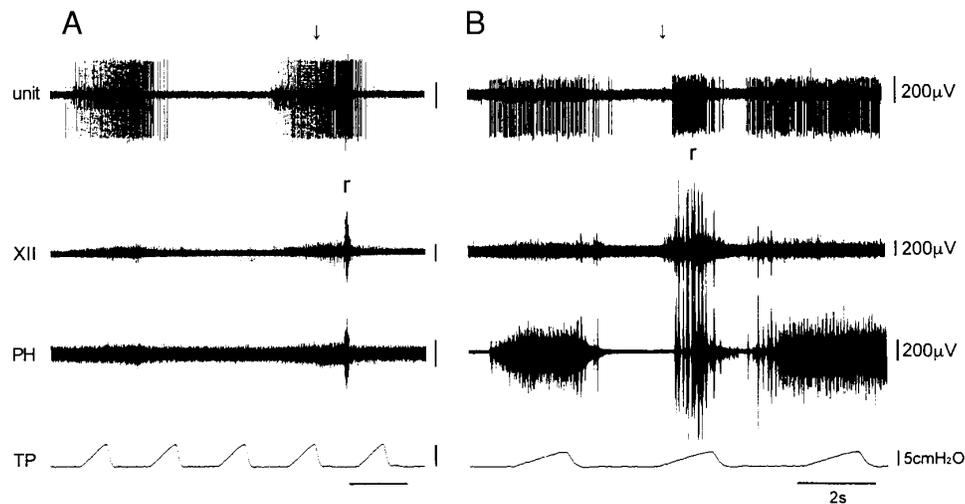


FIG. 4. *A* and *B*: activity of respiratory neurons directly projecting to XII Mns during fictive rejection. *A*: type-A neuron. *B*: type-B neuron. Simultaneous record of extracellular spikes of a respiratory neuron, XII nerve discharge, PH nerve discharge, and tracheal pressure are shown (from *top* to *bottom*). Downward arrows indicate the onset of water application. Two episodes labeled *r* denote rejection.

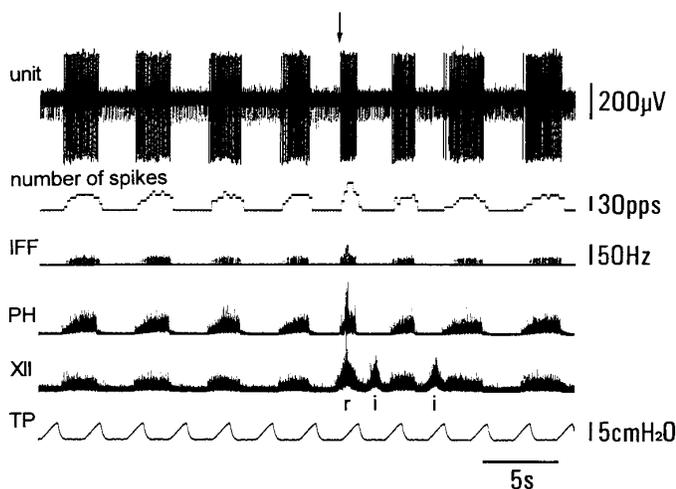


FIG. 5. Simultaneous record of extracellular spikes of the respiratory neuron shown in Fig. 3, number of spike potentials per second (200 ms/bin), instantaneous firing frequency of spike potentials, rectified PH nerve discharge, rectified XII nerve discharge, and tracheal pressure during fictive rejection and ingestion. Arrow indicates onset of water application. Two episodes labeled *i* and an episode labeled *r* denote ingestion and rejection, respectively.

burst activity in the XII nerve associated with weak activity in the PH nerve discharge (Fig. 6, asterisk) was observed. This episode was regarded as a fictive swallow-breath. During fictive swallow-breath, two spike discharges were observed in the type-B neuron. The type-B neuron resumed phasic inspiratory-related activity thereafter.

