Activity of bulbar respiratory neurons during fictive coughing and swallowing in the decerebrate cat

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1. The behaviour of medullary respiratory neurons was studied during fictive coughing and swallowing evoked by electrical stimulation of the superior laryngeal nerve (SLN) in decerebrate, paralysed and artificially ventilated cats. Fictive coughing, swallowing and respiration were monitored by recording activities of the phrenic, hypoglossal and abdominal nerves.

2. Extracellular recordings were made from respiratory neurons in the ventral respiratory group (VRG) and in the Bötzinger complex (BOT). The neuronal types analysed included decrementing inspiratory neurons (I-DEC), augmenting expiratory neurons (E-AUG) and decrementing expiratory neurons (E-DEC) from the BOT area, and augmenting inspiratory neurons (I-AUG) and augmenting expiratory neurons (E-AUG) from the VRG area.

3. During fictive coughing, all the inspiratory and expiratory neurons were active during the inspiratory and expiratory phases of coughing, respectively. The firing of both I-DEC and I-AUG neurons was increased and prolonged in association with the augmented inspiratory activity of the phrenic nerve. The activity of E-AUG neurons of the VRG did not parallel the abdominal nerve activity, suggesting the existence of additional neurons which participate in the generation of abdominal nerve activity during fictive coughing.

4. During fictive swallowing, half of I-DEC neurons fired transiently at the onset of hypoglossal bursts associated with swallowing; the firing was suppressed during the rest of the hypoglossal bursts. Other I-DEC neurons were silent during hypoglossal bursts. Some I-AUG neurons fired during the initial half of hypoglossal bursts, and others were silent. The brief phrenic activity accompanying the swallowing might have originated from this activity in I-AUG neurons. The discharges of all E-AUG neurons (BOT and VRG) and the majority of E-DEC BOT neurons were suppressed during swallowing.

5. We conclude that these five types of respiratory neurons of the BOT and VRG are involved in the generation of the spatiotemporally organized activity of coughing and swallowing, and that at least a part of the neuronal network for respiration is shared by networks for these non-respiratory activities.

Coughing and swallowing are closely related to respiration. We wished to determine to what extent medullary respiratory neurons are involved in these non-respiratory activities. Previous studies have shown that medullary respiratory neurons discharge and therefore participate in coughing (Jakuš, Tomori & Stránsky, 1985; Shannon, Morris & Lindsey, 1992) and swallowing (Hukuhara & Okada, 1956; Sumi, 1963; Umezaki, Hori & Kiyohara, 1988). Our understanding of medullary respiratory neurons, in particular their firing patterns, locations, projections, and interconnections, has improved since the reports of Hukuhara & Okada (1956) and Sumi (1963). Numerous recent reports have indicated that respiratory neurons with specific firing patterns in the Bötzinger complex (BOT) and the ventral respiratory group (VRG) play major roles in respiratory rhythm generation and pattern formation (Cohen, 1979; Feldman, 1986; Richter, Ballantyne & Remmers, 1986; Ezure, 1990).

In the light of these recent reports, information on the types of respiratory neurons involved in coughing and swallowing in the previous studies is insufficient.

Coughing and swallowing are initiated by activity in laryngeal afferents (Widdicombe, 1964; Tomori & Widdicombe, 1969). Electrical stimulation of the superior laryngeal nerve (SLN) is a useful method for studying these reflexes in animal experiments. A brief pulse train (100 Hz) applied to the SLN elicits inspiration-to-expiration phase switching (Iscoe, Feldman & Cohen, 1979), whereas continuous pulses at a lower frequency (3–30 Hz) evoke swallowing (Doty, 1951; Miller & Loizzi, 1974; Amri, Car & Jean, 1984) and coughing (Mori & Sakai, 1972; Iscoe & Grélot, 1992), as well as prolonged apnoea (Remmers, Richter, Ballantyne, Rainton & Klein, 1986). Recent studies have introduced methods to evoke fictive coughing (Grélot & Milano, 1991; Bolser, 1991), fictive swallowing (Nishino, Honda, Kohchi, Shirahata & Yonezawa, 1985; Dick, Oku, Romaniuk & Cherniack, 1993), or both (Grélot, Milano, Portillo, Miller & Bianchi, 1992) in paralysed and artificially ventilated animals. Since the use of paralysed and artificially ventilated animals is critical to the study of medullary neurons, these recent developments have facilitated our study of the behaviour of respiratory neurons during these reflexes.

In the present study, we analysed the behaviour of five specific types of respiratory neurons during coughing and swallowing. Respiratory neurons analysed included decrementing inspiratory (I-DEC) neurons of the BOT and VRG, augmenting inspiratory (I-AUG) and augmenting expiratory (E-AUG) neurons of the VRG, and E-AUG and decrementing expiratory (E-DEC) neurons of the BOT. All of these neurons are important members of the respiratory neuronal network that controls premotor neuronal activity (Feldman, 1986; Ezure, 1990). Laryngeal and pharyngeal motoneurons are located in the ambiguus and retrofacial nuclei and their locations overlap with the VRG and BOT. These motoneurons also have respiratory rhythm and discharge with various timings during both coughing and swallowing (Kawasaki, Ogura & Takenouchi, 1964*a*, *b*). However, we excluded such neurons from the present analysis, since our interest was in the respiratory neurons that are thought to participate in the generation of respiratory rhythm.

METHODS

General procedures

Experiments were conducted on seven adult cats of either sex. The animals were initially anaesthetized with thiopentone sodium (Ravonal, 25 mg kg^{-1}, I.P.) and maintained with halothane (0·5–3·0%) in 50% nitrous oxide and 50% oxygen. Dexamethasone (1 mg kg^{-1}, I.M.) and atropine (0·1 mg kg^{-1}, I.M.) were administered to minimize brain oedema and to reduce secretion in the airways, respectively. The trachea was intubated, and cannulae were placed in the femoral artery to monitor blood pressure and in the cephalic vein for drug administration. The animals were positioned in a stereotaxic frame, initially in the supine position. Both the medial and lateral branches of the hypoglossal nerve on one side were cut distally and mounted in a bipolar cuff electrode for recording and stimulation. Similar bipolar cuff electrodes were attached to the cut superior laryngeal nerves (SLN) on both sides for stimulation. The exposed hypoglossal and SLN nerves were covered with petroleum (Vaseline) jelly, a thin plastic film, and the skin flaps. The animals were then rotated to the prone position and supported by hip pins and a clamp on an upper thoracic vertebra. The head of the animal was slanted downward 30 deg from the Horsley-Clarke horizontal plane to facilitate recording from the medulla.

Intercollicular decerebration was performed after craniotomy and bilateral ($n = 1$) or unilateral ($n = 6$) ligation of the common carotid arteries. The brain rostral to the transection was destroyed by suction and anaesthesia was discontinued. The dorsal surface of the medulla was exposed for recording and stimulation by cerebellectomy. A pair of stainless-steel needles, insulated except for the tips, were fixed bilaterally in the ventrolateral quadrant (laterality, 2 mm; depth, 3 mm) of the C4–C5 spinal cord for monopolar stimulation. The C5 phrenic and L1 abdominal muscle nerves were cut distally, mounted on bipolar recording electrodes, and immersed in oil pools. The animals were paralysed with pancuronium bromide (Mioblock, 0·15 mg kg^{-1} h^{-1}) and artificially ventilated at a fixed volume and frequency with positive end-expiratory pressure (1–2 cmH_2O). Blood pressure was monitored and kept above 80 mmHg; pressor agents (Carnigen, Hoechst, 0·05 ml kg^{-1}; Inovan, Kyowa, 5 μg kg^{-1} min^{-1}) were intravenously administered when necessary. Tracheal pressure, end-tidal CO_2 (4–5%), and rectal temperature (36–37 °C) were monitored.

Recording, stimulation and data processing

Activities from the phrenic, abdominal and hypoglossal nerves were amplified, full-wave rectified, and low-pass filtered (τ, 10 ms; cut-off frequency, 16 Hz). Brainstem neuronal activity was recorded extracellularly with glass micropipettes filled with 3 M KCl solution saturated with Fast-Green FCF dye (DC impedance, 1–1·2 MΩ). Similar glass electrodes with a lower impedance (about 0·8 MΩ) were used for stimulation of the brainstem. Points of stimulation as well as locations of neurons were occasionally marked with dye for later histological examination.

Fictive coughing and fictive swallowing were evoked by electrical stimulation of the SLN with continuous trains of constant current pulses (0·15 ms duration). We used stimulus frequencies arbitrarily between 10 and 30 Hz. The intensity of the continuous stimulation was 2–3 times the threshold current above which brief stimulation of the SLN (10 pulses at 100 Hz) delivered during mid-inspiration reversibly inhibited the central inspiratory activity (as assessed by phrenic nerve discharge). The threshold current was usually less than 50 μA. Responses to single-shock stimulation of the SLN were studied by applying stimuli (2–3 times the threshold) during control respiration at intervals of 400–500 ms. To examine suppression of firing locked to the stimulation, sweeps in

which stimulation was delivered during an active period of each neuron were selected and superimposed. When more than ten such sweeps were available, we estimated the approximate latency of the suppression of firing.

