Lamina IV-VI neurones of the second sacral segment projecting to the sixth cervical segment of the cat's spinal cord

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Abstract. Cervical projections of neurones located in the second sacral segment of the spinal cord were electrophysiologically investigated in 12 adult cats under α-chloralose anaesthesia. Extracellular or intracellular antidromic potentials from 41 neurones following stimulation of their axons in the spinal grey matter in the C6 segment brought evidence of sacro-cervical connections. Cell bodies of the investigated neurones were found mainly in the medial part of laminae IV, V and VI. Axons of these neurones ran in lateral funiculi, mostly in their dorsal parts (dlf). Most of the axons (26) ascended bilaterally. Fibres of 15 cells ran unilaterally - on the ipsilateral (13) or the contralateral side (2). Conduction velocities of axons measured between S2 and C6 segments were in the range from 38.4 to 69.1 m/s. It is suggested that these neurones may play an important role in movement coordination between hindlimbs and forelimbs.

Key words: spinal cord, ascending spinal tracts, antidromic identification
INTRODUCTION

Many anatomical and electrophysiological studies have been performed to investigate the cells of origin of long ascending tracts in the sacral segments. The analysis of field potentials has demonstrated six separate long ascending tracts in the sacral segments. The analysis of field potentials has demonstrated six separate groups of neurones in this part of the cord, particularly in the S2 segment (Grottet et al. 1994). It has been supposed that long supraspinal tracts rather than long intraspinal tracts have their origin in these groups.

However, it has been known that the long intraspinal tracts connect the lumbar part of the cord (L1, L2) to the thoracic (Th1) or cervical (C8) levels. Axons of these cells run in lateral funiculi both bilaterally and unilaterally and terminate in the central part of lamina VII or the lateral part of laminae V-VII of the spinal grey matter in higher parts of the cord (Barilari and Kuypers 1969, Rustoni et al. 1971). Long ascending propriospinal neurones with their somas located in the lumbar cord and with axons terminating in the cervical regions have also been described by English et al. (1985). It has been shown that postsynaptic potentials could be recorded in motor nuclei for forelimb muscles following stimulation of hindlimb nerves. This fact suggests the existence of intraspinal links between motor centres controlling movements of the limbs (Miller et al. 1971, 1973). Riddel et al. (1994) have established that some cells from the S2 segment ascend only to the thoracic level of the cord and do not form supraspinal connections. These cells are recognized as propriospinal neurones but the precise target of their projection has not been identified.

The aim of the experiments presented in this paper was to investigate by electrophysiological methods the cervical projections of neurones of the S2 segment, as well as the localization of their cell bodies in the S2 grey matter and the course of their axons.

METHODS

Experiments were performed on 12 cats weighing between 1.6 and 2.9 kg. All animals were anaesthetized with an initial dose (25-40 mg/kg i.m.) of Ketamine hydrochloride supplemented during recordings with α-chloralose (up to 50 mg/kg i.v.). Bicarbonate solution (0.1 M NaHCO3 with 5% glucose) was continuously injected intravenously (1-2 mg/kg/h). Cats were immobilized with gallamine triethiodide (3 mg/kg/h i.v.) and artificially ventilated.

At the beginning of the experiment, the trachea was intubated. Respiratory movements were reduced by bilateral pneumothorax. Preparation of the left femoral artery enabled monitoring of blood pressure. The left femoral vein was also cannulated for drugs (α-chloralose and gallamine triethiodide) administration. Temperature of the body and systolic blood pressure were kept within physiological limits (38 ± 1°C) and 90-120 mmHg, respectively.

Laminectomies were performed at three levels of the spinal cord, between the following segments: L6 - S2, Th12 - Th13, C5 - C7. The dura was cut and removed in the cervical and sacral regions. At the thoracic level the dura was left intact. The pia was opened only in small areas of S2 and C6 segments where recording and stimulating electrodes were inserted. Antidromic stimulation of axons within the grey matter of the C6 segment was performed using a pair of tungsten monopolar electrodes, with tip diameters of 3-5 μm. They were inserted vertically, bilaterally to the depth of 2.5 mm (Fig. 1). The distance between tips of these electrodes amounted to 3 mm. In order to activate fibers antidromically, single stimuli of 0.2 ms duration and strength from 0.05 to 0.3 mA were applied at a frequency of 4-5 Hz (square pulse stimulator, mod. S88, GRASS Instruments). Such current parameters enabled stimulation of all axons in a region 1 mm in diameter (Ranck 1975, Bagshaw and Evans 1976). Two pairs of bipolar silver ball electrodes (tip diameters of about 0.5 mm, 3 mm apart) were placed bilaterally on the surface of dorsolateral funiculi at the thoracic level (Fig. 1). They were used for antidromic activation of axons of the neurones under investigation. Stimuli of 0.2 ms duration and 0.1-1.0 mA strength were used.

