ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1994, 54: 235-242

Antidromic field potentials recorded from cell groups of S2 spinal cord segment with axons in the opposite Th13 dorsolateral funiculus in the cat

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Abstract. Location of cell groups within gray matter of S2 spinal cord segment, whose axons are running in the opposite Th13 dorsolateral funiculus, were studied by means of antidromic field potentials recorded in chloralose anaesthetized cats. The cell bodies are forming six separate groups occupying medial laminae II and III, medial laminae IV and V, medial lamina VII, medial lamina VIII, lateral laminae V and VI and lateral laminae VIII and IX. Properties of main components in antidromic field potentials recorded in each of areas have been described. The presumable termination sites of axons of investigated cellular groups are considered.

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Key words: crossed ascending spinal tracts, S2 segment, antidromic field potentials, cat

INTRODUCTION

Many of the second sacral segment neurones of the mammalian spinal cord are cells of origin of long ascending pathways. Although some cellular groups have been described in neuroanatomical studies, their physiological properties were not investigated in details. Most of their axons are crossed and they reach the cerebellum (Matsushita et al. 1979, Grant et al. 1982,), thalamic nuclei and their adjacent areas (Willis 1983, Jones et al. 1987) or they can be fibers of propriospinal neurones (Molenaar and Kuypers 1978).

It seems likely that some of cells of origin of spinocervical tract (Craig and Tapper 1978, Brown 1981), postsynaptic dorsal column neurones (Brown and Fyffe 1981) and a group II-activated ascending tract neurones (Harrison and Riddell 1990) whose fibres project ipsilaterally are located in S2 segment.

The aim of this study was to find out the distribution and number of groups of crossed ascending tracts neurones at this level. We have chose the technique of antidromic field potentials recording which was widely used in similar studies (see e.g. Hirai et al. 1978). It will be shown that they could be evoked following excitation of long ascending fibres at the level of Th13 segment within the dorsal part of the opposite lateral funiculus. This paper constitutes an introduction to studies on influences from hindlimb afferents onto identified cellular groups in S2 segment.

METHODS

Experiments were conducted on eighteen cats weighing between 2.2 and 3.65 kg. During surgical preparation the animals were anaesthetized with halothane and later with α-chloralose during recordings (70 mg/kg initial dose, supplemented with additional doses up to the total dose of 5-7 mg/kg/h). About 40 ml of bicarbonate solution (100 mM-$NaHCO_3$ with 5% glucose) was repeatedly injected intravenously. Its volume was compared to volume of urine collected via catheter. The temperature and the arterial blood pressure were controlled and kept within physiological limits ($38^{o}\pm1^{o}$C and 90-120 mmHg, respectively). All cats were paralysed with gallamine thriethiodide (3 mg/kg/h) and artificially respired; ventilation was adjusted to keep end-tidal CO_2 near 4%. In order to minimize the respiratory movements, a bilateral pneumothorax was performed.

Laminectomies were performed to expose the spinal cord at levels of Th13 and L6-S2 segments. The dura was cut and torn away at the level of whole S2 segment while the pia was opened only in small regions to allow insertion of the recording microelectrodes. At Th13 dura was usually left intact to secure more stable conditions of stimulation. The spinal cord was covered with warmed paraffin oil ($37^{o}\pm1^{o}$C).

In order to antidromically activate the axons of neurones, rectangular current pulses of 0.2 ms duration and strength of 0.9-1.5 mA were applied through the bipolar silver ball-tipped electrodes (tip diameters about 0.5 mm, separated by 3.0 mm). They were placed in parallel at the surface of the opposite dorsolateral funiculus (codlf) of Th13 segment. Of the bipolar pair, the electrode nearest to the recording electrode was connected to the cathode. The rate of stimulation was kept below 5 Hz. The monopolar stimulation was additionally tested for comparison in five animals (see Results section). The cathode of a similar diameter as used for bipolar stimulation was placed on the surface of codlf, the indifferent electrode being inserted in the back muscle.

