Discharge Patterns of Hypoglossal Motoneurons During Fictive Breathing, Coughing, and Swallowing

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Roda, Fabrice, Christian Gestreau, and Armand Louis Bianchi. Discharge patterns of hypoglossal motoneurons during fictive breathing, coughing, and swallowing. J Neurophysiol 87: 1703–1711, 2002; 10.1152/jn.00347.2001. We performed a series of experiments to study the intracellular activity of 58 hypoglossal motoneurons (HMs) in decerebrate, paralyzed, and ventilated cats. Changes in membrane potentials (MP) and discharge activities were evaluated during fictive breathing (FB), swallowing (FS), and coughing (FC). FS and FC were elicited by electrical stimulation of the superior laryngeal nerves. FB, FS, and FC all exhibited characteristic discharge patterns of the phrenic, abdominal, pharyngeal branch of the vagus, and hypoglossal nerves. Thirty-nine HMs displayed respiratory modulation, and 19 were nonrespiratory modulated. Nine HMs did not exhibit MP changes during FB, FS, and FC. During FS, 49 HMs exhibited MP changes consisting of depolarization, hyperpolarization or hyperpolarization-depolarization. HMs involved in FS were either respiratory modulated (n = 38) or not (n = 11). Only 20 HMs displayed MP changes and/or discharge activity during FC. All but two HMs fired during the expiratory phase of FC or at the end of this reflex. All HMs involved in FC (n = 20) were also modulated during both FB and FS. Our results suggest that the XIIth nucleus is functionally divided into common and distinct subsets of HMs based on their spontaneous activities and responses observed during FS and FC. The changes in MP and discharge frequencies observed during the three behaviors also suggest that HMs are driven by specific premotor neurons during FS, whereas a common premotor pathway is involved during FB and FC.

INTRODUCTION

Little is known about the central mechanisms that drive upper airway motoneurons in response to sensory stimuli. How does the CNS generate different motor output behaviors involving the same group of muscles, according to the tasks to be served? Animal models used to study fictive behaviors provide a good opportunity to begin to understand the complexity of central mechanisms, allowing stable neuronal recordings in the absence of movement-related afferent feedback. For example, the synaptic drives to laryngeal motoneurons were recently analyzed during multiple behaviors using intracellular recordings in decerebrate, paralyzed and ventilated cat (Gestreau et al. 2000; Shibata et al. 1999). In the present study, we focused on the central command received by hypoglossal motoneurons (HMs) during fictive breathing (FB), swallowing (FS), and coughing (FC).

The tongue is involved in various basic motor controls including food intake, mastication, and swallowing. It also plays a major role in vocalization (Zhang et al. 1994). The tongue is also active in respiration and plays a crucial role in the control of upper airway aperture (Van Lunteren and Dick 1997). It is composed of intrinsic muscles (longitudinal, transverse, and vertical) concerned with its shape and of extrinsic muscles concerned with its protrusion and retraction (Lowe 1980). The genioglossus (GG) is the main tongue protrusor while the styloglossus (SG), the hyoglossus (HG), and the geniohyoid (GH) may be considered as the main retractors and the thyrohyoid (TH) as an elevator.

These extrinsic tongue muscles contract in various combinations, either synergistically or antagonistically, depending on the required movement. During inspiration, airway patency is increased due to increased activity of the GG (Bonora et al. 1985). During swallowing, respiration is inhibited to prevent aspiration of food into the airways, and the tongue propels the bolus of food toward the alimentary canal (Doty and Bosma 1956). The GH and TH act in synergy to close the laryngeal vestibule and elevate the entire larynx, allowing the upper esophageal sphincter to be opened (see references in Umezaki et al. 1998b). In contrast, the roles of the various tongue muscles during cough are not well documented.

Previous studies show that HMs do not constitute a homogeneous population but are made up of distinct pools based on their morphology (Withington-Wray et al. 1988); the topographic organization of the XIIth nucleus is related to the different tongue muscles innervated by HMs (Dobbins and Feldman 1995; Fay and Norgren 1997; Travers et al. 1995). Several groups have characterized the discharge pattern of the hypoglossal nerve or HMs during breathing (Hwang et al. 1983a; Pierefiche et al. 1997; Umezaki et al. 1998a; Withington-Wray et al. 1988), swallowing (Amri et al. 1991; Tomomune and Takata 1988), or various combinations of behaviors (Dick et al. 1993; Dinardo and Travers 1994; Hayashi and McCremon 1996; Ono et al. 1998a; Satoh et al. 1998; Travers and Jackson 1992; Umezaki et al. 1998a). Because the discharge patterns of HMs during FB, FS, and FC are unknown, we analyzed MP changes and discharge frequencies of HMs.
during FB, FS, and FC to determine if common subsets of HMs are activated during multiple behaviors. Both qualitative and quantitative results of this study may be important for understanding the central organization of these behaviors and allow inferences about the activity of premotor neurons.

