

# Electrophysiological investigation of spino - olivary projections originating from sacral segments of the cat spinal cord

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**Abstract.** Ascending projections of sacral spinal cord neurones (S1-S2) to the dorsal accessory olivary nucleus (DAO) were electrophysiologically investigated in 3 adult cats under deep  $\alpha$ -chloralose anaesthesia. Antidromic action potentials were recorded extracellularly from 19 cells following stimulation of their axons in both the contralateral dorsal accessory olivary nucleus (coDAO) and the contralateral lateral funiculus at the level of lower thoracic segments (Th13). Two groups of neurones were identified in the gray matter of S1-S2 segments: one distributed in the medial part of Rexed's laminae VI and VII ( $n = 5$ ), the other located in the ventromedial part of lamina VIII ( $n = 14$ ). Axonal conduction velocities of neurones investigated were comprised in the range 32-55 m/s. A significant decrease of conduction velocity was observed in each case when distal and proximal parts of the axon were compared. Our research confirmed anatomical data concerning spino-olivary neurones originating from sacral segments. However, we suggest that axons of this pathway give off collaterals to other spinal or supraspinal centres.

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**Key words:** sacral segments, dorsal accessory olivary nucleus, cat

Ascending tract neurones located in sacral segments of the cats spinal cord have been widely described in anatomical and electrophysiological reports. They receive afferent input from muscle, joint and skin receptors of hind limbs and transmit this sensory information to various spinal and supraspinal structures (Aoyama et al. 1988, Riddell et al. 1994). Some of these cells have dual or even triple projections to various centres. It has been demonstrated that axons of sacral neurones form pathways terminating in cervical segments of the spinal cord (Krutki et al. 1997b, Mrówczyński 1997), the cerebellum (Grant et al. 1982, Xu 1988), thalamic nuclei (Carstens and Trevino 1978, Jones et al. 1987) or the reticular formation (Huber et al. 1999). Dual projections of cells from S1-S2 segments to the cervical segments and the cerebellum (Krutki et al. 1997a, Mrówczyński et al. 1998a), the cervical segments and the reticular formation (Mrówczyński et al. 1998b), the cerebellum and the reticular formation (Krutki et al. 1999) or the cerebellum and the thalamus (Huber et al. 1994) have been described. Grottel et al. (1998) have also indicated triple projections of sacral neurones to the cervical segments, the cerebellum and the reticular formation.

However, ascending projections of S1-S2 segment neurones to the olivary complex have not been investigated in detail. The inferior olivary nucleus is commonly known as a relay nucleus to the contralateral cerebellum, which is a source of climbing fibres to Purkinje cells in the cerebellar cortex. Thus, the origin and the course of spino-olivary tracts are important in view of control of movements of the body and limbs. Anatomical studies based on horseradish peroxidase transport in the cat have shown that populations of sacral neurones projecting to the dorsal and medial accessory olives (DAO and MAO) have concentrated in the ventral horn of the S1 segment (Armstrong and Schild 1979). Also in the cat, Molinari (1984) and Molinari and Starr (1989) have found neurones ascending to the rostral part of the DAO, and both the caudal DAO and MAO in the ventral horn of S1, as well as cells projecting to the rostral DAO and the caudal MAO in the intermediate part of the gray matter of the S2 segment. Matsushita et al. (1992) have described somatotopic termination of sacral segments spino-olivary neurones in MAO and DAO. The anatomical studies cited above have shown the location of spino-olivary cells in the sacral region of the cord and the termination of their fibres in various parts of the olivary complex. However, the physiological properties of those neurones have not been determined. Only Molinari et al.

(1990) performed an electrophysiological investigation of spino-olivary neurones, but it was restricted to lumbar segments of the spinal cord.

Therefore, the aims of our investigation in the cat were: (i) to confirm electrophysiologically the projections of sacral neurones to the dorsal accessory olivary nucleus (DAO), (ii) to determine the course of spino-olivary fibers along the spinal cord and (iii) to calculate and compare conduction velocities of axons.