Spike histograms (see Fig. 11) were constructed by simultaneously sampling discriminated neural spikes (digital) and integrated hypoglossal activity (analog) at 2 ms intervals. Spike signals were further binned (40 ms width). Traces of both spike and hypoglossal activity were averaged over several consecutive hypoglossal bursts of swallowing. Most data were stored on magnetic tape (PCM recorder: RP-882S, NF Electronic Instruments, Tokyo). The digitization (sampling rate, 70 μs) of the signal transferred to the PCM recorder contributed to the variations in spike height in the figures.

Identification of respiratory neurons

Neurons were recorded in the vicinity of the retrofacial nucleus and the nuclei ambiguus and retroambigualis. Not all of the recorded neurons were marked by dye; instead the recording and stimulation sites were identified empirically as follows. The BOT can be identified by the existence of the field potential elicited by antidromic activation of SLN motoneurons (Ezure, 1990; Jiang & Lipski, 1990) and by the presence of E-AUG neurons by which the BOT itself is defined (Merrill, Lipski, Kubin & Fedorko, 1983). Most neurons of the BOT so defined were sampled in an area more than 3·5 mm from the medullary surface. The VRG rostral to the obex was characterized by a dense assembly of I-AUG neurons, many of which were antidromically activated from the spinal cord. The neurons of the VRG were sampled in an area more than 3 mm ventral to the medullary surface. The VRG caudal to the obex was represented by an assembly of E-AUG neurons along a longitudinal column extending from the obex to 5 mm caudal to the obex and about 3 mm lateral to the mid-line. Most neurons of the VRG caudal to the obex were sampled at a depth of 2–3·5 mm from the dorsal surface. At the level of the obex, the DRG was located 1·5–2·0 mm lateral to the mid-line and 1·5–2·5 mm ventral to the dorsal surface. Furthermore, stimulating electrodes were sometimes switched from stimulation to recording to confirm the characteristic respiratory activity of the above-mentioned areas. Respiratory neurons were classified based on their firing patterns; the terminology of this classification was adopted from our previous studies (see Ezure, 1990).

All I-DEC and E-DEC neurons were tested for their projections to the areas of medullary respiratory neurons, particularly the DRG and VRG, using an antidromic electrical stimulation technique (e.g. Oku, Tanaka & Ezure, 1992). The maximum current used was 150 μA. Collision tests were employed to confirm antidromic activation. Only those antidromically activated from the brainstem on either side were analysed. Axonal arborization was evident as abrupt changes in antidromic latencies accompanying displacement of the stimulating electrode in adjacent tracks or within a track (Oku *et al.* 1992). The projections to the spinal cord were tested (maximum stimulus current, 1 mA) for all neurons, but no attempts were made to stimulate selectively the axonal pathways specific for coughing in the spinal cord (Newsom Davis & Plum, 1972) by moving the stimulating electrodes. For I-AUG and E-AUG neurons of the VRG, only those antidromically activated from the spinal cord were analysed. Axonal projections of the respiratory neurons to the vagal or glossopharyngeal nerves were not tested except for projections to the SLN.

RESULTS

SLN-evoked fictive coughing and fictive swallowing

Continuous stimulation of the SLN at a current just above the threshold for inspiratory inhibition (see Methods) reduced both the amplitude of the phrenic nerve activity and the respiratory frequency, but did not activate either fictive coughing or fictive swallowing (hereafter the term 'fictive' will not necessarily be affixed). With increased stimulus current, coughing and swallowing were activated by stimulation of the SLN on either side (Figs 1 and 2). A current intensity of twice the threshold for inspiratory inhibition was sufficient to activate coughing and swallowing.

Coughing was characterized by increased and prolonged phrenic activity which was suddenly suppressed and overtaken by bursting activity of the abdominal nerve (Bolser, 1991; Grélot & Milano, 1991). During this phrenic augmentation, hypoglossal activity increased simultaneously, and abdominal nerve activity increased to some extent prior to the burst activity (coactivation of expiratory and inspiratory nerves) (Figs 1*A* and 13*A*). This coactivation was not observed when the levels of abdominal activity was low.

Swallowing was characterized by a large amplitude burst of hypoglossal activity lasting for 400–700 ms; we regarded this burst activity as an indication of swallowing. During hypoglossal bursts, both phrenic and abdominal nerve activities were suppressed. When swallowing was evoked in the expiratory phase of coughing, the increased abdominal activity of coughing was also suppressed (Fig. 2*B*). In general, swallowing was evoked repetitively during continuous SLN stimulation (Doty, 1951; Miller, 1972; Dick *et al.* 1993).

Swallowing was frequently accompanied by a brief burst of phrenic nerve activity (Figs 1 and 2), which corresponds to the minor diaphragmatic movement known as 'Schluckathmung' (Marckwald, 1889) or 'swallow-breath'. Swallow-breaths were observed in all cats: it accompanied 90–100% of swallowing in four cats and about 60% in the others. Swallow-breaths started after the onset of hypoglossal bursts and occurred either with (Fig. 1*B* and *C*) or without (Fig. 1*B*) preceding inspiratory activity of the phrenic nerve. In the former case, the swallow-breath was separated from the preceding phrenic activity by a brief quiescent period as shown in Fig. 1*B*

and C. This type of swallow-breath always occurred when the inspiratory activity was terminated by swallowing (6 cats). This swallow-breath, however, was not post-inspiratory activity because it only occurred with swallowing (Fig. 1C): note that the second phrenic burst in Fig. 1C was not accompanied by a brief phrenic burst separated by a gap.

When both respiration and swallowing were observed during continuous SLN stimulation, there was a coupling between the two (Fig. 2). In the present experiments with intact vagal afferents, this coupling was usually entrained by periodic lung inflation (Fig. 2A). However, phasic lung inflation was not necessary for the occurrence of swallowing, respiration, coughing or swallow-breath, or for the interactions between them (Fig. 2B).

Neural responses during coughing and swallowing

Seventy-eight medullary respiratory neurons were selected for the present analysis and their behaviour during fictive coughing and swallowing was studied. Their short-latency responses to single-shock stimulation of the SLN were also studied. The neuronal types analysed included: (1) twenty-two I-DEC neurons recorded from the BOT and the rostral part of the VRG, (2) fifteen I-AUG neurons from the VRG rostral to the obex, (3) nine E-DEC neurons from the BOT ($n = 8$) and the VRG caudal to the obex ($n = 1$), (4) eighteen E-AUG neurons of the BOT, and (5) fourteen E-AUG neurons from the VRG caudal to the obex. Recording sites of thirteen neurons were identified after histological processing. Figure 3 shows the locations of nine neurons in the caudal part of the BOT.

All I-DEC neurons were antidromically activated by electrical stimulation of at least one site in the DRG or VRG on either side (contralateral, $n = 20$; ipsilateral, $n = 2$). In sixteen I-DEC neurons, including an ipsilaterally activated one, evidence of axonal arborization in the DRG or VRG was obtained (see Methods). None was antidromically activated from the spinal cord. All I-AUG neurons studied were antidromically activated from the spinal cord. All E-DEC neurons were antidromically activated by electrical stimulation of at least one site in the DRG or VRG on either side (contralateral, $n = 7$; ipsilateral, $n = 2$). Five E-DEC neurons, including an ipsilaterally activated neuron, showed evidence of axonal arborization in the DRG or VRG. None were antidromically activated from the spinal cord. Ten of the E-AUG neurons of the BOT were antidromically activated from the spinal cord. All E-AUG neurons of the VRG were antidromically activated from the spinal cord (Fig. 13C).

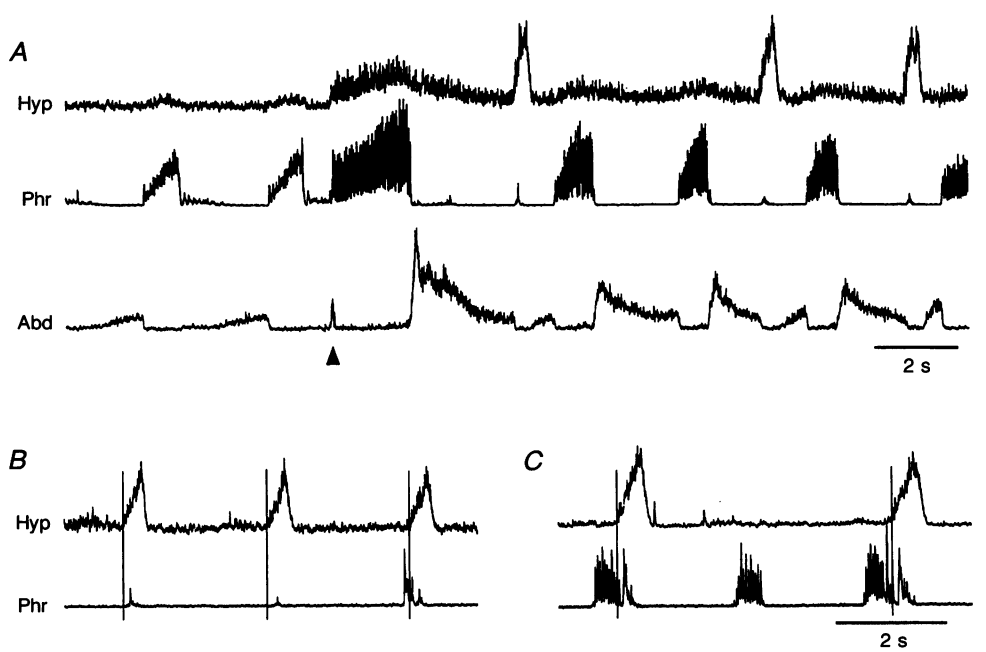

Figure 1. Fictive coughing, swallowing and swallow-breath (Schluckathmung)
The continuous stimulation of the SLN (onset indicated by arrowhead) lasted beyond the exnd of traces. *A*, coughing was followed by repetitive hypoglossal bursts (swallowing) during expiration. *B*, three consecutive hypoglossal bursts were accompanied by swallow-breaths (asterisks in Fig. 2A); the third burst was preceded by prematurely terminated inspiratory activity of the phrenic nerve. *C*, two hypoglossal bursts occurred at the end of inspiration and were accompanied by swallow-breaths. The second phrenic burst had no post-inspiratory activity. Vertical lines mark the onset of hypoglossal bursts. Hyp, hypoglossal; Phr, phrenic; Abd, abdominal nerve activity.

