Acta Neurobiol. Exp. 2002, 62: 271-276

Afferent input from distal forelimb nerves to branching spinoreticular-spinocerebellar neurones in the cat

Piotr Krutki and **Włodzimierz Mrówczyński**

Department of Neurobiology, University School of Physical Education, 55 Grunwaldzka St., 60-352 Poznań, Poland, Email: krutki@awf.poznan.pl

Abstract. Patterns of afferent connections from receptors of the distal forelimb were investigated in neurones located in C6-C7 segments of the spinal cord with branching axons projecting to the lateral reticular nucleus and the cerebellum. Experiments were made on five adult cats under α-chloralose anaesthesia. After antidromic identification, EPSPs and IPSPs were recorded from 22 neurones following stimulation of deep radial, superficial radial, median and ulnar nerves. Both excitatory and inhibitory effects were found in the majority of the cells, however, in 2 cases no synaptic actions were recorded. EPSPs were evoked from group I or II muscle, or cutaneous afferents – mostly monosynaptically. IPSPs from muscle, cutaneous or flexor reflex afferents were mostly polysynaptic. Seven various types of convergence were established in the cells investigated. Significance of parallel transmission of integrated information from various receptors of the distal forelimb to the reticular formation and cerebellum is discussed.

Key words: forelimb afferents, LRN, cerebellum, divergence, cat

Spinoreticular and spinocerebellar tracts originating from the cervical enlargement have been widely investigated in many anatomical or electrophysiological studies in the cat (Rosén and Scheid 1973a, Corvaja et al. 1977, Matsushita and Ikeda 1987). Recent studies performed in our laboratory have also revealed that a proportion of neurones giving rise to these tracts have branching axons that project both to the reticular formation and the cerebellum. The location, axonal course and conduction velocities of cells projecting to the ipsilateral lateral reticular nucleus (LRN) of the medulla oblongata, and to the inferior cerebellar peduncle (restiform body, RB), have been described (Grottel et al. 1999, Mrówczyński et al. 1999). It is commonly known that LRN neurones send axons to the cerebellum (Dietrichs 1983), so individual spinocerebellar--spinoreticular neurones form two parallel tracts projecting from the cervical segments of the spinal cord to the cerebellum – one direct and one indirect. The same information may be integrated in LRN neurones with signals from other spinal or supraspinal centres, e.g., the cerebral cortex (Bruckmoser et al. 1970), the red nucleus (Corvaja et al. 1977), the oculomotor region (Qvist and Dietrichs 1986), and then transmitted to the cerebellum. However, the physiological role of the cervical branching neurones is still hypothetical. So far, no data are available concerning the information processed by this group of cells. The aim of the presented investigations was to extend our previous findings and electrophysiological methods were used in order to establish the pattern of synaptic connections from peripheral afferents of the forelimb.

Experiments were carried out on 5 adult cats, weighing 3.350 to 4.100 kg. All the experimental procedures were approved by the local ethics committee and followed EU guidelines of animal care and Polish Law on the Protection of Animals. Anaesthesia was induced by ketamine hydrochloride (25-40 mg kg^{-1}, i.m.) and maintained by α-chloralose (in several doses, up to 50 mg kg^{-1}, i.v.). Monitoring of withdrawal and corneal reflexes during the operation or diameter of pupils, heart rate and arterial blood pressure during recordings controlled its adequacy. Cats were paralysed with gallamine triethiodide (1-3 mg kg^{-1} h^{-1}, i.v.) and immobilized in a stereotaxic frame. Ipsilateral forelimb nerves: deep radial (DR), superficial radial (SR), median (Med) and ulnar (Uln) were dissected and mounted in tunnel electrodes. C6-C7 segments of the spinal cord were exposed by a laminectomy, the cerebellum and the medulla oblongata were exposed by an occipital craniotomy on the ipsilateral side to the recording place. The detailed procedures were described in previous papers (Grottel et al. 1999, Krutki et al. 1999).

