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RESEARCH ARTICLE

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Spinal connections of ventral-group bulbospinal inspiratory neurons studied with cross-correlation in the decerebrate rat

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Abstract We examined the synaptic connections from ventral-group bulbospinal inspiratory neurons to upper-cervical inspiratory neurons and phrenic and intercostal motoneurons in decerebrate rats using cross-correlation. Inspiratory neurons were recorded in the medulla (n=28) at the level of the obex and from the upper-cervical segments (C1 and C2) of the spinal cord (n=29) in 18 vagotomized, paralyzed, ventilated, and decerebrated rats. The neurons were identified by their inspiratory firing pattern and antidromic activation from the spinal cord at C7. Whole-nerve recordings were made using bipolar electrodes from the central cut ends of the C5 phrenic nerve and the external and internal intercostal nerves at various thoracic levels. Cross-correlation histograms were computed between these recordings to detect short time scale synchronizations indicative of synaptic connections. Cross-correlation histograms (n=20), computed between the activities of ventral-group bulbospinal inspiratory neurons and the phrenic nerve, all showed peaks (mean half-amplitude width±SD, 1.1±0.3 ms) at short latencies (mean latency±SD, 2.0±0.6 ms) suggestive of monosynaptic excitation. Cross-correlation histograms (n=33), computed between the activities of ventral-group bulbospinal inspiratory neurons and upper-cervical inspiratory neurons, displayed four (12%) peaks (mean half-amplitude width±SD, 0.9±0.1 ms) at short latencies (mean latency±SD, 1.8±0.6 ms) suggestive of monosynaptic excitation, and six (18%) peaks (mean half-amplitude width±SD, 1.4±0.4 ms) at latencies near zero suggestive of excitation from a common source. Cross-correlation histograms (n=34), computed between the activities of ventral-group bulbospinal inspiratory neurons and the internal and external intercostal nerves at various thoracic levels (T2-8), showed six (18%) peaks (mean half-amplitude width±SD, 2.5±0.5 ms) at short latency (mean latency±SD, 4.5±1.1 ms) suggestive of oligosynaptic connections. Cross-correlation histograms (n=42) computed between activities of intercostal nerves at various levels of the thoracic spinal cord showed central peaks suggestive of excitation from a common source. Although the size of the peaks decreased with segmental separation, the displacement of the peaks from time zero did not increase with segmental separation (mean displacement±SD, 0.6±0.6 ms) as would be expected if the common excitation resulted from a descending monosynaptic excitation by a source such as the ventral-group bulbospinal inspiratory neurons. We conclude that all ventral-group bulbospinal inspiratory neurons make monosynaptic connections to phrenic motoneurons, a few make monosynaptic connections to upper-cervical inspiratory neurons, but connections to intercostal motoneurons are made via interneurons.

Key words Cross-correlation · Ventral-group bulbospinal inspiratory neurons · Upper-cervical inspiratory neurons · Intercostal motoneurons · Rat

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Introduction

Rats are emerging as the animal model of choice for studying the neurophysiology of respiration (Bianchi et al. 1995). However, much of our current knowledge about the neuronal organization of respiration has been derived from experiments on cats. It is not sufficient to assume that the neuronal organization is the same. As detailed below, investigations have revealed differences between rats and cats. It is therefore necessary to confirm similarities and differences in the neurophysiological organization of respiration between these two species. Such knowledge should enhance our understanding of the those aspects that are fundamental to respiratory rhythm generation and its transmission to the respiratory muscles.

Medullary inspiratory neuron groups

The medullary inspiratory neurons in rats (see Bianchi et al. 1995 for a review) lie in bilateral longitudinal columns at the level of the obex, corresponding to the dorsal and ventral respiratory groups already identified in cats. The dorsal respiratory group was first thought to be similar to that in cats (Saether et al. 1987), then absent (Ezure et al. 1988; Zheng et al. 1991). However, a recent study (de Castro et al. 1994) showed it to be present in rats, but with a much reduced spinal projection.

The ventral respiratory group in rats is extensive (Saether et al. 1987; Ezure et al. 1988; Ellenberger et al. 1990; Schwarzacher et al. 1991; Zheng et al. 1991, 1992), similar to that in cats, and is the main source of inspiratory drive to spinal respiratory neurons (see Monteau and Hilaire 1991 for a review). However, the bulbospinal expiratory neurons of this group appear to differ from those in cats by giving off intramedullary collaterals (Zheng et al. 1992). In addition, the Bötzinger complex of rats contains fewer expiratory neurons with an augmenting firing pattern, and they have fewer spinal projections (Bryant et al. 1993).

Upper-cervical inspiratory neurons

Upper-cervical inspiratory neurons form a column of neurons located near the lateral edge of the intermediate gray matter in rats (Lipski et al. 1993). In cats these neurons (Aoki et al. 1980) project mainly toward the intercostal motoneurons (Lipski and Duffin 1986; Hoskin et al. 1988), with a weaker projection toward the phrenic motoneurons (Lipski and Duffin 1986; Douse et al. 1992; Nakazono and Aoki 1994).

