Branching neurones in the cervical spinal cord with axons that reach sacral segments and the lateral reticular nucleus.
An electrophysiological study in the cat

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Abstract. Branching neurones in the cervical enlargement of the spinal cord were electrophysiologically studied in α-chloralose anaesthetized cats with the method of antidromic activation of axons. Stimulating electrodes were placed bilaterally at levels of lower thoracic and sacral segments and in the lateral reticular nucleus (LRN), ipsilaterally to the recording sites in C6/C7 segments. Thirty-nine out of a total one hundred neurones could be classified as bidirectional neurones with both descending and ascending collaterals. In the remaining cases only long descending projections to spinal segments were found. Comparison of conduction velocities measured in descending branches revealed no significant differences between individual neurones. On the other hand, descending collaterals of double direction neurones conducted impulses considerably faster than their axonal branches ascending to LRN. Our results suggest that parallel transmission of information to various, spinal or supraspinal centres of the nervous system is more common than reported before.

INTRODUCTION

Propriospinal neurones located in the cervical region with descending branches projecting to the lumbosacral enlargement have been identified anatomically or electrophysiologically in several studies in cats (Jankowska et al. 1974, Matsushita et al. 1979, Yezierski et al. 1980, Alstermark et al. 1987, Miller et al. 1998), monkeys (Skinner et al. 1979) and man (Meinck and Piesiur--Strehlow 1981). It has been shown in many behavioural experiments in animals that these neurones form essential parts of the system involved in the coordination of movements of forelimbs and hindlimbs (English 1980, Bem et al. 1995, Bélanger et al. 1996, Górska et al. 1996, Jiang and Drew 1996). So far, studies of the axonal trajectory of long descending propriospinal tract neurones originating from the cervical spinal cord have been focused mainly on direct projections to the lumbosacral enlargement. However, some studies have suggested the existence of collateral branches. In a some of these neurones a bilateral course of axons in the lateral funiculi of the spinal cord has been revealed (Verburgh et al. 1989, Krutki 1997, Krutki et al. 1997), as well as axonal branching at various levels of the spinal cord (Krutki et al. 1998). Alstermark et al. (1981) have demonstrated in the cat ascending collaterals to the ipsilateral lateral reticular nucleus (LRN) in a significant proportion of short C3-C4 propriospinal cells controlling forelimb motoneurones. These authors have also found such projections in some propriospinal neurones descending beyond Th9 segment.

The present work was undertaken to extend the above findings with respect to neurones with cell bodies located in C6 and C7 segments whose axons descend ipsilaterally, contralaterally or bilaterally to sacral segments (Krutki 1997). Electrophysiological methods were used in order to reveal ascending collaterals to LRN, to identify the location of neurones in the spinal grey matter of cervical segments, to find a proportion of neurones with bidirectional projections and to measure and compare conduction velocities of their axonal branches.

METHODS

Eleven adult cats of either sex were used for the experiments. The animals were initially anaesthetized with ketamine (25-40 mg/kg, i.m.) and after the preliminary surgery with α-chloralose (in several doses, sup-

plemented as required up to 50 mg/kg, i.v.), fixed in the stereotaxic frame and immobilized with gallamine triethiodide (1-3 mg/kg/h, i.v.). The depth of anaesthesia was monitored by controlling the diameter of pupils and withdrawal reflexes during preparation and by continuous heart rate and blood pressure recording (kept within physiological limits: 90-120 mmHg) during experiments. Occipital craniotomy and laminectomies were performed over the medulla, C6-C7, Th12-Th13 and S1-S2 segments of the spinal cord to expose required levels of the cord for stimulation and recording. Some forelimb nerve branches (ulnar, radial and median) were also dissected free for stimulation during collision tests. The full description of anaesthesia and surgical procedures was given in previous papers (Krutki 1997, Krutki et al. 1997).