The pattern of activity of a type-C neuron that was identified to directly project to both the XII and PH Mns during fictive swallow-breath is illustrated in Fig. 7. Application of water to the pharynx (Fig. 7, downward arrow) induced a fictive swallow-breath characterized by a transient burst activity in the XII nerve discharge and a weak coactivation in the PH nerve discharge (Fig. 7, asterisk). During fictive swallow-breath, the type-C neuron showed 5 spike discharges. However, during two episodes of fictive ingestion (Fig. 7, *i*), the type-C neuron showed no activity.

Patterns of activity for the 32 bulbar inspiratory neurons during fictive ingestion and rejection are summarized in Table 1. Of the 25 single projection neurons, 19 (76%, type-A) neurons showed a burst activity during fictive ingestion, whereas the remaining 6 (24%, type-B) neurons showed no activity during fictive ingestion. On the other hand, all of the seven (100%, type-C) dual projection neurons kept silent during fictive ingestion. No dual projection neurons showed firing activity during fictive ingestion. During fictive rejection, activities of five type-A, three type-B, and six type-C neurons were examined. All of five tested type-A, three tested type-B, and six tested type-C neurons showed the burst activity.

Numbers of analyzed episodes for three types of inspiratory neurons are shown in Table 2. Activities of the three types of inspiratory neurons during a total of the 113 episodes of fictive ingestion were analyzed. Of 113 episodes, 80 and 33 episodes were induced by repetitive SLN stimulation and by application of water to the pharynx, respectively. For the 19 type-A neurons, the number of spikes during fictive ingestion induced by repetitive SLN stimulation and

by application of water to the pharynx was  $14.8 \pm 4.27$  and  $11.0 \pm 1.41$  (mean  $\pm$  SD), respectively. There was no significant difference in the number of spikes during fictive ingestion induced by the two means. A total of 11 episodes of fictive swallow-breath occurred in 1 type-A, 4 type-B, and 1 type-C neurons. During fictive swallow-breath, burst activities were observed in a type-A and a type-C neuron. Of the four type-B neurons, three type-B neurons showed a transient burst activity during fictive swallow-breath, but one type-B neuron did not.

Activities of five type-A, three type-B, and six type-C neurons were further examined during fictive rejection induced by repetitive SLN stimulation and by application of water to the pharynx. In a total of 33 episodes of fictive rejection, 12 and 21 episodes were induced by repetitive SLN stimulation and by application of water to the pharynx, respectively. The number of spikes during fictive rejection induced by repetitive SLN stimulation was  $26.2 \pm 5.76$  for the type-A neurons,  $43.0 \pm 4.24$  for the type-B neurons, and  $44.8 \pm 5.89$  for the type-C neurons, respectively. There was a significant difference in the mean number of spikes during fictive rejection between the type-A and type-C neurons ( $P < 0.05$ ). The number of spikes during fictive rejection induced by application of water to the pharynx was  $40.7 \pm 26.1$  for the type-B neurons and  $56.3 \pm 26.3$  for the type-C neurons, respectively. No fictive rejection was induced for the tested five type-A neurons by application of water to the pharynx. There was no significant difference in the mean number of spikes during fictive rejection between the type-B and type-C neurons.

Figure 8 summarizes locations of three types of neurons recorded in the present study. Four neurons were located in the vl-NTS region around the obex, 26 neurons in the dm-AMB region from 1.5 mm caudal to 1.0 mm rostral to the obex, and 2 neurons in the intermediate region between the vl-NTS and dm-AMB regions at the level of 1.0 mm caudal

