EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, Vol. 37, pp. 380–392, 2013 doi:10.1111/ejn.12060

SYNAPTIC MECHANISMS

A survey of spinal collateral actions of feline ventral spinocerebellar tract neurons

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Keywords: ascending tract neurons, cerebellum, feedback, motor control, spinal cord

Abstract

The aim of this study was to identify spinal target cells of spinocerebellar neurons, in particular the ventral spinocerebellar tract (VSCT) neurons, giving off axon collaterals terminating within the lumbosacral enlargement. Axons of spinocerebellar neurons were stimulated within the cerebellum while searching for most direct synaptic actions on intracellularly recorded hindlimb motoneurons and interneurons. In motoneurons the dominating effects were inhibitory [inhibitory postsynaptic potentials (IPSPs) in 67% and excitatory postsynaptic potentials (EPSPs) in 17% of motoneurons]. Latencies of most IPSPs indicated that they were evoked disynaptically and mutual facilitation between these IPSPs and disynaptic IPSPs evoked by group Ia afferents from antagonist muscles and group Ib and II afferents from synergists indicated that they were relayed by premotor interneurons in reflex pathways from muscle afferents. Monosynaptic EPSPs from the cerebellum were accordingly found in Ia inhibitory interneurons and intermediate zone interneurons with input from group I and II afferents but only oligosynaptic EPSPs in motoneurons. Monosynaptic EPSPs following cerebellar stimulation were also found in some VSCT neurons, indicating coupling between various spinocerebellar neurons. The results are in keeping with the previously demonstrated projections of VSCT neurons to the contralateral ventral horn, showing that VSCT neurons might contribute to motor control at a spinal level. They might thus play a role in modulating spinal activity in advance of any control exerted via the cerebellar loop.

Introduction

A number of ascending tract neurons give off axon collaterals at a spinal level and may thus operate not only as projection neurons but also as spinal interneurons and contribute to the operation of spinal neuronal networks (for references see Discussion). For spinocerebellar neurons evidence was found for crossed spinal projections of ventral spinocerebellar neurons in midlumbar segments, known as Ib ventral spinocerebellar tract (VSCT) neurons, but not the spinal border VSCT neurons (Bras *et al.*, 1988), nor the dorsal spinocerebellar neurons (Randic *et al.*, 1981; Houchin *et al.*, 1983; Edgley & Gallimore, 1988). In cervical segments ipsilateral and crossed descending projections were found in ventrally but not in dorsally located rostral spinocerebellar neurons (Hirai *et al.*, 1978, 1984; see Mrowczynski *et al.*, 2001) the former considered as forelimb homologues of VSCT neurons (Hirai *et al.*, 1978, 1984; Bras *et al.*, 1988; Krutki & Mrowczynski, 2002). Evidence was also presented for ipsilateral, contralateral or bilateral ascending projections of neurons in the S1/S2 segments (Grottel *et al.*, 1998; Krutki *et al.*, 1998) which might be sacral homologues of lumbar VSCT neurons. However, both were only found to project to thoracic segments.

With respect to the spinal target cells of axon collaterals of ascending tract neurons only very preliminary data are available. Terminal projection areas of segmental collaterals of both spinocervical tract and postsynaptic dorsal column tract neurons (Brown *et al.*, 1977; Rastad *et al.*, 1977; Jankowska *et al.*, 1979; Brown & Fyffe, 1981) and VSCT neurons (Bras *et al.*, 1988) indicate that these neurons might modify activity of a variety of spinal neurons outside motor nuclei. However, attempts to find out which particular spinal neurons are their target cells were made only for spinocervical tract neurons (Jankowska *et al.*, 1979; Kahlat & Djouhri, 2012).

The aim of the present study was to identify spinal target cells of spinocerebellar neurons, in particular VSCT neurons, by stimulating axons of these neurons within the cerebellum and by making a survey of the resulting effects on spinal neurons. The premise was that any directly evoked spinal effects of stimuli applied within the cerebellum will be attributable to collateral actions of spinocerebellar neurons even though such stimuli will in addition activate a variety of other neurons and might be followed by synaptic actions relayed by these neurons. By choosing stimulation sites from which a high proportion of VSCT neurons were antidromically activated we could increase the probability of detecting their spinal actions. However, any synaptic actions revealed in this way could be ascribed to VSCT neurons only facultatively. Actions of VSCT neurons could be differentiated from potential actions of other spinocerebellar neu-

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Received 28 August 2012, revised 8 October 2012, accepted 16 October 2012

rons located within the lumbosacral enlargement because only VSCT neurons were found to give off initial axon collaterals in lumbar segments (see above). Nevertheless, any synaptic actions of lumbar VSCT neurons might be evoked in parallel with actions of their cervical and/or sacral homologues provided that these VSCT subpopulations have terminal projection areas in the lumbar segments and are co-activated from the same cerebellar regions.

Materials and methods

Ethical approval

All experiments were approved by the Ethics Committee for Animal Research at the University of Gothenburg (Göteborgs Djurförsöksetiska Nämnd) and comply with the USA National Institutes of Health and European Union guidelines for animal care. The animals were bred and housed under veterinary supervision at the Laboratory of Experimental Biomedicine at Sahlgrenska Academy where the experiments were carried out.

Preparation

The experiments were performed on eight deeply anaesthetized cats of both sexes weighing 2.2–3.0 kg. Anaesthesia was induced with sodium pentobarbital (Apoteksbolaget, Göteborg, Sweden; 40–44 mg/kg, i.p.) and maintained with intermittent doses of α-chloralose (Rhône-Poulenc Santé, France; doses of 5 mg/kg administered every 1–3 h, up to 55 mg/kg, i.v.). Additional doses of α-chloralose were given when motor reactions were evoked during dissection or when increases in the continuously monitored blood pressure or heart rate were evoked by the experimental procedures. During recordings, neuromuscular transmission was blocked by pancuronium bromide (Pavulon, Organon, Sweden; 0.3 mg/kg i.v.) and the animals were artificially ventilated. Neuromuscular relaxation was induced only after several hours of surgery and when the animal had reached a deep and stable level of anaesthesia and was thereafter maintained by adding pancuronium bromide at doses corresponding to about 0.2 mg/kg/h. Mean blood pressure was kept at 100–130 mmHg and end-tidal concentration of CO_2 at about 4–4.5% by adjusting the parameters of artificial ventilation and the rate of a continuous infusion of a bicarbonate buffer solution with 5% glucose (1–2 mL/h/kg). The core body temperature was kept at about 37.5 °C by servo-controlled infrared lamps. The experiments were terminated by a lethal dose of pentobarbital i.v. followed by formalin perfusion.

To increase the probability of activation of spinal interneurons, as well as to enhance any indirect actions of the tested stimuli, the potassium (K^+) channel blocker 4-aminopyridine (4-AP) was applied during recording in most of the experiments at doses of 0.1–0.2 mg/kg iv, as used in our previous studies (see e.g. Jankowska *et al.*, 2005). These doses were expected to result in a plasma concentration of 4-AP of about 1 μM and could be compared with clinically used doses of 10 mg, corresponding to 0.14 mg/kg in a 70-kg patient with minimal side effects (for the latest references see Alvina & Khodakhah, 2010).