In rats intracellular labeling of the upper-cervical inspiratory neurons revealed single short collaterals in the region of the phrenic nucleus and axons that descend as far as the rostral thoracic spinal segments (Lipski et al. 1993). We recently reported electrophysiological confirmation of the weak connection to phrenic motoneurons, but could not find evidence for connections to intercostal motoneurons (Tian and Duffin 1996).

Ventral-group bulbospinal inspiratory neuron spinal connections

To phrenic motoneurons

Ventral-group bulbospinal inspiratory neurons excite phrenic motoneurons in rats. Numerous studies using tracing techniques (Onai et al. 1987; Ellenberger and Feldman 1988, 1990; Yamada et al. 1988; Saji and Miura 1990; Goshgarian et al. 1991, 1993; Núñez-Abades et al. 1991; Portillo and Núñez-Abades 1992; Dobbins and Feldman 1994; Lipski et al. 1994) have demonstrated the anatomical basis for such connections, but few have provided electrophysiological confirmation. Both of these studies (Saether et al. 1987; Duffin and van Alphen 1995) used cross-correlation to demonstrate connections, the latter showing their bilateral nature. These results are confirmed here and the strength of this connection is demonstrated.

To upper-cervical inspiratory neurons

In cats, the activity of the upper-cervical inspiratory neurons results from excitation by medullary inspiratory neurons of both dorsal and ventral groups (Hoskin and Duffin 1987a, b). In rats, a weak projection from ventral-group bulbospinal inspiratory neurons to upper-cervical inspiratory neurons has been demonstrated using anatomical tracing (Lipski et al. 1994) and is confirmed here by cross-correlation.

To intercostal motoneurons

The connection from ventral-group bulbospinal inspiratory neurons to intercostal motoneurons is not known for rats, although the experiments of Lipski et al. (1994), using intracellular labeling, show that they project as far as the first thoracic spinal segment. For cats, the dorsal-group inspiratory neurons are known to monosynaptically excite intercostal motoneurons (Duffin and Lipski 1987), but the connection from ventral-group inspiratory neurons to intercostal motoneurons (Merrill and Lipski 1987) is probably via interneurons (Kirkwood et al. 1988). A similar conclusion for rats is reached here.

Materials and methods

Animal preparation

Experiments were performed on 18 adult male Sprague-Dawley rats weighing between 300 and 380 g. These experiments were a subset of a larger study, part of which has been previously reported (Tian and Duffin 1996). All procedures were reviewed and approved by the University of Toronto animal care committee. The rats were initially anesthetized with 2.0% halothane in oxygen, and anesthesia was maintained at surgical levels with 1.0–2.0% halothane before decerebration.

The trachea, femoral artery, jugular vein, and bladder were then cannulated. At this time the vagus nerves were cut bilaterally. A continuous infusion (2 ml 1 M $NaHCO_3$ and 10 ml 5% dextrose in 38 ml Ringer's) was established via the jugular vein (5–7 ml/h per kilogram) to stabilize the animal's fluid balance (Quintin et al. 1989) and help maintain femoral arterial blood pressure (Harvard Apparatus) between 75 and 110 mmHg (usually about 100 mmHg). In addition, dexamethasone (0.2 mg) and atropine (0.05 mg) were given intramuscularly to minimize brain edema and airway secretions, respectively. Rectal temperature was monitored and maintained at 37±1.0°C with a servo-controlled electric heating pad (Harvard Apparatus).

The animals were held in a stereotaxic frame (Kopf) and by clamps at rostral thoracic and sacral vertebrae with the head inclined at about 30°. They were then paralyzed with pancuronium bromide (0.4 mg/h i.v., Pavulon) and ventilated (Harvard Apparatus) via the tracheal cannula, with supplemental oxygen (40–80%) added to the inspiratory line. The end-tidal CO_2 was monitored

(Datex) and maintained between 4 and 5%. To minimize the motion of the brain stem, bilateral pneumothoraces were made with an end-expiratory pressure of 2–3 cm H_2O applied to prevent atelectasis.

Decerebration was carried out using a method similar to that described by Zheng et al. (1991). Bilateral burr holes were made in the parietal skull and vascular clips (Weck) applied to the central saggital sinus to allow removal of the central portion of bone between the burr holes. In this way a slot across the width of the skull approximately 5 mm wide was established. Brain tissue was then slowly and gently removed by suction, carefully avoiding damage to the large vessels on the floor of the cavity created. Small pieces of Gelfoam soaked in thrombin solution were used to control bleeding on the exposed surfaces of the brain. With this technique, a complete section of the brain at the precollicular level was achieved (confirmed by postmortem examination). Halothane was then discontinued.