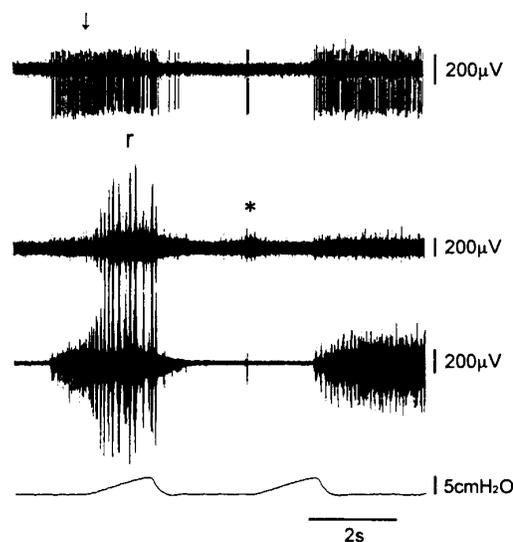


FIG. 6. Activity of respiratory neurons directly projecting to XII Mns during fictive swallow-breath. Simultaneous record of extracellular spikes of a type-A neuron, XII nerve discharge, PH nerve discharge, and tracheal pressure are shown (from top to bottom). Downward arrow and an episode labeled *r* indicate the onset of water application and swallow-breath, respectively. Asterisk denotes swallow-breath.

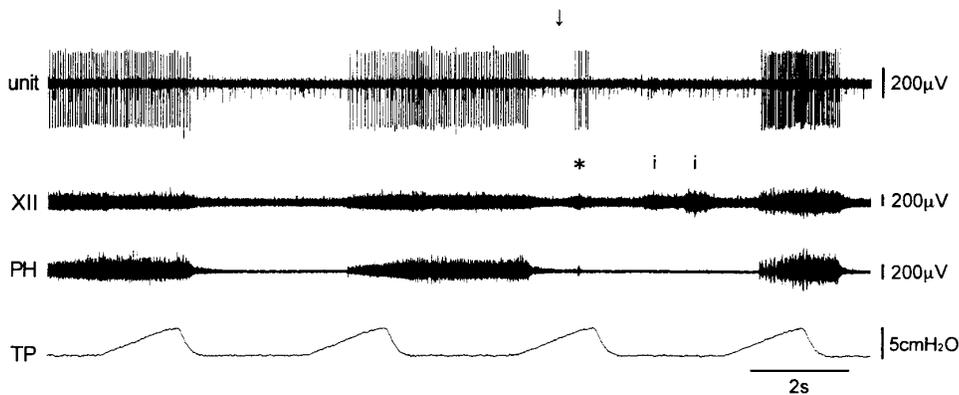


FIG. 7. Activity of a respiratory neuron directly projecting to both the XII and PH Mns during swallow-breath and ingestion. Simultaneous record of extracellular spikes of a type-C neuron, XII nerve discharge, PH nerve discharge, and tracheal pressure are shown (from top to bottom). Downward arrow indicates the onset of water application. Asterisk denotes swallow-breath. Two episodes labeled i denote ingestion.

to the obex. With regard to the behavior during fictive ingestion, three type-A neurons and one type-C neuron were found in the vl-NTS region. Two type-A neurons were found in the intermediate region. Three types of neurons that showed different patterns during fictive ingestion were intermingled in the dm-AMB region.

## DISCUSSION

### *Classification of XII premotor neurons with inspiratory-related activity*

In this study the behavior of 32 respiratory neurons in the regions vl-NTS and dm-AMB, which monosynaptically project either to XII Mns or to both the XII and PH Mns, was investigated during fictive ingestion and rejection. These respiratory neurons showed rhythmical burst activities in coincidence with the rhythmical discharge of the PH nerve. They antidromically responded to microstimulation of their respective motor nucleus. In addition, averaging of the rectified XII and PH nerve discharges with respect to spontaneous spikes of these respiratory neurons revealed an increase in the activity of either the XII nerve or both the XII and PH nerves. In the present study, we did not attempt to investigate modulation of respiratory neurons that projected to neither XII or PH Mns, nor those that projected only to PH Mns. Therefore it should be taken into account that the sampling of respiratory neurons was biased.

