The role of the nucleus retroambiguus in the neural control of respiration, vocalization and mating behavior
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Chapter 8

Functional heterogeneity among neurons in the nucleus retroambiguus with lumbosacral projections in female cats

ABSTRACT

Nucleus retroambiguus (NRA), in the caudal medulla, projects to all spinal levels. One physiological role is abdominal pressure control, evidenced by projections to intercostal and abdominal motoneurons from expiratory bulbospinal neurons (EBSNs) within NRA. The roles of NRA projections to the lumbosacral cord are less certain, though those to limb motoneurons may relate to mating behavior and those to Onuf's nucleus (ON) to maintaining continence. To clarify this we have physiologically characterized NRA projections to the lumbosacral cord.

Extracellular recordings were made in NRA under anesthesia and paralysis in estrus cats. Administered CO₂ gave a strong respiratory drive. Antidromic unit responses were recorded to stimulation of the contralateral ventrolateral funiculus of L6, L7 or sacral segments and to microstimulation in the region of semimembranosus motor nucleus or ON. All units were found at sites showing expiratory discharges. Units that showed collisions between antidromic and spontaneous spikes (all in late expiration) were identified as EBSNs. These were common from the VLF of L6 (42.5%) or L7 (32.9%), but rare from the sacral VLF or the motor nuclei. Antidromic latencies revealed a subthreshold respiratory drive in some non-EBSNs. This group had lower conduction velocities than the EBSNs. The remainder, with a negligible respiratory drive, had even lower conduction velocities.

A new population of NRA neurons has thus been defined. They are not active even with a strong respiratory drive, but may provide most of the synaptic input from NRA to lower lumbar and sacral segments and could subserve functions related to mating behavior.

INTRODUCTION

The nucleus retroambiguus (NRA) is a group of neurons located ventrolaterally in the most caudal part of the medulla oblongata. Functionally, the NRA is crucial for abdominal and intrathoracic pressure control in the context of respiration (Merrill, 1974), vomiting (Miller et al., 1987) and vocalization (Holstege, 1989) and probably also for the control of mating behavior (VanderHorst and Holstege, 1995). To produce these behaviors, the NRA receives a strong projection from the periaqueductal gray (PAG), but also from structures in the pons and medulla oblongata which are involved in the control of respiration, such as the parabrachial and Kölliker-Fuse nuclei, the retrotrapezoid body, the Bötzing complex and the nucleus of the solitary tract (Gerrits and Holstege, 1996). The NRA in turn sends its fibers mainly to the contralateral brainstem and spinal cord. In the brainstem
these fibers terminate in the nucleus ambiguus, which contains motoneurons of the pharynx, larynx and soft palate (Holstege, 1989). In the cervical, thoracic and upper lumbar cord they terminate on phrenic, intercostal and abdominal motoneurons (Holstege & Kuypers, 1982; Feldman et al., 1985), and in the lumbosacral cord on distinct groups of motoneurons, innervating hindlimb and pelvic floor muscles (Holstege and Tan 1987; VanderHorst and Holstege 1995). This NRA-lumbosacral motoneuronal projection is thought to be involved in mating behavior (VanderHorst and Holstege, 1996) and differs greatly in strength in estrus and non-estrus periods. For example, in the NRA semimembranosus motor nucleus, the density of NRA terminals is more than eight times greater in estrus than in non-estrus, a difference which is due to a process of growth (VanderHorst and Holstege, 1997b).

The NRA also constitutes the most caudal part of the ventral respiratory group and is the main site of expiratory bulbospinal neurons (EBSNs) (Merrill, 1970, 1974). EBSNs in the NRA have excitatory projections to motoneurons of muscles involved in respiration, such as intercostal and abdominal motoneurons (Iscoe, 1998; Kirkwood et al., 1999). Some of the EBSNs send their fibers more caudally, to mid and caudal lumbar levels and to the region of pelvic floor motoneurons in S1 and S2 (Miller et al., 1995; Sasaki et al., 1994), including pelvic floor motoneurons of Onuf’s nucleus, which innervate the external urethral and anal sphincters (Sato et al., 1978). The projection from the EBSNs to Onuf’s nucleus is thought to be involved in maintaining continence during increased intra-abdominal pressure, e.g. during coughing, but may also have a role in mating behavior. It is not yet known whether the NRA cells projecting to the lumbosacral motoneuronal cell groups controlling the mating posture differ from those projecting to motoneurons innervating the muscles controlling respiration and vocalization. Indeed, the possibility of there being a contribution to the NRA projections at any spinal cord level comprising neurons other than respiratory neurons (mostly EBSNs) has not been investigated. The aims of the present electrophysiological study are, firstly, to determine what are the functional properties of the neurons involved in the NRA projections to the lumbosacral spinal cord and, secondly, to determine whether the NRA projections to Onuf’s nucleus and the semimembranosus motor nucleus consist mostly of EBSNs or of other neurons. Preliminary reports have appeared (Ford et al., 2001; Boers et al., 2003; Kirkwood and Ford, 2004).

**METHODS**

The surgical procedures, pre- and postoperative care, and the handling and housing of the animals were carried out in accordance with UK legislation [Animals (Scientific Procedures) Act, 1986]. Nine female cats were bilaterally ovariectomized. After two to four weeks they were treated daily with estradiol benzoate (sc, 0.02 mg/kg) for 7 days prior to the terminal experiment. The animals were brought into estrus as a standardized condition in which it is thought that the projections
from the NRA to the spinal cord may be stronger than in non-estrous (VanderHorst et al., 1997b). After five days the cats showed signs of estrus behavior such as lordosis of the back, treading of hindlimbs and lateral deviation of the tail when the base of the tail was tapped or scratched. The terminal experiment was performed under sodium pentobarbitone (initial dose of 37.5 mg/kg IP, then IV as required) and paralysis with gallamine triethiodide. The cats were artificially ventilated via a tracheal cannula with oxygen-enriched air. \( \text{CO}_2 \) was added to the inspired gases to increase the end tidal values to high enough levels (7-8%) to give a strong respiratory drive, which was monitored as the inspiratory discharge in a recording from the external intercostal nerve of T6. These discharges and blood pressure, measured via a femoral cannula (mean above 80 mm Hg), were continuously monitored. During paralysis, adequate anesthesia was confirmed by the stability of these recordings, including absence of substantial responses to a noxious pinch of the forepaw. Rectal temperature was measured and maintained between 37 and 38°C by a heating blanket. The animal was mounted in a frame by vertebral clamps, a clamp on the iliac crest and a plate screwed on the skull. The head was moderately ventroflexed.