Varnished tungsten needle electrodes (tip diameter 5 μm, exposed for 10-20 μm) were inserted to the inferior cerebellar peduncle (restiform body, RB) and the lateral reticular nucleus (LRN), according to Horsley-Clarke's coordinates: P, 8.0 to 8.5; H, -3.5 to -4.0; L, 5.6 and, P, 15.0; H, -10.0 to -12.0; L, 3.1 to 4.1, respectively (Berman 1968). Cathodal stimuli (0.2 ms) were delivered through these electrodes with the maximal strength of 100 μA and only axons within a radius of 0.5 mm from an electrode tip could be activated (Bagshaw and Evans 1976). The locations of stimulation sites were verified histologically following each experiment by electrolytic lesions (10 mA for 15 s). Stimuli delivered for forelimb nerve fibres were of 0.1 ms duration and strength expressed in multiples of threshold for the most sensitive fibres in a nerve (Jack 1978).

Antidromic action potentials from RB and/or LRN as well as postsynaptic potentials (PSPs) from afferent fibres were recorded intracellularly in C6-C7 segments with glass micropipettes (tip diameter 1.5-2.5 μm, resistance 2-10 MΩ), filled with 2 M potassium citrate solution. An additional recording silver ball electrode was placed on the spinal cord surface close to the dorsal root area in order to record incoming afferent volleys. Identification of neurones was based on the criteria for antidromic activation, as described by Lipski (1981). All recorded signals were amplified, filtered, digitized (analog-digital converter PCL-818HD) and sent to a computer for further analysis. Antidromic or segmental latencies and amplitudes of recorded potentials were measured from 5 or 10 averaged subsequent signals.

Excitatory or inhibitory postsynaptic potentials (EPSPs or IPSPs) were identified as evoked from group I or II muscle, cutaneous or flexor reflex afferents (FRA) on the bases of nerve type and strength of stimulation (Jack 1978, Lundberg et al. 1987, Jankowska and Riddell 1993). Stimuli of 0.1 ms duration, with an intensity varied from 1.1 to 10 times threshold for the most excitable fibres in a nerve were delivered, at a frequency 3Hz. A synaptic linkage was assessed basing on central latencies of PSPs, measured from the fastest component of the incoming afferent volley (Jankowska and Roberts 1972, Edgley and Jankowska 1988).

Twenty-two neurones were investigated intracellularly in the grey matter of C6-C7 segments. Cells were included into the analysed sample only when amplitudes of their

action potentials exceeded 40 mV. They were distributed in medial parts of the dorsal horn, the intermediate grey and the ventral horn (Fig. 1E). Axonal conduction velocities measured for the distance between stimulation and recording sites were comprised in the range 29-74 m s^{-1}. In two cases no PSPs were recorded, in two cells only inhibitory effects and in four only excitatory effects could be found. In the remaining fourteen neurones both EPSPs and IPSPs were evoked from forelimb afferents. However, it should be noted that in the whole population excitatory actions from the nerves investigated were recorded slightly more than half as often as inhibitory ones (44 and 74 potentials, respectively). Amplitudes of recorded potentials varied from 0.8 to 3.1 mV and from -0.8 to -2.3 mV, for EPSPs and IPSPs, respectively. Examples of postsynaptic potentials evoked from a muscle nerve (DR), a skin nerve (SR) and a mixed nerve are presented in Fig. 1 A-C.

Figures. 2A and B show proportions of the neurones investigated with EPSPs or IPSPs evoked from various nerves. Most neurones responded to stimulation of DR, SR and Med. Excitatory actions from these nerves were recorded from 15, 12 and 10 neurones, respectively, while inhibitory action were recorded from 14, 11 and 14 neurones, respectively. The least effective was stimulation of Uln; EPSPs were recorded from 3 and IPSPs from 5 neurones.