A tungsten varnished needle stimulating electrode (tip diameter about 5 μm, exposed for 10-20 μm) was inserted into the ipsilateral LRN according to stereotaxic coordinates (Berman 1968): P, 15; H, -10 to −12; L, 3:1 to 4.1. The electrode was fixed at a position that yielded a field potential recorded in C6/C7 segments at the lowest threshold. Two stimulating electrodes of similar parameters were also inserted bilaterally into the grey matter of sacral segments of the spinal cord (perpendicular to the surface, 1-1.2 mm lateral from the midline, to a depth of 2.2-2.5 mm). Cathodal stimuli (0.2 ms) were delivered with a strength 50-200 μA in LRN and 40-150 μA in S1/S2. According to Bagshaw and Evans (1976), only axons not more than 0.5 mm distant from the stimulating electrode were expected to be excited by 100 μA pulses. After each experiment small electrolytic lesions (20 μA constant current for 15 s) were made before the electrodes were removed in order to verify histologically the locations of the stimulation sites. Two bipolar silver electrodes were placed on the surface of the spinal cord in contact with lateral funiculi at the level of Th13 segment, ipsilaterally (iTh13) and contralaterally (coTh13) to the recording site. Stimuli applied through these electrodes (0.2 ms; 0.2-1.0 mA) were used as the search stimuli during penetrations of the spinal cord with the recording microelectrode. Nerve branches were placed in separate tunnel electrodes mounted under the skin. They were stimulated with 0.1 ms pulses with an intensity expressed in multiples of threshold for the most sensitive nerve fibres in the given nerve.

Antidromic action potentials were recorded in C6 and C7 segments with glass micropipettes filled with 2M potassium citrate solution (tip diameter 1.5–2.5 μm, imped-

A

iTh13 800μA

coTh13 500μA

iS 80μA

coS 120μA

LRN 150μA

4 mV

5 ms (A)
10ms (B,C)