The medullary surface was exposed, first by removal of the occipital skull, then reflection of the dura bilaterally, and finally removal of the caudal part of the cerebellum by suction. The spinal cord was exposed from C1 to C7 by a dorsal laminectomy. All recording and stimulating sites were exposed by removing the arachnoid membrane and cauterizing blood vessels with a microcautery (Fine Science Tools).

Finally, the left C5 phrenic nerve was exposed, sectioned, desheathed, and placed in a paraffin oil pool for recording. The internal and external intercostal nerves were dissected free at various levels of the thoracic spinal cord, sectioned, and also placed in paraffin oil pools.

Recording and stimulation

Extracellular recordings were made using glass-coated tungsten microelectrodes (0.3–0.5 MΩ at 1 kHz). These signals were amplified (Neurolog NL104), filtered (0.125–8 kHz) using a 10-band stereo equalizer (Audio Reflex), and action potentials were discriminated with a time-amplitude window gate (Bak and Schmidt 1977). The discriminator trigger was set on the rising phase of the action potential and the amplitude window gate set at a minimum height that allowed detection of the action potential trajectory about 0.5 ms later. Antidromic activation of descending axons in the lateral and ventral funiculi at C7 used glass-coated tungsten stimulating microelectrodes. The stimulus pulses (0.2 ms, 5–50 μA) were obtained from a pulse buffer (Neurolog NL510) driven by a stimulus programmer (Digitimer 4030) via a digital width controller (Neurolog NL401).

Respiratory activity was recorded from the phrenic and intercostal nerves with bipolar silver wire electrodes and amplified (Neurolog NL104). Motoneuron axon action potentials were discriminated with amplitude gates (Bak) set to detect action potentials exceeding the noise level. The amplitude gates' output pulses were delayed by the same period as the window delay for the neuron action potential discriminators.

Signals were monitored with loudspeakers, displayed on oscilloscopes (Tektronics, Nicolet) and a thermal array chart recorder (Graphtec WR3600), and stored on video tape in a digitized form (Vetter).

Protocol

After establishing recordings of the phrenic and intercostal nerve activities, a search (Narishige micromanipulator) was made for a ventral-group inspiratory neuron and an upper-cervical inspiratory neuron. During initial identification the neurons were observed at wide bandwidths (0.05–20 kHz) to help distinguish them from axons, then filtered at limited bandwidths to obtain the best discrimination from noise. Both neurons were then antidromically activated (collision test; Lipski 1981) from the C7 spinal segment to establish their projections caudal to the phrenic motor nucleus.

Cross-correlation histograms were calculated, using the standard pulses generated from the discriminators, by a purpose-built cross-correlator (Anderson and Duffin 1976) set for 0.2-ms bin widths. The trigger pulses were delayed (Neurolog NL730) to enable the histogram to display periods before time zero. In this way we examined the synaptic connections from the ventral-group bulbospinal inspiratory neurons to the upper-cervical inspiratory neurons and the phrenic and intercostal motoneurons, as well as the synchronization of activities between various intercostal nerves.

Cross-correlation histogram peaks were characterized by calculating the k-ratio, the peak bin count divided by the mean bin count (Sears and Stagg 1976), and tested for significance (Graham and Duffin 1981) at $P<0.01$. For featureless cross-correlation histograms, we used the minimum k-ratio for significance (at the observed mean bin count and chosen level of significance) to calculate the maximum number of interaction events that may have been undetected for every 1000 driver events (see Graham and Duffin 1981 for details). For example, consider the case of the cross-correlation histograms computed between ventral-group bulbospinal inspiratory neurons and upper-cervical inspiratory neurons. The number of undetected interaction events is the number of upper-cervical inspiratory neuron action potentials resulting from a synchronizing processes (such as synaptic excitation by the ventral-group bulbospinal inspiratory neuron) unrecognized as a peak in the cross-correlation histogram. The driver events are the ventral-group bulbospinal inspiratory neuron action potentials.

All results are expressed as mean±standard deviation. The features measured for the cross-correlation histograms include the latency to the start of the peak and the half-amplitude width of the peak.

Results

General observations

In 18 experiments conducted as part of a larger study, portions of which have been previously reported (Tian and Duffin 1996), simultaneous recordings were made of the firing patterns of a ventral-group bulbospinal inspiratory neuron, an upper-cervical inspiratory neuron, phrenic nerve discharge, and the discharges of external and internal intercostal nerves at various levels of the thoracic spinal cord. Figure 1 shows an example of such recordings.

The ventral-group bulbospinal inspiratory neurons (n=28) were recorded about the level of the obex at locations read from the micromanipulator (Fig. 2). All were antidromically activated from the C7 spinal segment (22 ipsilaterally, 16 contralaterally, 10 of 15 bilaterally). Because we used a tungsten microelectrode mounted on a micromanipulator for antidromic activation, rather than a fixed position, we were able to search for locations of minimum threshold (typically less than 15 μA). At these low currents, we never observed two latencies indicative of antidromic activation of the axon from more than one location. We noted, from our observations of the locations of the antidromic stimulation electrode, that the ipsilateral axons were found mostly in the lateral funiculus, while the contralateral axons were found mostly in the ventral funiculus.