Experiments were performed on 3 adult cats of either sex weighing between 2.5 and 3.3 kg. Rules for animal experiments were respected according to the Polish Law on Protection of Animals. At the beginning of each experiment animals were anaesthetized with Ketamine hydrochloride (25-40 mg/kg i.m.) for the initial surgery and the anesthesia was maintained with injections of several doses  $\alpha$ -chloralose (supplemented as required, up to 50 mg/kg, i.v.). The depth of anaesthesia was continuously controlled by checking the withdrawal and pupillary reflexes during surgery as well as the heart rate and blood pressure during recordings. Cats were artificially ventilated and paralysed with gallamine triethiodide (3 mg/kg/h i.v.) during recording. The body temperature, blood pressure and the end tidal CO<sub>2</sub> were controlled and kept within physiological limits ( $38 \pm 1^\circ\text{C}$ , 80-120 mmHg and 2-4%, respectively). The detailed procedures were described in previous papers (Mrówczyński 1997, Mrówczyński et al. 1998a).

Laminectomies were made at lumbosacral (L7-S2) and thoracic (Th12-Th13) levels of the spinal cord. The lower part of medulla oblongata was also exposed. In order to allow insertion of recording and stimulating electrodes, the dura was cut and removed and small holes in the pia were made. Open regions of central nervous system were covered with warm paraffin oil ( $37 \pm 1^\circ\text{C}$ ). Some nerves of the hind limb (suralis, deep peroneal and superficial peroneal) were dissected free and prepared for stimulation during collision tests.

Antidromic stimulation of axons within the dorsal accessory olivary nucleus (DAO) was made using a single tungsten electrode (tip diameter of 3-5  $\mu\text{m}$ ) inserted into this structure contralaterally to the recording site, according to Horsley-Clarke's stereotaxic coordinates (Berman 1968): P: 10.0-11.0; L: 1.6-2.5; H: 10.0-11.0. Negative single stimuli of 0.2 ms duration, strength of 50-200  $\mu\text{A}$  at a rate of 3-5 Hz were applied through this electrode. Bagshaw and Evans (1976) have shown that stimulation with these parameters would excite axons in a spherical volume of about 1 mm in diameter around the

tip of the stimulating electrode. After each recording session the precise locations of electrode tips in coDAO were verified histologically by electrolytic lesions (10 mA cathodal current for 15 s). Axons of the neurones investigated were also stimulated along the spinal cord with a bipolar silver ball-tipped electrode placed bilaterally on the surface of dorsal parts of lateral *funiculi* at the Th13 level. Stimuli of 0.2 ms duration and 0.1–1.2 mA intensity were applied at a frequency of 3–5 Hz through this electrode.

Antidromic action potentials were recorded extracellularly from neurones using glass micropipettes (tips broken to 1.2–2.0  $\mu\text{m}$  diameter, impedance 3–5 M $\Omega$ , filled with 2M potassium citrate) introduced into the gray matter of S1–S2 segments of the cord. The positions of recording electrodes were verified histologically at the end of each experiment. The recorded signals were

sampled by an analog-digital converter (PCL-818HD) and stored on a computer disc for further analysis. Single or averaged (5–10 sweeps) responses were analyzed. The identification of antidromic potentials was based on criteria described previously by Lipski (1981), including the test of high frequency following ( $> 200\text{Hz}$ ) and a collision with orthodromically evoked potentials at the appropriate interval. The experimental arrangement is schematically presented in Fig. 1A.

Recordings were taken from 19 neurones located in the gray matter of S1/S2 segments. Figure 1B shows samples of extracellular antidromic potentials from S1–S2 neurones after excitation of their axons in the coDAO and at the level of coTh13. The analysis of antidromic responses clearly showed that the whole group of investigated neurones ascended contralaterally along the spinal cord, terminating in the contralateral