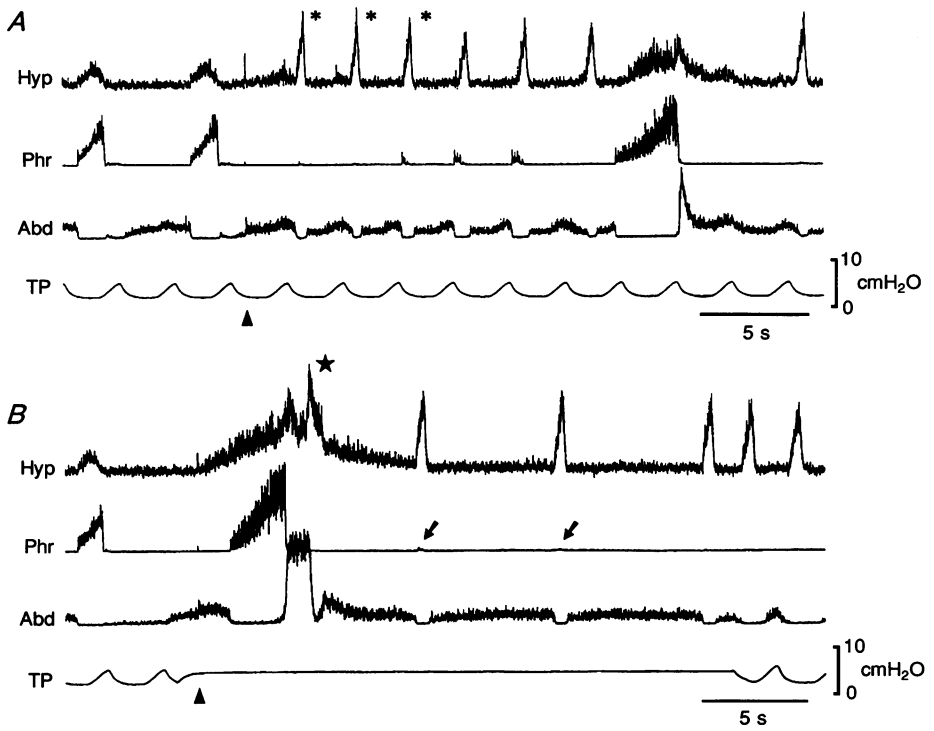

Figure 2. Coupling between swallowing, respiration and lung inflation
A, continuous SLN stimulation (onset at arrowhead) evoked swallowing first and then coughing. Repetitive hypoglossal bursts were entrained by lung inflation. *B*, the ventilator was stopped and then SLN stimulation was started at the arrowhead. During the course of coughing, a swallowing burst (star) suppressed abdominal activity. Arrows in *B* show swallow-breaths. Initial three swallowing bursts in *A* (asterisks) are enlarged in Fig. 1*B*. TP, tracheal pressure.

Details of neural responses to coughing and swallowing are described in the following sections. In brief, all the inspiratory and expiratory neurons were active during the inspiratory and expiratory phases of coughing, respectively. The duration of firing of expiratory neurons varied among neurons during the expiratory phase of coughing. During swallowing, half of the inspiratory neurons fired; the remaining inspiratory neurons and most expiratory neurons did not fire or their firing was suppressed. Single-shock stimulation of the SLN (see Fig. 7) either suppressed or did not affect the firing of most neurons. The responses to swallowing and single-shock stimulation of the SLN are summarized in Table 1.

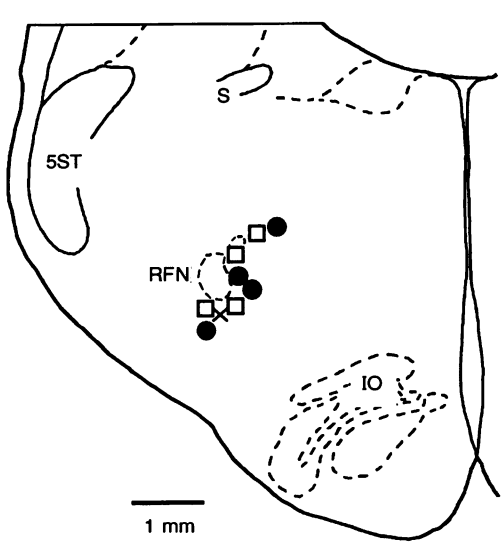

Figure 3. Distribution of respiratory neurons
Nine neurons were located based on dye marks and projected on a transverse plane at the caudal level of the retrofacial nucleus. ●, decrementing expiratory neurons (E-DEC); ×, augmenting expiratory neuron (E-AUG); □, decrementing inspiratory neurons (I-DEC); IO, inferior olivary nucleus; RFN, retrofacial nucleus; S, solitary tract; 5ST, spinal trigeminal tract.

Table 1. Responses of respiratory neurons to single-shock SLN stimulation and swallowing

	I-DEC ($n = 22$)		I-AUG ($n = 15$)		E-DEC ($n = 9$)	E-AUG (BOT) ($n = 18$)	E-AUG (VRG) ($n = 14$)	
	No effect	Misc.	Supp.	Misc.	Supp.	Supp.	Supp.	Misc.
SLN single shock	17	5	11	4	9	18	12	2
Swallowing								
Active	9	2	4	3	2	0	0	0
Inactive	8	3	7	1	7	18	12	2

Misc., miscellaneous responses; Supp., suppression.

I-DEC neurons of the BOT and VRG

Figures 4 and 5 show the behaviour of four I-DEC neurons during coughing and swallowing evoked by continuous stimulation of the SLN. During coughing, their decrementing firing patterns were preserved in most cases, but the firing was extended throughout the augmented inspiratory phase. The firing stopped before the abdominal burst of coughing and remained silent during the subsequent expiratory phase.

During swallowing, two types of responses were observed. Eleven I-DEC neurons fired during hypoglossal bursts (Fig. 4). The other eleven neurons did not fire (Fig. 5). All the swallowing-active neurons discharged briefly at the onset of hypoglossal bursts (Figs 4*A* and 6*C*). The firing started around 100 ms after the onset of hypoglossal bursts, lasted for 200 ms or less, and was then suppressed for the duration of the bursts. In two swallowing-inactive neurons, firing which had persisted during continuous SLN stimulation was suppressed during hypoglossal bursts (Fig. 5*B*).

The firing of swallowing-active neurons did not coincide with the activity of swallow-breath: the firing of swallowing-active neurons consistently preceded the activity of swallow-breath (Fig. 6*C*). When inspiration was terminated by swallowing, the firing of I-DEC