Antidromic volleys were recorded from the S2 dorsal root entry zone (for the control) with a monopolar silver ball electrode. Recordings of antidromic field potentials were made using glass micropipetts filled with 2 M potassium citrate solution or with 9% solution of horseradish peroxidase (HRP Sigma, type VI,in 0.2 M KCl solution in Tris buffer at pH 8.6). Their tips were broken to 1.5-2.0 μm. Sometimes it was possible to record large extracellular potentials. In these cases criteria for antidromic nature of excitation were the same as described previously (Grottel et al. 1991).

Photographs of 3-5 superimposed single recordings were analyzed. The recordings were taken at different depths of a series of microelectrode penetrations made at lateral angle of 4 deg through the S2 segment. At some locations, to measure precisely the timing of the onset of field potentials, the averager (ANOPS 105) was used (32 single sweeps for each average with a time resolution of 10 μs per adress).

The approximate conduction velocities of axons of ascending tract cells were calculated from the latencies of antidromic field potentials components evoked by stimulation of the thoracic funiculus and the corresponding conduction distances.

The sites of recordings with the microelectrode were determined on the basis of the angles of micromanipulator to which it was attached and depths of insertion from the surface of the spinal cord. Additionally, in areas where recordings were the most frequently performed, the microelectrodes were left in last tracks and positions of tips were histologically verified. The depths of identified fixed microletrode tracks observed under the microscope were compared to indications of the micromanipulator counter. In five experiments iontophoretic injections of horseradish peroxidase (by 200-250 ms long positive pulses of 15-30 nA, 2/s, total injection time up to 10 min) were made in areas where the largest antidromic extracellular potentials appeared (Fig. 1B). Serial transverse 60 μm sections of S2 segment were cut and HRP deposits were revealed according to the procedure of Mesulam (1978).

RESULTS

Altogether 179 antidromic field potentials were recorded in different areas of the white and grey matter of the second sacral segment during systematical penetrations. Figure 1A shows examples from one sample. Useful data were, however, derived from 159 recordings of the largest amplitudes (from 0.1 to 2.0 mV) which in general were found in six areas (Fig. 1A). They were encountered in medial laminae II and III (area "a", N = 22), medial laminae IV and V (area "b1", N = 30), medial lamina VII (area "c", N = 25), medial lamina VIII (area "d", N = 17), lateral laminae V and VI (area "b2", N = 50) and lateral laminae VIII and IX (area "e", N=15) of Rexed (1954). In most cases single (Fig. 1A and C) or series (see example of unitary spikes in Fig. 2C) of extracellular potentials emerged from focal potentials in these regions. This suggested aggregation sites of neurones sending their axons in codlf at Th13 segment. Accuracy of locations was confirmed by iontophoretic extracellular injections of HRP (11 cases). One example from area "b1" is shown in Fig. 1B.

In first five experiments attempts were undertaken to determine both the best arrangement of stimulating electrodes (see Methods) and the stimulus strengths adequate to evoke an antidromic response in the S2 segment. In our experimental conditions we found that the lowest threshold of stimulus applied with bipolar electrodes at the thoracic level to evoke a distinguishable antidromic volley recorded at the surface of S2 segment was about 0.9 mA. The amplitude of that response grew up and reached nearly the maximum, when the stimulus was graded from 1.0 to 1.5 mA (Fig. 2B). There were no particular changes in characteristics of recorded potentials when a monopolar arrangement was additionally tested. Basing on these results and on findings of Bagshaw and Evans (1976) we assumed, that the bipolar mode reduced the current spread insignificantly in comparison to a monopolar electrode. The effective radius of stimulation with a strength of 1.5 mA could be less than 1 mm in those experiments. Similar results concerned with applied stimulus strengths were observed during penetrations with microelectrodes. When the large negative focal potential was recorded, the current intensity at Th13 was adjusted downward and the minimal effective strength determined (below 0.9 mA, N = 17). On the other hand, when a group of neurones did not respond at a stimulus strength of 1.0 mA, the current intensity was progressively increased (up to 1.5 mA), to excite more remotely located axons in codlf (Fig. 2C).

Figure 2A exemplifies averaged records taken from areas "a-e" when the strenght of the stimulus applied to the surface at Th13 codlf was graded.