B

iTh13 250Hz
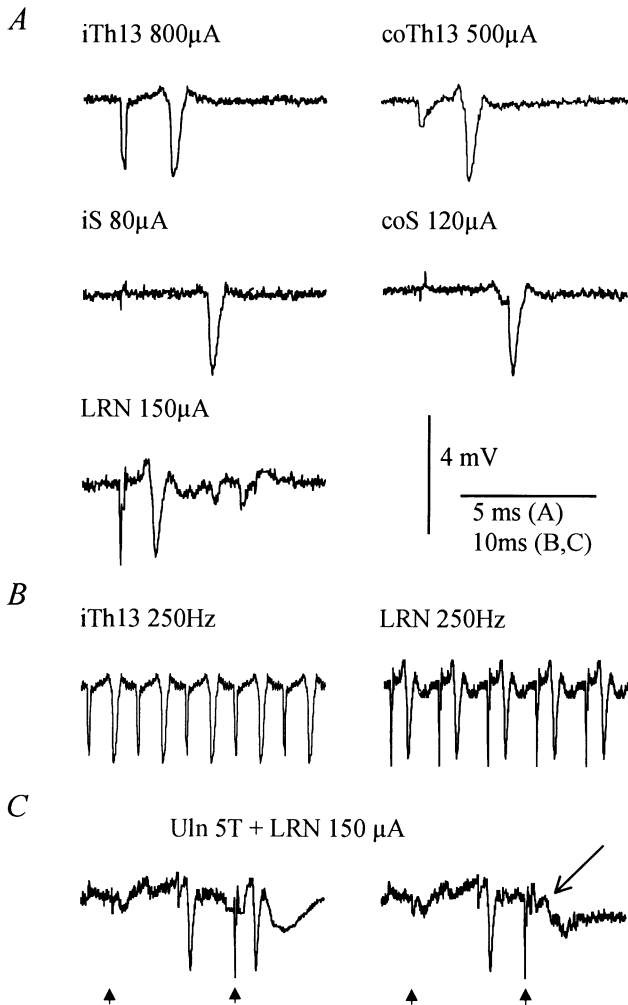
LRN 250Hz

C

Uln 5T + LRN 150 μA

Fig. 1. A, records of antidromic action potentials from a double direction neurone located in the C7 segment. Bilateral descending projections to sacral segments of the spinal cord and the ascending projection to LRN were established in this case. Averaged responses (5 sweeps) are presented, stimulation current is indicated above each record. Conduction distances in this case were 11.5 cm between C6 and LRN and 6.8 cm between C6 and Th13. Axonal conduction velocities calculated for ipsilateral ascending and descending branches of the presented neurone were the same and amounted to 62 m s^{-1}. B, records of high frequency stimulation tests performed in order to confirm the antidromicity of action potentials. No changes in the latency nor in the amplitude of the recorded action potentials were found while stimulating with 250 Hz. C, an example of the collision between the orthodromic potential from the ulnar nerve stimulated with an intensity of 5 times threshold (Uln 5T) and the antidromic spike obtained following LRN stimulation. Stimuli artifacts are indicated below records, the arrow points to the place of disappearance of the antidromic potential. The collision was successful when the delay between two potentials was minimum the double antidromic latency plus the refractory period.

ance 2-5 MΩ). The exact locations of recording sites were attributed to positions of micropipette tips which were defined from a depth from the surface of the cord, a distance from the midline and an angle of the micromanipulator.

Antidromicity of a potential was recognized from the following criteria: the all-or-none appearance, fixed latency, amplitude and threshold of stimulation, capability to follow repetitive stimuli at high frequencies (200 Hz) with no changes in the amplitude or the latency and a collision with orthodromic potentials evoked by stimulation of forelimb nerve branches. All the recorded signals were amplified and passed to a computer for storage and further analysis. Both single events or averaged responses (5–10) were recorded. Extracellular records obtained from one neurone which responded to stimulation of LRN as well as of all sites in the spinal cord and examples of high frequency stimulation tests and collision tests are given in Fig. 1.

RESULTS

A total of one hundred neurones were recorded extracellularly. They were located in the grey matter of C6 and C7 segments, at depths 2.7-5.9 mm from the cord dorsum that corresponded to laminae VII-VIII of Rexed (Rexed 1954). Locations of two neurones could be attributed to laminae IV and VI (Fig. 2A). Seventy three neurones were antidromically activated from sacral segments and in 27 cases antidromic potentials were evoked by stimulation of Th13 but not S1/S2. Neurones of the latter group most likely sent their axon terminals to lumbar segments. Descending projections were bilateral in most cases ($n = 61$), with the remainder ipsilateral ($n = 24$) or contralateral ($n = 15$).

Sixty one cells were classified as long descending propriospinal neurones. In the remaining 39 cells, ascending collaterals to the ipsilateral LRN were also revealed. Because both these collaterals (ascending and descending) ran in the spinal cord in opposite directions the term bidirectional neurones will be used to refer to these cells. In 28 cases of the latter category sacral projections were demonstrated, while 11 cells of this group projected to the Th13 level, but not to S1/S2. The contribution of particular types of neurones to the total sample is presented in Fig. 2B. It should be noted, however, that such a division into three groups is incomplete as regards detailed differences between ascending, descending, ipsilateral or contralateral projections of all
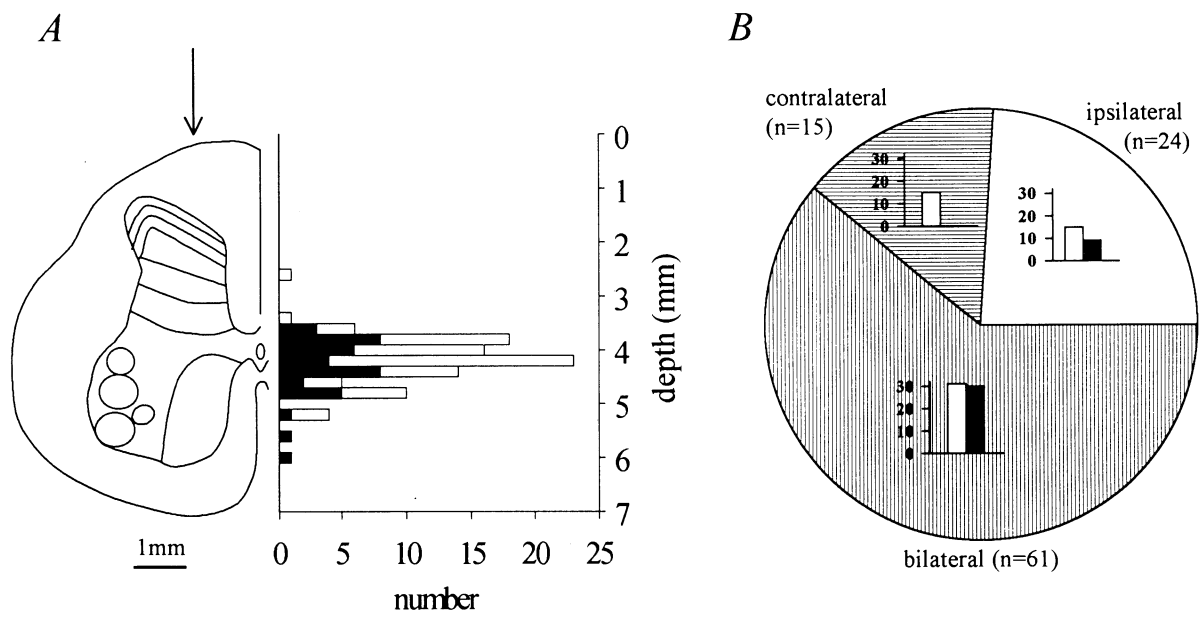
A