Synaptic linkage of PSPs recorded from various afferent fibres is presented in Fig. 2C. Excitatory actions were usually evoked monosynaptically (77.3%), either in the

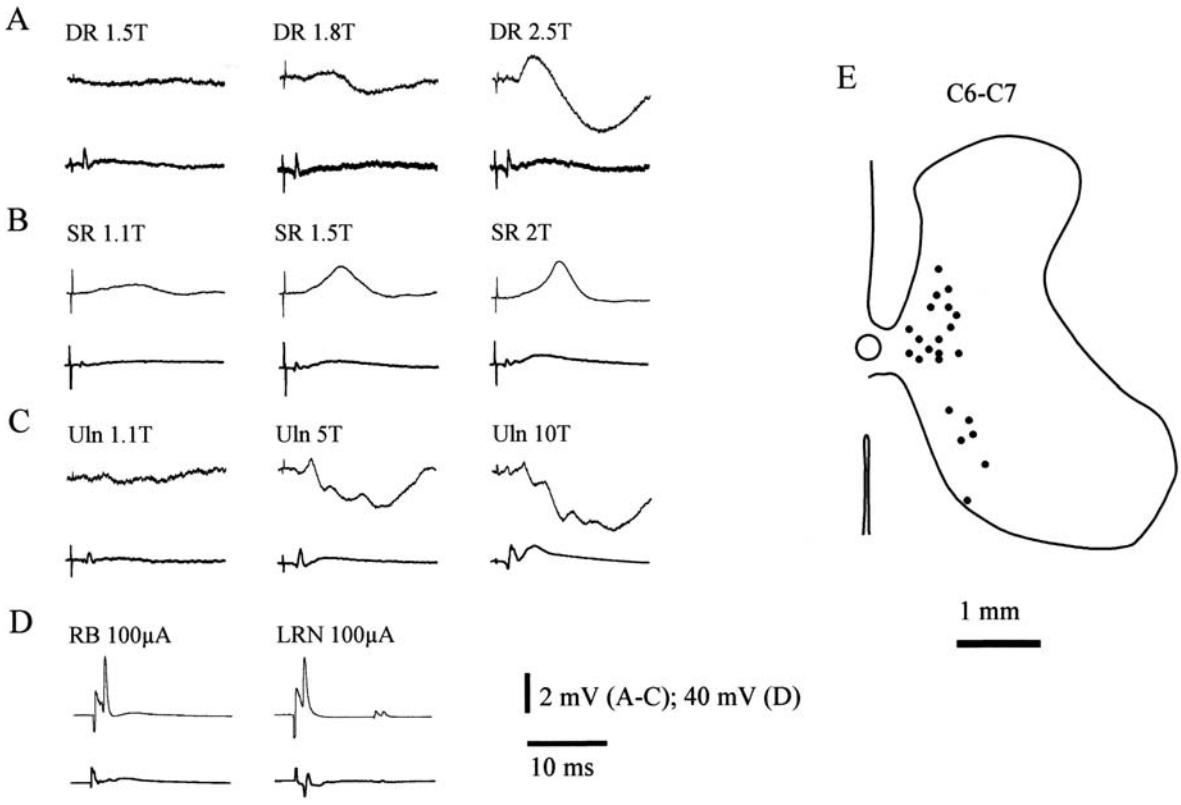

Fig. 1. A-C, Examples of postsynaptic potentials (upper traces, 5 averaged recordings) evoked in a branching neurone following stimulation of muscle, cutaneous or mixed afferent fibres in deep radial (DR), superficial radial (SR) and ulnar (Uln) nerves. A, EPSP identified as monosynaptic (central latency <1.7 ms) and IPSP evoked polysynaptically from group II muscle afferents. B, disynaptic EPSP from low threshold cutaneous afferents. C, IPSP evoked disynaptically (central latency <1.8 ms) from group I muscle afferents (Uln 1.1T) and polysynaptic IPSPs from flexor reflex afferents (Uln 5T and 10T). D, antidromic action potentials (upper traces, 5 averaged recordings) obtained from this neurone following stimulation of the restiform body (RB) and the lateral reticular nucleus (LRN). Lower traces in A-D are records from the dorsal roots entry zone. The intensities of stimuli are given above each record. E, distribution of neurones presented on the averaged outline of transverse sections of sixth and seventh cervical segments of the spinal cord (C6-C7). Recording sites were determined from depth of a micropipette introduction, its distance from the mid-line and the angle of the micromanipulator.