**Methods**

**Experimental animals**

Animal experiments were carried out in accordance with the European Community Council Directive (86/660/EEC) as well as the French law. Nineteen adult cats of either sex, weighing between 2.5 and 3.5 kg, were housed in temperature-controlled rooms with food and water available ad libitum. They acclimatized for ≥15 days before experimentation.

**Surgical procedures**

The various experimental procedures have been detailed elsewhere (Gestreau et al. 1996, 2000). Briefly, the animals were initially anesthetized with an intramuscular injection of 1.5 ml/kg of a mixture of Alfaxalone and Alfadone acetate (9 and 3 mg/ml, respectively; Saffan, Schering-Plough Ltd.) and then maintained at a surgical level of anesthesia using a mixture of 1.0–2.5% halothane (Fluothan, Coopers) in room air. The trachea, femoral vein, and artery and the bladder were cannulated. The external carotid arteries were ligated. The hypoglossal and superior laryngeal nerves (SLN) of both sides were dissected free from surrounding tissues, placed on bipolar silver electrodes, secured to the muscles, and isolated with a piece of Parafilm covered with a mixture of petroleum jelly (Vaseline) in mineral oil. The animals were then placed in a stereotaxic frame and decerebrated at the mid-collicular level. The right C4 phrenic and left L1 or L2 abdominal nerves were dissected and cut on a dorsolateral approach; both nerves were placed and cut on bipolar silver electrodes immersed in pools of mineral oil. After an occipital craniotomy was performed, the caudalmost aspect of the cerebellum was gently retracted. Anesthesia was discontinued and the animals were paralyzed using galamine triethiodide (Flaxedil, intravenous, supplemented as required) and artificially ventilated. Movements of the brain stem were reduced by a bilateral pneumothorax, and a positive end-expiratory pressure of 2–4 cm H2O was applied to prevent collapse of the lungs. Tidal volume and pump frequency were set (typically 20–30 ml, 25–30/ min, respectively) to find the end-tidal CO2 threshold, typically ≤5% (range: 4.5–5.5%), which elicited respiratory modulation of both hypoglossal and lumbar nerve activities, evident on the traces of integrated activity (time constant, 100 ms). In several animals, ventilator settings were satisfactory when weak respiratory modulation was present in hypoglossal nerve activity despite the absence of expiratory modulation of lumbar activity (e.g., Fig. 5A). Because the vagi were intact, stroke volume and frequency were adjusted to prevent synchronization of central respiratory rhythm to lung inflation. These conditions yielded a robust phrenic discharge (good signal-to-noise ratio, frequency and amplitude) and maintained the animals in normocapnic conditions (alveolar PCO2 between 32 and 39 mmHg). Mean blood pressure was maintained ≥90 mmHg using, if necessary, intravenous administration of metaraminol bitartrate (Aramine). Rectal temperature was maintained at 36–38°C using a servo-controlled heating pad.

**Intracellular and nerve recordings**

Intracellular recordings were made from HMs from the right medulla. The cells were located from 1.0 mm caudal to 1.5 mm rostral to the obex, 0.5–2.0 mm lateral from the midline, and 1.0–2.0 mm below the dorsal surface. Glass microelectrodes with tip diameters of ≤1 μm (impedances typically 15–20 MΩ at 100 Hz) were filled with 3 M KCl to determine if neuronal hyperpolarization observed in some HMs was chloride-dependent. Chloride ions were injected continuously into cells by iontophoresis until a reversal was obtained.

Intracellular potentials were amplified and filtered (DC to 10 kHz) through a high-impedance circuit incorporating capacity compensation and DC offset. For each neuron, the MP was defined as the difference between intracellular and extracellular potentials, using as reference a single grounded Ag-AgCl electrode inserted into the neck muscles. All measurements were corrected, if necessary, by measuring the extracellular potential close to the recorded motoneuron after the microelectrode was withdrawn from the cell. HMs were identified by antidromic potentials in response to stimulation of their axons by electrical stimuli (0.1-ms duration, 2.0–8.0 V) delivered to the ipsilateral hypoglossal nerve using a bipolar silver electrode. Extracellular antidromic conduction distance between the cathode of the stimulating electrode and the recording electrode (4 cm) was estimated by dissection of the hypoglossal nerve between the site of stimulation and the medulla. Nerve activities were recorded from the central end of the right C4 phrenic root, left L1 or L2 lumbar (iliohypogastric), pharyngeal branch of the vagus, and left hypoglossal nerve using bipolar silver electrodes. Nerve activities were amplified and filtered (band-pass 0.01–10 kHz). Data were simultaneously displayed on a chart recorder (Gould TA-2000) and oscilloscopes and stored on video tape after digital conversion (Neurocor DR-890, sampling frequencies of 11 kHz for nerve recordings and 22 kHz for intracellular recordings) for subsequent analysis.