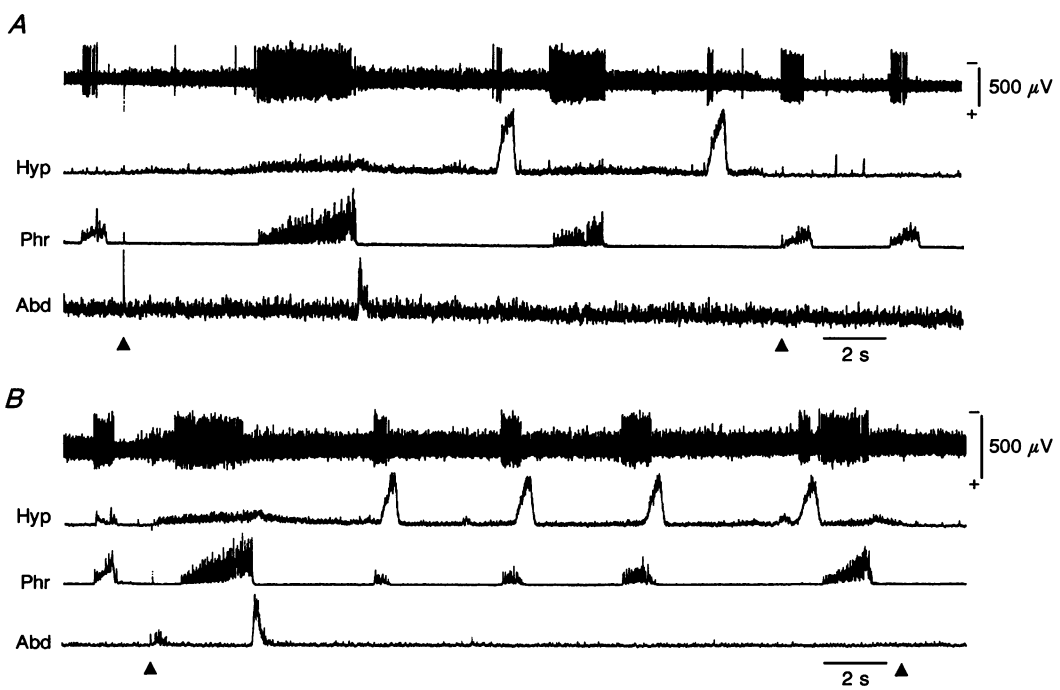

Figure 4. Behaviour of I-DEC neurons during fictive coughing and swallowing
Extracellular activities from two swallowing-active neurons are shown in the top traces of *A* and *B*. Continuous stimulation of the SLN was applied during the period between arrowheads. *A*, two hypoglossal bursts have no preceding phrenic activity. *B*, the first three hypoglossal bursts are associated with prematurely terminated inspiratory activity of the phrenic nerve, and the firing linked to inspiration and that linked to swallowing overlapped.

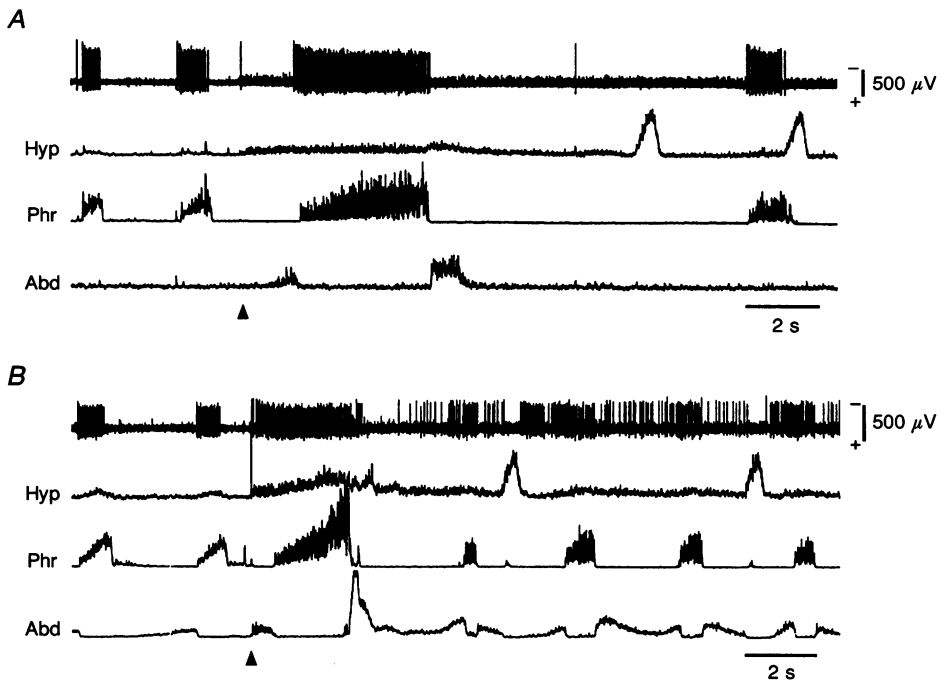

Figure 5. Swallowing-inactive I-DEC neurons
Two I-DEC neurons (*A* and *B*) are shown. *A*, this neuron fired only in the inspiratory phase, but not during hypoglossal bursts. *B*, this neuron was activated by continuous SLN stimulation (onset at arrowheads, continuing beyond the end of traces) and the firing was suppressed during hypoglossal bursts. This neuron was also activated by single-shock SLN stimulation (Fig. 7*A*).

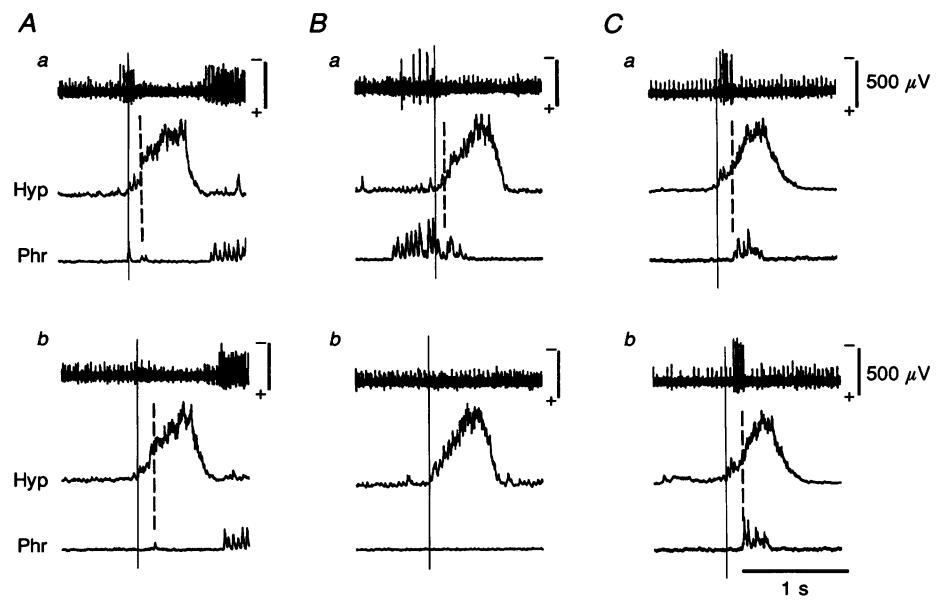

Figure 6. Firing of I-DEC neurons during swallowing with swallow-breath
Two swallowing-inactive I-DEC neurons (*A* and *B*) fired only in association with prematurely terminated inspiratory activity of the phrenic nerve (*Aa* and *Ba*), but not in the absence of such activity (*Ab* and *Bb*). *C*, a swallowing-active neuron at two swallowing events, in which the firing precedes the swallow-breath. Vertical lines represent the onset of hypoglossal burst; vertical dashed lines represent the onset of swallow-breath. Variation in spike height was caused when spikes occurred on the field potentials locked to individual SLN stimulation (see also Figs 9 and 10).

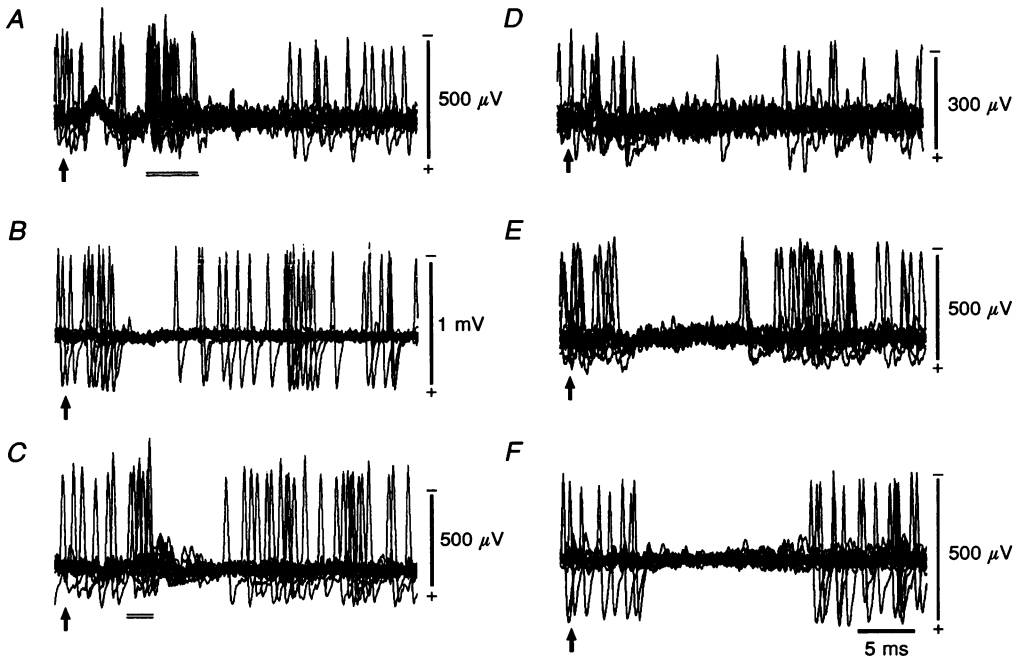

Figure 7. Responses of respiratory neurons to single-shock stimulation of the SLN
SLN stimulation (arrow) was delivered during control respiration. *A*, I-DEC neuron; *B* and *C*, I-AUG neurons; *D*, E-DEC neuron; *E*, E-AUG neuron of the BOT; *F*, E-AUG neuron of the caudal VRG. Each panel consists of 20 sweeps, at least 10 of which were made during the neuron's discharge phase. Double lines in *A* and *C*, facilitation of firing.