The monosynaptic projection from these respiratory neu-

TABLE 1. *Patterns of activity of the inspiratory neurons during fictive ingestion and rejection*

	Active	Nonactive	Total
XII	19 (5/5)	6 (3/3)	25
XII+PH	0	7 (6/6)	7
Total	19	13	32

In parentheses, numbers of inspiratory neurons that showed burst activities during fictive rejection along with numbers of tested neurons were indicated; all of 5 tested type-A, 3 tested type-B, and 6 tested type-C neurons showed a spike burst activity during fictive rejection. XII, neurons that directly project to XII motoneurons (Mns); XII+PH, neurons that directly project to both the XII and PH Mns; Active, neurons that showed activities during fictive ingestion; Nonactive, neurons that showed no activities during fictive ingestion. "Active XII," "Non active XII," and "Nonactive XII+PH" correspond with type-A, type-B, and type-C neurons, respectively.

rons to XII and/or PH Mns was identified by comparing the sum of the latency of antidromic activation, the utilization time, and the synaptic delay versus the latency of an increase in the rectified activity of the XII and/or PH nerves that were averaged with respect to spontaneous spikes of these respiratory neurons (Ono et al. 1994). The inspiratory neuron shown in Fig. 2 responded with antidromic spike potentials after a constant latency of 0.5 ms to stimulation of the contralateral XII nucleus. The conduction time between the soma of the inspiratory neuron and the stimulating electrode in the contralateral XII nucleus was estimated to be 0.3 ms, after 0.2 ms was subtracted as the utilization time (Jankowska and Roberts 1972a). The conduction time from the somata of XII Mns to the recording electrode on the XII nerve was estimated to be 1.0 ms, on the basis of the latency of the negative deflection of the antidromic field potential in the XII nucleus evoked by stimulation of the XII nerve minus 0.2 ms as the utilization time. If we assume a synaptic delay to be 0.3–0.4 ms (Jankowska and Roberts 1972b), the sum of conduction times in the inspiratory neuron (0.3 ms), the XII nerve (1.0 ms), and one synaptic delay (0.3–0.4 ms) amounts to 1.6–1.7 ms. Indeed, facilitation was observed in the XII nerve discharge 1.6 ms after the onset of the negative deflection of triggering spikes of the inspiratory neuron. Therefore the calculated latency corresponds well with the observed latency, within a difference too small for another neuron to be intercalated between the recorded inspiratory neuron and XII Mns. In contrast, no monosynaptic projection was found from this inspiratory neuron to PH Mns: neither antidromic spike response to stimulation in the PH nucleus nor facilitation in the PH nerve discharge was present. With respect to the remaining 24 inspiratory neurons that demonstrated facilitation in the XII nerve discharge, the latency of facilitation corresponded with the calculated monosynaptic latency within a difference of  $\leq 0.2$  ms. Thus we are led to conclude that there are monosynaptic excitatory projections from the recorded inspiratory neurons to XII Mns. A similar example of an inspiratory neuron that projected to XII Mns was shown in Fig. 4 in our previous study (Ono et al. 1994).

With regard to the inspiratory neuron shown in Fig. 3, the conduction time to XII Mns was estimated to be 0.6 ms, by subtracting the utilization time (0.2 ms) from the latency of antidromic spikes evoked by stimulation of the contralateral XII nucleus (0.8 ms). The sum of 0.6 ms, 0.3–0.4 ms as a synaptic delay, and 1.0 ms as a conduction time from the XII Mn to the recording electrode on the XII nerve

TABLE 2. Numbers of analyzed episodes of fictive ingestion and rejection for three types of inspiratory neurons

	Ingestion			Rejection			
	<i>n</i>	SLN-induced	Water-induced	Swallow-Breath	<i>n</i>	SLN-induced	Water-induced
Type-A	19	35 (35)	7 (7)	1 (1)	5	5 (5)	0
Type-B	6	23 (0)	9 (0)	9 (6)	3	8 (8)	4 (4)
Type-C	7	22 (0)	17 (0)	1 (1)	6	5 (5)	17 (17)
Total	32	80	33	11	14	12	21