**Stimulation**

Several periods of iterative stimulation, each lasting 10–20 s, of both SLN nerves were used to test the responses of each HM during FC and FS. These periods were also used to analyze the postsynaptic responses of HMs. The first period of stimulation was delivered at 5 Hz, the second at 10 Hz. When the number of induced reflex activities (FC or FS) was <3, additional stimulation was applied using a broad range of frequencies (2–30 Hz). Each period of stimulation was separated by a recovery of at least five respiratory cycles.

**Laryngeal-induced fictive coughing and swallowing**

Characteristics of FC and FS have been detailed elsewhere (Bolser 1991; Gestreau et al. 1996, 2000; Grèlot and Milano 1991; Grèlot et al. 1992). Briefly, fictive behaviors were typically evoked by electrical stimulation (0.1- to 0.3-ms pulses, 2–5 V) of both SLN, at 2–5 Hz for FC and 10–30 Hz for FS; occasionally, however, both FC and FS were obtained with the same stimulation parameters. FS was characterized by a burst of hypoglossal activity lasting 300–500 ms and coincident with residual phrenic nerve activity lasting ~150 ms, also defined as “phrenic breakthrough” (Jodkowski and Berger 1988). This burst corresponds to the buccopharyngeal stage of swallowing, i.e., the sequential activation of the oro-pharyngo-laryngeal muscles. FC was characterized by enhanced phrenic nerve activity (the inspiratory phase), immediately followed by activity of the abdominal nerve (the expiratory phase) (Bolser 1991; Gestreau et al. 1997; Grèlot and Milano 1991; Grèlot et al. 1992; Shannon et al. 1998).

**Data analysis**

Cells were included in this study when the recording lasted ≥5 min, stable resting MPs and firing rates were observed throughout the recording session, resting MPs were less than −40 mV, and they were tested during both FS and FC. Mean and peak discharge frequencies as well as MP changes were measured during FB, FS, and FC. For each HM, the amplitude and duration of MP changes were measured during each behavior. Five successive respiratory cycles were used to average the respiratory-modulated depolarization and discharge frequencies of phasic HMs. For cells displaying tonic firing, the integrated phrenic nerve activity allowed cycle-triggered histograms to be
constructed, and mean discharge frequencies were compared during the inspiratory and expiratory phases for \( \geq 20 \) respiratory cycles using a paired \( t \)-test. For FS and FC, average values were calculated using all MP changes (depolarization and hyperpolarization) and/or bursts of action potentials produced during episodes of a given behavior. Postsynaptic potentials (PSPs) in response to SLN stimulation were examined in most HMs, digitized (sampling interval, 100 \( \mu \)s) with appropriate software (ComputerScope, RC Electronic), and superimposed or averaged (20–100 sweeps). \( \chi^2 \) tests were used to analyze the distributions of responses according to the level of resting MPs of HMs and compare the patterns of responses during multiple behaviors (FB and FS, FB and FC, and FS and FC). Comparisons of averaged MP values, duration of MP changes, and mean and peak discharge frequencies during different behaviors were performed using a one-way ANOVA. When appropriate, the Fisher’s protected least-significant difference (PLSD) post hoc test was used to define which group contributed to the statistical differences measured by the ANOVA. In addition, correlations between MP changes and the resting MPs of HMs were evaluated using linear regression analyses. Averaged data are expressed as means \( \pm \) SE. Differences were considered statistically significant at \( P < 0.05 \). All statistical comparisons were made with Statview for Windows. Mean and peak discharge frequencies during FB, FC, and FS were analyzed using P-Clamp6 software (Axon Instruments), and graphical representations were made with Excel and Powerpoint software (Microsoft).

**RESULTS**

Data were obtained from 58 HMs. During FB, HMs were inspiratory-modulated (I), expiratory-modulated (E), or non-respiratory-modulated (No-FB). During FS, HMs were depolarized (D), hyperpolarized (H), hyperpolarized-depolarized (HD), or displayed no response (No-FS). During FC, HMs exhibited two types of responses (type 1 and type 2) or did not change their MPs (No-FC). Resting MPs of HMs averaged \(-54.7 \pm 0.8 \) mV (range \(-42 \) to \(-75 \) mV). Because intrinsic properties might have been altered in cells with depolarized resting MP and depolarization may have affected MP trajectories during FB, FS, and FC, we compared the qualitative responses of HMs (Table 1) with resting MP greater (\( n = 42 \)) or less than (\( n = 16 \)) \(-50 \) mV (Mifflin 1997). We observed similar distributions of membrane potential changes in HMs during FB (I, E, and No-FB), FS (D, HD, H, and No-FS), and FC (type 1, type 2, and No-FC) regardless of resting MP (\( \chi^2 \) tests; Table 1). Therefore data from the two groups of cells were pooled for subsequent analysis.