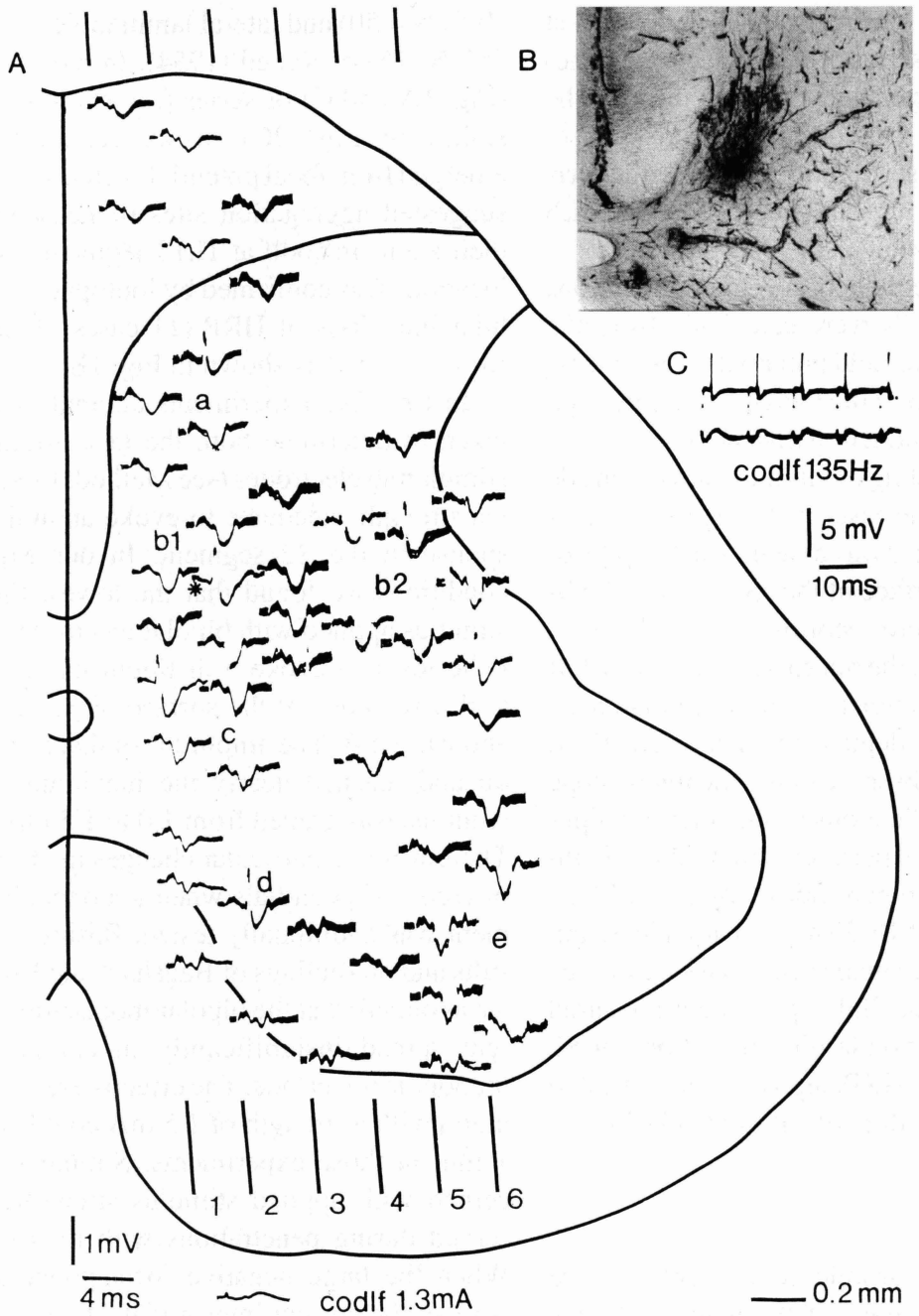

Fig. 1. A, distribution of antidromic field potentials evoked following the excitation of Th13 contralateral dorsolateral funiculus (codlf) (with the stimulus strength of 1.3 mA), at the transversal plane of S2 spinal cord segment. All recordings (3-5 superimposed sweeps) are made from one preparation by subsequent microelectrode penetrations. Lines labelled 1-6 show the direction and positions of microelectrode tracks. Distances from the midline are 1: 0.1mm, 2: 0.25 mm, 3: 0.45 mm, 4: 0.65 mm, 5: 0.8 mm and 6: 1.0 mm, respectively. The recording at the bottom of the Figure is from the surface of the spinal cord close to the microelectrode. Note the presence of large extracellular potentials in described areas labelled with letters "a-e". B, the photomicrograph of HRP deposit in area "b1". Its location is marked in A with the asterix. Injection has been performed after extracellular recording from the neurone in (C), following repetitive stimuli of 1.3 mA applied at Th13 codlf. Further details for this and the next figure, see text.