Following the initial vein, artery and tracheal cannulation a laminectomy exposed the 5th to 7th lumbar (L5–L7) segments of the spinal cord. A number of hind limb muscle and skin nerves were dissected free, transected and prepared for stimulation. The femoral nerve branches of quadriceps (Q) and sartorius (S) muscle nerves were mounted in subcutaneous cuff electrodes, while branches of the sciatic nerve – the posterior biceps and semitendinosus (PBSt), sural (Sur), gastrocnemius soleus (GS), plantaris (Pl), flexor digitorum and hallucis longus (FDL), superficial peroneal (SP) and/or deep peroneal (DP) – were mounted on pairs of silver electrodes in paraffin oil pool.

The caudal part of the cerebellum was exposed by a craniotomy and two tungsten electrodes (impedance 30–150 kΩ) were positioned within the white matter rostral to the fastigial and anterior interpositus nuclei. In view of bilateral terminal projection areas of VSCT neurons within the anterior lobe the electrodes were positioned bilaterally, targeting an area at Horsley–Clarke coordinates 1.5–2 mm lateral to the midline, 0.5–1.5 mm horizontal and 7.5–8.0 mm posterior, at an angle of 25° from vertical (tip directed rostrally). In some experiments a third tungsten electrode was introduced into the left medial longitudinal fascicle (MLF) at Horsley–Clarke coordinates P9, L0.6, H-5, the red nucleus (RN) at coordinates A3, L1.5, H-3.5 or the lateral vestibular nucleus (LVN) at coordinates P8, L3.5, H-3.

The final positions of these electrodes were adjusted on the basis of records of descending volleys evoked by single stimuli from the surface of the lateral funiculus in the thoracic (Th) 12–13th segments. The electrodes were left at locations from which distinct descending volleys were evoked at stimulus intensities of 20 μA or less. A further adjustment of the placement of the electrodes was also made by taking into account thresholds of antidromic activation of the first VSCT neurons encountered in the 4–6th lumbar (L) segments.

At the end of the experiments the stimulation sites were marked with electrolytic lesions (0.2 mA constant current for 10–15 s). Locations of these stimulation sites were subsequently verified on 100-μm-thick frontal or sagittal sections of the brain, cut using a vibratome, mounted on slides, counterstained with cresyl violet and scanned (Fig. 1B).

Stimulation and recording

Peripheral nerves were stimulated with constant voltage stimuli (0.2 ms duration, intensity expressed in multiples of threshold, *T*, for the most sensitive fibres in the nerve). For activation of fibres stimulated within the brain, constant current cathodal stimuli were used (0.2 ms, 20–100 μA).

Descending volleys were recorded transdurally from the cord dorsum. During placement of the stimulating electrodes the volleys were recorded monopolarly from the border zone between the left lateral funiculus and the dorsal columns at the cervical (C) 3 or Th 11–12 level. During recording the cord dorsum electrodes were placed in contact with the surface of the dorsal columns a few millimetres away from the cells recorded from.

Glass micropipettes filled with 2 M solution of potassium citrate were used for recording; they had tips broken to about 1.5 μm and impedance of 1.5–6 MΩ.

Motoneurons were identified by antidromic activation following stimulation of muscle nerves and by the pattern of synaptic potentials evoked by low-threshold muscle afferents. They were searched for primarily in the L7 segments but some were recorded in the L5 or L6 segments. VSCT neurons were identified by antidromic activation following stimuli applied in the cerebellum at locations indicated in Fig. 1B, as well as to the contralateral lateral funiculus at the level of the most caudal thoracic segments. They were searched for within the intermediate zone of the L5/L6 segments (within or just ventral to the area where focal field potentials were evoked from group I and II afferents). Interneurons were identified by characteristic features of their various functional populations (see results).

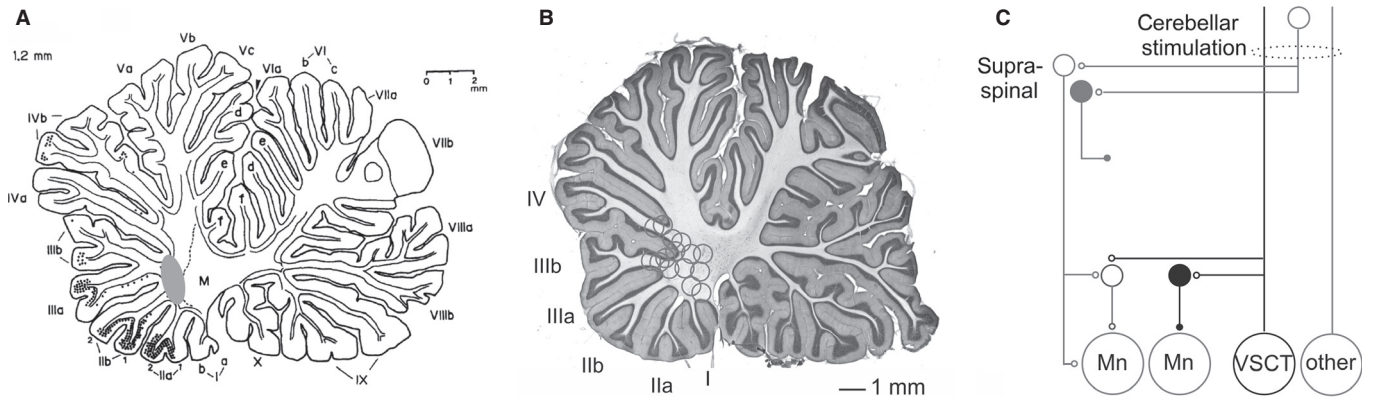

FIG. 1. Stimulation sites in the cerebellum. (A) Diagram of distribution of terminals of spinocerebellar tract fibres labelled by WG-HRP injected into the L7–S1 segments by Matsushita and collaborators (modified from Matsushita, 1988; fig. 5B. The targeted area of the stimulating electrodes is shaded. (B) Location of the stimulation sites (both ipsilateral and contralateral) projected on a sagittal section from one of the experiments. (C) Diagram of the pathways activated by cerebellar stimulation. Open and closed circles represent excitatory and inhibitory neurons, respectively, considering that they might include both spinal and supraspinal neurons. Black lines indicate the pathways via which monosynaptic excitation of interneurons and disynaptic inhibition of motoneurons could be attributed to collateral actions of VSCT neurons and their homologues. For other comments see text.

Analysis

Direct or indirect coupling between SCT neurons and their potential spinal target neurons was analysed on the basis of intracellular records from hindlimb motoneurons, from VSCT neurons and from two populations of spinal interneurons. Monosynaptically evoked PSPs were differentiated from disynaptically and polysynaptically evoked ones as described in the Results.

Indications of which premotor interneurons might be affected by collateral actions of SCT neurons were obtained from the analysis of changes in disynaptic PSPs evoked in motoneurons. Those facilitated following cerebellar stimulation were considered to be mediated by interneurons contacted by SCT axon collaterals, provided that the facilitated potentials were larger than the sum of potentials evoked by separately evoked test and conditioning stimuli (see, for example, Lundberg, 1979; Burke, 1999). The comparisons were made on digitalized records of PSPs, using the analog to digital converter Digidata (Axon Instruments) at a resolution of 30 μs per address. Both original data and averages of 10–40 single PSPs were stored on line. Changes in the recorded potentials were estimated offline by comparing areas of the averaged potentials within selected time windows (using a sampling and analysis system designed by E. Eide, T. Holmström and N. Pihlgren, University of Gothenburg). The time windows within which the areas were measured included the most directly evoked components, usually within the first 2–3 ms and generally between the onset and the first third of the declining phase (see Fig. 5G–J). The differences between the compared potentials were considered to be genuine in a given neuron if they exceeded 10% of control records and were consistently found in at least 2–3 stimulus combinations. Differences between samples of neurons were assessed for statistical significance using Student's *t*-test for unpaired or paired data, assuming equal variance at 5% confidence level.