**Comparison of axonal conduction velocities**

Axonal conduction velocities of HMs averaged 33.9 \( \pm \) 1.2 m/s (range 22.2–57.1). The data were tested for normality and were considered to follow a Gaussian distribution. Values of conduction velocities of the I, E, and No-FB HMs averaged 34.6 \( \pm \) 1.5 (range 22.2–50.0), 37.1 \( \pm \) 1.6 (range 28.6–50.0), and 30.7 \( \pm \) 2.6 m/s (range 22.2–57.1), respectively. These values did not differ statistically. Similarly, axonal conduction velocities from HMs that responded during FS or FC did not differ from those measured in No-FS or No-FC HMs, respectively.

**Synaptic responses to SLN stimulation**

Postysynaptic responses to SLN stimulation were studied in 47 HMs, which exhibited MP changes during FS or FC. In 43 HMs, these responses consisted mainly of long-duration (40–100 ms) inhibitory postsynaptic potentials (IPSPs) preceded in 26 HMs by a brief (\( \sim 10 \) ms) excitatory postsynaptic potential (EPSPs). The other four HMs exhibited EPSPs of \( \sim 100 \) ms in duration in response to SLN stimulation. The IPSPs could be reversed by iontophoresis of chloride ions in three of five HMs (see Fig. 1E), indicating the involvement of a chloride-dependent mechanism. No attempt was made to fully analyze the effect of stimulation intensity on the synaptic responses of HMs as done by Mifflin and collaborators (1997). However, we noted that shocks applied at high frequencies induced an attenuation of IPSP amplitudes in 20 HMs in which a broad range of stimulation frequencies was applied (see METHODS). In addition, increases in the amplitudes of EPSPs were observed in HMs that exhibited EPSP-IPSP sequences (data not shown).

**Spontaneous activity of HMs**

The discharge patterns of the 58 HMs during FB are provided in Table 2. Nineteen HMs were not recruited during FB (Fig. 1A). Of the 26 HMs with inspiratory modulation (I HMs), 19 had phasic MP changes with (\( n = 14 \); Fig. 1B) or without (\( n = 5 \); Fig. 4A) firing and 7 had tonic firing with weak or no changes in MP. Depolarization in phasic I HMs averaged 7.8 \( \pm \) 0.9 mV (range, 3.1–15.1 mV). Data from both tonic and phasic I HMs were pooled for analysis of discharge frequencies. Mean and peak discharge frequencies of I HMs averaged 25.6 \( \pm \) 2.7 (range, 7.8–49.9) and 44.5 \( \pm \) 5.4 spikes/s, respectively. All inspiratory-modulated HMs (E HMs, \( n = 13 \)) displayed phasic MP changes associated with phasic (\( n = 4 \); Fig. 1C) or tonic (\( n = 9 \); Fig. 5A) firing. Depolarization in E HMs averaged 6.1 \( \pm \) 0.8 (range, 2.1–11.5) mV, a value not statistically different from that measured in phasic I HMs. Data from both tonic and phasic E HMs were pooled for analysis of discharge frequencies. Mean and peak discharge frequencies of E HMs averaged 38.7 \( \pm \) 2.7 (range, 21.8–48.7) and 72.4 \( \pm \) 5.3 spikes/s.

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<th>Fiber</th>
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<td>No-FB</td>
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1. I, E, and No-FB refer to inspiratory, expiratory, and non-respiratory-modulated hypoglossal motoneurons (HMs), respectively. Depolarized (D), hyperpolarized (H), hyperpolarized-depolarized (HD), and no response (No-FS) refer to the different responses of HMs observed during fictive swallowing (FS). Type 1, type 2, and No-FC refer to the types of responses observed during fictive coughing (FC). See the text for precise descriptions of patterns. FB, fictive breathing; MP, membrane potential.

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TABLE 2. Spontaneous activities of HMs as related to their activity in FS and FC

<table>
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<tr>
<th>Spontaneous Activities</th>
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<td></td>
<td>D</td>
<td>HD</td>
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<tr>
<td>Type 1 in FC</td>
<td>9</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Type 2 in FC</td>
<td>0</td>
<td>7</td>
<td>2</td>
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<tr>
<td>No-FC</td>
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n = 26, 13, and 19 for I, E, and No-FB, respectively.