A paired t-test comparison showed that the ipsilateral latencies were significantly (P=0.04) shorter than the contralateral latencies. Assuming a linear axonal trajectory and allowing 2 mm extra distance for the contralateral projection, the single-point stimulation estimate of

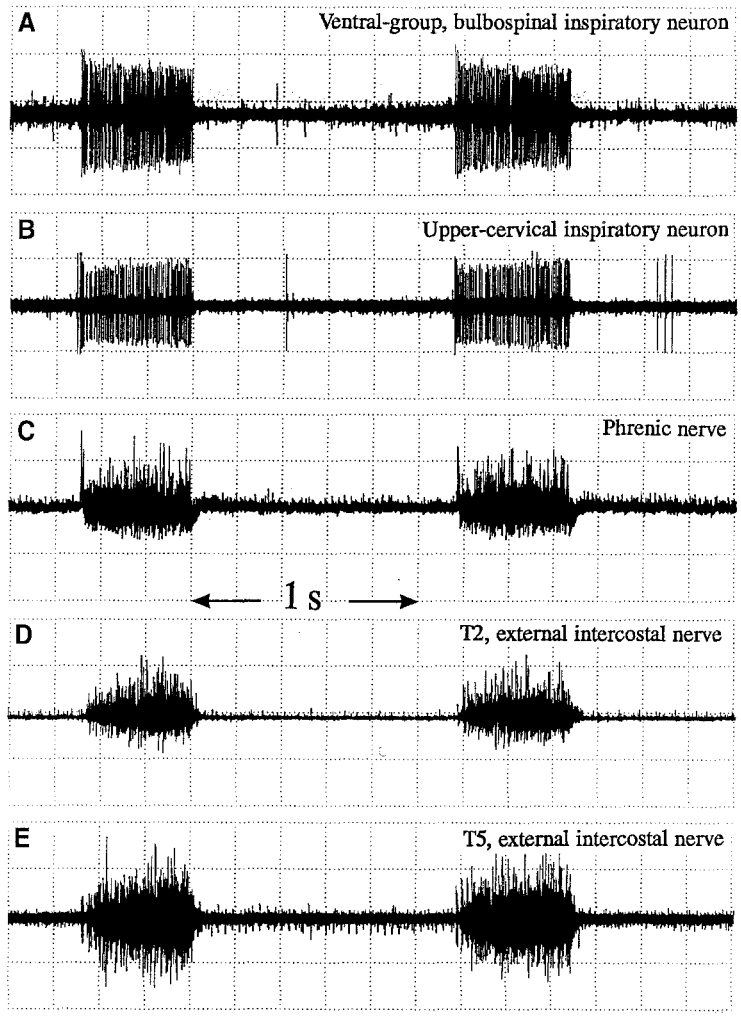

Fig. 1 Typical experimental recordings of: **A** firing patterns of ventral-group bulbospinal inspiratory neuron; **B** firing patterns of upper-cervical inspiratory neuron; **C** discharge of phrenic nerve; **D** discharge of T2 external intercostal nerve; and **E** discharge of T5 external intercostal nerve. The grid marks intervals of 0.2 s

the mean axonal conduction velocities was 11.2±1.4 m/s ipsilaterally and 11.4±2.1 m/s contralaterally. A possibly closer estimate of the mean conduction velocities, using the antidromic latencies less 0.2 ms assumed for an activation period, was 12.8±1.8 m/s ipsilaterally and 12.9±2.6 m/s contralaterally.

The firing patterns for the ventral-group bulbospinal inspiratory neurons were confined to the inspiratory phase, with an augmenting firing pattern that displayed a progressive decrease in action potential amplitude as firing frequency increased. The autocorrelation histograms for 18 neurons (64%) showed cyclic peaks indicative of regular interspike intervals and, from the interval histograms, we estimated that the mean firing frequency during the inspiratory phase was 18.4±0.5 Hz. The other 36% did not show cyclic peaks, indicating that the interspike intervals tended to be irregular.

The upper-cervical inspiratory neurons (n=29) were recorded in spinal segments C1 and C2, ventral to the dorsal root entry zone, at a mean depth of 1.1±0.2 mm read from the micromanipulator (Fig. 2). They were a subset of those described in a previous report (Tian and Duffin 1996). All were antidromically activated from the ipsilateral C7 spinal segment. Using these antidromic latencies and assuming a linear axonal trajectory, the single-point stimulation estimate of the mean axonal conduction velocity was calculated as 9.8±1.7 m/s. Using the antidromic latencies less 0.2 ms, the mean conduction velocity was calculated as 11.4±2.4 m/s.