case of muscle afferents (group I and group II, Fig. 1A) or cutaneous afferents. Ranges of measured latencies were 0.6-1.0 ms for group I muscle, 1.2-1.5 ms for group II muscle and 0.6-1.1 ms for cutaneous afferents. Disynaptic EPSPs from cutaneous fibres (Fig. 1B), with latencies 1.4-1.8 ms, or polysynaptic EPSPs from muscle afferents, with latencies 8.3-10.2 ms, were found only in few neurones. No excitatory effects could be evoked from high threshold afferents, classified as FRA. IPSPs were evoked from group I or II muscle (Fig. 1A, C), cutaneous or flexor reflex afferents (Fig. 1C) and they were polysynaptic in most cases (94.6%, latencies 2.5-6.8 ms, 3.6-5.5 ms, 3.6-7.2 ms and 3.5-12.0 ms, respectively). In few neurones disynaptic IPSPs from group I muscle afferents were also found. Their latencies varied between 1.5 and 1.7 ms.

Seven different patterns of convergence of PSPs were revealed in the neurones studied, with respect to various types of nerve fibres activated. Responses could be evoked from: 1) group I muscle afferents, 2) group I muscle afferents + FRA, 3) group I + II muscle afferents + FRA, 4) group I + II muscle + cutaneous afferents, 5) group I muscle + cutaneous afferents + FRA, 6) group I + II muscle + cutaneous afferents + FRA, 7) group II muscle + cutaneous afferents. Except two previously mentioned neurones without postsynaptic potentials recorded, in all remaining cases muscle afferents from at least one of the forelimb nerves were effective in exciting or inhibiting a neurone. PSPs evoked from cutaneous afferents or FRA in no cases appeared independently.

In our previous reports it has been demonstrated that branching neurones in cervical segments form a significant proportion of neurones projecting either to the cerebellum or LRN and they are distributed in similar regions of the spinal grey matter as other spinocerebellar or spinoreticular tract cells (Grottel et al. 1999, Mrówczyński et al. 1999). It has also been confirmed that these neurones have similar conduction velocities in ascending axonal branches. Illert and Lundberg (1978) have measured 10-80 m s^{-1} for neurones projecting to LRN, while Hirai et al. (1976) reported 24-67 m s^{-1} for spinocerebellar tract neurones.

In the present study we have planned to answer the question of whether afferent input from forelimb nerves also reveals any similarities to neurones investigated previously. Electrophysiological studies concerning C3-C4 propriospinal neurones with axonal branches ascending to LRN have shown that they are monosynaptically activated from cutaneous and group I muscle afferents (Illert and Lundberg 1978, Illert et al. 1978). Other experiments have displayed effects from high threshold cutaneous and muscle afferents (Ekerot et al. 1979). Rosén and Scheid (1973a) have also investigated neurones located in LRN. They have revealed responses to stimulation of DR or SR. Both excitatory and inhibitory synaptic effects from DR had thresholds in the range of group II muscle afferents and only in few cases of group I afferents. In most neurones effects from cutaneous afferents in SR accompanied those obtained from muscle afferents. These authors have also found a convergence of responses from different types of cutaneous receptors (Rosén and Scheid 1973b). Among spinocerebellar tract neurones located in the cervical enlargement of the cat spinal cord two groups of neurones