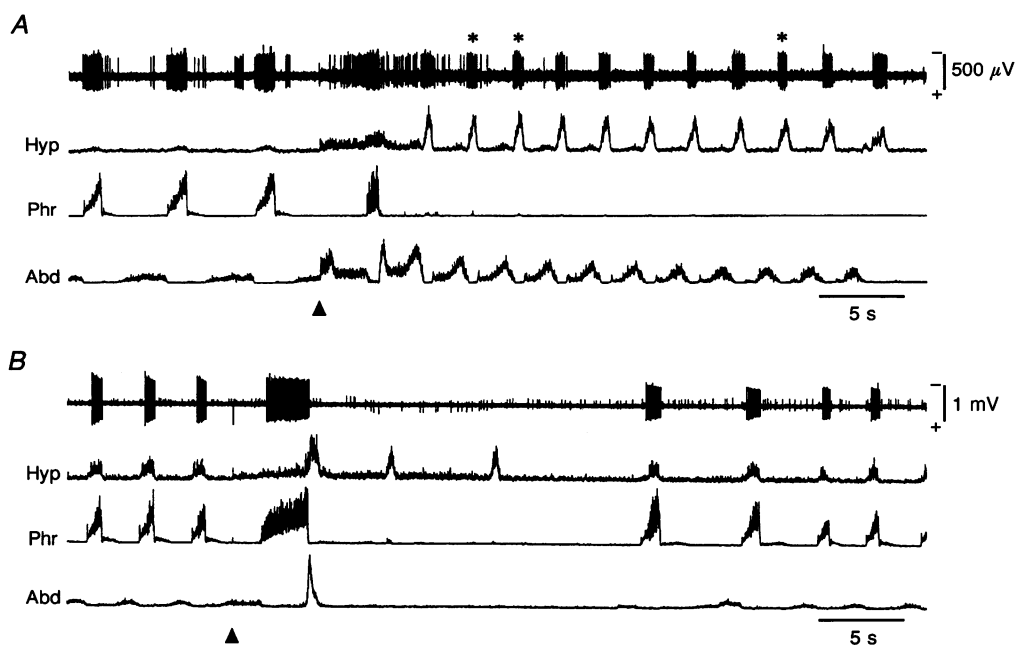

Figure 8. Behaviour of I-AUG neurons of the VRG
A, swallowing-active neuron. *B*, swallowing-inactive neuron. Repetitive hypoglossal bursts in *A* were synchronized with lung inflation. Three bursts (asterisks) are enlarged in Fig. 9*A*.

neurons that started before the onset of hypoglossal bursts consistently corresponded with that of the phrenic nerve (Figs 4*B*, 5*A* and 6*A* and *B*). This type of firing pattern was observed in both swallowing-active and swallowing-inactive neurons. On the other hand, when swallowing was not preceded by phrenic activity, swallowing-active neurons fired after the onset of hypoglossal bursts (Figs 4*A* and *B*, and 6*C*), and swallowing-inactive neurons did not fire at all (Figs 5*A* and 6*A* and *B*).

Single-shock stimulation of the SLN elicited no detectable short-latency effects in seventeen of the I-DEC neurons. In the other five, firing was evoked with latencies ranging from 6·5 to 9·1 ms and then suppressed (Fig. 7*A*). Two of these five neurons were swallowing-active and the other three were swallowing-inactive.

I-AUG neurons of the VRG

During coughing, all I-AUG neurons fired in parallel with the phrenic nerve discharge, i.e. firing increased together with the augmentation of the phrenic nerve discharge, and suddenly stopped at the transition from the inspiratory to the expiratory phase of coughing.

During swallowing, seven neurons were active (Fig. 8*A*) and eight were inactive (Fig. 8*B*). In the swallowing-active neurons, the firing occurred during the augmenting phase of hypoglossal activity, i.e. the firing started after the onset of hypoglossal bursts and generally did not continue through the peak of the hypoglossal bursts. Onset and duration of the firing varied in all swallowing-active neurons. Figure 9 shows two neurons, one with a long duration of the firing (Fig. 9*A*) and the other with a short duration (Fig. 9*B*, rightmost trace) when swallowing was not preceded by inspiratory phrenic activity. When swallow-breaths were observed, the firing occurred during the period of swallow-breath (Fig. 9). Unlike I-DEC neurons, suppression of firing during swallowing was not evident in either swallowing-active or swallowing-inactive neurons.

When inspiration was terminated by swallowing, the firing of I-AUG neurons that started before the onset of hypoglossal bursts was consistently associated with the terminated inspiratory activity of the phrenic nerve (Fig. 9*B*). Both the neuronal firing and phrenic activity that corresponded to the terminated inspiration continued

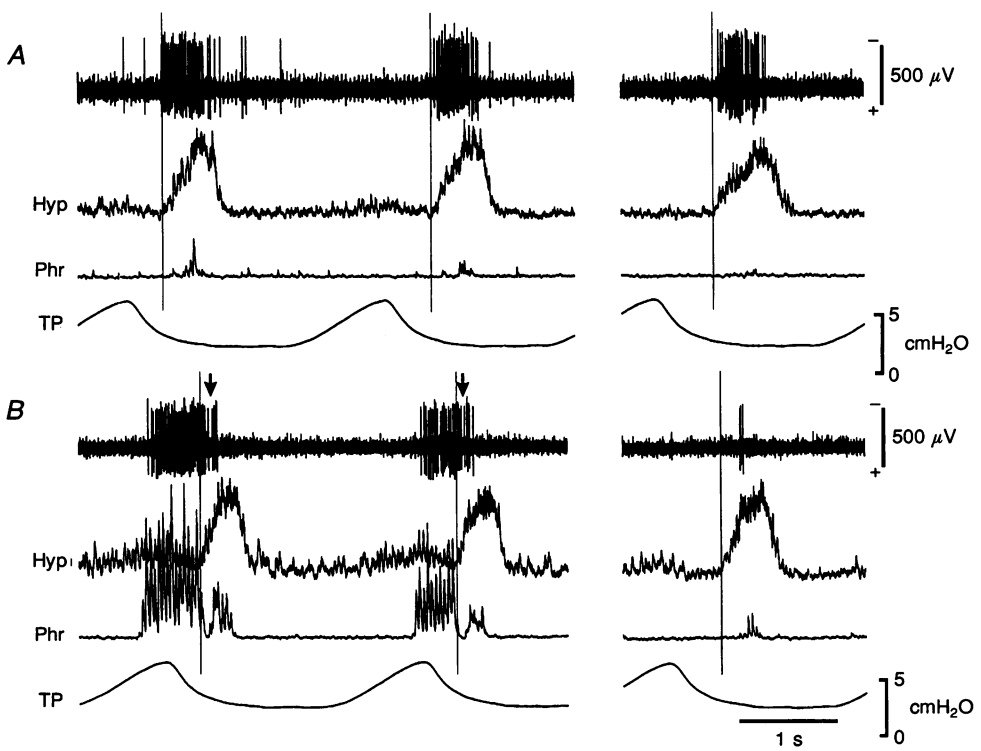

Figure 9. Relation between firing of swallowing-active I-AUG neurons and swallow-breath
Two neurons (*A* and *B*) are shown. *A*, three bursts (from Fig. 8*A*) with small but clear swallow-breaths (the first two) and an unclear swallow-breath (the third). *B*, swallow-breaths with and without preceding inspiratory activity of the phrenic nerve. Firing linked to inspiration and that linked to swallow-breath are consistently separated by transient pauses (arrows). When swallowing was not preceded by inspiratory phrenic activity, neurons had long (*A*) or short (*B*) durations of firing. Vertical lines represent the onset of hypoglossal bursts.