In parentheses, numbers of episodes in which inspiratory neurons showed burst activities are indicated. *n* is number of neurons. Activities of the 3 types of inspiratory neurons were analyzed during a total of 113 episodes of fictive ingestion. In addition, 11 episodes of swallow-breath were observed. Five of 19 type-A, 3 of 6 type-B, and 6 of 7 type-C neurons were further tested during a total of 33 episodes of fictive rejection. Type-A neurons, single projection neurons that showed a burst activity during fictive ingestion. Type-B neurons, single projection neurons that showed no activity during fictive ingestion. Type-C neurons, dual projection neurons that kept silent during fictive ingestion.

amounts to 1.9–2.0 ms. This calculated monosynaptic latency corresponded with the observed latency of facilitation (1.9 ms). Likewise, the conduction time from the stimulating electrode in the contralateral PH nucleus to the soma of the recorded inspiratory neuron was 1.4 ms, by subtracting the utilization time (0.2 ms) from the latency of antidromic spikes evoked by stimulation of the contralateral PH nucleus (1.6 ms). The negative deflection of the antidromic field potential in the PH nucleus evoked by stimulation of the PH nerve started 1.1 ms after the stimulus artifact; the subtraction of 0.2 ms from 1.1 ms yielded 0.9 ms. If we add 1.4, 0.9, and 0.3–0.4 ms as a synaptic delay, we get 2.6–2.7 ms in a total. In Fig. 3, the facilitation of the PH nerve discharge is seen to start 2.6 ms after triggering spikes. It is thus concluded that the recorded inspiratory neuron makes a direct excitatory projection to both the XII and PH nerves. Similar calculations revealed that the observed latencies of facilitation matched well with the calculated monosynaptic latency from these neurons to XII and PH Mns within a difference of  $\leq 0.2$  ms. Accordingly, it is concluded that these inspiratory neurons make monosynaptic excitatory projections to both XII and PH Mns. A

similar example of an inspiratory neuron that projected to both XII and PH Mns was demonstrated in Fig. 5 in our previous study (Ono et al. 1994).

Synaptic connections between medullary respiratory neurons and respiratory motoneurons have been extensively studied by spike-triggered averaging (Fedorko et al. 1989; Kirkwood and Sears 1973; Lipski et al. 1983) and cross-correlation (Cohen et al. 1974; Davies et al. 1985; Mateika and Duffin 1989). These studies revealed monosynaptic connections by demonstrating the peak with short latency (1.5–2.4 ms in Cohen et al. 1974; 2.5–4.5 ms in Mateika and Duffin 1989), short rise time (0.4–1.4 ms in Cohen et al. 1974;  $0.5 \pm 0.2$  ms in Lipski et al. 1983), and short half-width ( $2.0 \pm 0.7$  ms in Lipski et al. 1983) in the nerve discharge. In Fig. 2, the rise time was 1.1 ms and the half-width was 3.1 ms. In Fig. 3, the rise time and half-width for the XII nerve discharge were 1.4 and 1.2 ms, respectively, whereas the rise time and half-width for the PH nerve discharge were 1.2 ms and 1.4 ms, respectively. These short rise time and half-width as well as the short latency comparable with those in previous studies are suggestive of presence of monosynaptic connection between inspiratory neurons and XII and PH Mns.