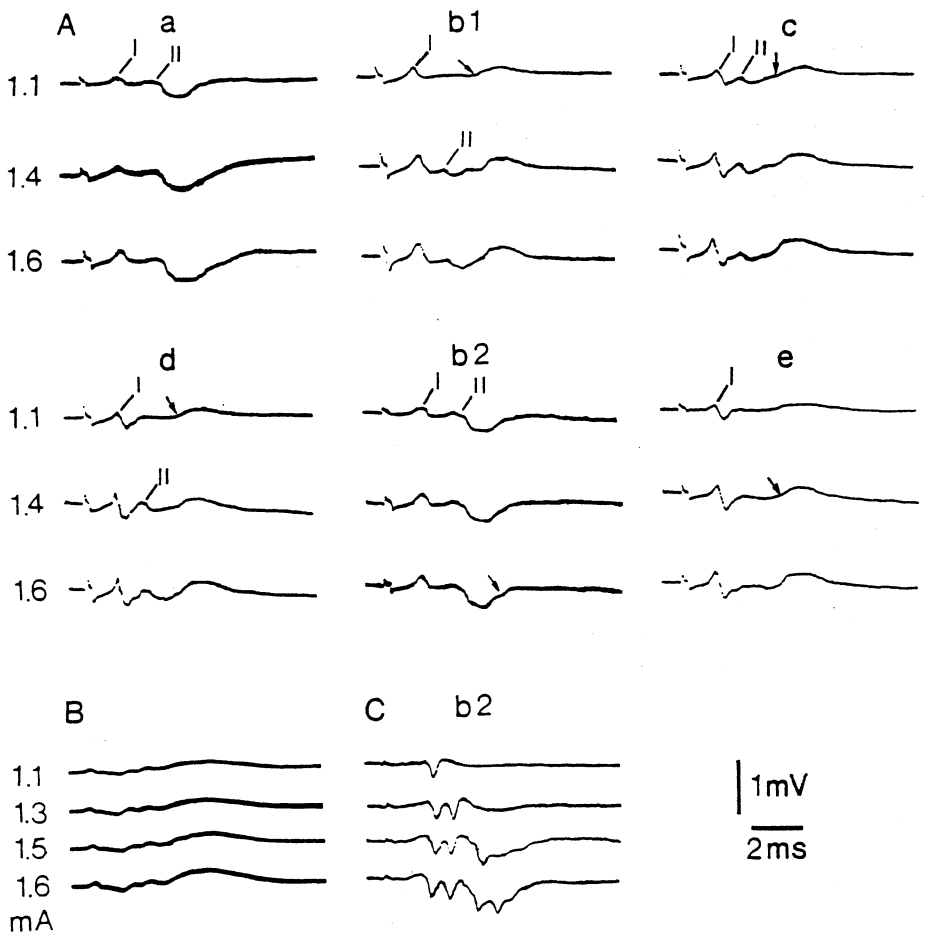

Fig. 2. A, averaged recordings of field potentials from areas "a-e" of S2 segment, evoked with gradation of the stimulus strength at Th13 codlf. Roman numerals refer to the antidromic components of potentials. Arrows show presumably onsets of late components. In B and C averaged recordings were taken from the surface and area "b2" respectively, of the S2 segment during gradation of the stimulus. Note the presence of unitary spikes in compound field potentials.