Justification of experimental approaches used in this study

Aiming at a survey of actions of spinocerebellar neurons at a spinal level the study focused on a qualitative rather than a quantitative analysis of these actions. Hence, the study focused on finding as many indications for actions of spinocerebellar neurons on their potential spinal target cells as possible, rather than on estimating the strength of these actions. The survey was therefore based on different kinds of mutually supporting evidence for the proposed actions rather than aiming to collect larger material for any of them. In addition it was considered more important to stimulate a fraction of spinocerebellar neurons, resulting in weaker effects, rather than a greater proportion of neurons by using stronger stimuli which would have increased a risk of co-activation of other neuronal systems.

As most of the terminals of crossed spinocerebellar neurons labelled by anterograde transport from the L5–S1 segments were found in sub-lobules II–IV of the anterior lobe of the cerebellum (Matsushita, 1988; Matsushita & Yaginuma, 1989), the stimulating electrodes were placed within the cerebellar white matter at locations where axons of spinocerebellar neurons should pass on their way to these sub-lobules (see Fig. 1A), as indicated in Fig. 1B. In addition, as the ipsilateral and contralateral terminal projection areas found by Matsushita & Yaginuma (1989) were reported to be similar and axons of individual VSCT neurons branching within the cerebellum might target both the ipsilateral and the contralateral projection areas, the stimuli were applied bilaterally and effects obtained from the left and the right cerebellar stimulation sites pooled together. However, we will denote both the spinal and the cerebellar sides as 'ipsilateral' or 'contralateral' with respect to the left side of the spinal cord from where all of the spinal neurons were recorded.

Monosynaptic excitation evoked by cerebellar stimuli in the lumbar segments of the spinal cord would be obviously due to direct actions of the stimulated axons. Disynaptic inhibition would be likewise due to direct actions of the stimulated fibres, on spinal interneurons mediating the inhibition as outlined in Fig. 1C, in view of a lack of evidence for projections of supraspinal inhibitory neurons in the cat to the lumbar spinal segments (Wilson & Yoshida, 1969; Peterson *et al.*, 1979). However, disynaptic excitation of spinal neurons might be mediated not only by spinal but also by supraspinal neurons, provided that the potential supraspinal relay neurons activated by cerebellar stimuli target these spinal neurons (see Discussion). Trisynaptic excitation and trisynaptic inhibition could be likewise relayed by supraspinal as well as by spinal neurons. The conclusions drawn regarding spinal target cells of spinocerebellar neurons had thus to be restricted to monosynaptic and disynaptic inhibitory actions of cerebellar stimuli.

The differentiation between target cells of lumbar VSCT neurons and of target cells of cervical and sacral spinocerebellar neurons demonstrated to project to lower thoracic segments but with undefined, if any, projection areas in lumbar segments was another issue. The study was designed to optimize finding target cells of VSCT neurons, for example by selecting cerebellar regions from which the majority of VSCT neurons could be antidromically activated at reasonably low thresholds. However, if the cervical and/or sacral spinocerebellar neurons were co-activated by the same stimuli the conclusions of this study might apply not only to lumbar VSCT neurons but also to their cervical and sacral homologues as further discussed at the end of Discussion.

Results

Unilateral or bilateral projections of individual VSCT neurons and latencies of their antidromic activation

Of 54 VSCT neurons encountered in seven cats, all recorded from on the left side of the spinal cord, 14 (26%) neurons were activated from both the ipsilateral and the contralateral cerebellar stimulation sites, 29 (34%) from only the ipsilateral sites and 11 (20%) from only the contralateral sites when stimulus intensities did not exceed 100 μA. Axons of all these neurons ascended contralaterally, as verified by antidromic activation by stimulation of the right lateral funiculus at the Th 12–13 level. Latencies of activation of VSCT neurons from the cerebellum ranged between 2.9 and 7.0 ms from the stimuli. The shortest of these latencies corresponded to the latencies of descending volleys induced by the stimuli (example in Fig. 2A), while the longest ones were delayed with respect to the onset of these volleys by up to 3–4 ms. The test of collision of responses following cerebellar stimuli was therefore used to ensure that even the spikes with the longest latencies were compatible with antidromic activation of VSCT neurons. The neurons tested included in particular nine VSCT neurons with spikes evoked at latencies of 4–6 ms from the stimuli, which were > 1 ms longer than the conduction time of the fastest spinocerebellar neurons. These spikes were prevented from appearing after spontaneous spikes at proper collision intervals of up to twice the latent periods of activation of the neurons by cerebellar stimuli (example in Fig. 2B). The collision was also tested with spikes evoked by stimulation of the lateral funiculus at the thoracic level (Fig. 2C).

The range of latencies of antidromic activation of VSCT neurons (Fig. 3A, black) was unexpectedly large, several neurons displaying much slower conduction velocities than the fastest conducting neurons. The range of these latencies was nonetheless in keeping with the range of latencies of consecutive components of descending volleys following cerebellar stimuli (see bottom records in Fig. 4). Considerable differences in the latencies would be compatible with effects of stimulation of not only stem axons, or major axonal branches of these neurons within the cerebellum, but also of smaller and slower conducting collaterals. For these reasons the longest synaptic delays of antidromic activation of VSCT neurons in Fig. 3B might be overestimates as they are related to the earliest components of the descending volleys.

Estimates of latencies of likely monosynaptic and disynaptic actions of fibres stimulated within the cerebellum based on latencies of antidromic activation of VSCT neurons

Considering the shortest latencies of antidromic activation of VSCT neurons, *monosynaptic* actions evoked via their axon collaterals in the same spinal segments would be expected to be evoked at minimal latencies of about 3.4 ms from the stimuli, as indicated in Fig. 3C. They would exceed latencies of the descending volleys by about 0.5 ms (with an additional about 0.2 ms for the conduction time along the spinal collaterals of VSCT neurons and 0.3 ms for the synaptic delay). However, monosynaptic actions could also be evoked at latencies exceeding these minimal latencies if evoked by slower conducting neurons. It is thus virtually impossible to set the upper limit of these latencies but another 1 ms might reasonably be allowed. If so, they would be up to latencies compatible with disynaptic coupling or would even overlap with latencies of disynaptic PSPs (as indicated by continuous and dashed lines respectively in Fig. 3C).

Disynaptic actions would be evoked at latencies of at least 1 ms from the volleys, i.e. at least 4 ms from the effective stimuli, as indicated in Fig. 3C. These would be longer than the minimal latencies of monosynaptic actions by at least 0.6–1.0 ms, including (i) about 0.2 ms delay for generation of spike potentials in the relay interneurons, (ii) about 0.1 ms for conduction time along axons of these interneurons and (iii) 0.3 ms for the synaptic delay between the interneurons and motoneurons. Disynaptic actions could nevertheless be evoked at latencies > 4 ms because latencies of not only monosynaptic actions of VSCT neurons but also delays (i) and (ii) might exceed their minimal values. As indicated by grey boxes in Fig. 3C their range would therefore overlap with the range of latencies of both monosynaptic and trisynaptic actions. Other features of disynaptic potentials, in particular the low probability of being evoked by single stimuli and temporal facilitation of distinct components of these potentials might thus be more important for their differentiation from monosynaptic and polysynaptic actions than the mere latencies (see Jankowska *et al.*, 2003). Trisynaptic actions would be likely only about 0.8–1.0 ms after the earliest disynaptic actions, as indicated in Fig. 3C, i.e. at latencies exceeding 5 ms.