Consequently, these respiratory neurons were classified into two groups, excitatory inspiratory neurons that monosynaptically projected to XII Mns (25 single projection neurons) and those to both the XII and PH Mns (7 dual projection neurons). This proportion (7/32, 22%) of dual projection neurons is similar to that reported of dual projection neurons to both the trigeminal and XII Mns (8/38, 21%) (Amri et al. 1990). However, it may not show the real proportion of neurons with bifurcating axons, because it is possible that single projection neurons and dual projection neurons may project to cranial and spinal Mns other than XII and PH Mns. In our previous study, 93% (25/27) and 7% (2/27) of the single projection neurons were antidromically activated by stimulation of contralateral and ipsilateral XII nucleus, respectively (Ono et al. 1994). Likewise, 86% (6/7) and 14% (1/7) of the dual projection neurons were antidromically activated by stimulation of contralateral and ipsilateral XII and PH nuclei, respectively. This is in accord with the previous study that showed contralateral dominance of monosynaptic projection of medullary inspiratory neurons to the PH Mns (Cohen et al. 1974). The above findings led us to investigate the contralateral projection of the inspiratory neurons in the present study. Nevertheless, the possibil-

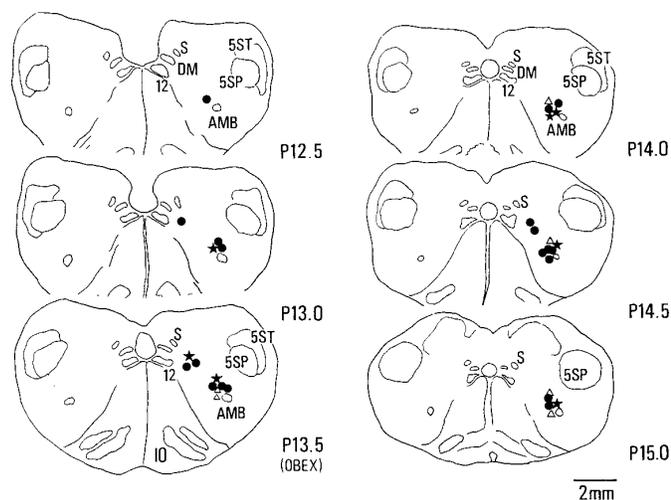


FIG. 8. Schematic drawings of coronal sections of the brain stem, showing locations of neurons recorded in the present study. ●, type-A neurons (single projection neurons that showed a burst activity during fictive ingestion); Δ, type-B neurons (single projection neurons that showed no activity during fictive ingestion); ★, type-C neurons (dual projection neurons that kept silent during fictive ingestion). AMB, nucleus ambiguus; DM, dorsal motor nucleus of the vagus; S, solitary tract; 5SP, spinal trigeminal nucleus; 5ST, spinal trigeminal tract; 12, hypoglossal nucleus.

ity could not be ruled out that the single projection neuron projects to the ipsilateral PH Mns because the projection to the contralateral spinal cord was only examined in the present study.

*Patterns of activity of subpopulations of XII premotor neurons during fictive ingestion and rejection*

Identification of motor activity elicited by repetitive SLN stimulation and water application to the pharynx before the animal was immobilized was somewhat difficult to interpret. Nevertheless, it is highly likely that the ingestive response with coactivation of the GG, the geniohyoid, and the styloglossus muscles represents buccopharyngeal phase of swallowing (Doty and Bosma 1956). What kind of motor act that accompanied spasmodic EMG activity in the tongue muscles as well as the diaphragm by applying a volume of water to the pharynx represent? We believe that it represents rejection (gape), because the animal shows a large mouth opening with a retrusive movement of the tongue with the tip pointing downward (DiNardo and Travers 1994; Travers and Norgren 1986). After the animal was immobilized, a volume of water applied to the pharynx elicited a rejection response followed by two episodes of ingestive response (Fig. 5). We believe that the rejection response indicates a fictive gape, because the PH nerve activity maintained a ramp-shaped discharge characteristic of respiration as opposed to other expulsive oropharyngeal movements such as coughing, vomiting, and sneezing (DiNardo and Travers 1994; Widdicombe 1986; Widdicombe et al. 1988).