FIG. 1. Changes in membrane potential (MP) of hypoglossal motoneurons (HMs) in relation to fictive breathing (FB; A–C), and during electrical stimulation of the XIIth nerve (D) or the superior laryngeal nerves (E). A: HM with no respiratory-modulated MP. B: Inspiratory HM with MP depolarization and firing associated with phrenic nerve activity. C: Expiratory (or postinspiratory) HM with an abrupt MP depolarization and decrementing firing associated with the postinspiratory phase of FB. D: Electrical stimulation of the XIIth nerve elicited antidromic spikes (bottom) or no response (collision test, top) when an orthodromic spike occurred shortly before the antidromic stimulation. E: 3 superimposed responses of an HM to superior laryngeal nerves stimulation before (top) and after (bottom) chloride injection by iontophoresis. This motoneuron exhibited a short excitatory postsynaptic potential (EPSP) followed by a long inhibitory postsynaptic potential (IPSP). Chloride injection reversed the IPSP to an EPSP, indicating the involvement of a chloride-dependent mechanism. PHR, phrenic nerve; XII, hypoglossal nerve; ABD, abdominal nerve.

Range, 51.3–96.0 spikes/s, respectively. Values of mean and peak discharge frequencies of E HMs were significantly greater (P < 0.01 and P < 0.05, respectively) than those of I HMs. Linear regression analysis revealed no correlation between resting MPs of respiratory HMs (I and E) and their amplitudes of depolarization during FB.

Activity of HMs during FS

Apart from nine HMs exhibiting no change in MP (No-FS), three types of responses were distinguished during FS (n = 49, Table 1). Most HMs (n = 34) were subjected to a bell-shaped depolarization (D, Fig. 2B) in phase with the hypoglossal burst. Depolarization of D HMs during FS averaged 11.9 ± 0.7 (range, 4.2–21.1) mV, lasted 482 ± 241 (range, 290–800) ms, and was associated with firing (Figs. 2B and 4B). Mean and peak discharge frequencies of D HMs averaged 61.0 ± 2.4 (range, 40.0–84.3) and 91.9 ± 5.1 (range, 39.6–152.4) spikes/s, respectively. The second type of response (in 6 HMs) was a hyperpolarization (H, Fig. 2C) that averaged 5.7 ± 0.7 (range, 2.9–7.2) mV and lasted 310 ± 229 (range, 200–680) ms. Reversal of swallowing-induced hyperpolarization to depolarization (Fig. 2, E and F) was tested and obtained in five HMs by intracellular iontophoresis of chloride ions, indicating the involvement of a chloride-dependent inhibitory mechanism. The third type of response (in 9 HMs) consisted of a hyperpolarization concomitant with burst activity in the hypoglossal nerve followed by an abrupt depolarization associated with firing (HD, Fig. 2D). Hyperpolarization of HD HMs averaged 4.5 ± 0.4 (range, 2.6–8.2) mV and lasted 400 ± 120 (range, 260–690) ms and did not differ from that observed in H HMs. Depolarization of HD HMs averaged 5.3 ± 0.5 (range, 3.3–12.2) mV, lasted 300 ± 196 (range, 210–500) ms, and gave rise to firing activity with mean and peak discharge frequencies of 56.4 ± 3.1 (range, 34.5–82.1) and 90.6 ± 6.5 (range, 70.4–128.6) spikes/s, respectively. When compared with the values measured in HD HMs, D HMs exhibited larger (P < 0.01) depolarizations, whereas mean and peak discharge frequencies did not differ. Linear regression analysis showed a significant correlation (P < 0.05) between the resting MPs of HMs activated during FS (D + HD) and their amplitudes of depolarization. In addition, HD HMs had more hyperpolarized resting MPs than D, H, or No-FS HMs (P < 0.01 for all comparisons).

Activity of HMs during FC

Most HMs (n = 38) did not respond during FC (No-FC). In the remaining HMs (n = 20), two types of responses were distinguished during FC (Table 1). Ten HMs (type 1, Fig. 3A) gradually hyperpolarized during the inspiratory and expiratory phases of FC and depolarized abruptly toward the end of the abdominal nerve discharge. Hyperpolarization of type 1 HMs averaged −3.0 ± 0.5 (range, −1.9 to −5.7) mV and lasted 2,670 ± 134 (range, 2,010–3,150) ms. Depolarization of type 1 HMs averaged 5.8 ± 0.6 (range, 3.0–8.7) mV and lasted 1,237 ± 107 (range, 698–1,690) ms. Mean and peak discharge frequencies associated with this depolarization averaged 36.3 ± 6.5 (range, 29.5–70.1) and 60.5 ± 10.2 (range, 39.1–111.6) spikes/s, respectively. The other 10 HMs (type 2, Fig. 3B) successively hyperpolarized and then depolarized during the inspiratory and expiratory phases of FC, respectively. In this group, however, two HMs also displayed a depolarization...
HMS averaged 42.2 