Extracellular and intracellular antidromic potentials were recorded from neurones located in the S2 segment (Fig. 1) using glass micropipettes (tip diameters 1.5-2.0 μm, resistance 2-5 MΩ, filled with 2 M potassium citrate solution,) and amplified (isolated low-noise preamplifier, mod. Iso-DAM8, World Precision Instruments, Inc.). Records were analysed on the photographs of 3-5 superimposed single sweeps from the oscilloscope screen.

In all experiments the following right hindlimb nerves were prepared: anterior biceps and semimembranosus (ABSm), posterior biceps and semitendinosus (PBS1), suralis (Sur), medial gastrocnemius (MG) and peroneus communis (Per. com.). The nerves were sectioned and their proximal parts placed on bipolar stimulating electrodes. The hindlimb nerves were stimulated with single pulses of 0.1 ms duration at a frequency of 4-5 Hz. The strength of stimulation pulses was expressed in relation...
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registered after stimulation of axons by tungsten electrodes inserted bilaterally into the grey matter of the C6 segment of the spinal cord. Axons were also stimulated by surface electrodes placed bilaterally on dorsal parts of lateral funiculi at the Th13 level. Antidromic excitations were recorded from 41 neurones, extracellularly from 34 cells (Fig. 2A and B) and intracellularly from 9

to the threshold of the most excitable fibres in the nerves. The potentials elicited by the stimulation at the C6 or Th13 levels as well as by stimulation of hindlimb nerves were also recorded from the surface of the sacral cord using a monopolar silver electrode placed on the root entry zone (Fig. 1).

The antidromic response was recognized according to the following criteria: the constant latency of a spike, its "all - or - none" appearance, its following of the high frequency stimulation (150-300 Hz) and its collision at appropriate intervals with a synaptically evoked potential (Lipski 1981, Grottel et al. 1991).

RESULTS

Antidromic potentials were recorded from neurones located in the grey matter of the S2 segment. They were

Fig. 1. The arrangement of the experiment. A, a pair of stimulating tungsten electrodes inserted into the grey matter of the C6 segment. B, a pair of bipolar stimulating electrodes placed on the surface of the Th13 segment. C, recording micropipette inserted into grey matter of the S2 segment and the cord dorsum surface recording electrode.

Fig. 2. A, extracellular records: from the neurone of bilateral projection to the C6 segment - antidromic potentials were recorded bilaterally from both the C6 and Th13 levels (4 sites of stimulation). B, extracellular records from the neurone of ipsilateral projection to the C6 segment with the contralateral branch to the Th13 segment - antidromic potentials were recorded ipsilaterally from the C6 level and bilaterally from the Th13 level (3 sites of stimulation). C, records of the collision of the antidromic potential evoked after stimulation of ipsilateral C6 segment (ant.) with synaptically evoked potential (syn.) resulting from the stimulation of the Sural nerve (Sur 6 x threshold). In A, B and C the upper records present extracellular potentials recorded from neurones, the lower records present cord dorsum potentials from the surface of the spinal cord.
neurones. In some neurones high frequency stimulation tests and collision tests (Fig. 2C) were made. Evidence of the existence of the sacro-cervical connection was obtained this way.

The locations of cell bodies were determined according to the micropipette position and its depth from the cord surface. The micropipettes directed 4°-13° medio-laterally and 5°-10° rostro-caudally were inserted into the S2 segment at a distance of 0.1-0.6 mm from the mid-line, to a depth 1.3-2.23 mm from the surface of the spinal cord. These three parameters were noted for every single neurone and its position into grey matter of the S2 segment was indicated. The investigated neurones were located in the middle part of the dorsal horn of the S2 segment, mainly in lamina V (20 cells). Some of the neurones were also located in the central parts of laminae IV (9 cells) and VI (12 cells) (Rexed 1954). The distribution of the investigated neurones within the S2 segment is shown in Fig. 3