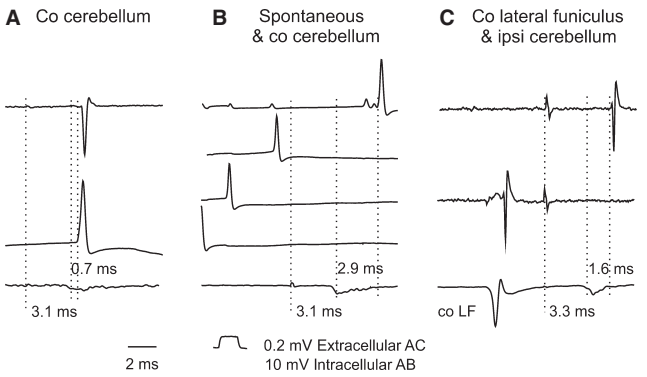

FIG. 2. Examples of different latencies of antidromic activation of VSCT neurons. Records from three VSCT neurons in three preparations (top traces) and records of descending volleys from the cord dorsum at the L5 segment (bottom traces). (A) Single extracellular and intracellular records from a neuron activated at one of the shortest segmental latencies. (B) Series of records showing that cerebellar stimuli activated the neuron only when no spontaneous (or synaptically evoked) spikes were evoked in it within 6 ms preceding its antidromic activation. (C) Records illustrating that activation of the neuron by stimulation of the contralateral lateral funiculus (LF) prevented its activation from the cerebellum by collision of the centrifugal and centripetal spikes. In each panel first dotted lines indicate the timing of the cerebellar stimuli, the second dotted lines the onset of the descending volley (with its latency indicated below the cord dorsum record) and the third dotted lines the onset of the spikes evoked by cerebellar stimuli. The figures between the second and third dotted lines give segmental latencies of the spikes.

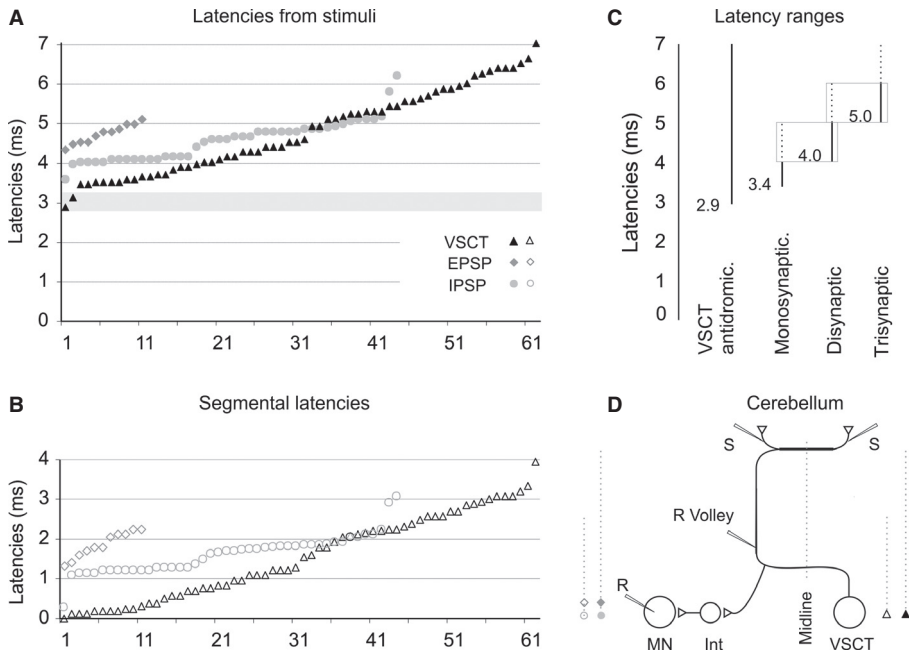

FIG. 3. Comparison of latencies of EPSPs and IPSPs in motoneurons and of antidromic activation of VSCT neurons. (A and B) Latencies of IPSPs (n = 44) and EPSPs (n = 11) evoked in motoneurons together with latencies of spike potentials antidromically evoked in VSCT neurons (n = 62) by cerebellar stimuli; all are ranked from the shortest to the longest. The VSCT neurons were activated by stimuli applied either contralaterally or ipsilaterally. (A) Latencies from stimulus artefacts; the grey strip indicates the range of latencies of the descending volleys evoked by cerebellar stimuli. (B) Latencies measured with respect to the descending volleys recorded at the level of location of VSCT neurons (segmental latencies). (C) Ranges of latencies of synaptic actions evoked monosynaptically, disynaptically and trisynaptically, predicted as described in the text with respect to latencies of antidromic activation of VSCT neurons by cerebellar stimuli. Dashed lines indicate the probable extent of latencies of synaptically evoked potentials overlapping with latencies of potentials evoked by additional relay neurons. Grey boxes indicate ranges where overlap might occur. (D) Diagram showing the stimulation and recording sites. VSCT cells projecting to the cerebellum were stimulated inside the cerebellum. The antidromically conducted volley was recorded at the L5 level. The VSCT cells were recorded either extracellularly or intracellularly and the motoneurons (MN) were recorded intracellularly. Dotted lines with filled and open symbols represent delays plotted in A and B, respectively.

Do cerebellar stimuli evoke any monosynaptic or disynaptic PSPs in hindlimb motoneurons?

The most frequent synaptic actions of stimuli applied in the cerebellum were found to be IPSPs; they were seen in 44/66 (67%) of motoneurons tested. They displayed the characteristics of disynaptically evoked IPSPs, appearing in only 29% of motoneurons after the first stimulus and as a rule being very small, but increasing in size after the second, third and successive stimuli (such as those illustrated in Fig. 4C and D), thus displaying strong temporal facilitation. In the remaining motoneurons similarly temporarily facilitated IPSPs appeared only after the second or third stimuli, as illustrated in Fig. 4A and B. Higher frequencies of stimuli in the train (300 and 400 Hz) were more effective than the lower frequencies (200 Hz).

The IPSPs were evoked at latencies 3.7–6.1 (4.58 ± 0.07; mean ± SEM) ms from the stimuli and 0.5–3.0 (1.62 ± 0.07) ms from the cerebellar descending volleys recorded at the lumbar level. As shown in Fig. 3A and B, the range of latencies of all but two of these IPSPs was therefore compatible with latencies of disynaptic actions relayed by single spinal interneurons that were monosynaptically activated via axon collaterals of VSCT neurons, as specified in the preceding section. Only two longest latency IPSPs might, but would not necessarily, have been evoked trisynaptically.

Excitatory postsynaptic potentials with a sufficiently reliably definable onset were found in only 11/66 (17%) motoneurons. In seven of these motoneurons the EPSPs were the earliest effects of the cerebellar stimuli (as in the motoneurons illustrated in Fig. 4E, F, H and I) while in the four other motoneurons EPSPs were preceded by shorter latency IPSPs. EPSPs were evoked from either ipsilateral stimulation sites (e.g. those illustrated in Fig. 4) or from contralateral sites.