In four cats, to locate the semimembranosus (Sm) motoneuron group in the lower lumbar cord, the Sm nerve was prepared and stimulated via platinum wire electrodes, as were the semitendinosus (St), tibial and common peroneal nerves. The motoneurons supplying these other nerves are located respectively caudal, dorsal, and lateral to the Sm motor nucleus (VanderHorst and Holstege, 1997b). A laminectomy was made to expose the spinal cord from L5 to L7. The exposed spinal cord and the tibial, common peroneal, St and Sm nerves were submerged in paraffin oil pools made from skin flaps. The dura mater was opened and small patches of the pia mater were removed. A movable glass-insulated tungsten microelectrode (typical impedance 1 MΩ) was inserted in the gray matter of the lumbar cord at an angle of 5-26°. Motoneuron antidromic field potentials recorded via this electrode and resulting from stimulation of the Sm nerve defined the location of the Sm motor nucleus. The field potentials resulting from stimulating the other nerves were used as a guide. An electrode track was selected which passed through Sm motor nucleus, and subsequently used for microstimulation to identify units in the NRA with axon collaterals in this region. In 3 of these cats two tracks passing through the motor nucleus were selected 0.5 mm apart rostro-caudally. The white matter in the left ventrolateral funiculus (VLF) of L6 was stimulated by a fixed pair of low impedance needle electrodes (cut-back tungsten microelectrodes, about 0.3 mm tip exposed, separation 1.5 mm arranged so that one tip was in the lateral funiculus, one in the most ventral white matter). Stimulators for nerves or spinal cord were either Type 2533 (Devices Ltd UK) or DS2 (Digitimer Ltd, UK), both constant voltage (0.05 ms pulses for nerves, amplitude 10 × nerve threshold, measured with reference to an afferent volley recorded via a platinum wire electrode from the cord dorsum close to dorsal root entry of the appropriate segment; 0.2 ms pulses for the spinal cord, voltages up
to 30V, either polarity, according to which gave the lower threshold for the unit concerned. Currents for microstimulation (0.2 ms cathodal pulses) were measured as the voltage across a 100 Ω resistor in the reference line.

In another five cats the left pudendal nerve was prepared and stimulated to find the location of Onuf’s nucleus in the sacral cord. In addition to the pudendal nerve, the left tibial, distal to calcaneus, and posterior biceps semitendinosus (PBSt) nerves were prepared and stimulated. The tibial and PBSt nerves were chosen because their motoneuron groups are located dorsolateral and rostral to Onuf’s nucleus respectively. The pudendal and PBSt nerves were stimulated by platinum wire electrodes and the tibial nerve by needles through the plantar skin. In these animals, a similar procedure to that described above (Sm motor nucleus) was used, except that the laminectomy exposed the spinal cord from L6 to S2.

![Motoneuron antidromic field potentials recorded on a selected electrode track in the sacral cord. Nerves were stimulated, in order, on each sweep: pudendal (Pud); posterior biceps semitendinosus (PBSt); tibial, distal to the calcaneus (Tib). Five sweeps are superimposed, at each of the two indicated depths. On selected tracks, the PBSt fields were always either small or absent. If a PBSt antidromic field potential were present in recordings from exploratory tracks, the electrode was moved caudally. The tibial field potential, prominent in A (Tib field), defined the dorsolateral border of the ventral horn (see also Fig. 8). The pudendal field potential (Pud field), prominent in B was used to define the length of the track passing through Onuf’s nucleus. Note the typical, temporally-dispersed form. The slower, probably synaptic, field potential which followed the very small antidromic PBSt field in B, was common at this depth.](image)

**Fig. 1.** Motoneuron antidromic field potentials recorded on a selected electrode track in the sacral cord. Nerves were stimulated, in order, on each sweep: pudendal (Pud); posterior biceps semitendinosus (PBSt); tibial, distal to the calcaneus (Tib). Five sweeps are superimposed, at each of the two indicated depths. On selected tracks, the PBSt fields were always either small or absent. If a PBSt antidromic field potential were present in recordings from exploratory tracks, the electrode was moved caudally. The tibial field potential, prominent in A (Tib field), defined the dorsolateral border of the ventral horn (see also Fig. 8). The pudendal field potential (Pud field), prominent in B was used to define the length of the track passing through Onuf’s nucleus. Note the typical, temporally-dispersed form. The slower, probably synaptic, field potential which followed the very small antidromic PBSt field in B, was common at this depth.
The lumbosacral spinal cord and the pudendal and PBSt nerves were submerged in paraffin oil pools made from skin flaps. The tungsten microelectrode was inserted in the gray matter of the sacral cord at an angle of 10-20° to record the motoneuron antidromic field potentials. Onuf’s nucleus was always found caudal to the PBSt motor nucleus and ventral to the tibial motor nucleus (Fig. 1). An electrode track was selected which passed through Onuf’s nucleus but was not too close to the lateral white matter, and subsequently used for microstimulation, as for L6. The white matter in the left VLF of L7 or more caudal in S1 and S2 (one experiment) was stimulated by a fixed pair of fine needle electrodes.

In all cats the caudal part of the medulla oblongata was prepared, the dura mater opened and part of the pia mater removed. The right side of the medulla was stabilized with a pressure plate and a glass microelectrode filled with 3M NaCl (external tip diameter 3.0 - 3.2 μm) was inserted for recording the activity of the neurons in the NRA (custom-made FET unity-gain amplifier plus D160 amplifier, Digitimer Ltd, UK). The record was bandpass filtered at 300 Hz and 10 kHz.

![Fig. 2. Drawing showing the arrangements of stimulating and recording electrodes in the lumbosacral spinal cord and caudal medulla. Record Insp., recording of inspiratory discharge in an external intercostal nerve; Sm. or Pud., diagrammatic location of semimembranosus motor nucleus or Onuf’s (pudendal nerve) nucleus.](image-url)
Electrode positions were defined by the rostrocaudal and mediolateral coordinates of the manipulator with respect to the position of Obex. Fig. 2 gives an overview of the arrangements of the electrodes in the lumbosacral cord and medulla. Data were acquired for computer storage using Spike2 software and a 1401 interface (Cambridge Electronic Design, UK). Two to five minutes of data, including the medullary and external intercostal nerve recordings and the stimulus times were recorded for each unit. For the units activated from the tungsten electrode in the gray matter, this was usually done with the electrode at a stimulation site where the threshold was minimal. For all units the axonal conduction velocity was calculated from the mean antidromic latency and the conduction distance.