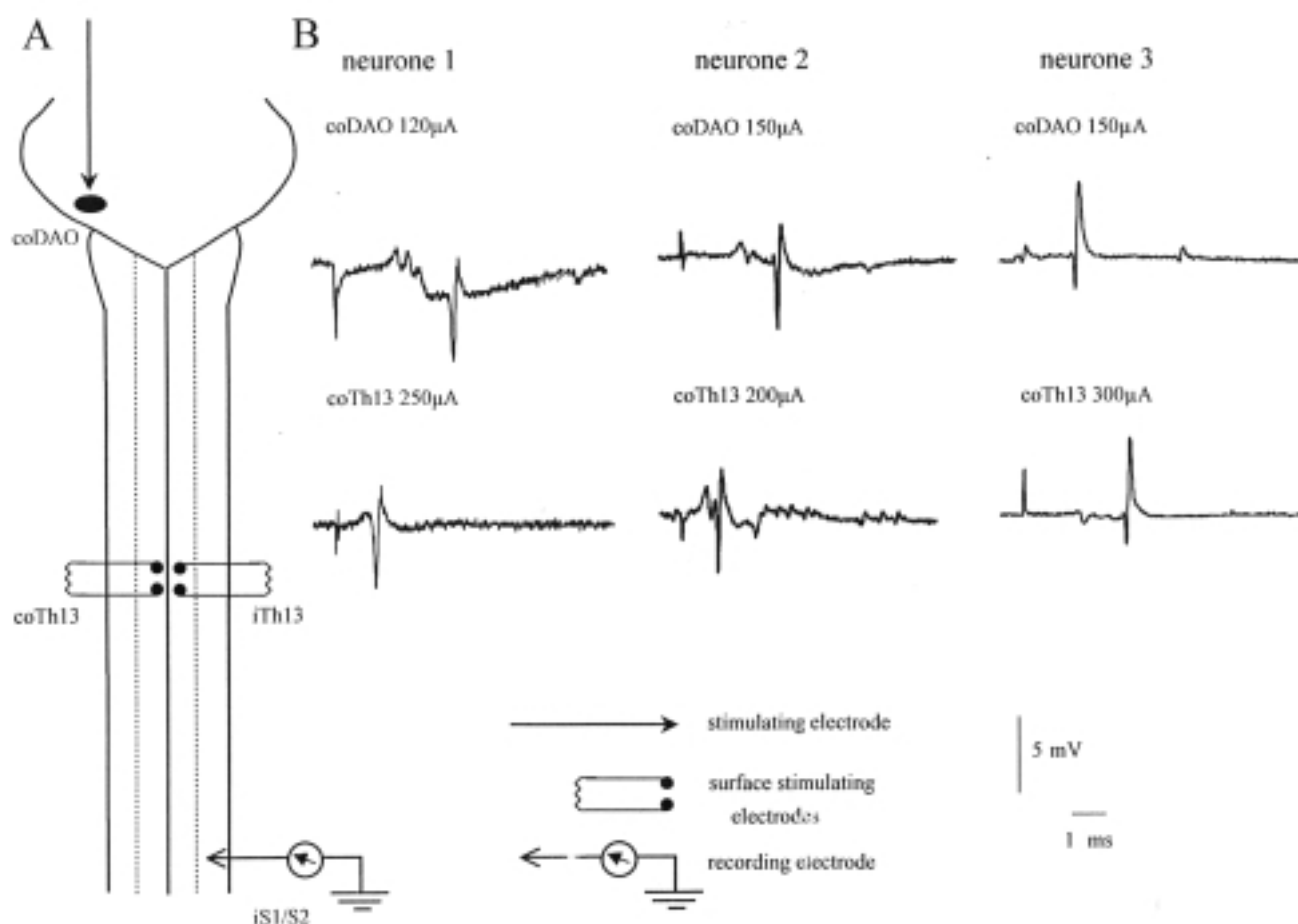


Fig. 1. A, a scheme illustrating the arrangement of experiments with stimulating and recording sites indicated. B, samples of extracellular records from sacral neurones obtained after stimulation of axons in the coDAO and at the level of the coTh13 segment. The values of current used for stimulation are given above records.

dorsal accessory olivary nucleus (no antidromic responses were obtained following iTh13 stimulation). Additionally, the values of the current used for stimulation at the Th13 segment indicated that spino-olivary axons ran in the lateral funiculus of the spinal cord (Fig. 2B).

The positions of micropipette tips were determined from angles of micromanipulator direction ( $4^{\circ}$ – $12^{\circ}$  mediolaterally and  $0^{\circ}$ – $10^{\circ}$  rostrally), distances from the midline (0.1–1.2 mm) and the depth from the dorsal surface of the cord (2.31–3.93 mm), which enabled us to establish the location of each neurone in the grey matter of the S1–S2 segments. Cells were distributed in two distinct groups. The first one which contained only 5 neurones, was located in the intermediate zone, in the medial part of laminae VI–VII (Rexed 1954). However, the second group was found in the ventral horn

(the ventromedial region of Rexed's laminae VIII) and consisted of 14 neurones (Fig. 2A).