Excitatory postsynaptic potentials appeared at latencies 4.3–5.1 ms (4.74 ± 0.07 ms) from the stimuli and 1.3–2.2 ms (1.85 ± 0.09 ms) from the descending volleys. These latencies were very close to the latencies of the IPSPs, indicating a similar coupling of EPSPs and IPSPs. As shown in Fig. 3A and B the range of latencies of EPSPs corresponded to the mid-range of latencies of IPSPs and no statistically significant differences were found between them. Similar latencies of EPSPs and IPSPs in individual motoneurons are illustrated in Fig. 4 E and H by overlying EPSPs and IPSPs evoked in two motoneurons. In one of these EPSPs evoked from the ipsilateral stimulation site appeared at 0.1 ms longer latency than IPSPs from the contralateral side (Fig. 4E), while in the other one they appeared at latencies 0.5 ms shorter (Fig. 4H). Temporal facilitation of EPSPs evoked by successive stimuli (with faster raising phase in Fig. 4B and increase in amplitude in Fig. 4F) together with the shortest segmental latencies of 2 ms similarly show that they were more compatible with disynaptic than monosynaptic coupling. In addition in other motoneurons, EPSPs evoked at the same latencies appeared only after the third, fourth or fifth stimuli. However, even if the earliest of these EPSPs were evoked disynaptically, trisynaptic or polysynaptic coupling of the later ones could not be excluded.

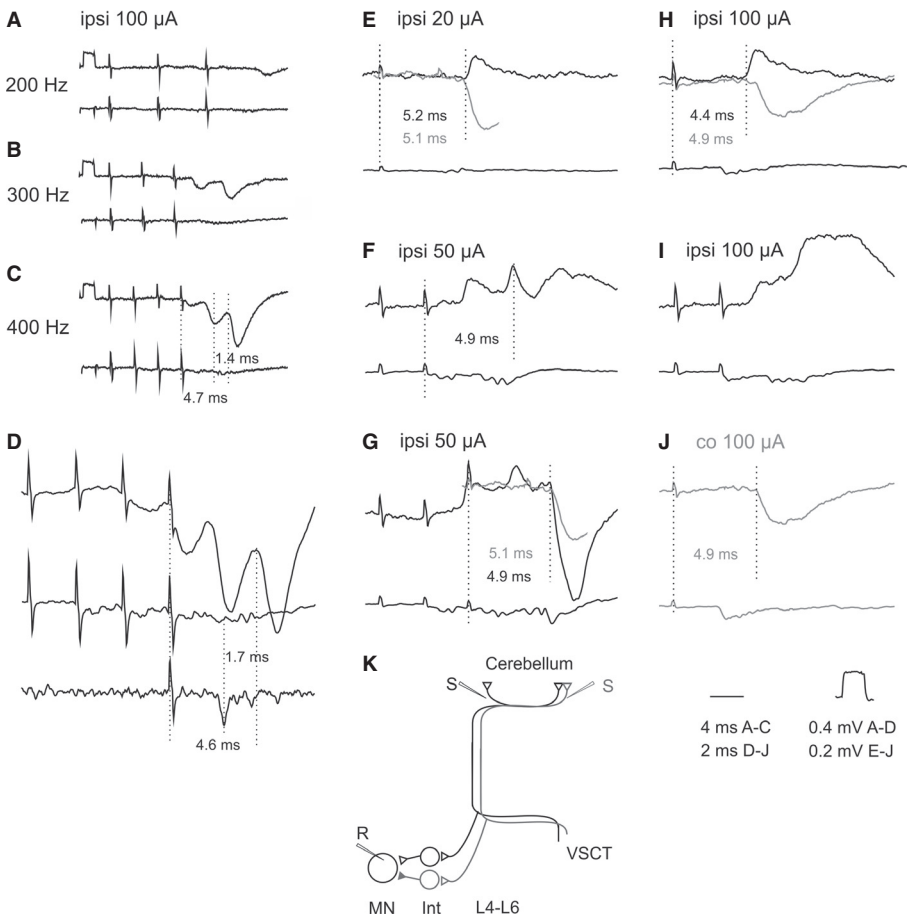

FIG. 4. Examples of IPSPs and EPSPs evoked by cerebellar stimuli and their timing. Intracellular records from four motoneurons (upper traces) and records from the cord dorsum (lower traces) at the L5 level obtained from three experiments. A–C illustrate a greater effectiveness of an increasing number of stimuli applied at increasing frequencies in an unidentified extensor motoneuron. Note distinct IPSPs following successive stimuli, temporal facilitation of these IPSPs and segmental latencies $\leq$ 2 ms, as required for disynaptic coupling. (D) Record from an anterior biceps-semimembranosus motoneuron at the same frequency of cerebellar stimuli as in C but at a twice faster time base, and two records from the surface of the spinal cord, obtained concurrently with the intracellular record and with single stimuli and at a higher amplification. (E–J) Intracellular records from two Q motoneurons in the same experiment illustrating similar timing of EPSPs and IPSPs evoked from ipsilateral and contralateral cerebellar stimulation sites (indicated as ipsi for ipsilateral and co for contralateral). Superimposed early part of the IPSPs in E and G, aligned with respect to the stimulus artefacts, and the whole length of the IPSPs in H are used to compare timing of EPSPs and IPSPs. Dotted lines in C and D indicate shock artefacts, the onset of the descending volleys and the onset of the IPSPs (figures between the records giving latencies from the volleys and those at the bottom latencies from the stimulus). Dotted lines in H and J indicate shock artefacts and the onset of the IPSPs (figures below intracellular records giving latencies from the stimulus. In this and in the following figures the negativity in intracellular records is downward and in records from the cord dorsum upwards. Note different time and voltage calibrations in J. (K) Diagram indicating that both EPSPs and IPSPs are relayed by single interneurons. In this case an inhibitory interneuron is excited from the contralateral cerebellum stimulation site and an excitatory interneuron from the ipsilateral site.

Which disynaptic actions of peripheral afferents on motoneurons are facilitated by cerebellar stimuli; which premotor interneurons mediating reflex actions are affected by these stimuli?

Ia inhibitory interneurons

Facilitation of actions of Ia inhibitory interneurons by VSCT neurons was not considered likely because these interneurons are located much more ventrally than the reported axonal projection areas of VSCT neurons. Nevertheless, in 10/12 (83%) of posterior biceps - semitendinosus (PBST) motoneurons IPSPs evoked from Q group Ia afferents were found to be facilitated when Q stimulation was preceded by cerebellar stimulation (Fig. 5A–J). The facilitation (expressed as the difference between the test and conditioned IPSPs minus any IPSPs evoked by stimuli applied in the cerebellum) ranged between 6 and 331% (221 ± 28%; mean ± SEM) of the areas of the test IPSPs (Fig. 5L). The facilitation occurred independently of whether the cerebellar stimuli evoked any IPSPs by themselves. At least four or five conditioning stimuli were needed but intervals between the last cerebellar volleys and Q volleys at which facilitation was obtained were only about 1 ms, compatible with direct actions on the interposed interneurons and connections depicted in Fig. 5M.