The NRA column was sampled between 1.0 and 6.3 mm caudal to obex, 1.5 - 3.0 mm lateral to the midline (Fig. 9A). Tracking was made at intervals of 0.1 or 0.2 mm in the rostrocaudal and mediolateral directions. Not all positions in the NRA column were tested in every cat. To find the units in the NRA, first, spontaneously active expiratory units were sought, at a depth of 1.7 mm or more from the surface. Second, units antidromically activated from the contralateral lumbosacral cord, possibly including the expiratory units, were sought in the same or nearby regions. The procedure included tracking through the region of expiratory activity, in both dorsal and ventral directions, but the regions medial and lateral to the expiratory column were not systematically sampled for antndromically activated units. Only a few units with large spikes were isolated as single units. In general, our aim was to sample as many units as possible, including small units and those not spontaneously active. We therefore chose electrodes that would record multiunit discharges. Attempts were made to maximize the spike amplitude(s), but not at the cost of losing other units. For each unit we used the basic standard criteria for antidromic identification, a sharp threshold and a latency that was relatively constant and independent of stimulus strength. In the course of each electrode track in the medulla, units were sought by stimulating first from the VLF electrodes and then from the tungsten electrode. Stimuli were applied to the latter at intervals of about 100 µm along the electrode track through the whole distance within the gray matter. Once a unit was found antidromically activated from this electrode, thresholds for the unit were measured every 100 µm (sometimes 50 µm) along the track. Generally currents up to 100 µA were used for tracking, but occasionally larger currents were used once a unit had been found.

It would be expected that any unit identified from the tungsten electrode should also be identified from the more rostrally placed VLF electrodes. In fact only a few units were identified from both sites. In general, with multiple units often being excited from the VLF electrodes, the identification of which of these units, if any, was the one excited from the tungsten electrode was difficult and time-consuming and was most often not attempted. Units from the different sites are therefore treated below as separate populations.
Plots of activation threshold vs. depth along the selected track or tracks for the tungsten electrode (depth-threshold plots) were constructed for units antidromically activated from this electrode, to locate as far as possible the site of the axon stimulated and thus to determine whether the axon had collateral branches in or near Sm motor nucleus or Onuf’s nucleus. Units were classified as being activated from a collateral if they had a threshold minimum in the depth-threshold plot within the gray matter region of the selected track. We were guided in our interpretation by the descriptions of Merrill (1974), Lipski (1981) and, in particular Davies and Kubin (1986). Minima with rather high values (>40 µA) were only accepted if they were located well away from the white matter and the depth-threshold plot was steep (i.e. a narrow minimum). The occurrence of multiple minima, and/or multiple latencies of activation also allowed otherwise doubtful collateral identifications to be confirmed. If a unit showed only a minimum in the white matter region of the track or in the gray matter near to the white matter, it was classified as being stimulated in its stem axon and it was added to the VLF population of either L6 (Sm motor nucleus tracks) or L7 (Onuf’s nucleus tracks). Units were classified as having collaterals in Sm motor or Onuf’s nucleus if they had threshold minima ≤20 µA at a depth corresponding to the region of Sm motor or Onuf’s nucleus (see Results).

At the end of the experiment the animals were killed with an overdose of sodium pentobarbitone. The lumbosacral cord was removed and fixed in 4% paraformaldehyde. After the fixation the tissue was stored in 0.1M phosphate buffer with 25% sucrose at 4°C. The tissue containing the electrode tracks was cut on a freezing microtome into 50 µm transverse sections. One in two sections were mounted on gelatin-coated slides and dried overnight. The next day the slides were stained with neutral red and coverslipped with Depex mounting medium. The electrode track used for stimulation was always readily identified by holes in the tissue or gliosis, presumably because of repeated tracking at the one site, the identity of this track being confirmed by its position (usually the most medial or lateral relative to the other exploratory tracks). Depths along this track were assessed relative to positions of motor nuclei (see Results), and were confirmed by absolute depth measurements (mm in the section), which always corresponded well to the other measurements, within 0-10% allowance for shrinkage.

RESULTS

General properties of antidromically identified units
All antidromically activated units were found close to the spontaneously active expiratory units, whose location was clearly identified by Merrill (1970) as being coincident with the NRA. This observation also included a few situations where there was an apparent gap in the rostro-caudal cell column, at which no spontaneous units were observed. At these sites there were also no antidromically-activated units observed.
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At many of the sites in the NRA, stimulation in the VLF of L6, L7 or S1/S2 evoked antidromically-activated responses from multiple units, just as was reported by Merrill and Lipski (1987) for stimuli at thoracic levels. Fig. 3A shows superimposed sweeps in which the spikes from six units in the NRA can be seen, with different antidromic latencies (arrows). These units were successively activated with increasing stimulus strength in the VLF of L7. Despite the presence of many spontaneously occurring spikes at this location, for at least 3 of the units (2nd, 3rd and 4th in order of latency), there are no signs of collisions between the spontaneous and the antidromically-evoked discharges. However, for the 6th unit, the antidromically-evoked spike was clearly absent for many of the sweeps. There are two possible causes for the absence of an antidromically-evoked spike (assuming that the stimulus is sufficiently suprathreshold). These two are illustrated in Fig. 3B, where 5 units with well-separated latencies were antidromically activated from the VLF of rostral L6. In the upper record, 5 successive sweeps during expiration are superimposed. The unit with the shortest latency shows collision with a spontaneous spike, which occurred just before the stimulus in one of the sweeps. However the units with the second and fourth shortest latencies were also absent at times, as shown in the lower record. Here, 3 successive sweeps were superimposed during inspiration, when no spontaneous spikes were present and so these absences cannot represent collision. We therefore assume that the absence in this case was a result of failure of antidromic invasion of the cell’s soma, due to inhibition during inspiration, as is commonly the case for EBSNs (Merrill, 1974). This assumption was generally confirmed by antidromic latency measurements (see below).

Identification of EBSNs

The definition of an EBSN is a neuron active preferentially during expiration and possessing a spinal axon, i.e., in these experiments, antidromically identified from the lumbosacral segments. For the few units with large, well-isolated spikes, this definition was easy to apply, and a conventional collision test was done to confirm that the spontaneously active unit was the same unit as the antidromically-identified unit. In general, however, where relatively small spikes were recorded in multi-unit recordings, this was not possible and the identification order was reversed. The units were identified first by being antidromically-activated, then subsequently and often off-line, as showing collisions with spontaneous spikes and therefore as having an expiratory firing pattern. The procedure was first to find examples of sweeps during expiration where the spike from the unit concerned was absent, then to examine each of these sweeps to check that a spontaneous spike of similar amplitude was present in the period ± t from the stimulus time, where t was the antidromic latency and also that the absence of a spike was not due to superimposition with a spontaneous spike from another unit. The occurrence of such apparent collisions as rarely as only once or twice per respiratory cycle was accepted as a criterion for the unit to be defined as an EBSN, but it had to be fulfilled for several respiratory cycles, with these apparent collisions occurring
Fig. 3. Examples of recordings with multiple units activated antidromically. A, six different units recorded at one site in the NRA and activated successively as the stimulus to the VLF of L7 was increased (about 35 sweeps superimposed at each of the stimulus values indicated). B, five units similarly activated from the VLF of L6. The upper record (E) shows five superimposed sweeps during expiration; the lower (I) shows three superimposed sweeps during inspiration. Note that the first unit in E collides with a preceding spontaneous spike on one sweep, and the second and the fourth unit are not present in each of the three sweeps during inspiration.
Chapter 8

A

B

Stim. VLF L6
NRA
Ext. intercostal nerve (insp.)