They consisted of two main antidromic components (labelled with I and II) of different latencies, amplitudes and durations (see Table I). Usually stimuli of the intensity of about 1.0-1.1 mA (N = 38) were strong enough to evoke the first components, those of the intensity of 1.2-1.3 mA (N = 66) the first and second components while the 1.4-1.5 mA stimuli (N = 38) resulted in large compound antidromic field potentials. However, in areas "a", "c", and "b2" already stimuli of 1.1 mA could occasionally evoke both components I and II.

In areas "b1","c","d","b2" and "e" antidromic components were accompanied by positive waves (Fig. 2A, onsets indicated by arrows). Sometimes they appeared with low stimuli and had latencies from 2.9 to 4.0 ms with a mean of 3.5 ms (n = 22). Other could be observed only with stimuli close or above to 1.5 mA and their latencies were included in a range 4.6-7.0 ms (mean of 5.5 ms, n=19). Because of their long latencies we did not try to relate these waves to any of antidromic components and consequently stimuli of above 1.5 mA were not used to evoke them.

A summary of component I and II properties in potentials from different areas is given in Table I. The largest amplitudes of component I in areas "b1" and "c" differed significantly (P<0.01, Student's t-test), in comparison to the smallest amplitudes which appeared in regions "b2" and "a". The smallest amplitudes of component II were recorded in areas "d" and "e". They differed significantly (P<0.01) from those observed in all other regions or even in some cases were difficult to distinguish (Fig.2A). Other notable properties between component I and II amplitudes at different locations have been not found.

The fastest conduction velocities of axons estimated on the basis of component I latencies for

TABLE I

Characteristics of component I and II of antidromic field potentials recorded in six areas of S2 segment. All were evoked by stimulation of the Th13 codlf with stimulus strengths between 0.9 and 1.5 mA

		Component I		Component II	
Area "a"	N = 22	*n* = 19		*n* = 20	
	latency (ms)	0.7-1.6	(1.08±0.2)	1.7-3.8	(2.51±0.5)
	amplitude (mV)	0.1-0.5	(0.37±1.5)	0.3-1.8	(0.99±4.7)
	duration (ms)	1.1-2.0	(1.33±0.2)	2.0-6.0	(3.36±1.1)
	c.v. (m/s)	59.3-135.0	(103.7±24.8)	29.0-64.0	(43.7±10.7)
Area "b"	N = 30	*n* = 20		*n* = 24	
	latency (ms)	0.8-1.8	1.26±0.3)	1.8-4.2	(2.34±0.5)
	amplitude (mV)	0.1-1.7	(0.59±4.1)	0.2-1.3	(0.99±4.1)
	duration (ms)	0.6-1.9	(1.08±0.3)	1.8-4.8	(2.84±0.9)
	c.v. (m/s)	57.5-133.3	(94.6±25.1)	27.4-66.7	(51.9±10.7)
Area "c"	N = 25	*n* = 24		*n* = 18	
	latency (ms)	0.7-2.4	(1.14±0.3)	1.8-4.0	(2.26±0.6)
	amplitude (mV)	0.2-1.0	(0.53±2.5)	0.2-1.0	(0.79±3.1)
	duration (ms)	0.8-2.4	(1.26±0.4)	1.4-4.0	(2.52±0.7)
	c.v. (m/s)	54.1-135.0	(107.3±22.5)	31.6-63.9	(53.8±9.5)
Area "d"	N = 17	*n* = 16		*n* = 14	
	latency (ms)	0.8-1.2	(1.00±0.1)	1.8-2.6	(2.1±0.2)
	amplitude (mV)	0.2-0.6	(0.38±1.9)	0.2-0.4	(0.38±0.8)
	duration (ms)	0.8-1.4	(1.06±0.2)	1.4-3.8	(2.47±0.7)
	c.v. (m/s)	95.8-133.0	(119.3±12.0)	36.5-63.9	(56.0±6.5)
Area "b2"	N = 50	*n* = 40		*n* = 44	
	latency (ms)	0.7-1.8	(1.15±0.3)	1.4-4.6	(2.51±0.6)
	amplitude (mV)	0.1-0.6	(0.38±1.6)	0.3-2.0	(1.16±5.7)
	duration (ms)	0.7-2.2	(1.18±0.4)	1.5-7.0	(3.36±1.1)
	c.v. (m/s)	69.5-135.0	(112.6±20.0)	28.3-85.7	(52.1±11.1)
Area "e"	N = 15	*n* = 11		*n* = 9	
	latency (ms)	0.8-1.8	(1.26±0.4)	1.6-2.6	(1.98±0.3)
	amplitude (mV)	0.1-0.7	(0.47±2.0)	0.1-0.4	(0.36±1.0)
	duration (ms)	0.6-2.0	(1.28±0.5)	1.2-3.2	(2.33±0.5)
	c.v. (m/s)	66.7-133.0	(105.4±26.5)	50.0-81.2	(62.3±10.4)