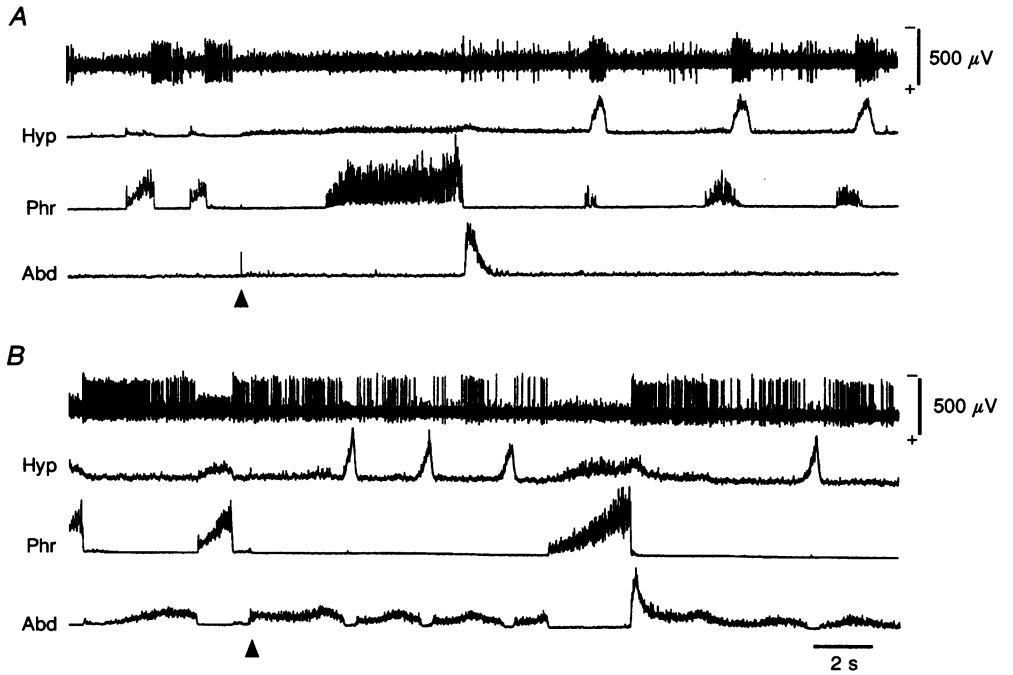

Figure 10. Behaviour of E-DEC neurons of the BOT during coughing and swallowing
Two E-DEC neurons (*A* and *B*) are shown. The neuron in *A* shows less intense firing during coughing than during control respiration. The firing during swallowing occurred at the peak of hypoglossal bursts. *B*, firing during coughing was not clearly changed from that during respiration. Firing was suppressed during hypoglossal bursts (see Fig. 11*A*). Continuous SLN stimulation was started at arrowheads.

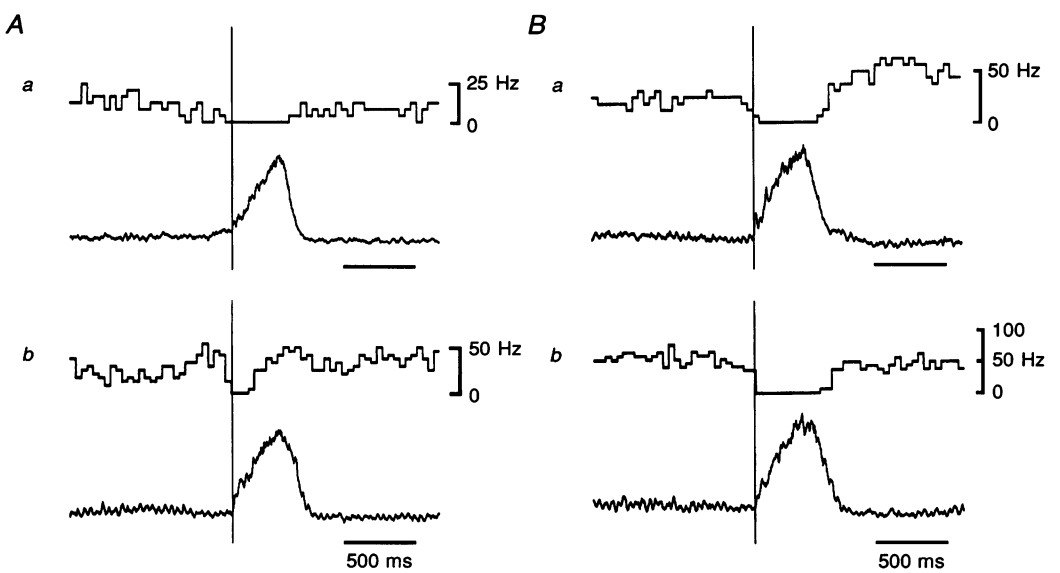

Figure 11. Suppression of firing of E-DEC and E-AUG neurons during swallowing
Spike histograms (upper traces) of swallowing-inactive E-DEC (*Aa* and *Ab*) and E-AUG (*Ba*) neurons of the BOT and E-AUG neuron of the caudal VRG (*Bb*) are shown with integrated hypoglossal nerve activity (lower traces). Six (*Aa* and *Ab*) and four (*Ba* and *Bb*) consecutive cycles were aligned and averaged at the onset of hypoglossal bursts. Firing of some E-DEC neurons was suppressed transiently at the onset of hypoglossal bursts and then recovered during the rest of the burst (*Ab*). In contrast, the firing of all E-AUG neurons of the BOT and VRG was suppressed throughout the hypoglossal burst. The neurons in *Aa* and *Bb* are shown in Figs 10*B* and 13*A*, respectively.

for about 100 ms beyond the onset of hypoglossal bursts (see Fig. 1*C*). In two swallowing-active neurons, a transient decrease in their activity was consistently observed between the firing associated with inspiration and that associated with swallowing (Fig. 9*B*).

Single-shock stimulation of the SLN suppressed the firing of eleven I-AUG neurons (Fig. 7*B*). This suppression started at around 3·5–5·0 ms. In the remaining four, this suppression was absent ($n = 2$) or occurred later ($n = 2$) (Fig. 7*C*); facilitation of firing before the suppression was observed in the case shown in Fig. 7*C*. Three of these four I-AUG neurons were swallowing-active and the other one was swallowing-inactive.

During control respiration, the firing onsets of the I-AUG neurons varied, although their discharges always stopped simultaneously with that of phrenic activity. Five I-AUG neurons were recruited within 100 ms of the onset of phrenic activity (early-recruited, Fig. 8*A*), six neurons were recruited more than 300 ms after the onset of phrenic activity (late-recruited, Fig. 8*B*), and the remaining four fell into the intermediate range. The early-recruited neurons included the four neurons with firing which was not suppressed or was suppressed late by single-shock stimulation of the SLN. Four of the five early-recruited neurons were swallowing-active and five of the six late-recruited neurons were swallowing-inactive: this difference was significant ($P < 0·05$, χ^2 test).

E-DEC neurons of the BOT and VRG

During coughing, all E-DEC neurons fired during the expiratory phase of coughing, but none fired during the preceding inspiratory phase. In three neurons, the firing during the expiratory phase was obviously less than that during the corresponding phase of normal respiration (Fig. 10*A*). In the other neurons, the firing either increased or did not change (Fig. 10*B*).

During swallowing, two neurons were active and the rest were inactive. Firing spanned the peak of hypoglossal bursts (Fig. 10*A*) in one neuron with an ipsilateral axon, and occurred during the initial half of the burst in the other neuron with a contralateral axon. The firing of five swallowing-inactive neurons was completely suppressed during hypoglossal bursts (Fig. 11*A a*) and that of two swallowing-inactive neurons was suppressed transiently at the onset of hypoglossal bursts (Fig. 11*A b*). During coughing two swallowing-active neurons decreased their firing as shown in Fig. 10*A*.

Single-shock stimulation of the SLN suppressed the firing of all E-DEC neurons (Fig. 7*D*). In five E-DEC neurons, the estimated latency of this suppression ranged from 5·0 to 6·0 ms.

E-AUG neurons of the BOT

All E-AUG neurons, both antidromically activated and non-antidromically activated, discharged similarly during coughing and swallowing.

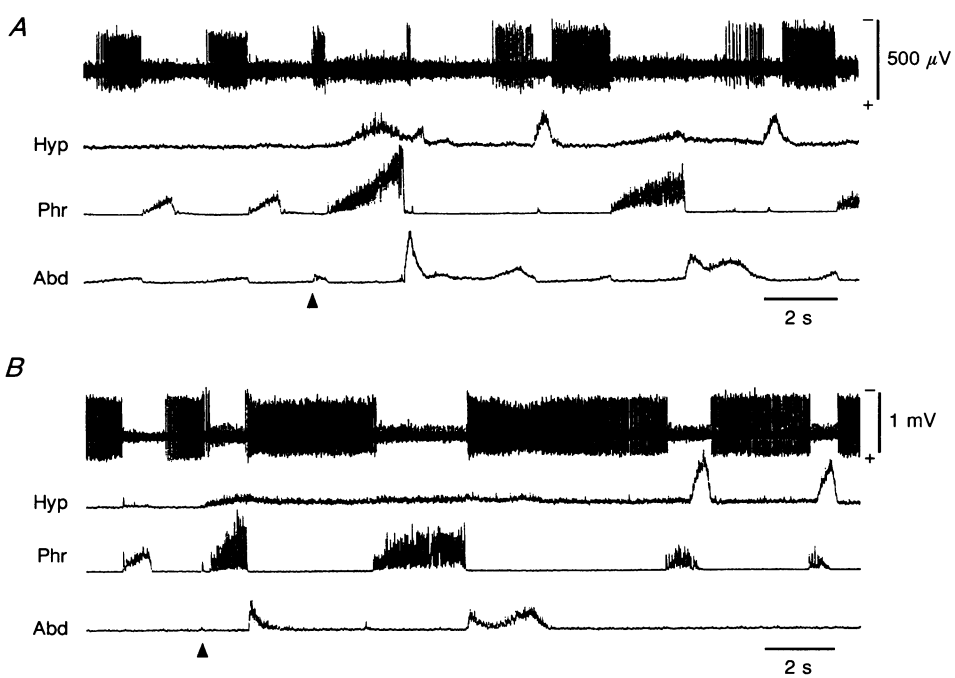

Figure 12. Behaviour of E-AUG neurons of the BOT
During the expiratory phase of coughing, the neuron in *A* fired briefly at the onset of expiration and stopped firing. In contrast, the neuron in *B* fired throughout the expiratory phase, despite low levels of abdominal activity. Firing of both neurons was suppressed during hypoglossal bursts. Continuous SLN stimulation started at arrowheads.