B

Fig. 2. A, left side, the outline of the averaged C6/C7 segments of the spinal cord with the grey matter divided into laminae according to Rexed. Right side, the diagram illustrating recording depths of all cells investigated. Open bars represent neurones classified as propriospinal only, filled bars represent double direction neurones with ascending and descending branches. The arrow indicates the direction of the insertion of a micropipette into the spinal cord. B, the slice diagram illustrating the relative contribution of ipsilateral (open slice), contralateral (horizontal hatch) or bilateral (vertical hatch) course of descending branches of all neurones investigated. Diagrams inside slices reflect numbers of exclusively propriospinal (open bars) or spinoreticular--propriospinal neurones (filled bars) in each group.

TABLE I

Various patterns of divergence of neurones located in C6 and C7 segments of the spinal cord

Type	Antidromic activation					Number
	iLRN	iTh13	coTh13	iS1/S2	coS1/S2	($n = 100$)
1	+	+	−	−	−	4
2	+	+	+	−	−	8
3	+	+	−	+	−	5
4	+	+	+	+	−	2
5	+	+	+	−	+	3
6	+	+	+	+	+	17
7	−	+	−	−	−	7
8	−	−	+	−	−	5
9	−	+	+	−	−	3
10	−	+	−	+	−	8
11	−	−	+	−	+	9
12	−	+	+	+	−	1
13	−	+	+	−	+	6
14	−	+	+	+	+	22

neurones investigated. Analysis of antidromic responses recorded in the cervical cord following stimulation of five spinal or supraspinal centres (cf. Methods) allowed us to distinguish fourteen types of neurones, different with respect to their pattern of divergence. The full classification is given in Table I.

Conduction velocities were measured for descending as well as ascending collaterals, on distances between C6/C7 and Th13 or between C6/C7 and LRN, respectively. The mean values of conduction velocities in axons descending in the spinal cord were 60 ± 13 m s^{-1} (mean$\pm SD, n = 85$) and 61 ± 13 m s^{-1} ($n = 76$) for ipsilateral and contralateral branches, respectively (difference insignificant, $P>0.05$, Student's t-test). However, comparison of conduction velocities measured in bidirectional neurones revealed significant differences ($P<0.01$) between mean values for ascending and descending collaterals on the ipsilateral side of the spinal cord (Fig. 3A). Conduction velocities of the ascending axonal branch were 41 ± 20 m s^{-1} ($n = 39$) while for the descending one 59 ± 13 m s^{-1} ($n = 39$) The diagram in

Fig. 3B illustrates the relationship between conduction velocities measured in reticular and prospriospinal collaterals of individual neurones. In the majority of cells the ascending axonal branch conducted slower than the descending one (differences up to 40 m s^{-1}), although in a proportion of neurones the values of conduction velocities were almost the same for both collaterals. The ratio of mean values for ascending to descending branches amounted to 0.685.