The firing patterns for the upper-cervical inspiratory neurons varied. In most cases, activity was confined to inspiration, but some had an additional tonic component at a lower firing frequency during expiration, as reported previously (Tian and Duffin 1996). All neurons displayed an augmenting firing pattern during inspiration. The autocorrelation histograms for 30% showed cyclic peaks indicative of regular interspike intervals and the interval histograms showed that the firing frequency during the inspiratory phase was 18.3±0.4 Hz. The other 70% did not show cyclic peaks, indicating that the interspike intervals tended to be irregular.

Cross-correlation histograms: ventral-group bulbospinal inspiratory neurons to phrenic nerve

Cross-correlation histograms, computed between the activities of ventral-group bulbospinal inspiratory neurons

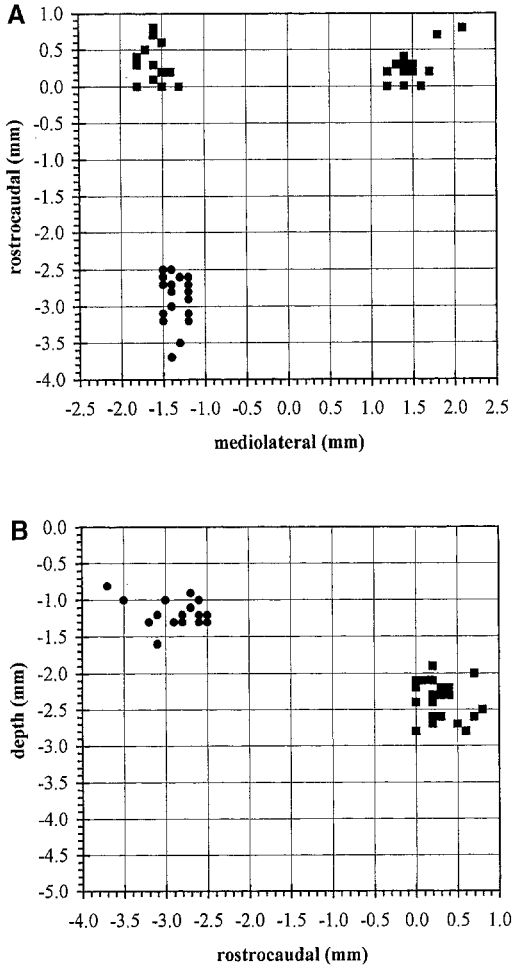

Fig. 2A, B The recording sites for the inspiratory neurons. The coordinates relative to the obex and the tissue surface were read from the micromanipulators. The sites for ventral-group bulbospinal inspiratory neurons are indicated with *filled squares* and those for upper-cervical inspiratory neurons with *filled circles*. **A** The dorsal view. **B** The saggital view shows both sides of the medulla