N, total number of potentials recorded in each area; n, number of components observed in potentials recorded from each area; (±) mean values and standard deviations; c.v. approximate axonal conduction velocities of investigated cellular groups

neurones located in areas "d" and "b2" differed significantly ($P<0.01$) from the velocities of fibres with cell bodies in area "b1". On the other hand, fibres of area "a" neurones were in general the slowest ($P<0.01$), as determined by latencies of component II. Those velocities differed significantly

from higher conduction velocities of axons originating in regions "b2", "c", "d" and "e". Ranges of conduction velocities for both components within each of group were, in general, separated rather than superimposed.

If components of antidromic field potentials were related to excitation of neurones with different conduction velocities of their axons, it would suggest the presence of subgroups within one recorded cellular group.

DISCUSSION

The results of this study revealed six foci in S2 segment which could be antidromically driven at the opposite Th13 dorsolateral funiculus. An analysis of potential properties recorded in each of them indicates sites with separated neuronal populations. Their final projections are unknown, but by their location they can be classified into five anatomically different cellular groups.

Neurones in area "b1" and "b2" overlap with a spinocerebellar cells of "group 5" revealed by Matsushita et al. (1979). However, the amplitudes of component I in medial (b1) and lateral (b2) neuronal groups or conduction velocities of their axons were different and pointed to separate populations in our study.

On the other hand, sites of antidromic foci in areas "c" and "e" roughly respond to location of "group 2" and "group 6" neurones, respectively. Both of them also project to the cerebellum (Matsushita et al. 1978, see Grant et al. 1982, as well). Similar properties of potentials recorded within neighbouring "b1" and "c" regions would suggest that cell groups at those locations were not well separated. In a previous paper (Grottel et al. 1991), we studied a population of spinocerebellar cells in S1 segment of similar location to those of area "e". Nevertheless, S1 neurones were distributed in the ventral horn more laterally and maximal values of axonal conduction velocities were considerably lower. It is unlikely that some of area "e" cells under this study constitute their caudal extension.

Some of the cells of origin of spinothalamic tract in lumbosacral segments with fibres in the opposite dorsolateral funiculus (Jones et al. 1987), could be identified with area "a" and "d" neurones. However, the conduction velocities of classical spinothalamic fibres are slower (26.0-64.0 m/s, mean of 39.3 m/s; Meyers and Snow 1982), compared to conduction velocities of axons for neurones in two above mentioned regions (29.0-135.0 m/s, mean of 73.7 m/s and 36.5-133.0 m/s, mean of 87.6 m/s, respectively). Fibres of long crossed propriospinal system in the lateral funiculus whose cell bodies were occasionally found in intermediate zone of sacral segments (Molenar and Kuypers 1978) should be mentioned, too.

Considering location of descending fibres within Th13 segment funiculi (Basbaum and Fields 1977, Coulter and Jones 1977) and sites of their termination in a gray matter of sacral segments (Basbaum and Fields 1977, Basbaum et al. 1978), it seems likely that their actions could be observed at some locations in this study. This assumption comes from observation on the threshold of 1.6 mA, most frequently used to evoke late waves in components of antidromic focal potentials. It is quite possible that intensities of stimuli in a range from 1.0 to 1.5 mA applied at Th13 were adequate enough, to excite most of ascending and only a few of descending fibres.

ACKNOWLEDGEMENTS

Our thanks are due to Miss A.Siebert for excellent technical assistance. Participation in experiments of Mr. W. Mrówczyński is gratefully acknowledged. This study was supported by the KBN, Project No. 4 1387 91 01.

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Received 2 February 1993, accepted 6 June 1994