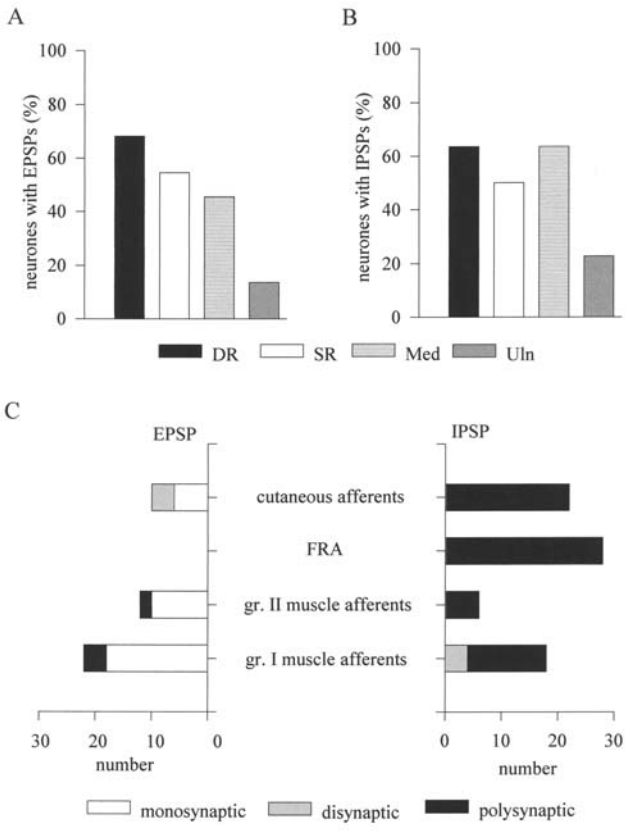

Fig. 2.A-B, Relative contribution of the nerves stimulated in the excitation (A) and inhibition (B) of the neurones tested, shown as a percentage of the whole population of cells. DR, deep radial; SR, superficial radial; Med, median; Uln, ulnar nerves. C, number of monosynaptic, disynaptic and polysynaptic excitatory effects (left side) or inhibitory effects (right side) evoked from various groups of afferent fibres in the neurones studied. FRA, flexor reflex afferents.

have been distinguished with respect to convergence patterns of forelimb input (Hirai et al. 1976, 1984). Neurones of the first group are monosynaptically excited by group I muscle afferents; neurones of the second one are polysynaptically inhibited by flexor reflex afferents.

Thus, our results are partly consistent with those obtained for spinoreticular as well as for spinocerebellar tract cells. Monosynaptic excitatory actions evoked from group I and II muscle afferents (both from flexors and extensors) and polysynaptic inhibitory actions from muscle, cutaneous and high threshold afferents have been the most characteristic features. As in reports of authors cited above, PSPs resulting from DR, SR and Med stimulation have been commonly found, while stimulation of Uln has been less effective. However, previous investigators have reported neither predominant inhibitory effects in neurones, nor so many different patterns of convergence as presented in this paper. It indicates that branching neurones under study form to some extent a distinct population of cells projecting from the cervical spinal cord to LRN and the cerebellum. It is suggested that different patterns of afferent input from forelimb receptors are processed by different subpopulations of spinal branching neurones. It is possible that various types of branching neurones are activated during subsequent phases of locomotion or voluntary movements. This information reaches the cerebellum, which is important for the feedback control of a performed movement. Parallel transmission to LRN provided by the same neurones makes possible the early integration in this nucleus of changing signals from muscle, cutaneous or joint receptors of the forelimb with signals from other spinal or supraspinal centres.

This study was supported by the State Committee of Scientific Research grant.

Bagshaw E.W., Evans M.H. (1976) Measurement of current spread from microelectrodes when stimulating within nervous system. Exp. Brain Res. 25: 391-400.

Berman A.L. (1968) The brain stem of the cat. A cytoarchitectonic atlas with stereotaxic coordinates. The University of Wisconsin Press, Madison, Milwaukee.

Bruckmoser P., Hepp-Reymond M.C., Wiesendanger M. (1970) Cortical influence on single neurons of the lateral reticular nucleus of the cat. Exp. Neurol. 26: 239-252.

Corvaja N., Grofova I., Pompeiano O., Walberg F. (1977) The lateral reticular nucleus in the cat – I. An experimental anatomical study of its spinal and supraspinal afferent connections. Neuroscience 2: 537-553.