During FS and FC are detailed in Table 2. Eight HMs exhibited no changes in MP during FB, FS, and FC. Twelve HMs were activated only during one fictive behavior. 18 HMs responded during two fictive behaviors, whereas 20 HMs responded to all three fictive behaviors. Strikingly, most (n = 18) of the latter were I HMs. Also, all HMs involved in FC were respiratory modulated, whereas 11 of 49 HMs involved in FS were not. This indicated significant correlations of responses (P < 0.001 for all comparisons, $\chi^2$ tests) among FB, FS, and FC. In addition, all type 1 HMs exhibited a D pattern during FS (Fig. 4), whereas all type 2 HMs had a H or HD pattern during FS (see Table 2 and Fig. 5).

Comparisons of depolarization and mean and peak frequencies during FB, FS, and FC were made using the values obtained in the 18 HMs that depolarized and discharged during all three behaviors. Depolarization was greater during FS than during FB and FC ($P < 0.001$ and $P < 0.001$, respectively) but did not differ between FB and FC. More specifically, this increase in depolarization during FS was due to D but not to HD HMs. Mean and peak discharge frequencies increased during FS compared with FB and FC ($P < 0.001$ and $P < 0.05$, respectively).

**Comparison of responses during FB, FS, and FC**

Responses of HMs during FB as related to their activities during FS and FC are detailed in Table 2. Eight HMs exhibited no changes in MP during FB, FS, and FC. Twelve HMs were activated only during one fictive behavior. 18 HMs responded during two fictive behaviors, whereas 20 HMs responded to all three fictive behaviors. Strikingly, most (n = 18) of the latter were I HMs. Also, all HMs involved in FC were respiratory modulated, whereas 11 of 49 HMs involved in FS were not.

This indicated significant correlations of responses (P < 0.001 for all comparisons, $\chi^2$ tests) among FB, FS, and FC. In addition, all type 1 HMs exhibited a D pattern during FS (Fig. 4), whereas all type 2 HMs had a H or HD pattern during FS (see Table 2 and Fig. 5).

Comparisons of depolarization and mean and peak frequencies during FB, FS, and FC were made using the values obtained in the 18 HMs that depolarized and discharged during all three behaviors. Depolarization was greater during FS than during FB and FC ($P < 0.001$ and $P < 0.001$, respectively) but did not differ between FB and FC. More specifically, this increase in depolarization during FS was due to D but not to HD HMs. Mean and peak discharge frequencies increased during FS compared with FB and FC ($P < 0.001$ and $P < 0.05$, respectively).
In this study, we analyzed the MP changes and firing activities of HMs during FB, FS, and FC. Various subsets of HMs were distinguished based on their responses during the three fictive behaviors. These responses will be discussed in relation to the relative contributions of both extrinsic inputs and intrinsic properties of HMs.

**FIG. 5.** Comparison of MP changes and discharge frequencies of a HM during FB, FS, and FC. A: this motoneuron depolarized during the postinspiratory phase of FB. B: during FS, the HM hyperpolarized and then depolarized (HD pattern) in parallel with the XIIth nerve discharge (†). This HM exhibited a type 2 pattern during FC with MP trajectories corresponding to a hyperpolarization followed by a depolarization during the inspiratory (I) and expiratory (E) phases of FC, respectively. Note that depolarization in FS was similar to that measured in FB and FC. Compared to values measured during FB (C), peak discharge frequencies (the highest instantaneous frequency within each burst) increased during FS, but were similar during FC (D). Time bar: 2 s. For abbreviations, see Fig. 1.

respectively) but did not differ between FC and FB. These increases were due to the discharge activity of both D and HD HMs.

**DISCUSSION**

In this study, we evaluated the MP changes and firing activities of HMs during FB, FS, and FC. Various subsets of HMs were distinguished based on their responses during the three fictive behaviors. These responses will be discussed in relation to the relative contributions of both extrinsic inputs and intrinsic properties of HMs.