Single projection neurons were subdivided into two types based on their behavior during fictive ingestion. Type-A neurons showed a burst activity during fictive ingestion, whereas type-B neurons did not. Alternatively, all the recorded type-C neurons kept silent during fictive ingestion. Thus the type-A neurons are likely to be involved in the central pattern generation of ingestion as well as respiration. They participate in switching from respiration to ingestion by converting their activities from respiratory-related activities to ingestion-related ones. With regard to the response of the type-A neuron during ingestion by the two means of induction, there was no significant difference. A given type-A neuron always showed a burst activity during fictive ingestion regardless of how it was induced. On the contrary, the type-B neurons are involved in the central pattern generation of respiration, but not of ingestion. Tomomune and Takata (1988) studied intracellular activity of XII Mns during fictive swallowing in the cat. They found excitatory postsynaptic potentials that were followed by inhibitory postsynaptic potentials in some GG Mns and excitatory postsynaptic potentials in some styloglossus Mns. The type-A neurons may participate in eliciting these excitatory postsynaptic potentials. None of type-C neurons showed activity during fictive ingestion, suggesting that they do not appear to be involved in ingestion.

During swallow-breath, which is not typical ingestion because of the presence of PH nerve coactivation, a different behavior from that during typical ingestion was observed. One of the 19 type-A and 1 of the 7 type-C neurons showed a burst activity during swallow-breath. Nine episodes of swallow-breath were observed in four of six type-B neurons.

Among these four type-B neurons, three type-B neurons showed a burst activity during fictive swallow-breath, whereas one type-B neuron showed no burst activity. This suggests that the central motor commands related to swallow-breath is relayed by both the type-A and type-C neurons as a population. On the other hand, the type-B neurons may not be involved in swallow-breath as a population. This assumption should be justified by further studies with a larger sample size.

All of the six tested type-C neurons showed a burst activity during fictive rejection as well as inspiration, indicating that they were involved in the central pattern generation of rejection as well as respiration. During rejection, the type-C neurons may receive a rejection-related patterned input from the CPG for rejection and send their output simultaneously to both the XII Mns innervating the GG muscle that protrude the tongue to secure the patent upper airway and to PH Mns to contract the diaphragm to reject the aversive stimulus (Milano et al. 1992; Monges et al. 1978). In contrast, among the eight tested single projection neurons, all of five type-A neurons and all of three type-B neurons showed a burst activity during fictive rejection. This suggests that as a population both the type-A and the type-B neurons may participate in rejection. If this is the case, the type-A neurons may project to the XII Mns that are involved in producing the inverse behavioral functions, ingestive and rejection responses. Alternatively, the type-B neurons may project to the XII Mns that are involved in rejection but not in ingestion. This assumption is in accord with the existence of multifunctional neuronal network serving distinct functions such as breathing, coughing, and swallowing (DiNardo and Travers 1994; Gestreau et al. 1996).

The pattern of response of given XII premotor neurons during both fictive ingestion and rejection was the same, irrespective of the method of inducing these activities. This may indicate that central motor commands with regard to ingestion and rejection were already preprogrammed and issued to the three types of premotor neurons.

*Relationship between subpopulations of XII premotor neurons and XII Mns*

Among extrinsic tongue muscles innervated by XII Mns, the GG, the styloglossus, and the geniohyoid muscles exhibit rhythmical discharges coincident with the inspiratory phase, whereas the hyoglossus muscle is more or less active during expiration in the cat (Horner 1996; Ono et al. 1990). On the other hand, during swallowing the styloglossus and the geniohyoid muscles are active, whereas the hyoglossus muscle is inactive (Fig. 1). Several EMG investigations in humans have demonstrated that the GG muscle participates in swallowing (Cunningham and Basmajian 1969; Lowe et al. 1977; Vitti et al. 1975). However, it is still controversial whether the GG muscle is obligatory for swallowing in animals. It was reported that the GG muscle was generally inactive during swallowing in the sheep (Amri et al. 1989). Miller and Bowman (1974) suggested that GG motor units were recruited by the respiratory CPG, and intermittently by the swallowing CPG. Because the GG muscle contains muscle fibers that run spokewise in the tongue, different parts of the GG muscle that are innervated by different subsets