Latency (ms)
Collision

Time (ms)
Time (s)
preferentially at a similar time in expiration in each cycle. Confirmation that the procedure was reliable was derived from the observations that the collisions thus identified were always more frequent towards the end of expiration, corresponding to the well known, usual incrementing pattern of excitation previously reported for EBSNs (Merrill, 1974). This also corresponded to the time course of excitation of the individual units, as indicated by antidromic latency measurements (see section below). Any absences of antidromic spikes during inspiration were also checked for possible collisions: this was usually an easy task because only rarely were any spontaneous spikes present in the recordings during inspiration. For many units the antidromic spikes were absent throughout inspiration.

Care was also taken to ensure that the excitation of the units was clearly suprathreshold, because for some units the threshold changed with time, leading to intermittent firing. Such units were excluded from the analysis. This was a particular problem for the slowest conducted units, where a sizable part of the conduction path (including terminal branches) might have been unmyelinated or thinly myelinated. Such units also sometimes showed a considerable latency shift with time when stimulated at a steady rate. The problem could be minimized by the use of a lower stimulus repetition rate, but since we also wished to monitor the variation in antidromic latency during the respiratory cycle, this rate could not be set too low. The lowest rate used was about 3/s, but the most common rate was 5/s and sometimes 10/s. There was probably therefore a selection bias against the slowest conducting units. There may also have been a bias to identify the most slowly firing units as non-EBSNs, since if their spikes were too infrequent, a sufficient number of collisions would not occur. However, the fact that a long conduction distance was involved helped to increase the probability of seeing collisions for a given firing rate and thus minimized this bias.

**Antidromic latency measurements**

Variation in antidromic latency during the respiratory cycle was investigated for all units, as an indication of excitability changes consequent to synaptic excitation or inhibition (Merrill, 1974; Lipski, 1981). Fig. 4 is an example of a unit demonstrating clear variation. The raster display in A shows successive single sweeps during 2.5 respiratory cycles for a unit antidromically activated from the VLF of rostral L6.

**Fig. 4.** Variation in antidromic latency for an EBSN. A, left panel, raster display of an NRA recording showing successive responses to stimuli in the VLF of L6. Time between the sweeps runs from bottom to top, as indicated by the same recording shown on a compressed time scale in the right panel. Stimulation rate 10/s. Also shown is the efferent discharge in the external intercostal nerve of T6, which indicates the timing of inspiration. B, the same data as in the right panel of A, now shown together with the timing of the stimuli (top trace) and a plot of the antidromic latency (bottom, filled symbols), measured for the unit activated with a latency of around 10 ms in A. Note that in many sweeps in A the unit does not fire at the expected latency. In all of these sweeps during expiration, collision could be deduced (open circles, see text for explanation), allowing definition of the unit as an EBSN.
Note that for several sweeps during late expiration in each cycle, when there are many spontaneous spikes of similar amplitude, there is no antidromic spike. Note also that there are a few sweeps at the start of inspiration, where there are no spontaneous spikes (two sweeps for the first full inspiration and one for the second). The antidromic latency also showed a very clear systematic variation between these periods, as is illustrated in the latency plot in B. In such plots a cursor was used to measure spike times at a constant level on the spike rising phase. If possible, the level was chosen to allow the inclusion of any variation in initial segment/soma-dendritic spike segmentation that might be present. Each sweep was inspected to ensure that the spikes from the same unit were counted and to avoid artefacts such as spike superposition. The absences of antidromic spikes at the beginning of inspiration occurred at a time in the cycle when the latency was longest, consistent with these absences being the result of inhibition, whereas the absences at the end of expiration occurred when the latency was shortest, corresponding to the greatest excitation, and therefore consistent with the occurrence of collisions with spontaneous

The gradual decrease in latency as inspiration progresses was reported by Merrill (1974) and corresponds to the decrementing pattern of inhibition often displayed in EBSNs (Ballantyne and Richter, 1986). The continued decrease in latency as expiration progresses is then a further sign of incrementing excitation during expiration, presumably before the unit reaches firing threshold. Note that in referring to this as incrementing excitation we do not exclude the possibility of a contribution from waning inhibition, which can occur during phase I expiration in EBSNs (Ballantyne & Richter, 1986). During the period of firing in late expiration, the antidromic latency of this unit showed a great deal of variation, including large jumps of latency to values of about 1 ms shorter than the antidromic latency. The time course of the latency variation of this unit is typical of the EBSNs, though the extent of the variation was often less.

Not all these features were restricted to EBSNs. Fig. 5 shows two units (a and b) evoked by stimuli to the VLF of caudal L7. It is obvious from the raster display of Fig. 5A that unit a shows latency variability, but it is less obvious for unit b. However, the latency plots in B indicate that both units showed clear latency

**Fig. 5.** Variation in antidromic latency for two units activated from the VLF of L7. Same arrangement as in Fig. 4, except two antidromically activated units (a, b) are visible in the recording. Stimulation rate 5/s. Different respiratory cycles are shown in the raster display (A) and the antidromic latency plots (B). Note that, for unit a, the same pattern is visible in both A and B, namely absence of the unit’s antidromically activated spikes for some sweeps during inspiration and for one or two sweeps (collision deduced for each one) during expiration, together with a rather variable latency during late expiration. The collisions allowed this unit to be classified as an EBSN. The second unit (b), with antidromic spikes throughout expiration and no collisions, was classified as a non-EBSN, but nevertheless showed a variation in latency during the respiratory cycle and an absence of antidromic spikes during inspiration. Note different latency ranges for units a and b in B.
Functional roles for NRA-lumbosacral projecting neurons

A

Stim. VLF

NRA

Ext. intercostal nerve (insp.)

Latency (ms)

Collision

Unit a

Unit b

B

Stim. VLF L7

NRA

Ext. intercostal nerve (insp.)

Latency (ms)

Collision

Unit a

Unit b

Time (s)
variation (notice the different latency scales on the ordinates). Unit \( a \) showed a few collisions during late expiration, together with jumps of latency in this period, and was classified as an EBSN. It also showed a few absences of spikes during inspiration and a clear latency maximum at the start of inspiration, followed by a ramp of excitation during early expiration, just like the unit of Fig. 4. No collisions were seen for unit \( b \), which was therefore classified as a non-EBSN, but it is worth noting that for this unit, the blockade of spike invasion, presumably due to inhibition extended through almost all of inspiration. There was also a ramp of excitation evident during expiration, corresponding to an average latency shift per cycle of about 0.15 ms.