In support of this conclusion, EPSPs fulfilling criteria of monosynaptic EPSPs were found to be evoked by cerebellar stimuli in 7/22 (32%) intracellularly recorded Ia inhibitory interneurons with input from Q (see example in Fig. 5K). All of these EPSPs were evoked at latencies $\leq$ 1 ms from the descending volleys. In addition, EPSPs at segmental latencies of 1.1–1.7 ms were found in seven interneurons and IPSPs at the same latency range in 8/22 interneurons. The longer latency EPSPs and IPSPs had characteristics of disynaptically evoked ones.

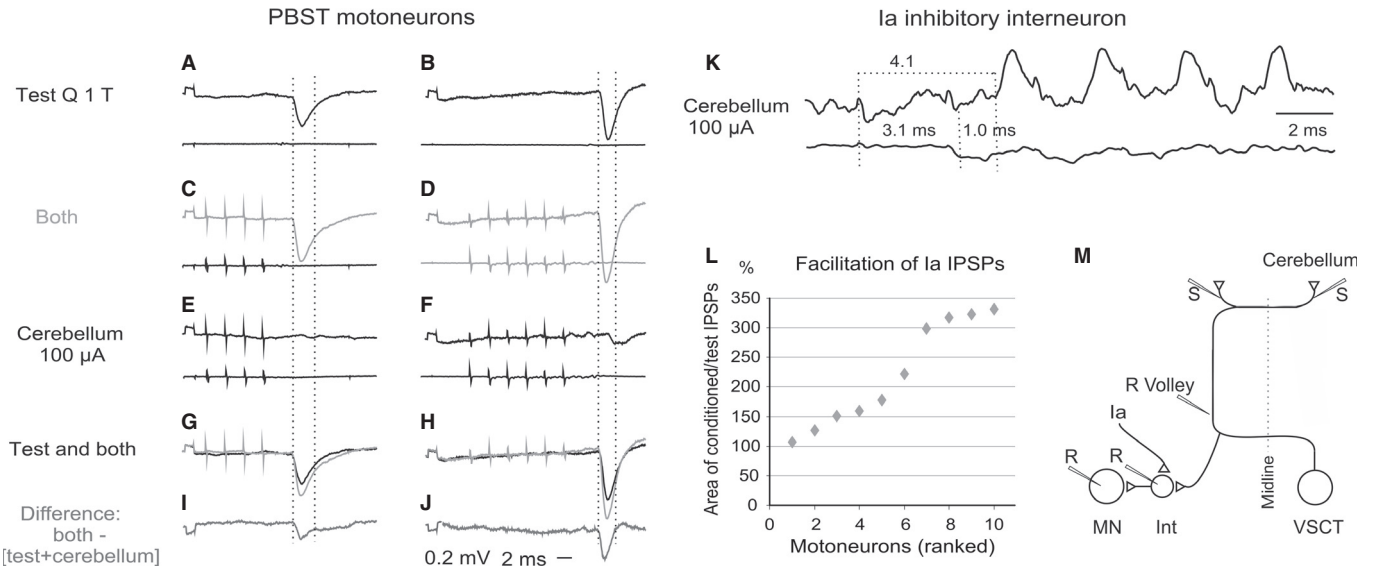

FIG. 5. Examples of facilitation of reciprocal inhibition in flexor motoneurons and of records from Ia inhibitory interneurons. Upper traces in A–F are intracellular records from two PBST motoneurons in two preparations (left and right columns, respectively). The test IPSPs from Q (A and B) and IPSPs following a conditioning train of cerebellar stimuli (C and D) are superimposed in G and H for comparison and differences between these IPSPs are shown in I and J. The dotted lines indicate the time windows within which the areas of the PSPs were measured. In L, the areas of the most effectively facilitated IPSPs in the whole sample of Ia IPSPs recorded in ten motoneurons are expressed as a percentage of the areas of the test IPSPs. They are ranked from the smallest to the largest. (K) Records from an Ia inhibitory interneuron and from the cord dorsum illustrating monosynaptic EPSPs following the successive stimuli applied in the cerebellum. Dotted lines indicate the shock artefact of the first stimulus, the onset of the descending volley initiated by it (at a 3.07 ms latency) and the onset of the EPSPs following this volley after an additional delay of 1.07 ms, or totally after 4.14 ms after the stimulus. (M) Diagram indicating that VSCTs could act on motoneurons via interneurons coexcited by Ia afferents (Ia inhibitory interneurons). Stimulation (S) and recording (R) sites from motoneurons and interneurons are indicated.

Group I/II (Ib) inhibitory interneurons

Facilitation of inhibitory actions from group Ib afferents was tested on IPSPs evoked in extensor motoneurons by stimulation of extensor group I afferents. It was considered more likely than facilitation of reciprocal inhibition in view of the most extensive branching of the so far investigated axon collaterals of VSCT neurons in the intermediate zone.

The effects of a train of 4–6 conditioning stimuli applied in the cerebellum were tested at intervals of 0–2 ms between the last cerebellar volley and the volleys induced by the test stimuli. IPSPs from Ib afferents were found to be increased in 14 (74%) of 19 motoneurons tested (examples in Fig. 6A–C). When the areas of the test and conditioned IPSPs were compared, the facilitation (expressed by the difference between the test and conditioned IPSPs from which any IPSPs evoked from the cerebellum were subtracted) ranged between 4 and 315% of the areas of the test IPSPs, the conditioned IPSPs being increased to an average of 185 ± 29% (mean ± SEM; $n = 11$) of the test IPSPs. The degree of facilitation of IPSPs from group Ib afferents based on these figures thus appears to be weaker than that of Ia IPSPs. However, the strongest cases of facilitation of Ib IPSPs could not be quantified in eight motoneurons in which test stimuli evoked IPSPs superimposed on EPSPs from the same nerve (Fig. 6A) or evoked hardly any early IPSPs (Fig 6B). In such cases the differences could be noted but without expressing them in the percentage of control IPSPs. Facilitation of Ib IPSPs may thus be considered as similarly or even more marked than facilitation of Ia IPSPs and similarly evoked by direct actions of VSCT neurons on interposed interneurons.

Facilitation of IPSPs from group II afferents was tested on IPSPs evoked by lowest threshold group II afferents using stimuli activating both group I and group II afferents. IPSPs evoked by group II afferents were therefore differentiated primarily by their latencies, as IPSPs of group II origin were considered those evoked at latencies $\geq$ 2 ms. IPSPs of such latencies, or longer latency components of IPSPs evoked by stimuli supra-threshold for group II efferents, were found to be facilitated in 11/20 tests in extensor motoneurons (examples in Fig. 6D–F). However, increases in several of the IPSPs were not measurable as the IPSPs attributable to group II afferents followed Ib IPSPs (as in records illustrated in Fig. 6D) and the areas of the test IPSPs could therefore not be quantified. Measurable facilitation evoked at optimal stimulus parameters found in five motoneurons did, however, fall within the same ranges as facilitation of Ib IPSPs (triangles in Fig. 6G). As in the case of facilitation of IPSPs evoked by group Ia and Ib afferents, intervals between the cerebellar and afferent volleys were 0–2 ms.