of the GG Mns (Sauerland and Harper 1976) may serve differently in several respiratory-related activities. The EMG activity may not always represent the activity of a single muscle because the activity recorded from a given electrode site is indicative of the electrical activity of muscle fibers in the region of the electrode (English and Weeks 1989). Variations in experimental technique such as the difference in locations of electrode placement in the GG muscle may account for some of the different findings with respect to involvement of the GG muscle in swallowing. Although the present study has not been able to determine to which XII Mns the recorded inspiratory neurons project, the type-A neurons may be assumed to project to those XII Mns that innervate tongue muscles involved in both ingestion and rejection as well as inspiration, i.e., tongue muscles such as the GG, the geniohyoid, and the styloglossus muscles. In contrast, both the type-B and type-C neurons may project to the XII Mns innervating tongue muscles participating in rejection and inspiration, i.e., a part of the GG muscle that does not participate in ingestion. However, because the size of our sample is small, the patterns of projection and the possible functional differences between the type-C neurons and previously reported bulbospinal inspiratory neurons as well as between the type-A and type-B neurons require further investigation.

#### *Functional significance of XII premotor neurons with inspiratory-related activity*

The type-A neurons ceased their inspiratory-related rhythmic activities at the onset of SLN stimulation, and kept silent during repetitive SLN stimulation until the advent of fictive ingestion. It can therefore be assumed that the type-A neurons are inactivated oligosynaptically by SLN stimulation, by their postsynaptic inhibition (Ballantyne and Richter 1991) and/or by their disfacilitation due to abolition or decrease in excitatory inputs from the respiratory CPG, which is polysynaptically inactivated. This inactivation may be more or less sustained during repetitive SLN stimulation. In addition, afferent impulses evoked by SLN stimulation would activate the swallowing CPG that is postulated to be located in the hindbrain (Jean 1972a,b; Jean and Car 1979; Kessler and Jean 1985; Miller 1982; Roman 1986). When the activity of the CPG for ingestion reaches a certain level, patterned excitatory commands for ingestion may be issued to the type-A neurons, resulting in a transient burst activity in XII Mns unaccompanied by the PH Mn activity. This assumption is in accord with the respiratory-related intracellular activity of XII Mns during repetitive SLN stimulation in the cat (Withington-Wray et al. 1988). They reported depolarization of the XII Mn in the absence of the PH nerve activity. It is also in agreement with the simultaneous record of jaw movements and muscle activities, in which the interaction between respiratory and ingestion CPGs has been shown (McFarland and Lund 1993). Larson et al. (1994) demonstrated that some of the respiratory neurons in the ventral medulla were involved in coordination between swallowing and vocalization. They classified respiratory neurons by patterns of discharge; however, whether these neurons were interneurons or motoneurons was not studied. Recently, Gestreau et al. (1996) recorded intracellular potentials from

inspiratory neurons in the NTS region during fictive coughing and swallowing. Although some of them were bulbospinal premotor neurons, their possible projection to medullary motoneurons including XII Mns were not studied. With regard to the activity of XII Mns during ingestion and rejection, DiNardo and Travers (1994) have shown functional overlap between subsets of XII Mns producing ingestion and rejection, and consequently, they have emphasized the involvement of premotor substrates in the medullary reticular formation in the production of ingestion and rejection.

Although the exact details of the neuronal mechanisms and organization of CPGs or the mode of their interactions are not clear yet, we conclude that the excitatory XII premotor neurons with inspiratory-related activity receive activating commands from the respiratory CPG. In addition, some of them receive temporary commands from the CPGs for ingestion and/or rejection. These neurons would participate in switching the inverse behavioral functions of the tongue.

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