Figure 12 shows the activity of two E-AUG neurons. During coughing, all E-AUG neurons fired during the expiratory phase. Some E-AUG neurons fired only briefly after the inspiratory termination (Fig. 12*A*) and some fired throughout the expiratory phase (Fig. 12*B*). However, the duration of firing during this expiratory phase varied from neuron to neuron but not within a given neuron. Quantitative evaluation of this firing was not made. The firing of all E-AUG neurons was suppressed during hypoglossal bursts of swallowing (Figs 11*Ba* and 12).

Single shock stimulation of the SLN suppressed the firing of all E-AUG neurons (Fig. 7*E*). In four E-AUG neurons, the onset latency of this suppression could be estimated (range: 3·8–5·0 ms).

E-AUG neurons of the VRG

The responses of E-AUG neurons of the VRG to coughing and swallowing were similar to those of E-AUG neurons of the BOT.

During coughing, all E-AUG neurons examined fired during the expiration phase. The firing started after the transition from inspiration to expiration and the extent of firing varied (Fig. 13). Although the activity of the E-AUG neurons paralleled the abdominal activity during control respiration, the firing during coughing was dissociated. None fired during the inspiratory phase (coactivation phase) of coughing in spite of visible abdominal nerve activity (Fig. 13*A* and *B*). Furthermore, either the firing was suppressed at the onset of the expiratory phase of coughing or the firing that had started was suppressed during the continuation of enhanced abdominal nerve activity as shown in Fig. 13*A* and *Ba*. This type of suppression was observed in six neurons. During swallowing, all E-AUG neurons were suppressed in a manner similar to that of E-AUG neurons of the BOT (Fig. 11*Bb*).

Single-shock stimulation of the SLN suppressed the firing of twelve E-AUG neurons (Fig. 7*F*). No effects were observed in the other two neurons. The suppression started between 4·8 and 7·0 ms, as estimated from seven E-AUG neurons.

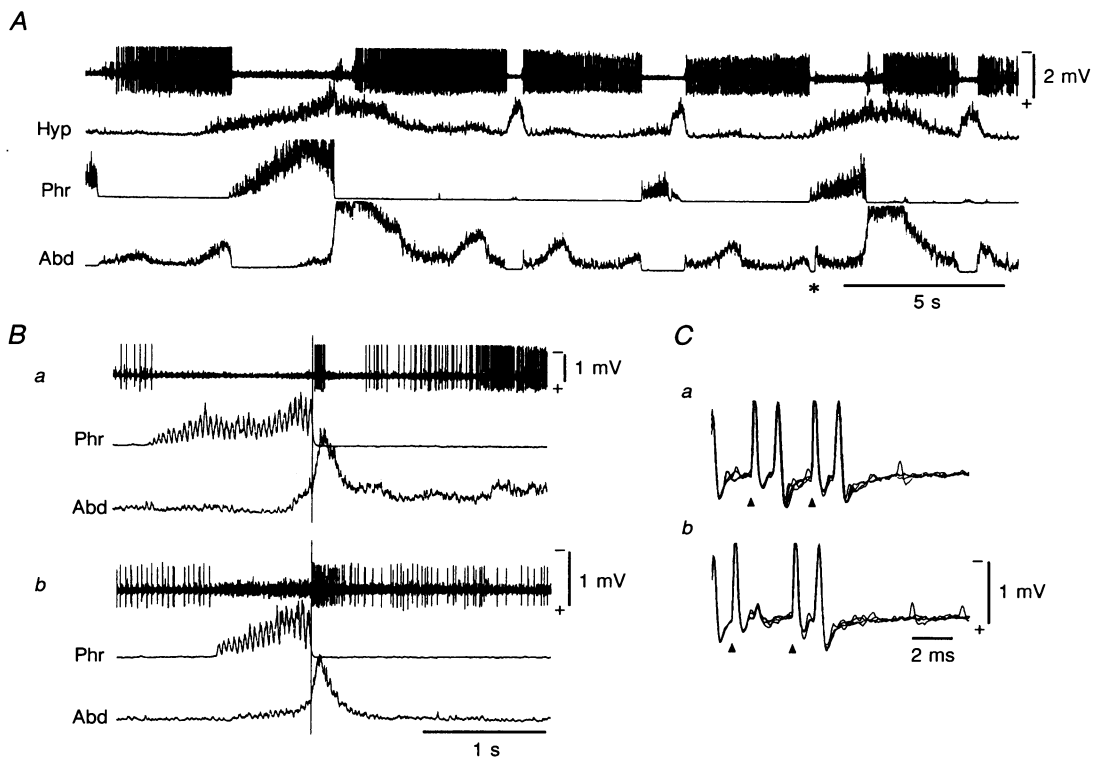

Figure 13. Behaviour of E-AUG neurons of the VRG caudal to the obex
A, firing of this neuron was suppressed transiently for about 300 ms at the onset of expiration. No spikes (initial cough) and only a few spikes (second cough) were seen at the onset of the expiratory phase. Traces in *A* are truncated because of saturation and changes in spike amplitude. Stimulation was switched from the contralateral to the ipsilateral SLN at asterisk. *B*, discharge patterns were dissimilar (*a*) or similar (*b*) to abdominal discharge. Vertical lines indicate the onsets of expiratory abdominal bursts and correspond to the cessation of phrenic discharge. E-AUG discharges started after the phase transition. No E-AUG neurons of the VRG fired during the inspiratory phase in spite of abdominal activity (*A* and *B*). *C*, antidromic activation of the neuron (shown in *Ba*) from the contralateral spinal cord. Double shocks (arrowheads) were applied 2 ms (*a*) and 1 ms (*b*) after spontaneous spikes. The first shock in the lower trace elicited no spikes because of collision.

DISCUSSION

This report is the first to describe the behaviour of the five subtypes of respiratory neurons (I-DEC, I-AUG, E-DEC, and two types of E-AUG) of the BOT and VRG during both coughing and swallowing. During the prolonged and augmented inspiratory phase of coughing, I-DEC and I-AUG neurons discharged with the same patterns as during normal respiration. In contrast, E-AUG neurons of the BOT and VRG started firing at the onset of the expiratory phase of coughing, but their firing did not parallel abdominal nerve activity. During swallowing, activities of E-AUG neurons of the BOT and VRG and most E-DEC neurons were suppressed. During swallowing, half of the I-DEC neurons fired briefly at the onset, and about half of the I-AUG neurons had discharges associated primarily with swallow-breath. The results provide a database for analysing the roles of these respiratory neurons in the generation of coughing and swallowing.

Respiratory neurons analysed

The projection sites of the neurons studied have been elucidated in earlier studies (see Long & Duffin, 1986; Ezure, 1990). I-DEC neurons located in the area from the BOT to the rostral part of the VRG inhibit bulbospinal and propriobulbar inspiratory neurons in the VRG and DRG (Ezure, Manabe & Otake, 1989), as well as bulbospinal expiratory neurons in the caudal VRG and BOT (Ezure, 1990; Ezure, Manabe, Otake & Sasaki, 1990). I-AUG and E-AUG neurons of the VRG are premotor neurons that drive spinal inspiratory and expiratory neurons, respectively (Cohen, 1979; Long & Duffin, 1986). E-AUG neurons of the BOT inhibit a variety of medullary respiratory neurons (Merrill *et al.* 1983; Ezure & Manabe, 1988; Fedorko, Duffin & England, 1989; Jiang & Lipski, 1990) and phrenic motoneurons (Merrill & Fedorko, 1984). E-DEC neurons of the BOT inhibit neurons of the BOT, VRG, DRG and caudal VRG (Ezure & Manabe, 1988; Ezure, 1990).

The locations of the currently sampled neurons suggest that they belong to the same populations previously studied. Most neurons analysed were antidromically activated from the spinal cord or the medulla. Only a few E-AUG neurons of the BOT were not antidromically activated from the spinal cord and were not tested for their medullary projections. However, the BOT itself is characterized by E-AUG neurons and these are probably propriobulbar, based on previous electrophysiological and anatomical studies (Merrill *et al.* 1983; Ezure & Manabe, 1988; Jiang & Lipski, 1990). The similarity of responses to single-shock stimulation of the SLN in this study and those of previous studies (e.g. Jiang & Lipski, 1992) constitutes additional evidence that the neurons in our study belong to the same populations as those previously studied.

Because we did not stimulate the vagal and glossopharyngeal nerves, two possibilities should be considered. First, the neurons antidromically activated from the spinal cord or the medulla may also have axons in the vagal or glossopharyngeal nerves. We believe this is unlikely, because our antidromic mapping and horse radish peroxidase-staining studies of respiratory neurons of the BOT and VRG have never shown such multiple projections (see, for example, Ezure, 1990). Second, some of the antidromic activation from the medulla, especially from the ipsilateral medulla, may have been due to the stimulation of intramedullary axons of the motoneurons by current spread. Although this is possible for the two I-DEC and two E-DEC neurons activated ipsilaterally, this too is unlikely since the sites of activation in the VRG and DRG were sufficiently distant from the neurons (about 3 mm rostrocaudally).