To verify a coupling between fibres stimulated within the cerebellum and interneurons mediating reflex actions of group Ib and II afferents, intracellular records were obtained from 11 intermediate zone interneurons with group I or both group I and II input which were antidromically activated from the GS and/or PBST motor nuclei in two cats. EPSPs from the cerebellum were evoked in five of these interneurons. Latencies of these EPSPs corresponded to 1 and 1.4 ms segmental latencies in two interneurons (example in Fig. 6H), while in the remaining three interneurons EPSPs were evoked at segmental latencies of 2.6, 3 and 4 ms. Both the shortest and the longest latency EPSPs followed successive stimuli in a train of 3–4 stimuli at constant latencies and amplitudes of early components of all these EPSPs remained unchanged, as required for monosynaptically evoked EPSPs. However, their later components displayed increasing amplitudes, so that they might represent combined monosynaptic and disynaptic actions evoked by cerebellar stimuli.

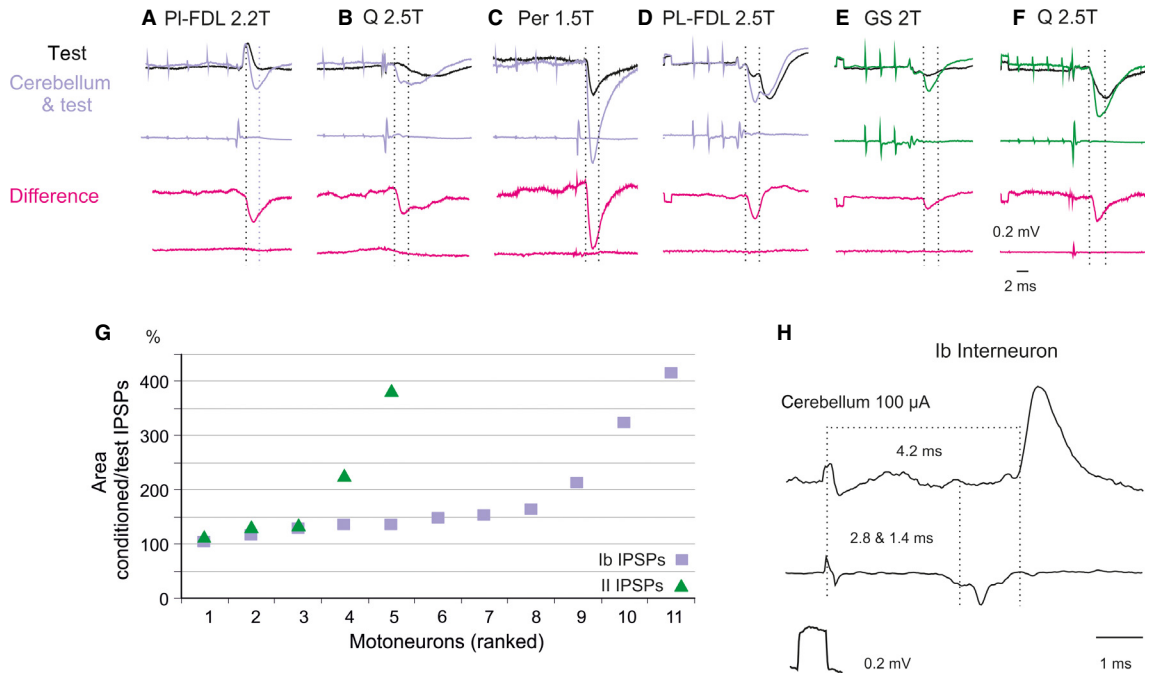

FIG. 6. Examples of facilitation of inhibition of extensor motoneurons from group Ib and II afferents. In A–F upper traces are intracellular records from four motoneurons, Pl or FDL (A–C), ABSM (D), ABSM (E) and unidentified (F) in two preparations while lower traces are from the cord dorsum. The test IPSPs (black traces) were evoked from the nerves indicated above. They are superimposed on IPSPs evoked by the test stimuli preceded by a train of stimuli applied in the cerebellum for comparison. Differences between these IPSPs are shown below. Note that components of the illustrated IPSPs delayed with respect to the afferent volleys by 1.4–1.5 ms (A–D) or between 2.2 and 2.8 (E and F) either emerged or were increased following conditioning stimuli but the areas of the difference traces could be related to the areas of the test IPSPs only in C, D and F because the areas of the test IPSPs in A, B and E were not measurable. The dotted lines indicate the time windows within which the areas were measured. In the plot in G, the most effectively increased IPSPs attributable to group Ib (squares) or group II afferents (triangles) are expressed as a percentage of the measurable areas of test IPSPs. The increases are ranked from the smallest to the largest. (H) Records from an intermediate zone interneuron with input from GS group I afferents and the corresponding descending volley. Dotted lines indicate a shock artefact of the cerebellar stimulus, the positive peak of the descending volley and the onset of the EPSP. Other indications are as in Fig. 5. The diagram shown in Fig. 5M may apply here after having replaced the Ia inhibitory interneurons by intermediate zone interneurons mediating reflex actions of group Ib and II afferents.

Excitatory interneurons

Facilitation of EPSPs induced in motoneurons by cerebellar stimuli was tested on EPSPs evoked from group II afferents and from reticulospinal neurons (by stimulation of the MLF). Facilitation of synaptic actions of group II afferents was indicated by increases of EPSPs (n = 7) evoked by stimulus intensities at 2.5–5 T and at segmental latencies of 2.5–4 ms, at least the earliest of which might have been evoked disynaptically. The degree of facilitation appeared to be comparable with the degree of facilitation of the IPSPs but could not be quantified because all of the tested EPSPs were preceded by IPSPs evoked by group Ib and/or II afferents so that the areas of the test EPSPs evoked from group II afferents were not measurable.

Facilitation of EPSPs evoked from the MLF (n = 5) was easier to evaluate as it involved disynaptic EPSPs not preceded by IPSPs, as illustrated in Fig. 7A–C. However, the facilitated components of these EPSPs appeared at fairly long latencies from the third or fourth stimuli applied to the MLF (1.1–1.5 ms from the MLF descending volley). They might thus have been mediated by either transynaptically activated reticulospinal neurons or excitatory spinal interneurons.

Although only very few examples of facilitation of disynaptically evoked EPSPs by cerebellar stimuli were found, they are nonetheless reported as further indications for collateral actions of VSCT neurons on not only inhibitory but also excitatory premotor interneurons, supplementing indications based on disynaptic EPSPs evoked in motoneurons by cerebellar stimuli themselves (Fig. 4E, F, H and I).

Does any coupling occur between spinocerebellar neurons?

If any collaterals of VSCT or other spinocerebellar neurons contact VSCT neurons, they might serve to coordinate actions of subpopulations of these neurons. This might be possible in view of VSCT crossed terminal projection areas overlapping with the areas of location of VSCT neurons and could be tested by using cerebellar stimulation subthreshold for antidromic activation of these neurons.