The axonal conduction velocities of neurones studied were calculated from the antidromic latencies and distances between the recording site in S1–S2 segments and the stimulating site in coDAO. Generally, 19 spino-olivary neurones conducted with mean velocities ranging from 32 to 55 m/s. No differences were found between neurones of the two groups of different locations. Axonal conduction velocities were also calculated separately for proximal (between sacral segments and the Th13 level) and distal (between the Th13 level and DAO) parts of each axon. It was shown that all axons conducted considerably faster in their proximal parts (56–74 m/s and 43–89 m/s) in comparison to their distal parts (27–45 m/s and 25–48 m/s), respectively (Fig. 2C).

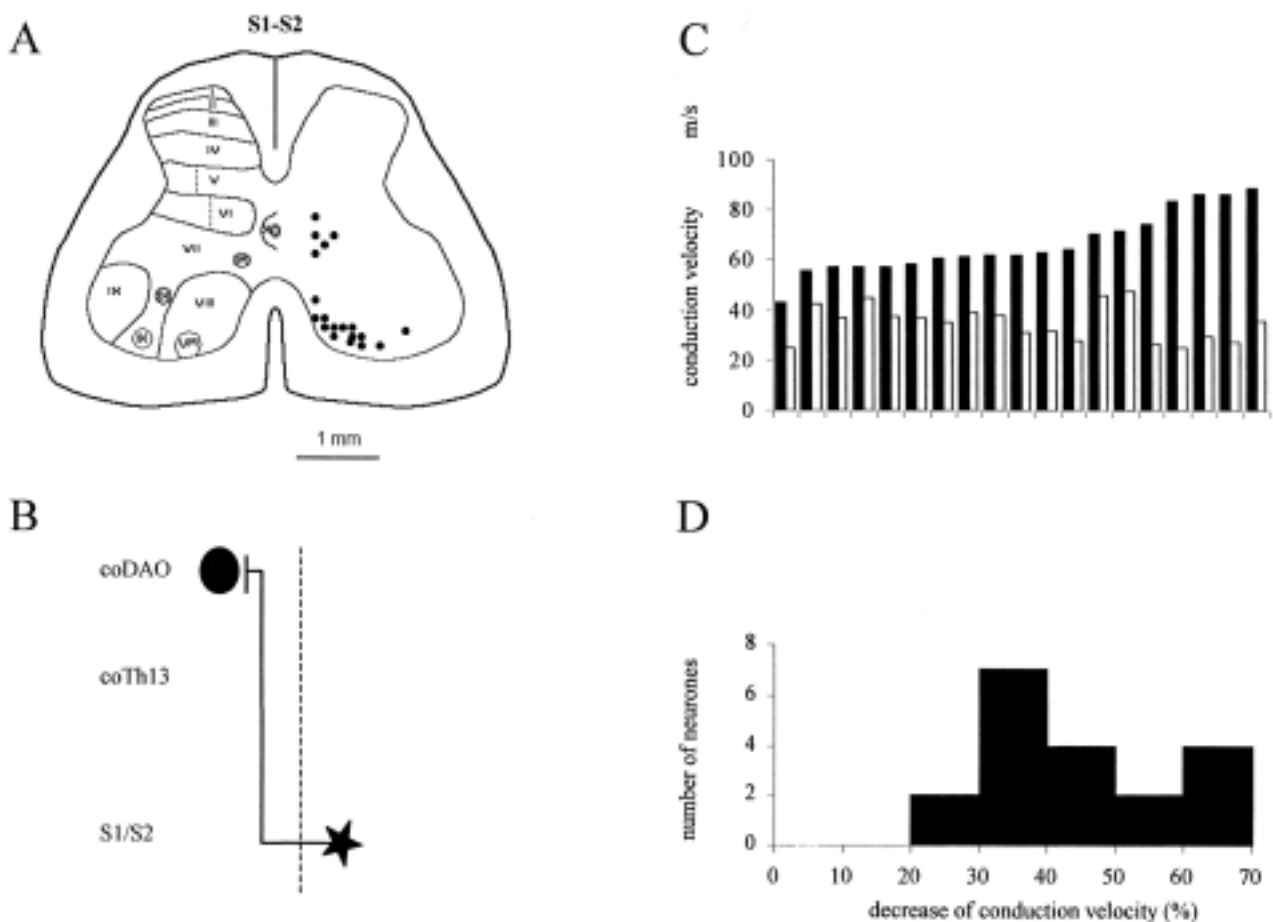


Fig. 2. A, distribution of the neurones studied shown on the averaged outline of the S1–S2 segments of transverse sections of the spinal cord. B, schematic drawing of projection and axonal course of neurones in the study. C, diagram presenting axonal conduction velocities of individual neurones as measured separately for proximal (between S1–S2 and Th13 segments, filled bars) and distal (between Th13 and DAO, open bars) parts of axons. D, histogram of the percentage of decrease in conduction velocities in distal parts of axons.

When differences between conduction velocities in proximal and distal parts were presented as a percentage of the initial value, the decrease of conduction velocity ranged from 20% to more than 60%. The histogram of these values for the population studied is presented in Fig. 2D.

Our results electrophysiologically confirm the existence of spino-olivary neurones in S1-S2 segments of the cat spinal cord that project to the dorsal accessory olivary nucleus (Armstrong and Schild 1979, Molinari 1984, Molinari and Starr 1989, Matsushita et al. 1992). The distribution of spino-olivary neurones in our study is in agreement with previous investigations performed by Armstrong and Schild (1979) who have demonstrated one group of cells projecting to the DAO in the medial lamina VII and the dorsomedial part of lamina VIII in the S1 segment. In studies of Molinari (1984) spino-olivary tract neurones have been located in the ventromedial part of the lamina VIII in the S1 segment or in the medial part of lamina VII in the S2-S3 segments. Spino-olivary neurones ascending to DAO were also identified by Matsushita et al. (1992) in the medial part of lamina VII of S1 segment.