In addition to being classified as either EBSNs or non-EBSNs, units were therefore further classified as showing (i) inhibition during inspiration (either blockade of invasion or a latency at the start of inspiration at least 0.1 ms greater than at the start of expiration), (ii) a ramp of excitation during expiration (a decrease of latency of at least 0.1 ms) and (iii) latency jumps of more than 0.5 ms during late expiration. The definition of expiration was made from the external intercostal nerve discharge, as is conventional from a phrenic recording, including phase I expiration (post-inspiration, Richter, 1982). The non-EBSN population was therefore divided into two groups, respiratory modulated non-EBSNs or non-respiratory modulated non-EBSNs. The latter showed neither inhibition during inspiration, nor a ramp of excitation during expiration, as defined above with the 0.1 ms limits. Fig. 3B, largest spike is an example of one such unit. The classification of the majority of units in terms of latency variation was made simply from raster displays such as in Figs. 4A and 5A, printed out on appropriate time scales, with cursors to show fixed latencies. Only for selected units, either when chosen as examples or when judgement from the raster displays were uncertain, were formal measurements made as in Figs 4B, 5B. Automatic spike detection was not used.

No antidromically identified units were seen with any other consistent respiratory firing patterns or patterns of antidromic latency variation, with the exception of 2 units showing a barely detectable latency minimum in early expiration. Some units with inspiratory or early-expiratory firing patterns were encountered while tracking in the NRA, but none of these was antidromically activated.

*Units antidromically activated from the VLF of L6*

Forty units were identified from stimulation of the VLF of rostral L6, of which 17 were classified as EBSNs. Table 1 shows the properties of these units. Notice that nearly all of the EBSNs (15) showed a ramp of excitation during expiration. All of them showed inhibition during inspiration and 6 showed latency jumps. Of the 23 non-EBSNs, only one, from this L6 VLF group, showed an excitatory ramp during expiration, though 15 were inhibited in inspiration. The remaining 8 showed no modulation with respiration.
Functional roles for NRA-lumbosacral projecting neurons

Eighty-two units were antidromically activated from the VLF of caudal L7 and 17 from the VLF of S1/S2 (Table 1). Twenty-eight of these units were classified as EBSNs, 27 from L7 and 1 from S1/S2. Of the EBSNs activated from the VLF of L7 nearly all (26) showed inhibition during inspiration and 7 of them showed a ramp of excitation during expiration. Six EBSNs showed a jump in latency during expiration. The one EBSN that was activated from the VLF of S1/S2 showed inhibition during inspiration, but did not show a ramp of excitation in expiration.

Thirty-five of the 55 non-EBSNs activated from the VLF of L7 showed inhibition during inspiration, 5 of them showed a ramp of excitation during expiration. Twenty of these units did not show any signs of excitation during expiration or inhibition during inspiration. Most of the non-EBSNs (14) activated from the VLF of S1/S2 showed inhibition during inspiration, and 2 of them did not show modulation with respiration.

Conduction velocities

Conduction velocities for the units identified from the VLF are summarized in Fig. 6. The EBSNs showed a wide variation in conduction velocity, ranging from 8.5 to 59.8 m/s. The range of conduction velocities of the respiratory modulated non-EBSNs was generally less, 4.2 to 44.8 m/s, but with two outliers (62 and 66.3 m/s). The non-respiratory modulated non-EBSNs showed even less variation, with

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**Table 1.** Characterisation of respiratory drive in antidromically-activated units
The table shows properties derived from antidromic latency variation during the respiratory cycle (see text).

<table>
<thead>
<tr>
<th>Region</th>
<th>Type</th>
<th>Inhibitory inhibition</th>
<th>Expiratory ramp</th>
<th>Latency jumps</th>
<th>Modulation &lt; 0.1 ms</th>
<th>Total</th>
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<td>15</td>
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<td>17</td>
</tr>
<tr>
<td></td>
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<td>0</td>
<td>1</td>
<td>-</td>
<td>1</td>
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<td>1</td>
<td>-</td>
<td>1</td>
</tr>
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<td>7</td>
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Chapter 8

values ranging from 3.7 to 31.3 m/s. The median conduction velocities for the EBSNs, the respiratory modulated non-EBSNs and non-respiratory modulated non-EBSNs were 27.9, 22.1, and 11.4 m/s respectively. These conduction velocity populations differed significantly (Kruskal-Wallis, p < 0.05), with significant differences identified both between EBSNs and respiratory modulated non-EBSNs and between respiratory modulated and non-respiratory modulated non-EBSNs.

One possible factor that could have influenced these comparisons is slowed conduction in the more caudal segments, especially as one group (those activated from S1/S2) was predominantly non-EBSN. While individual axons are highly likely to show slowing towards their terminations (Sasaki et al., 1994), such an effect was not detected in the populations as a whole (Fig. 6B), the median conduction velocities being 22.8, 20.9 and 21.8 for L6, L7 and S1/S2 respectively (not significantly different, Kruskal-Wallis, p > 0.05).

Units antidromically activated from the gray matter of L6
Eleven units were antidromically activated from the gray matter of caudal L6. Fig. 7 shows an example. The depth-threshold plot (B) has three minima at depths of 1.75, 1.9 and 2.3-2.5 mm. At each of these depths the unit gave different latencies, 8.9, 8.8 and 8.5 ms respectively. Such a plot is an indication of a branched terminal collateral field (Merrill, 1974; Lipski, 1981). The broad minimum (threshold as low as 1.0 μA) at a depth of 2.3-2.5 mm corresponds to the depth of the Sm motor nucleus as determined by the motoneuron antidromic field potentials (Fig. 7C). A clear motoneuron cell group was visible in the neutral red stained sections at this location (Fig. 7A), corresponding to the location of Sm motor nucleus in the
A comprehensive description by VanderHorst and Holstege (1997b). From these data it can be concluded that this unit has collateral branches in the Sm motor nucleus as well as in the area dorsolateral to this nucleus, which contains motoneurons innervating tibialis anterior, flexor digitorum longus, extensor digitorum longus and peroneus longus (VanderHorst and Holstege, 1997b).

Three units were classified as having collaterals within the Sm motor nucleus (threshold minima 1, 6, 10 µA). Five others had threshold minima within 0.2 mm of the nucleus (values 17, 40, 40, 80 and 110 µA). Two more had threshold minima at 0.65 and 0.5 mm dorsal (values 5, 40 µA) and one more showed a

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**Fig. 7.** Identification of a unit with a collateral in Sm motor nucleus. A, track of stimulating electrode in caudal L6, reconstructed from histological sections. Gray area shows Sm motor nucleus. B, depth-threshold plot for stimulation at points along this track. The different latencies (indicated by the different symbols) represent the values read off the oscilloscope screen at the time of the recording. C, amplitude of Sm motoneuron antidromic field potential at the same depths, giving a physiological definition of the position of the Sm motor nucleus.
threshold minimum within the nucleus, with a value of 120 µA, but a very narrow plot (width 0.27 mm at a value of 240 µA).