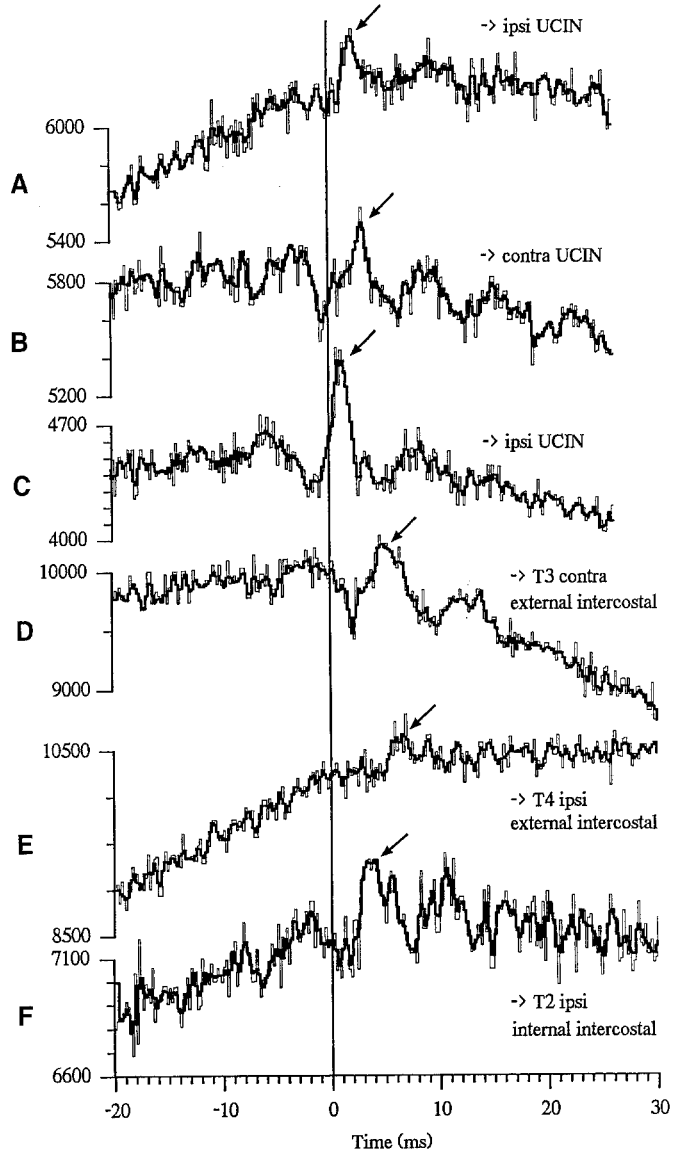

Fig. 3A-F Peaks (*arrows*) in cross-correlation histograms computed between the activities of ventral-group bulbospinal inspiratory neurons and the activities of upper-cervical inspiratory neurons (*UCIN*) and intercostal nerves. All of the bulbospinal inspiratory neurons showed cross-correlation peaks (not shown) suggestive of monosynaptic connections with phrenic motoneurons. **A** A peak at short latency, interpreted as evidence for an ipsilateral, monosynaptic excitatory connection to a UCIN. **B** A peak at short latency, interpreted as evidence for a contralateral, monosynaptic excitatory connection to a UCIN. The peak is superimposed on periodic fluctuations in the baseline, interpreted as evidence for high-frequency oscillations. **C** A peak close to time zero, interpreted as evidence for an ipsilateral common activation of a ventral-group bulbospinal inspiratory neuron and a UCIN. **D-F** Peaks at short latency, interpreted as evidence for paucisynaptic excitatory connections to the T3 external, T4 external, and T2 internal intercostal nerves, respectively. Bin widths 0.2 ms; vertical axes, counts/bin

(20/28) and the discharge of the phrenic nerve (11 ipsilateral and 9 contralateral), all showed significant peaks, with a mean latency of 2.0±0.6 ms and a mean half-amplitude width of 1.1±0.3 ms.

Cross-correlation histograms: ventral-group inspiratory neurons to upper-cervical inspiratory neurons

Cross-correlation histograms (n=33), computed between the activities of ventral-group bulbospinal inspiratory neurons and upper-cervical inspiratory neurons (57% ipsilateral) displayed a variety of features.

Figure 3A, B shows two examples of the four (12%) cross-correlation histograms (three ipsilateral) displaying a peak. All were significant at the chosen level (P<0.01). The peaks (0.9±0.1 ms half-amplitude width) were found at short latencies (1.8±0.6 ms) that exceeded the conduction times between the ventral group inspiratory neuron and the upper-cervical inspiratory neuron by at least 0.5 ms.

Figure 3C shows an example of the six (18%) cross-correlation histograms displaying a peak (1.4±0.4 ms half-amplitude width) at or near time zero. In addition,

11 (33%) cross-correlation histograms displayed periodic peaks and troughs, indicative of high-frequency oscillations (HFO) at a frequency of 135±13 Hz.

The remaining cross-correlation histograms (13, 39%) were featureless. The degree of interaction which may have gone undetected was calculated for these cross-correlation histograms as 1.9±0.5 interaction events per 1000 driver neuron events.

Table 1 The distribution of intercostal nerves used for computing cross-correlation histograms between the activities of ventral-group bulbospinal inspiratory neurons and intercostal nerves

Spinal segment	External intercostal		Internal intercostal	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
T2	–	1	2	–
T3	2	5	–	2
T4	2	1	–	–
T5	5	1	–	–
T6	3	2	–	–
T7	–	3	–	–
T8	–	2	–	–

Cross-correlation histograms: ventral-group inspiratory neurons to intercostal nerves

Cross-correlation histograms (n=34) were computed between the activities of ventral-group inspiratory neurons and the discharges of the ipsilateral and contralateral internal and external intercostal nerves at levels T2-8 of the thoracic spinal cord as detailed in Table 1.

Figure 3D-F shows examples of the six (18%) cross-correlation histograms displaying a peak (2.5±0.5 ms half-amplitude width) at short latency (4.5±1.1 ms). Only five (15%) of the cross-correlation histograms displayed HFO at a frequency of 124±18 Hz. The remaining cross-correlation histograms (23, 68%) were featureless. The degree of interaction which may have gone undetected was calculated for these cross-correlation histograms as 9.1±2.0 interaction events per 1000 driver neuron events.

Cross-correlation histograms: intercostal nerve to intercostal nerve

Cross-correlation histograms (n=42) were also computed between the discharges of external intercostal nerves