**Functional heterogeneity of HMs in relation to FB, FS, and FC**

MP changes and firing activities of HMs during FB, FS, and FC provide evidence for distinct functional groups within this motor pool. One group of HMs was not recruited during FB, FS, or FC. This subset may correspond to motoneurons involved in oro-motor functions other than those considered herein, such as suckling, licking, or mastication (Amri et al. 1991; Thexton et al. 1998; Travers et al. 1997). Most HMs (39 of 58) fired or displayed MP changes in phase with FB, whereas fewer (19) exhibited no respiratory modulation. This finding corroborates previous observations (Hwang et al. 1983a; Pierrefiche et al. 1997; Umezaki et al. 1998a; Withington-Wray et al. 1988) and indicates that under normocapnic conditions, not all HMs receive input from the respiratory central pattern generator (CPG). However, synaptic drives to HMs are subjected to a state-dependent modulation (Woch et al. 2000; Yamuy et al. 1999), and central respiratory drive potentials are affected by hypercapnia, hypoxia, or anesthesia (Bonora et al. 1984; Hwang et al. 1983b; Lowe 1980; Pierrefiche et al. 1997). Our results confirm previous work showing that most HMs are active in inspiration, although discharges in either the inspiratory-to-expiratory transition or during early expiration (postsinspiratory) have been also described (Hwang et al. 1983a; Lowe 1980; Umezaki et al. 1998a; Withington-Wray et al. 1988). Another group of HMs was only recruited during FS, a result similar to those of several reports showing HM activity specific to swallowing (Amri et al. 1991; Sumi 1969; Tomomune and Takata 1988). Other HMs had MP changes related to FB and FS. This subset of HMs could be driven by both the respiratory and swallowing CPGs, an observation consistent with previous work (Ono et al. 1998a).

Last, some HMs were recruited during the three behaviors. Cells from this subset were mainly I HMs, and exhibited various patterns during nonrespiratory behaviors. This novel finding indicates that they received converging excitatory and inhibitory inputs from the CPGs responsible for breathing, swallowing, and coughing. In addition, the heterogeneity of responses observed in this group resembles that described for other motoneurons innervating respiratory muscles, i.e., phrenic (Grélot et al. 1992) and laryngeal (Gestreau et al. 2000; Shiba et al. 1999). Although this kind of heterogeneity led several authors to consider the laryngeal motoneurons as “multifunctional” (Larson et al. 1994; Shiba et al. 1999; Yajima and Larson 1993), the various responses of I HMs observed in the present study is likely explained by the diversity of the motor tasks in which the tongue is involved and is consistent with their serving as the “final common pathway.”

Multifunctional properties of neurons have been demonstrated in invertebrates (Meyrand et al. 1991, 1994), and the results of several studies led to proposals that neurons with similar functions exist in mammals, although it must be stressed that this term is applied to neurons thought to participate in the generation of several motor functions rather than to the motoneurons themselves (Gestreau et al. 1996, 1997, 2000; Grélot and Bianchi 1996; Grélot et al. 1996; Shannon et al. 1996, 1998, 2000).

**Synaptic inputs in FB, FS, and FC vs. intrinsic properties of HMs**

Important intrinsic properties such as resistance and rheobase were not measured in this study. However, axonal conduction velocities and resting MPs provided indirect measures of the passive properties of the recorded HMs and allowed us to gauge the relative contributions of intrinsic properties to the various responses observed during FB, FS, and FC. HMs had a unimodal distribution of axonal conduction velocities, and no differences were found between HMs with distinct responses during FB, FS, and FC. This suggests that the functional heterogeneity of HMs is unrelated to differences in soma size. Also, the qualitative responses of HMs were not dependent on the initial level of resting MP, indicating that apparently func-
tionally equivalent motoneurons were recorded at different resting MPs. However, a correlation was found between the resting MPs of HMs and the amplitudes of depolarization only during FS not during FB or FC. This suggests that the patterns of depolarization observed during FS resulted from a combination of both intrinsic properties and extrinsic inputs, whereas intrinsic properties contributed less to the responses of HMs during FB and FC. An important determinant of firing properties of HMs is the $I_h$ current, an inwardly rectifying cationic current activated on hyperpolarization from resting MP (Bayliss et al. 1994; Berger 2000). Thus the $I_h$ current may have contributed to the changes in MP in HD HMs during FS. Furthermore, the amplitude of depolarization of HD HMs during FS was less than that in D HMs, although both types of HMs exhibited similar discharge frequencies. Therefore the $I_h$ current may also participate in the increase in discharge frequency of HD HMs during FS.