The unit of Fig. 7 was a non-EBSN, inhibited in inspiration and with excitation during expiration shown by a latency ramp of about 0.1 ms (very similar to unit b in Fig. 5). However, the majority of the units with collaterals in the gray matter of L6 did not show signs of expiratory modulation (Table 1). Only one was classified as an EBSN (the one with a threshold minimum of 120 µA within Sm motor nucleus), and only two showed a ramp of excitation. Seven units were inhibited during inspiration. Four were classified as non-respiratory modulated non-EBSNs.
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Units antidromically activated from the gray matter of S1

Fifteen units were activated from the gray matter of S1. Fig. 8B shows an example of a depth-threshold plot of such a unit. This plot has two minima at depths of 2.1 and 2.4 mm in the sacral cord, both within Onuf’s nucleus, as shown by comparison with the amplitude plot for motoneuron antidromic field potentials from the pudendal nerve (Fig. 8C, filled circles). The second amplitude plot in Fig. 8C (open circles) relates to the motoneuron antidromic field potentials from the tibial nerve and shows that this motoneuron group is located at 1.4-1.6 mm deep. These motoneurons are located at the dorsolateral edge of the ventral horn (VanderHorst and Holstege, 1997b), a feature which confirmed the depth calibration for the track, as did the occurrence of a clear cell group identifiable as Onuf’s nucleus in the neutral red stained sections (VanderHorst and Holstege, 1997b) at 2.0-2.6 mm deep (Fig. 8A). The field potentials from stimulation of the pudendal nerve had a complex, dispersed time course (Fig. 1B) presumably representing slowly conducting motor axons, so the measured amplitude in Fig. 8C may not be closely related to the numbers of activated motoneurons. Nevertheless, the whole distance of the electrode track over which these potentials were detected corresponds well to the histological location of Onuf’s nucleus. Together these data indicate that this unit had collateral branches in Onuf’s nucleus.

All of the units in S1 classified as having collaterals showed two or more minima in their depth-threshold plots (including, in at least 5 instances, one minimum in the white matter). Eight units were classified as showing collaterals within Onuf’s nucleus (minimum thresholds 1-20 µA). Two others had threshold minima within 0.2 mm dorsal to the nucleus (values of 20, 80 µA ). Three more had threshold minima within Onuf’s nucleus (values 30, 40, 70 µA) and two more showed threshold minima 0.4 mm dorsal (values of 10, 18 µA).

The latency plot of the unit in Fig. 8 indicated no signs of excitation during expiration, but did show a small latency increase during inspiration, just qualifying as ≥ 0.1ms. Similar to the situation in L7, only two of the units with collateral branches in or near Onuf’s nucleus were classified as EBSNs (both minima within Onuf’s, values 13, 30 µA). These two plus six other units were inhibited during inspiration (Table 1). Seven units were classified as non-respiratory modulated non-EBSNs.

Conduction velocities for the units having collaterals at L6 or S1 were calculated from the measured conduction distance and latencies. The conduction velocities ranged from 3.7 to 41.1 m/s. The median was 16.3 m/s for the EBSNs and 11.7 for the non-EBSNs, a difference that is not significant (Wilcoxon rank sum test, p > 0.05).

Proportion of EBSNs

The proportion of units activated from the VLF which were EBSNs was a little lower
for L7 than for L6 (27/82, 32.9% vs 17/40, 42.5%) (Table 1), but the proportion projecting to the VLF of S1/S2 was much lower (1/17, 5.9%) and was significantly less than for L6, but not than for L7 ($\chi^2$, $p < 0.025$, equivalent to $p < 0.05$ with Bonferroni correction). The equivalent proportions of the units giving collaterals at either L6 (1/11) or S1 (2/15) are similar to this, but not individually significantly different to the proportions in the VLF of L6 or L7. However if considered together (3/26, 11.5%), their proportion is significantly less than (44/122, 36%), the total

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**Fig. 9.** Positions of units in the caudal medulla. The positions of all the units in this study, measured with respect to obex, are plotted on both of two standard dorsal views of the medulla. All sites were within the NRA, as defined by the nearby presence of expiratory neural activity. A, different symbols indicate units with different sites of stimulation for antidromic activation: first three, electrodes in the white matter; last two, movable tungsten electrode on a track passing through either Sm motor nucleus or Onuf’s nucleus. Note that the apparent concentration of units activated from the VLF of S1/S2 in the mid/caudal range of this plot, and that of units from Onuf’s nucleus in the rostral region, reflects the restricted range of sites sampled in the experiments concerned rather than any somatotopy. B, Units classified as EBSNs (gray filled circles), respiratory modulated non-EBSNs (squares) and the non-respiratory modulated non-EBSNs (black triangles).
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VLF proportion ($\chi^2$, $p < 0.05$). If units with threshold minima > 40 $\mu$A were excluded from those counted as showing collaterals, and therefore included with those activated from the VLF, these proportions would become 2/21 and 44/126 and would still be significantly different ($\chi^2$, $p < 0.05$).

Locations of units in different categories
The locations of the units in the medulla are plotted in Fig. 9, identified either by their identified projections (A) or their main physiological categories (B). Note that, in A, the rostrocaudal distribution of the units identified from the region of each motor nucleus is indistinguishable from that pertaining to the VLF for the same group of animals (i.e. the distribution for Sm corresponds to that for L6 VLF and the distribution for Onuf’s corresponds to that for L7 VLF). Note also that, in B, units in each physiological category were found along the whole length of the column, with no indication of segregation. This is just what might be expected from the examples of Fig. 3, where in each case, all three types of unit were found at a single recording site. This latter also indicates that, although there was uneven sampling across the experiments in terms of rostrocaudal positions, the uneven sampling cannot be the cause of the low proportions of EBSNs found in the units projecting to the motor nuclei or to S1/S2 VLF.

DISCUSSION

The principle outcome of this study is the identification of a group of neurons in the NRA, which project to the lumbosacral spinal cord and which are distinct from EBSNs. In these experiments, in the barbiturate-anesthetized cat, they are distinguishable by 1) their lack or virtual lack of discharges, 2) their weaker respiratory modulation as compared to EBSNs (fewer of the non-EBSNs showing an expiratory ramp or inspiratory inhibition) and 3) their slower conduction velocities.

Functional heterogeneity
Note that our definitions of unit categories are not absolute. The absence of collisions, as recognized here, does not completely rule out the possibility that some of the units defined as non-EBSN could have been firing at low rates or intermittently during expiration. However, given that a strong chemical drive to breathe was involved, and that many EBSNs may in any case be considered to be multifunctional (Grélot et al., 1996) it is not unreasonable that neurons with only a minor expiratory discharge should be categorized as having some other primary function. Miller et al. (1985) noted a few neurons, located within the respiratory column and antidromically activated from the upper lumbar cord, which were not spontaneously firing, but which showed antidromic latency variation with the respiratory cycle. This small group of neurons (11, as compared to 70 expiratory neurons) was classified as “silent expiratory”, but they were recorded at eupneic levels of $CO_2$. They may or may not have been recruited into activity under the
conditions of high levels of CO\textsubscript{2} used here, so this group probably includes some neurons corresponding to EBSNs here and some corresponding to respiratory modulated non-EBSNs.