Respiratory neuronal discharges during coughing

Since coughing is a respiration-related activity, the behaviour of respiratory neurons during coughing may not differ greatly from that during normal respiration. As expected, all of the inspiratory neurons fired in the inspiratory phase of coughing and likewise the expiratory neurons in the expiratory phase of coughing, without exception. This is consistent with previous studies in chloralose-anaesthetized (Jakuš *et al.* 1985) and decerebrate cats (Shannon *et al.* 1992). During coughing induced by mechanical stimulation of the tracheobronchial tree, the firing of both inspiratory and expiratory neurons of the medulla is enhanced during their respective phases of coughing (Jakuš *et al.* 1985). This has also been confirmed in bulbospinal neurons and putative propriobulbar neurons of the VRG (Shannon *et al.* 1992). These results, as well as those of the present study, clearly indicate that the respiratory neural network participates in the generation of motor activity during coughing.

I-AUG neurons of the VRG, which are the main sources of excitation to spinal inspiratory motoneurons, increased and prolonged their firing during the augmented inspiratory phase of coughing as expected. However, I-DEC neurons also had increased activity. This enhanced activity of I-DEC neurons may function to decelerate and shape the phrenic activity, since these I-DEC neurons inhibit I-AUG neurons of the VRG (Ezure *et al.* 1989). Orem & Trotter (1993) observed an unexpected conversion of I-DEC neurons to I-AUG neurons during augmented breaths in intact and unanaesthetized cats, a result, they suggested, inconsistent with the postulated role of I-DEC neurons in respiratory rhythm generation. We do not yet fully understand why the firing of I-DEC neurons is enhanced during augmented breaths (Orem & Trotter, 1993) or during coughing.

During normal respiration, most E-AUG neurons of the BOT do not fire in early expiration. Nevertheless, they start firing immediately after inspiratory termination during coughing, although the duration of firing varies. E-AUG neurons display similar firing patterns during augmented breaths (Orem & Trotter, 1993). The firing of E-AUG neurons of the BOT, in concert with E-DEC neurons, may inhibit medullary inspiratory neurons during the expiratory phase of coughing. However, E-AUG neurons of the BOT are inactive during sneezing (Orem & Brooks, 1986). These findings suggest that E-AUG neurons of the BOT, major constituents of the respiratory network (Merrill *et al.* 1983; Jiang & Lipski, 1990), participate differently in the generation of coughing and sneezing.

Furthermore, our results suggest the existence of unidentified neurons responsible for the burst of abdominal activity during coughing. Bulbospinal E-AUG neurons of the caudal VRG excite spinal expiratory motoneurons (Kirkwood & Sears, 1973; Merrill, 1974) and are thought to be the primary source of abdominal activity in normal respiration. Indeed, all the E-AUG neurons of the VRG in the present study had spinal axons. However, their firing was inadequate to explain the burst of abdominal nerve activity during coughing. Since spinal stimulation was applied at C4–C5, these E-AUG neurons did not necessarily project to motoneurons of the abdominal nerves. However, the same argument holds true even if the E-AUG neurons studied projected to intercostal motoneurons, since expiratory intercostal and abdominal muscles are activated similarly during coughing (Grélot & Milano, 1991; Iscoe & Grélot, 1992). Moreover, none of the E-AUG neurons fired during the coactivation phase of coughing, i.e. during the period when both abdominal and phrenic activity are present. Therefore, neurons other than the E-AUG neurons of the caudal VRG must have been activated and driven the expiratory motoneurons during this phase. Jodkowski & Berger (1988) suggested the existence of still unidentified inputs to spinal expiratory motoneurons because prolonged and occasionally enhanced internal intercostal activity during SLN stimulation could not be explained by the activity of bulbospinal E-AUG neurons of the VRG. Price & Batsel (1970) observed that putative bulbospinal E-AUG neurons of the VRG caudal to the obex are excited in patterns and at frequencies appropriate to the pattern and strength of the active expiration during sneezing. It would be of interest to study whether bulbospinal E-AUG neurons of the VRG in the same preparation function differently during coughing and sneezing.

In summary, all five types of respiratory neurons analysed participated in the generation of coughing. Furthermore, we believe that some unidentified cough-specific neurons excited expiratory motoneurons and modified the firing pattern of E-AUG neurons.

Respiratory neuronal discharges during swallowing

Hukuhara & Okada (1956) were the first to report the existence of medullary inspiratory neurons which fire at the onset of swallowing evoked by pharyngeal instillation of water in decerebrate spontaneously breathing cats. Sumi (1963), using a similar preparation, recorded from both inspiratory and expiratory neurons of the brainstem and showed that both expiratory and inspiratory neurons consist of swallowing-active and swallowing-inactive types. Both studies indicate that swallowing-active inspiratory neurons discharge in the early phase of swallowing and are silent during the rest of swallowing. Similar transient activations during swallowing have been reported in 38% of the inspiratory neurons of the VRG and the DRG in ketamine–urethane-anaesthetized cats (Umezaki *et al.* 1988). Although the results of the present and previous studies are not directly comparable because of differences in the preparation and identification of respiratory neurons, a common finding has been the presence of inspiratory neurons that are transiently active in the early phase of swallowing.

I-DEC neurons, which seem homogeneous during normal respiration, were classified as swallowing-active and swallowing-inactive neurons. Swallowing-active neurons fired for a brief period at the onset of hypoglossal bursts. This firing pattern suggests that the firing of swallowing-active neurons is suppressed after this brief discharge by either active inhibition or disfacilitation. The suppression may be similar to that observed in swallowing-inactive neurons whose firing persisted during SLN stimulation (see Fig. 5*B*). Although this suppression was not observed in the swallowing-inactive neurons whose firing stopped during SLN stimulation, those neurons may also receive similar inputs during swallowing. This implies that not only swallowing-active neurons but also swallowing-inactive I-DEC neurons receive inputs from the swallowing central pattern generator (see, for example, Amri *et al.* 1984). Swallowing-active I-DEC neurons may participate in the control of the sequence of motoneuronal activation during swallowing. This may be accomplished by inhibiting some types of medullary respiratory neurons, since I-DEC neurons inhibit medullary inspiratory and expiratory neurons (Ezure *et al.* 1989; Ezure, 1990). Two types of I-DEC neuron groups, which behave similarly during respiration but differently during vomiting, have also been reported (Miller & Ezure, 1992). SLN-evoked swallowing provides another opportunity to identify subgroups of I-DEC neurons and to study the functional organization of I-DEC neurons. However, the observed differences in short-latency responses to SLN stimulation of I-DEC neurons in our study do not explain the differences in their behaviours during swallowing.

I-AUG neurons of the VRG were also classified as swallowing-active or swallowing-inactive neurons. Early-

recruited neurons tended to be swallowing-active, and late-recruited neurons tended to be swallowing-inactive. Swallowing-active I-AUG and I-DEC neurons may behave differently after activation during swallowing, since the suppression of firing after activation seems absent in I-AUG neurons. As shown in Fig. 1*B* and *C*, swallow-breath is separated from inspiratory phrenic activity, and the activity of I-AUG neurons, which are inspiratory premotor neurons, is also interrupted between the preceding inspiration and the subsequent swallow-breath (Fig. 9*B*). Therefore, the behaviour of swallowing-active I-AUG neurons is consistent with their being the primary source of excitation to phrenic motoneurons during swallow-breaths. Swallow-breaths are sometimes observed clearly, but are barely visible on other occasions. Many I-AUG neurons fire during swallowing even when swallow-breaths are weak. On these occasions, phrenic motoneurons receive inputs from active I-AUG neurons but may not reach firing threshold, or exhibit only limited firing. This has been clearly shown by Grélot *et al.* (1992), who recorded intracellularly from phrenic motoneurons and showed that all phrenic motoneurons exhibit a short-lasting swallow-related depolarization. Therefore, there may be no basic difference in the mechanisms underlying discharges accompanying distinct and indistinct swallow-breaths.

The firing of all E-AUG neurons of both the BOT and the VRG and that of most E-DEC neurons was suppressed during hypoglossal bursts associated with swallowing. This suppression may function to shape the swallowing activity by disinhibiting (E-AUG and E-DEC neurons of the BOT) and disfacilitating (E-AUG neurons of the VRG) other respiratory neurons, especially when swallowing occurs during the expiratory phase when these expiratory neurons fire. Without this suppression, I-AUG and I-DEC neurons may not fire (Ezure, 1990). At present, we do not know of any respiratory neurons that cause this suppression, whether due to inhibition or disfacilitation. This suppression, as well as the firing of I-DEC and I-AUG neurons during swallowing, is produced by inputs possibly from the swallowing central pattern generator.

In summary, the firing of all five types of respiratory neurons analysed was modulated during swallowing; therefore, these respiratory neurons may participate in the generation of swallowing activity. This implies that the respiratory network is involved, at least partly, in the network responsible for the production of the spatio-temporally co-ordinated activity of swallowing.

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