**Premotor command of HMs in relation to FB, FS, and FC**

Patterns of MP changes reveal the central drive received by HMs, thus allowing inferences about the activity of premotor neurons (Gestreau et al. 2000; Grélot et al. 1992; Shiba et al. 1999). Three patterns of responses were observed during FS, suggesting that HMs were differentially driven by the swallowing CPG. Therefore in addition to the coordination of the activity of several motor nuclei during FS (Jean 2001), these data and others from laryngeal motoneurons (Gestreau et al. 2000; Shiba et al. 1999) provide evidence for a divergence of synaptic inputs from the swallowing CPG within the same motor nucleus. The premotor neurons involved in FS are located in medullary regions surrounding the nucleus tractus solitarius (NTS) and the nucleus ambiguus (Amri and Car 1988; Amri et al. 1990; Cunningham and Sawchenko 2000; Fay and Norgren 1997; Kessler and Jean 1985; Ono et al. 1998b; Sang and Goyal 2001; Travers and Norgren 1983). Inhibitory premotor neurons to HMs activated by stimulation of SLN afferents are located in the region around the hypoglossal nucleus including the Roller nucleus (Ono et al. 1998b). Such premotor neurons may be responsible for both the SLN-evoked IPSPs and the inhibitory drive observed in HMs during FS. In contrast, the origin of the excitatory synaptic inputs from the swallowing CPG to HMs is unclear (Kessler 1993; Ono et al. 1994). Two subsets of HMs were recruited during both FB and FS, illustrating the complex organization necessary to coordinate breathing and swallowing (Dick et al. 1993; Jean 2001). Hypoglossal premotor neurons exhibiting inspiratory modulation also exist in the region surrounding the NTS (Ono et al. 1994). However, whether or not the same premotor hypoglossal neurons display respiratory- and swallowing-related activities remains an open question. HMs received a stronger excitatory drive during FS than during FB or FC, suggesting that the excitatory premotor pathway from the swallowing CPG to the XIIth nucleus is independent of that used for breathing or coughing. However, previous studies suggest the existence of common premotor elements shared by respiratory and swallowing CPGs (Bianchi and Grélot 1994; Gestreau et al. 1996).

All HMs active in FC also displayed respiratory-modulated activity. In addition, both discharge frequencies and depolarization amplitudes during FC were similar to those observed in FB. These results suggest that the respiratory and coughing CPGs share the same premotor neurons to the XIIth nucleus, an idea consistent with the view that the respiratory CPG is reconfigured to produce the cough motor pattern (Grélot and Bianchi 1996; Oku et al. 1994; Shannon et al. 1996, 1998). Indeed, premotor neurons to phrenic (Gestreau et al. 1996; Oku et al. 1994; Shannon et al. 1996) and laryngeal (Baekey et al. 2001) motoneurons are also activated during FC. However, some premotor neurons may be involved only in FC. This conclusion is supported by recent findings suggesting specific involvement in FC of neurons within nonrespiratory areas (Gestreau et al. 1997) and by the demonstration that the cough reflex can be abolished without major effects on breathing pattern (Jakus et al. 2000). Another striking observation is that most inspiratory HMs that responded during FC were activated during the expiratory but not the inspiratory phase of FC. Interestingly, external intercostal muscles exhibit similar switches in their phase-relation to diaphragmatic discharge in the transition from breathing to coughing (Iscoe and Grélot 1992). Therefore if breathing and coughing CPGs share the same premotor pathways, this converse phase-relation should also be present at the level of the premotor neurons to the hypoglossal and intercostal motoneurons. This hypothesis remains to be investigated.

**Functional implications**

The cells of the present study were recorded in the middle of the XIIth nucleus, a region known to contain HMs innervating intrinsic and several extrinsic tongue muscles such as protrators and retractors (Dobbins and Feldman 1995; Fay and Norgren 1997; Travers et al. 1995). Therefore the HMs characterized by their different responses during FB, FS, and FC may innervate distinct tongue muscles.

HMs depolarized during FS may innervate tongue retractor; retraction during swallowing ensures propulsion of the food bolus (Amri et al. 1989; Jean 2001). The SG is activated during inspiration (Fregosi and Fuller 1997; Fuller et al. 1998), is inactive during the inspiratory and expiratory phases of cough (Satoh et al. 1998), and HMs innervating the SG depolarize during FS (Tomomune and Takata 1988). Thus I HMs with D pattern during FS and type 1 response during FC may innervate the SG.

The GG, the main protrusor of the tongue, is active during eupneic breathing and contributes to the maintenance of airway patency (Adachi et al. 1993; Fregosi and Fuller 1997; Fuller et al. 1998; Lowe 1980; Van Lunteren and Dick 1992). HMs innervating this muscle exhibit a sequence of depolarization-hyperpolarization during swallowing (Tomomune and Takata 1988). We hypothesize that I HMs with either H or HD pattern during FS innervate the GG, although the pattern of MP changes observed during FS differed from that described by Tomomune and Takata. The reasons for these differences remain unclear.

In conclusion, we have provided a detailed analysis of changes in MP and discharge frequencies of HMs during FB, FS, and FC. According to these changes, we divided HMs into subsets that may be functionally different. Our results suggest that HMs are driven by specific premotor neurons during FS, and intrinsic properties of these HMs influence the changes in MP and discharge activities observed during this behavior.
contrast, we hypothesized that extrinsic inputs are responsible for the changes in MP and firing rate observed during FB and FC, and a common premotor pathway is involved in these responses.

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REFERENCES