Dietrichs E. (1983) Cerebellar nuclear afferents from the lateral reticular nucleus in the cat. Brain Res. 288: 320-324.

Edgley S.A., Jankowska E. (1988) Information processed by dorsal horn spinocerebellar tract neurones in the cat. J. Physiol. 397: 81-97.

Ekerot C-F., Larson B., Oscarsson O. (1979) Information carried by the spinocerebellar paths. In: Reflex control of posture and movements. Prog. Brain Res. 50: 79-90.

Grottel K., Krutki P., Mrówczyński W. (1999) Bidirectional neurones in the cervical enlargement of the cat spinal cord with axons descending to sacral segments and ascending to the cerebellum and the lateral reticular nucleus. Exp. Physiol. 84: 1059-1071.

Hirai N., Hongo T., Kudo N., Yamaguchi T. (1976) Heterogenous composition of the spinocerebellar tract originating from the cervical enlargement in the cat. Brain Res. 109: 387-391.

Hirai N., Hongo T., Sasaki S. (1984) A physiological study of identification, axonal course and cerebellar projection of spinocerebellar tract cells in the central cervical nucleus of the cat. Exp. Brain Res. 55: 272-285.

Illert M., Lundberg A. (1978) Collateral connections to the lateral reticular nucleus from cervical propriospinal neurones projecting to forelimb motoneurones in the cat. Neurosci. Lett. 7: 167-172.

Illert M., Lundberg A., Padel Y., Tanaka R. (1978) Integration in descending motor pathways controlling the forelimb in the cat. 5. Properties of and monosynaptic excitatory convergence on C3-C4 propriospinal neurones. Exp. Brain Res. 33: 101-130.

Jack J.J.B. (1978) Some methods for selective activation of muscle afferent fibres. In: Studies in neurophysiology (Ed. R. Porter). Cambridge University Press, Cambridge, p. 155-176.

Jankowska E., Riddell J.S. (1993) A relay for input from group II muscle afferents in sacral segments of the cat spinal cord. J. Physiol. 465: 561-580.

Jankowska E., Roberts W.J. (1972) Synaptic actions of single interneurones mediating reciprocal Ia inhibition of motoneurones. J. Physiol. 222: 623-642.

Krutki P., Grottel K., Mrówczyński W. (1999) Divergence of lamina VII and VIII neurones of S1 and S2 segments of the cat's spinal cord to the cerebellum and the reticular formation. Acta Neurobiol. Exp. 59: 81-88.

Lipski J. (1981) Antidromic activation of neurones as an analytic tool in the study of the central nervous system. J. Neurosci. Methods 4: 1-32.

Lundberg A., Malmgren K., Schomburg E.D. (1987) Reflex pathways from group II muscle afferents. 1. Distribution and linkage of reflex actions to α-motoneurones. Exp. Brain Res. 65: 271-281.

Matsushita M., Ikeda M. (1987) Spinocerebellar projections from the cervical enlargement in the cat, as studied by anterograde transport of wheat germ agglutinin – horseradish peroxidase. J. Comp. Neurol. 263: 223-240.

Mrówczyński W., Grottel K., Krutki P. (1999) The electrophysiological study of axonal branching of the C6-C7 neurones of the cat's spinal cord projecting to the lateral reticular nucleus and the cerebellum. Arch. Ital. Biol. 137: 279-288.

Qvist H., Dietrichs E. (1986) Afferents to the lateral reticular nucleus from the oculomotor region. I. The Edinger-Westphal nucleus. Anat. Embryol. 175: 261-269.

Rosén I., Scheid P. (1973a) Patterns of afferent input to the lateral reticular nucleus of the cat. Exp. Brain Res. 18: 242-255.

Rosén I., Scheid P. (1973b) Responses of spino-reticulo-cerebellar pathway to stimulation of cutaneous mechanoreceptors. Exp. Brain Res. 18: 268-278.

Received 21 May 2002, accepted 25 October 2002