DISCUSSION

Branching neurones in the cervical enlargement with axons reaching lower spinal segments and ascending to the reticular formation, the tectum, the thalamus or the cerebellum have been previously revealed anatomically in the rat (Verburgh and Kuypers 1987, Verburgh et al. 1990) and the cat (Skinner et al. 1989, Verburgh et al. 1989). According to these studies, the relative number of double direction neurones in comparison to exclusively proprios-
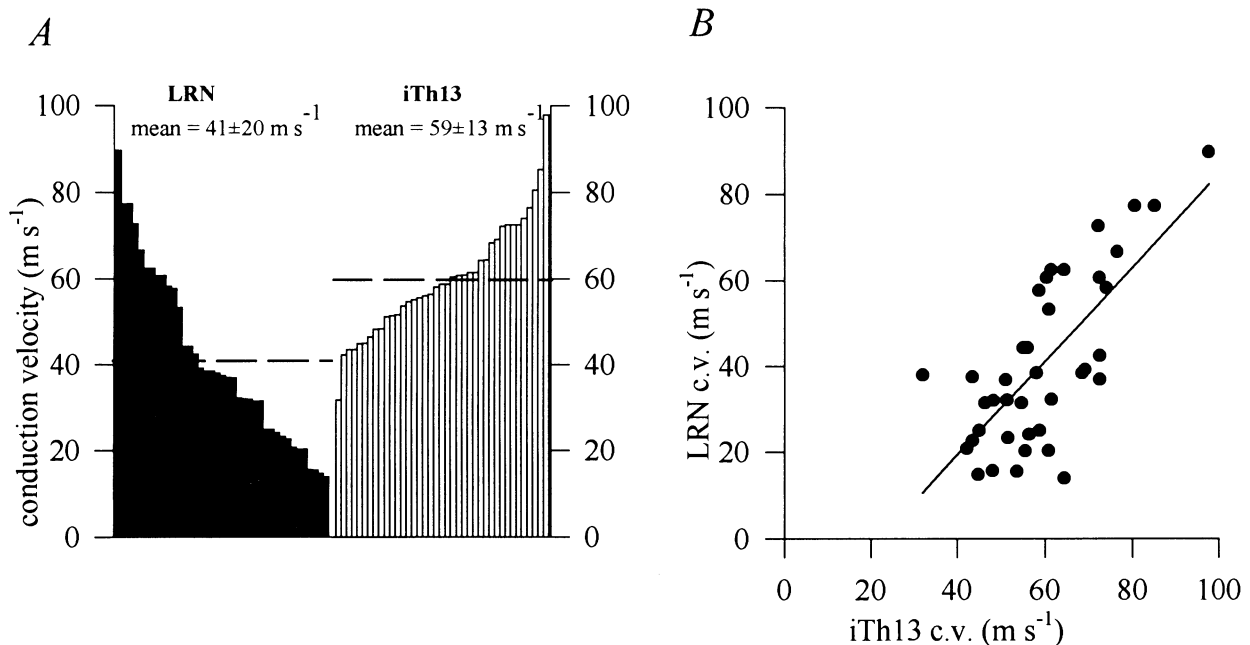

Fig. 3. A, the comparison of conduction velocities measured separately in axonal branches ascending to LRN (filled bars, left) and those descending to iTh13 (open bars, right) of double direction neurones. Horizontal dashed lines in the diagram indicate mean values of conduction velocities calculated for respective collaterals. B, the relationship between conduction velocities (c.v.) of ascending (measured between the recording site in C6/C7 and LRN) and descending collaterals (measured between the recording site in C6/C7 and iTh13) of individual branching neurones. The regression line presented on the diagram was calculated using linear regression analysis and the regression equation was: $y = -24.067 + 1.088x$. The correlation coefficient: $r = 0.528$.