Some of the non-EBSNs still appeared to receive some expiratory excitation, as shown by some decreasing ramps of latency. However, consistent with their absence of discharge, the occurrence of these ramps was lower than for the EBSNs. Also, none of the non-EBSNs showed latency jumps. We are not certain what these represent. As far as we know, they have not been reported previously. They may be considered separately from the latency ramp during early expiration. Merrill (1974), who first described the antidromic latency variation for EBSNs described a pattern with very little excitatory ramp, though Barillot and Bianchi (1979) showed some examples with similar ramps to those described here. Bainton and Kirkwood (1979) noted that near the CO\textsubscript{2} threshold for the respiratory rhythm, very little expiratory ramp was present in the firing rate of EBSNs. The presence of the ramps here may be the result of using a strong chemical drive and therefore a stronger synaptic drive than pertained for Merrill (1974). A possibility that must therefore be considered is that the jumps represent more facilitated invasion simply because of a greater depolarization. However, the amplitude of the jumps (up to 1 ms) makes this unlikely: it is hard to see how facilitated antidromic invasion by this amount, longer than the duration of an axonal or initial segment spike, could occur. A second possibility to be considered is that the jumps might represent a switch to orthodromic firing, evoked synaptically via electrical stimulation of an unidentified ascending or descending tract. The very variable latency associated with the jumps could be taken as suggestive of synaptic excitation. However two observations argue against this. Firstly the jumps always occurred at about the time the cells were observed or deduced to start firing spontaneously and jumps could not be produced at other times by increasing the stimulation strength to the spinal cord electrodes. Secondly, the jumps did not occur to some relatively constant latency, which would represent the conduction time of a postulated afferent pathway; rather they always represented a relatively small change from the "normal" antidromic latency of the unit concerned.

We suggest that in the present experiments the jumps were the result of two factors. The first is the long conduction distance (254 - 316 mm), which is probably 10 times longer than used by previous authors, with stimulation in the cervical cord. At some delays after a spontaneous orthodromic impulse the antidromic impulse would have traveled for some time in the partial refractory period, at others for some time (perhaps for the whole time) in the supernormal period. In the former there is not only a raised threshold compared to in an inactive axon, but slowed conduction, in the latter a decreased threshold and faster conduction, probably sufficient over this conduction distance to lead to the observed jumps (Bergmans, 1970). The second factor is that electrical stimulation could have been close to the terminations of the axons, where multiple branches, with
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various conduction velocities may have been present (Kirkwood, 1995; Kirkwood et al., 1999). Thus, for instance, it could be possible that at some intervals after a spontaneous impulse a larger diameter branch, further from the electrodes and therefore normally of a higher threshold, could show supernormality at a time a smaller branch was still relatively refractory. The effective stimulation site could then jump from the smaller to the larger branch. Both the jumps and the accompanying latency variability, could thus be explained by these factors and the absence of these features in the non-EBSNs is consistent with the definition of these units as non-firing.

If the new group of cells are not separated absolutely from the EBSNs, is it correct to divide the population into two (or three) groups, or should the whole population be regarded as a continuum? The issue comes down to a question of an operational definition. The EBSN population is in any case defined operationally as neurons firing with an expiratory pattern in particular circumstances, i.e. in the anesthetized or decerebrate preparation. However, these neurons are known to be multifunctional, being active also in vomiting (Miller et al., 1987), defecation (Fukuda and Fukai, 1988) and vocalization (Katada et al., 1996), and probably also in sneezing (Price and Batsel, 1970) and in cough (Jakuš et al. 1985). These motor acts require higher intensity expiratory muscle discharges than in normal expiration (e.g. Miller et al., 1987). Thus, in favor of describing the population as a continuum, it might be argued that recruitment of a higher threshold population of bulbospinal neurons, otherwise identical to the EBSNs could be expected, and that our new groups could represent this expectation. However, such recruitment has not been demonstrated. Price and Batsel (1970) observed recruitment of expiratory neurons during both sneeze and with expiratory loading, as did Jakuš et al. (1985) in coughing, but it is not known whether these neurons, which were recruited from eupnea, almost all in non-paralyzed cats, would have already been recruited in the circumstances of our experiments, fictive respiration at high CO₂. Nor is it known how many of these neurons were bulbospinal. The increased drive in the motor output could alternatively arise via reconfiguration of spinal interneuron circuits, or by the recruitment of other bulbospinal neurons outside the NRA (e.g. Miller et al., 1996). A final validation of functionally heterogeneous groups in the NRA, such as we are proposing, would depend on recording the activity of each neuron in turn in each of the possible motor acts and assigning it to a group by its spectrum of activity in these motor acts. Such detailed experiments have not been done, so we can only conclude here, that the simple observation of a group of bulbospinal neurons which are not active with expiration, despite the use of a high respiratory drive, is, for the present, sufficient evidence of heterogeneity in the bulbospinal population in the NRA, thus supporting the hypothesis to this effect put forward by Holstege (1989).

Collateral projections
If the different functional categories are accepted, it is of great interest that almost
all of those units giving collaterals to the motor nuclei belonged to the non-EBSN category, particularly with respect to the Sm motor nucleus. The anatomical study of VanderHorst and Holstege (1997b) showed that the bulbospinal projection from the NRA to this motor nucleus is very likely to have a role related to reproductive behavior, since the strength of the projection increased almost ninefold during estrus. Whatever other roles the neurons whose collaterals we have demonstrated might have (e.g., in vomiting, which itself must involve a whole body synergy), we suggest that at least some of our non-EBSN group are likely to be specialized for a role in the postures of mating.