On the other hand, the differences between spino-olivary neurones presented in this paper and in previous studies have been also found. They concern mainly conduction velocities of fibres ascending to DAO. The conduction velocities measured in this study comprised in the range 32-55 m/s. Conduction velocities of spino-olivary cells that reached the DAO from the lumbar level of the cord were 24-30 m/s or 18-28 m/s in studies of Armstrong et al. (1968) and Molinari et al. (1990), respectively. So, they were considerably lower than those reported here. However, it should be noted that conduction velocities of spino-olivary pathways originating from the sacral region of the cord have not been determined previously.

It has also been indicated in this study that individual axons of neurones projecting to the DAO conduct significantly slower in their distal parts and this slowing in conduction exceeds 20% ( $n = 19$ ) and in some neurones is even higher than 40% ( $n = 10$ ) (see Fig. 2D). This property of spino-olivary axons has not been observed previously, though it has been described for some other ascending tracts originating from this level of the cord. A significant decrease of conduction velocity in distal parts of axons ascending from the spinal cord has been found for spino-cerebellar, spino-reticular and long ascending propriospinal tracts (Krutki et al. 1997a, b, 1999). This

feature suggests that spino-olivary projections of the neurones investigated are not the only target. These neurones may in fact give off collateral branches to the spinal cord (at the thoracic or cervical levels), to the cerebellum or to brain stem centres.

The location in gray matter, the contralateral axonal course in the lateral funiculus and values of conduction velocities measured between S1-S2 and Th13 segments (43-89 m/s) are especially similar to references described for a part of neurones of spino-cerebellar tracts originating from sacral segments. It has been shown anatomically and electrophysiologically that they are distributed in laminae VI-VII and VIII, their axons cross the midline and ascend in the lateral *funiculus* and conduction velocities are comprised in the range 48-96 m/s (Grant et al. 1982, Xu 1988, Krutki et al. 1997). It cannot be excluded that at least some neurones of these two groups may be in fact cells with axons branching to both the cerebellum and the olivary complex. The existence of such a divergence could modify our view on transmission of information from lower spinal cord segments to supraspinal centres involved in the motor control. However, this suggestion must be confirmed and the relevant experiments are now in progress.

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