pinal cells is rather low. The proportions of neurones with axonal branches descending to the lumbosacral enlargement and ascending to supraspinal centres have been estimated in the cat to range from 4% for cells with spinocerebellar collaterals (Verburgh et al. 1989) to 7% for those with spinoreticular collaterals (Skinner et al. 1989). In our study much higher proportions of branching neurones are presented (39%, cf. Results). It must be stated, however, that our data are in agreement with other physiological studies concerning ascending and descending collaterals of spinal neurones. Hirai et al. (1978) found descending collaterals in 43% of spinocerebellar neurones, and Alstermark et al. (1981) have reported that collaterals to LRN were present in 84% of short C3-C4 propriospinal neurones and in 11% of those projecting beyond the Th9 segment.

The present results show that mean conduction velocities are significantly lower in ascending collaterals when compared to those measured in descending branches. There is also a general tendency observed in individual cells that the ascending branch of the axon conducts slower than the descending one (Fig. 3B). This finding which suggests differences in thickness between two branches of the axon has also been demonstrated previously by Alstermark et al. (1981) for C3-C4 propriospinal neurones (means 44 and 26 m s^{-1}, for descending and ascending branches, respectively). It seems obvious that substantial differences in conduction velocities should also have functional consequences. However, we can only speculate that the time of the signal conduction from the investigated cells should be synchronized with the information transmitted to LRN by other spinal neurones, especially those involved in the same reflex pathways.

In our experiments LRN was stimulated only on one side, thus only ipsilateral ascending collaterals could be revealed in the neurones investigated - although ipsilateral, contralateral and bilateral axons descending to sacral segments were found. No ascending projections were established in nine neurones that projected to S1/S2 exclusively contralaterally nor in five cells identified as contralaterally descending beyond the Th13 segment, so they were classified as propriospinal (see Table I). However, we cannot exclude the possibility that some of the above neurones give off collaterals to the contralateral LRN. The existence of such collaterals is likely in neurones with branches descending bilaterally as well, though a lack of bilateral projections of individual spinal neur-

ones to LRN has been reported in the rat (Koekkoek and Ruigrok 1995). This problem needs to be addressed in future experiments.

The functional significance of double direction neurones also remains to be elucidated, however, the most likely role of these neurones is an integrative one. Previous studies have revealed that long descending propriospinal neurones in the cervical enlargement receive synaptic input from a variety of descending pathways and peripheral afferents. Alstermark et al. (1987a,b) have recorded in C3-C5 neurones excitatory postsynaptic potentials from cortico-, rubro-, tecto-, interstitio-, fastigio- and trigeminospinal fibres and inhibitory postsynaptic potentials from reticulospinal fibres. They have also demonstrated both excitatory and inhibitory inputs from neck and forelimb afferents. Other propriospinal neurones, located in lower cervical segments, are effectively influenced by forelimb afferents and from the brainstem as well (Schomburg et al. 1978, Skinner et al. 1980). Many similarities between the above cells and neurones examined in the present study (as concerns the location of cell bodies, the ipsilateral and/or contralateral course of descending axonal branches, conduction velocities) suggest the possibility that they also receive extensive input from various descending and peripheral afferents. Thus, they might mediate an integrative response to the lumbosacral enlargement and LRN, subserving forelimb-hindlimb coordination. On the other hand, the information transmitted to the LRN might serve as a feedback signal reaching one of the important centres involved in motor control. More detailed studies of synaptic input onto these neurones are now in progress.

In conclusion, our data show that a significant number of C6-C7 neurones with long descending propriospinal projections have also ascending collaterals to the lateral reticular nucleus. Combining the results of this study with those of previous morphological and electrophysiological investigations suggests that double direction projections of spinal neurones are more common than has been reported so far. Moreover, more rostral projection sites in the brainstem or cerebellum, not investigated in this study, cannot be excluded.

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