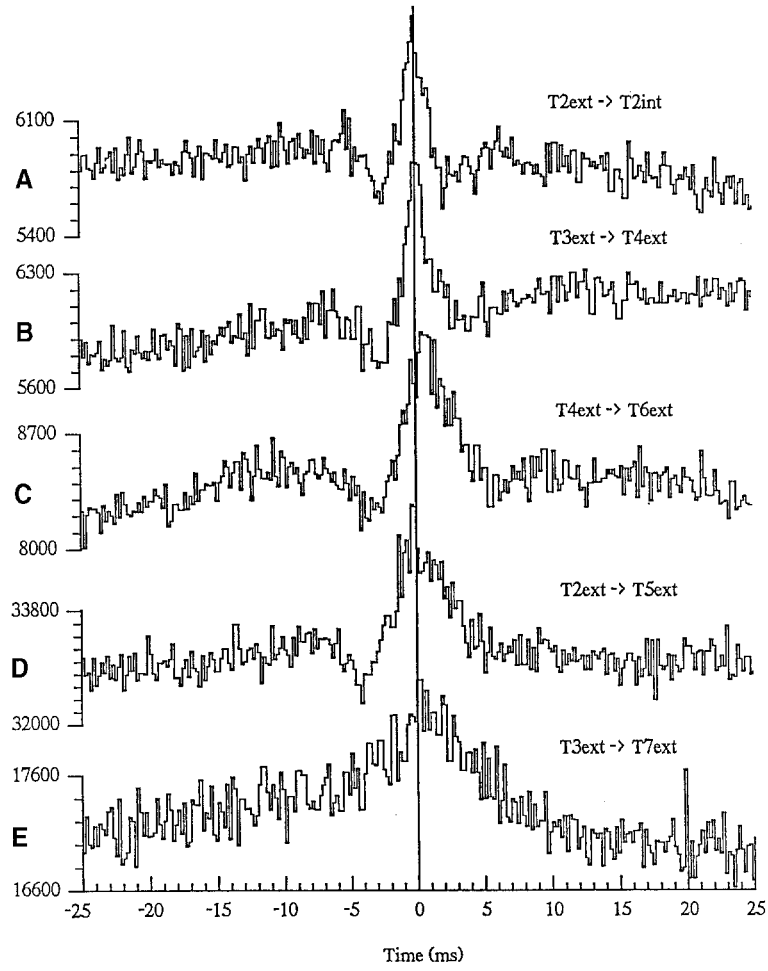

Fig. 4A-E Examples of cross-correlation histograms computed between the discharges of various intercostal nerves with different intersegmental separations (as indicated). Broad peaks at time zero were interpreted as evidence for common activation of both groups of motoneurons. Bin widths are 0.2 ms; vertical axes, counts/bin

from different ipsilateral levels of the thoracic spinal cord as well as between the discharges of external and internal intercostal nerves of the same segment. They showed central peaks with the size of the peak generally varying inversely with the segmental separation as shown in Fig. 4. The displacement of the peaks from time zero (0.57±0.59 ms) did not vary with segmental separation.

Discussion

General observations

Ventral-group bulbospinal inspiratory neurons

The ventral-group bulbospinal inspiratory neurons were found about the level of the obex, in agreement with the observations of others (Saether et al. 1987; Ezure et al. 1988; Schwarzacher et al. 1991; Zheng et al. 1991, 1992). As Lipski et al. (1994) noted from their anatomical tracing experiments, the contralateral axons of these neurons were generally found in the ventral funiculus, while the ipsilateral axons were found in the lateral funiculus. Their firing patterns were always augmenting and confined to the inspiratory phase, as observed by other investigators. We noted that these bulbospinal neurons were characterized by a decreasing action potential amplitude as firing frequency increased, in contrast to other inspiratory neurons observed.

Although others have noted differences between ipsilateral and contralateral antidromic activation times (Zheng et al. 1992), they did not find the difference to be significant as we did. However, our estimate of the mean conduction velocity was 11.2 m/s ipsilaterally and 11.4 m/s contralaterally, using single-point activation and assuming linear axon trajectories. A possibly more accurate estimation of the conduction velocity was obtained by using the antidromic latencies less 0.2 ms allowed for the axonal activation time; it yielded conduction velocities of 12.8 m/s and 12.9 m/s for ipsilateral and contralateral projections, similar to that reported by Saether et al. (1987) for contralateral axons. In either case, these results indicate that the conduction velocities of the ipsilateral and contralateral axons are similar and considerably slower than that reported for cats (Dick and Berger 1985).

Upper-cervical inspiratory neurons

The upper-cervical inspiratory neurons were located in spinal segments C1 and C2 beneath the dorsal root entry zone at a mean depth of 1.2 mm and with firing patterns similar to those described by Lipski et al. (1993). They are a subset of those described in our previous report (Tian and Duffin 1996). Our single-point stimulation estimate of mean conduction velocity of 9.8 m/s is similar to our previous measurements on 79 neurons (11.6 m/s), which included these neurons and compares favorably to the value of 11.7 m/s obtained by Lipski et al. (1993) for rats, but is considerably slower than the 32 m/s found in cats (Lipski and Duffin 1986).

Cross-correlation histograms

We examined the peaks of the cross-correlation histograms in order to reach conclusions about neuronal connections, treating the periodic patterns of peaks and troughs interpreted as indicating HFO as a cyclic fluctuation in baseline. The HFO frequencies (135 Hz and 124 Hz) are similar to our previously reported values (Tian and Duffin 1996) and slightly higher than HFOs (50–100 Hz) for cats (Cohen et al. 1987).