Analysis of the sites of stimulation and values of current showed that all of the neurones studied sent their axons in dorsal parts of lateral funiculi. However, these axons differed in their projections to the C6 segment. From the majority of these cells (26 neurones) antidromic potentials were recorded following ipsilateral as well as contralateral stimulation at both the C6 and Th13 levels. In this type of cells antidromic excitations were recorded from all 4 sites of stimulation (Fig. 4A - 1) therefore their axonal courses to the cervical cord were bilateral. In next 12 cells, antidromic potentials appeared only from 3 sites of stimulation following ipsilateral stimulation at the C6 segment, as well as ipsilateral and contralateral stimulation at the Th13 level. Apparently,

![Fig. 3](image-url)

Fig. 3. Location of neurones investigated in the present study indicated on the cross-section of the S2 segment: black squares represent neurones of bilateral projection to the C6 segment; crosses - neurones of ipsilateral projection to the C6 segment (with the contralateral branches to the Th13); white square, neurone of ipsilateral projection to the C6 segment; black rhombs, neurones of contralateral projection to the C6 segment.

![Fig. 4](image-url)

Fig. 4. A, scheme of the course of axons of four types of investigated neurones. B, mean conduction velocities measured between the sacral and cervical segments for the above four groups of neurones. C, number of neurones with significantly decreased conduction velocity (CVD) above the Th 13 segment (see text): no CVD, no decrease or decrease less than 10%; CVD 10%, decrease in the range of 10 - 20%; CVD 20%, decrease in the range of 20 - 30%; CVD 30%, decrease more than 30%.
the axons of these neurones reached segment C6 on the ipsilateral side only. However, these axons ascended also contralaterally, at least up to the thoracic level (Fig. 4A - 2). Two neurones showed an exclusively contralateral projection. In this type of cells antidromic potentials resulted from the contralateral stimulation at the level of C6 and Th13, thus from 2 sites of excitation only (Fig. 4A - 3). One neurone sent its axon to the C6 segment only on the ipsilateral side and antidromic potentials were recorded after exclusively ipsilateral stimulation at the level of both the C6 and Th13 segments (also 2 sites of excitation, but from opposite side of the cord than in the previous case - Fig. 4A - 4). Summarizing, 26 neurones had bilateral projections to the C6 segment, whereas axons of 15 neurones reached the C6 segment unilaterally: thirteen on the ipsilateral side and two on the contralateral side.

The conduction velocities of the investigated axons were calculated from the antidromic latencies measured between the S2 and C6 segments (Fig. 4B). The axonal conduction velocities for bilaterally projecting neurones ranged for the ipsilateral branch from 38.4 to 68.9 m/s with a mean of 50.9 ± 9.0 m/s (± SD, n = 26) and for the contralateral branch from 39.8 to 67.1 m/s with a mean of 50.2 ± 8.0 m/s (± SD, n = 26). Neurones sending their axons ipsilaterally to the C6 (with a contralateral branch at the Th13 level) conducted with a velocity of 45.2-69.1 m/s (53.9 ± 7.0 m/s, n = 12). The conduction velocities of the two axons with a contralateral course only were 39.8 and 48.0 m/s. The axon ascending to C6 ipsilaterally conducted at 53.8 m/s.

The rates of conduction in the proximal (from the S2 to the Th13 segment) and distal (from the Th13 to the C6 segment) parts of axons were also calculated and compared. In 27 out of 41 axons (65%), the conduction velocity decreased by 10%-40%. No changes, or changes of less than 10% of the mean value of the conduction velocity were found in the remaining 14 axons (Fig. 4C).

**DISCUSSION**

It is supposed that the investigated tract is an element of interconnections between hindlimb and forelimb motor centres. This type of pathway is often called propriospinal because neurones and their axons pass within the spinal cord and give off collaterals at various levels (Kostyuk et al. 1971, Kostyuk and Mayski 1972). Neurones described in this paper are similar to those located below the first lumbar segment with axons running bi-

laterally in dorsal funiculi and terminating in the grey matter (laminae V-VIII) at higher levels of the cord (Barilari and Kuypers 1969). Rustioni et al. (1971) have suggested that fibres from the lumbar level can ascend also in dorsolateral funiculi and terminate in the central parts off laminae VII or lateral parts of laminae V-VII in higher spinal segments. The results presented here are in agreement with those of Matsushita and Ueyama (1973), who indicated that motor nuclei in the cervical region (between C7 and Th1) received projections from lower spinal cord. Miller and Reitsma (1971, 1973) investigated connections carrying excitatory and inhibitory impulses to different groups of motoneurones controlling forelimb muscles after stimulation of hindlimb nerves. However, the location of cells of origin of these tracts was not precisely described in these papers.