On teleological grounds it might be expected that a high proportion of the units projecting to Onuf’s nucleus might be EBSNs. Sasaki et al. (1994) suggested that they might help in maintaining continence during the high intra-abdominal pressures of vomiting and other expulsive acts and Miller et al. (1995) provided experimental evidence in favor of this. We found only a few such projections. Of course the few that we did find might well have supported this role. In addition, by the same logic as above, these acts might also involve the recruitment of other neurons including some of the non-EBSNs whose projections were demonstrated here. The involvement of these would also be consistent with the abolition of the pudendal nerve discharge during fictive vomiting following midline section of the caudal medulla (Miller et al., 1995). Alternatively, it is possible that only a few fibers might be sufficient for such a role if a small input to motoneurons can be suitably amplified, such as by the operation of persistent inward currents, as has recently been suggested for another respiratory input (of unknown origin) to hindlimb motoneurons (Kirkwood et al., 2002; Kirkwood and Ford, 2004). In fact the rare occurrence of EBSN collaterals in the present study may still be consistent with previous observations. The collateral projections shown by Sasaki et al. (1994) for EBSNs at lower lumbar or sacral levels appeared to be relatively widely spaced, as compared to their spacing at upper lumbar levels or, indeed, for EBSNs at thoracic levels (Kirkwood et al., 1999). It is therefore possible that these projections represent a minor extension of what could be regarded as the main projections of these neurons to the motor nuclei of the thoracic and upper lumbar cord (Kirkwood et al., 1999). In contrast, the more commonly found collateral projections of the non-EBSN group suggest that these may represent the principal projections for these neurons. However, both of these suggestions need confirmation by direct comparison of the terminations of each type of neuron at different levels of the neuraxis.

Precise comparisons between the collateral projections demonstrated here and the collateral projections of EBSNs from previous studies (Sasaki et al., 1991, 1994) are not possible, because of the different methods of sampling involved. For Sasaki et al. (1994), as in many antidromic mapping studies, it was possible to record from a single unit activated from many different stimulation sites and to ensure that measurements were made on the same unit simply by maintaining
a continuous recording of its spontaneous firing. Here it was considered unsafe to attempt antidromic mapping in this way because of the difficulty of ensuring (for most units) that the same unit was evoked from different electrode tracks, when the unit concerned was either not spontaneously active, or was part of a multi-unit recording. Different sites along the one electrode track were considered safe because of the continuity of measurements of threshold or latency along the track (Figs. 7, 8). If sudden changes of latency occurred along a track (Fig. 7), it was clear that the same unit was involved because of the disappearance of the long latency spike whenever a higher threshold short latency spike was evoked. Because our maps were restricted to single electrode tracks, we cannot estimate directly how widespread the collateral projections were from the units that were recorded. We chose to sample along tracks passing through either Sm motor nucleus or Onuf’s nucleus because these two regions are known to have focal projections from the NRA (VanderHorst and Holstege, 1995). For the region of Onuf’s nucleus, Sasaki et al. (1994) found that 3/9 EBSNs with lumbosacral projections gave indications of collaterals. Because the proportions of non-EBSNs giving collaterals to this region here was higher than the proportion of non-EBSNs activated from the VLF, it is likely that more than 3/9 of non-EBSNs in the NRA give collaterals to this region, and also that they have more widespread projections than the EBSNs. Our data are also consistent with Sasaki et al. (1994) in that very few EBSNs appear to project below S1.

Some comment is required on the rather high thresholds accepted as evidence for the existence of collaterals for some units. An essential element for such acceptance was a narrow parabola in the depth-threshold plot. For instance, the highest value accepted for a threshold minimum was 120 μA, but for this unit the A coefficient in the best fit parabola (Davies and Kubin, 1986) was about 4200 μA.mm⁻², predicting that the axonal branch excited should be within about 0.17 mm of the stimulus point for 120 μA. Since this point was within the Sm motor nucleus, with the nearest white matter at a distance of about 0.8 mm, this excitation was accepted as evidence for the existence of a collateral. This A coefficient further predicts a very low conduction velocity for the excited fiber, about 2 m/s (Davies and Kubin, 1986), which also suggests the excitation of a collateral branch. However, note that even if those units with the highest thresholds are eliminated from consideration, the conclusion that collateral projections are more common from non-EBSNs than from EBSNs remains valid.

Location of units in the NRA
The NRA is an anatomically defined entity. In the cat its caudal part can readily be observed in Nissl-stained sections. Our identification of antidromically activated units being located in the NRA depends on their close association with the column of expiratory neurons, which itself was clearly demonstrated to correspond to the caudal part of the NRA by Merrill (1970, 1974). The experience in this laboratory over many years is entirely consistent with Merrill’s description: the column is very
compact and always recognizable in the recordings by the multi-unit expiratory discharges with an incrementing pattern and a sudden termination at the start of inspiration. The width of the column in either transverse dimension is about 0.2 mm, though if the recording of low amplitude spikes is included, it could be extended to 0.4 mm. The observation here that antidromically activated units could only be recorded within this column therefore gives them the same degree of association with the anatomically-defined NRA as the expiratory neurons and certainly puts them within the extent of the injection sites used by VanderHorst and Hostege (1995, 1997b) in their studies of anterogradely labeled projections to the lumbosacral cord. Further it is confirmation of the anatomical observation of VanderHorst and Holstege (1995), using retrograde labeling and an ipsilateral cervical spinal cord hemisection, that the only crossed fibers in this region with long spinal axons are those originating in the NRA.

No somatotopy was observed in the location of the neurons of different groups, either in terms of their projections, or in terms of their physiological properties. However, this conclusion must be regarded as provisional, given the relatively restricted sample available, in particular the samples available from individual animals. With more extensive recordings it is possible that an effect could be detectable, similar to the weak effect detected (all within the caudal NRA) in the rostro-caudal location of EBSNs projecting, or not, to L4-5 as compared to L1 (Miller et al., 1985).

Conduction velocities
The conduction velocities of the EBSNs measured here are lower than those previously reported from this laboratory for thoracic levels (Kirkwood, 1995), but quite consistent with the slowing of the conduction towards the terminations of individual axons in lumbosacral regions demonstrated by Sasaki et al. (1994). The main value of the conduction velocity observations here is that they give more weight to the separation of the three groups of units. Whether or not the separation between the groups is evident in the conduction velocities of the more proximal parts of their axons remains to be investigated.

Further functional considerations
A likely role for some of the non-EBSN units in the production of the mating postures has already been suggested, based on the collateral projections to the region of Sm motor nucleus. However, it is of interest that the non-EBSN category is also the dominant category with projections to sacral levels, including caudal to S1. Deductions about the role of NRA projections in mating behavior have previously been made by reference to the projections to more rostral levels (Vanderhorst and Holstege 1995, 1997b). Indeed the projections below S1 are not fully described, though Vanderhorst and Holstege (1995) showed one illustration from S2 and one from S3, including possible projections to tail motoneurons (their Fig. 8). There is a high concentration of tail motoneurons in the lower sacral and
cocygeal segments (Wada et al., 1990; Ritz et al., 1992), so these are obvious candidate targets for the most caudally projecting non-EBSNs. Stereotyped tail movements occur in variety of activities in the cat, such as mating, defecation, territorial marking and display (Michael, 1961; Kiley-Worthington, 1976), in some of which NRA projections are likely to participate or EBSNs have been shown to be active. All of these come under the heading of “survival behavior” and therefore might be expected to be controlled by the emotional motor system, to which the NRA has been assigned (Holstege 1991). There are therefore a variety of possible functional roles (specific, or non-specific) for these caudally projecting non-EBSNs.