Interpretation of the peaks in the cross-correlation histograms was based upon separating them into two categories; those displaced from time zero by a latency to the start of the peak and those which either straddled time zero or began at times too short for synaptic transmission. The latter were considered to result from excitation from a common source. Peaks with latencies at least 0.5 ms greater than the conduction times calculated for transmission were considered to result from synaptic connections (Moore et al. 1970) and interpreted as evidence for monosynaptic and paucisynaptic connections according to their half-amplitude widths (Kirkwood 1979).

Connections to phrenic motoneurons

We interpreted the peaks at short latencies in the cross-correlation histograms as evidence for monosynaptic excitatory connections from ventral-group bulbospinal inspiratory neurons to phrenic motoneurons. The connection is strong, since all of the cross-correlation histograms showed such peaks and they were easily discernible (mean k-ratio=1.12±0.04). This finding confirms our previous observation (Duffin and van Alphen 1995) and that of Saether et al. (1987) using cross-correlation, and substantiates the conclusions of others using tracing experiments (e.g., Dobbins and Feldman 1994).

We also took this observation as an assurance that the ventral-group bulbospinal inspiratory neurons studied with respect to their connections to upper-cervical inspiratory neurons and to intercostal motoneurons were indeed transmitters of inspiratory excitation to the spinal cord.

Connections to upper-cervical inspiratory neurons

We found very few indications of synaptic excitation of upper-cervical inspiratory neurons by ventral-group bulbospinal inspiratory neurons (12%), and although those peaks observed were considered to result from synaptic connections, they were not easily discernible (mean

k-ratio=1.08±0.04). The narrow half-amplitude widths of these peaks suggested that they were the result of monosynaptic connections (Kirkwood 1979); and they are the first electrophysiological evidence for such connections. They confirm the anatomical tracing evidence for such a connection obtained by Lipski et al. (1994).

Based on the evidence for such connections in cats (6%; Hoskin and Duffin 1987a) this connection appears to be stronger in rats. In both cases the estimate may be lower than actual, not because the cross-correlation histograms were calculated for an insufficient time - the undetected degree of interaction in the featureless cross-correlation histograms was estimated at only 1.9 interaction events per 1000 driver events - but because of the limits of the cross-correlation technique itself, such as its low sensitivity to slower postsynaptic potentials (Kirkwood 1979; Aertsen and Gerstein 1985).

Connections to intercostal motoneurons

Despite finding evidence for monosynaptic connections from all the ventral-group bulbospinal inspiratory neurons to phrenic motoneurons, few peaks (18%) could be discerned in the cross-correlation histograms for the same neurons to the intercostal nerves. The peaks were not easily discernible (mean k-ratio=1.06±0.02) and were sufficiently wide (2.5 ms) to suggest that the connections were not made directly but via interneurons. Although thoracic interneurons have not been described in rats, they are known to exist in cats (Kirkwood et al. 1988).

Other observations support the idea that connections were made via interneurons. The absence of peaks with latencies close to zero indicated that the cross-correlation process detected no common excitation of ventral-group bulbospinal inspiratory neurons and intercostal motoneurons, a conclusion similar to that made for cats by Davies et al. (1985), also based on cross-correlation. However, we suggest that common activation is likely to be present, and that the absence of peaks indicating common activation results from the inability of the cross-correlation process to detect such common activation when it is transmitted via interneurons (Kirkwood 1979). An additional argument for the presence of interneurons is also suggested by the characteristics of the cross-correlation histograms for the intercostal nerves as discussed below.

The cross-correlation histograms computed between intercostal nerves usually showed peaks about time zero and we interpreted these as indicative of a common source of excitation. Although common inhibition could also have been responsible (Moore et al. 1970), we considered this possibility less likely. If the shared excitation source was from the distributed collaterals of ventral-group bulbospinal inspiratory neurons then we would have expected the displacement of the peaks from time zero to have increased with segmental separation as is observed in cats (Kirkwood and Sears 1991).

In cats, the distributed collaterals from dorsal group inspiratory neurons (Duffin and Lipski 1987) provide the monosynaptic excitation that is a basis of such common excitation; the connection from ventral-group bulbospinal inspiratory neurons is not direct (Davies et al. 1985; Merrill and Lipski 1987). In rats the spinal projections of dorsal-group inspiratory neurons may be weak (de Castro et al. 1994) and therefore ineffective in providing a distributed monosynaptic excitation.

We therefore interpret the observation that the displacement of peaks in cross-correlation histograms computed between intercostal nerves did not vary proportionately with segmental separation, while the strength of the connection did, as indicating that the common drive is distributed by interneurons such that the transmission delays are similar for their projections to rostral and caudal extremes.

Conclusion

We conclude that in rats all ventral-group bulbospinal inspiratory neurons make monosynaptic connections with phrenic motoneurons, a few make such connections to upper-cervical inspiratory neurons, and connections to intercostal motoneurons are made via interneurons.

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