The present results demonstrate direct interconnections between spinal enlargements (S2 and C6 segments) containing motor centres for hindlimbs and forelimbs. Thus, these connections may contribute to coordination of movements of the limbs. However, this hypothesis requires more experimental research. The described tract is very similar to the propriospinal tract originating from rostral lumbar regions (laminae IV-VI) and terminating in the grey matter of cervical segments (English et al. 1985).

It has not been clearly established in our experiments whether axonal terminations of propriospinal fibres or only collaterals of fibres running to supraspinal structures were stimulated at the C6 level. In the latter case the stimulated collaterals might be a part of the long ascending pathways of dual propriospinal as well as supraspinal projections. This is very likely because several different tracts have their origin in the S2 segment. Three separate groups of cells of spino cerebellar tracts (SCT) have been encountered in the S2 segment. They are situated in lateral parts of laminae IV and V (group 5), in the medial part of lamina VII (group 2) and in the central part of laminae VIII and IX (group 6) (Matsushita and Hosoya 1979, Matsushita et al. 1979, Xu 1988). Crossed axons of these cells run mainly in the dorsolateral funiculus and most of them ascend to the cerebellar cortex through the restiform body (Grant 1982, Grant et al. 1982, Matsushita and Hosoya 1982, Kitamura and Yamada 1989). The S2 cells of origin of spinothalamalic tracts (STT) are widely distributed in lamina I (Craig and Kniffki 1985) and in laminae IV, V or VII, VIII (Trevino et al. 1972, Carstens and Trevino 1978, Jones et al. 1987). Axons of these tracts ascend in lateral or ventral funiculi and terminate
in lateral and medial parts of the thalamus. Dual projections of laminae VI and VII neurones to the thalamus and the cerebellum are also known (Huber et al. 1994). The neurones forming connections to the reticular formation (SRT) of the brain stem have been found in the S2 segment mainly in laminae V, VII and VIII (Fields et al. 1975, Maunz et al. 1978). Their axons project in ventral funiculi to the contralateral brain stem. Some neurones are also located in the central area of lamina VIII and their axons run to gigantocellular nuclei in the opposite dorsolateral funiculus (Huber et al. in press). The olivary complex receives sensory information from the lower cord cells located in laminae IV, V and VI (Armstrong and Schild 1979, Molinari 1984). A small number of them are situated in the central part of lamina VII, especially at the level of lumbar segments. Olivary fibres have been detected in all three funiculi (Armstrong and Schild 1979, Matsushita et al. 1992). Evidences that lamina IV, V and VI neurones of lumbo-sacral enlargement may project to the lateral cervical nucleus has been presented by Hongo et al. (1968), Bryan and Trevino (1973) and Brown (1981). The fibres of these tracts run in dorsolateral funiculi.

Before the present experiments, it has been supposed that the axons of cells of various supraspinal tracts located in laminae IV-VI of S2 segment may give off collaterals on their spinal course, also at the level of C6. The investigated projection may be a part of these tracts. This does not exclude the possibility that in the sacral cord propriospinal neurones also project to the C6 segment.

The spectrum of axonal conduction velocities in each of the described groups is rather wide. This suggests that the described tract consists of axons of various diameters. A significant decrease of axonal conduction velocities on their course is present in 65% of neurones. This may imply that axonal collaterals are branching off at various spinal levels (Riddel et al. 1994).

It is difficult to find any similarities in conduction velocities of the described neurones to cells of other afferent pathways. The fibres ascending from the sacral cord to the cerebellum conducted impulses rather faster: 61-100 m/s (Grottel et al. 1991). Most of spino-cervical axons conduct within a range of 53-89 m/s. It has been shown, however, that some of them conduct impulses significantly more slowly - below 44 m/s (Riddel et al. 1994). The spino-thalamic axons that originate in the same spinal cord region also have conduction velocities in a range from 27 to 64 m/s (Meyers and Snow 1982) or from 48 to 80 m/s (Huber et al. 1994). Moreover, spino-reticular tracts originating in the sacral cord show a wide range of conduction velocities: 16-96 m/s (Maunz et al. 1978). However, the results presented in this paper are similar to those described by Miller et al. (1973) who report conduction velocities of propriospinal afferent axons from 33 to 57 m/s.

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