The effects of cerebellar stimuli were therefore tested on 23 intracellularly recorded VSCT neurons in the L5 and L6 segments. EPSPs following cerebellar stimuli were found in six of these neurons while IPSPs were evoked in ten neurons. However, the negative results in the remaining neurons were inconclusive because these neurons were activated antidromically at stimulus intensities lower than needed for conditioning stimuli in other neurons. The EPSPs were evoked at minimal latencies of 3.8–5.2 ms from the stimuli (segmental latencies of 0.5, 0.8, 1.0, 1.3, 1.9 and 2 ms), of which the four earliest EPSPs fulfilled criteria of monosynaptically evoked actions of cerebellar stimuli. As illustrated in Fig. 8A–D the early components of these EPSPs followed all successive stimuli with only marginal increases in peak amplitude, consistent with monosynaptic coupling, but the third and fourth stimuli in addition evoked EPSPs (at a latency indicated by the fourth dotted line in B)

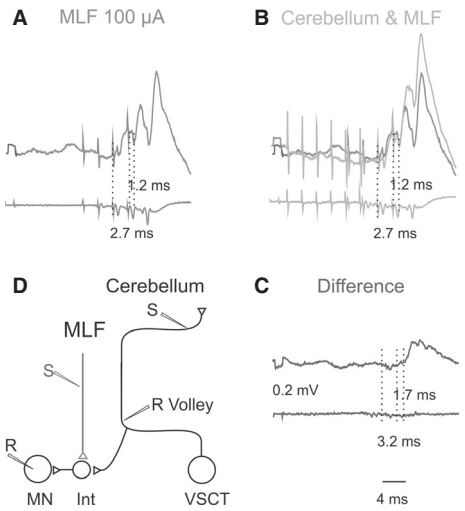

FIG. 7. Facilitation of EPSPs evoked from the MLF. (A–C) Intracellular records from a Q motoneuron (top traces) and records from the cord dorsum (bottom traces). (A) PSPs following stimulation of the MLF alone. (B) PSPs evoked by MLF stimulation preceded by a train of cerebellar stimuli, superimposed on those evoked from the MLF alone. The difference between them is shown in C. Dotted lines in A and B indicate the timing of the third stimulus and the onset of the descending volley and the EPSP evoked by this stimulus. The dotted lines in C indicate the timing of the stimulus, the onset of the descending volley and of the facilitated component of the EPSP. The figures below and above the cord dorsum records give latencies of the MLF descending volley with respect to the third stimulus and of the EPSP evoked by this stimulus with respect to the volley. Note in C that the facilitation involved not the earliest but somewhat later components of the EPSP. These were delayed by 4.3 ms from the last volleys following cerebellar stimuli. (D) Diagram showing sites of the stimulating and recording electrodes. It indicates that mutual facilitation of VSCT and MLF actions on motoneurons (MN) is compatible with convergence on the same interneurons (Int) which synapse on motoneurons recorded from.

or IPSPs (at latencies indicated by the fourth dotted lines in C and D).

Inhibitory postsynaptic potentials evoked in VSCT neurons (Fig. 8E–G) appeared at a similar range of latencies (4.9–6.1 ms from the stimuli) as latencies of later components of the EPSPs. They were evoked within the range of di- or trisynaptically evoked IPSPs evoked in motoneurons (see Fig. 3C), although towards their upper values. However, in view of the effectiveness of single stimuli (Fig. 8E) the distinct IPSPs following successive stimuli might be compatible with disynaptic rather than trisynaptic coupling despite their relatively long latencies.

Control experiments

As indicated in the section on justification of experimental approaches used in this study, only some effects of stimuli applied in the cerebellum could be unconditionally attributed to direct actions of spinocerebellar neurons on spinal neurons: monosynaptic excitation, as evoked without any interposed neurons; and disynaptic inhibition because of a lack of evidence that any potentially interposed inhibitory supraspinal neurons project as far caudally as mid lumbar segments in the cat. However, in contrast to disynaptic inhibition, disynaptic excitation could be potentially mediated by supraspinal neurons unintentionally activated by stimuli applied in the cerebellum and trisynaptic inhibition as well as excitation by both supraspinal and spinal neurons. In control experiments we therefore examined the probability of the involvement of descending tract neurons to the above described effects.

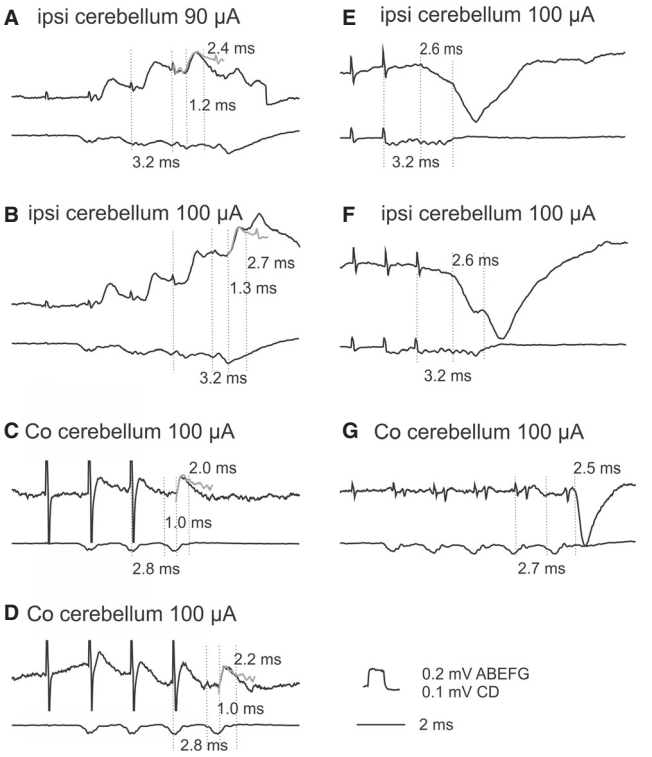

FIG. 8. Examples of PSPs evoked in VSCT neurons. Averaged intracellular records (upper traces) from four VSCT neurons (A–B, C–D, E–F and G, respectively) in three preparations and cord dorsum potentials (lower traces). A–D illustrate the most likely monosynaptically evoked EPSPs followed by longer latency IPSPs (A and D) or EPSPs depending on the intensity of cerebellar stimuli and their number. The longer latency PSPs appeared at the time of deviation between the original traces and the superimposed traces of PSPs evoked by the first or second stimuli. (E–G) Examples of IPSPs following either each successive stimuli or only the later stimuli in a stimulation train. The first dotted lines in the various panels indicate timing of one of the stimuli, the second and third dotted lines indicate the onset of the descending volleys and of the shortest latency EPSPs or IPSPs while the fourth dotted lines in A–D indicate additional longer latency PSPs. Figures below cord dorsum potentials give latencies of descending volleys with respect to the stimuli while the remaining figures give latencies of PSPs with respect to the descending volleys.

Could some effects of cerebellar stimulation be relayed by interposito-rubral and rubro-spinal neurons?

To address this question we tested the effects of cerebellar stimuli not only on spinal neurons but also on supraspinal neurons. In two experiments, 100-μA stimuli applied in the cerebellum failed to evoke any responses of neurons in the contralateral red nucleus (RN). Similar stimuli were similarly without effect on neurons in the ipsilateral RN in one experiment but in the second experiment in which the cerebellar electrode was closer (within about 0.5 mm) to the border of the nucleus interpositus, they evoked responses at a latency of 1.5 ms, corresponding to the latency of responses evoked from nucleus interpositus (Eccles *et al.*, 1975). Some effects of the cerebellar stimuli applied contralaterally could thus be mediated by rubrospinal neurons but only at latencies exceeding minimal latencies of excitation evoked from the RN (4.0–4.5 ms; Hongo *et al.*, 1969, 1972) by 1.5 ms, i.e. exceeding 5.5–6.0 ms from the effective stimuli.

However, when effects evoked from the cerebellum and from the RN were compared in the same motoneurons no indications were found for rubrospinal neurons relaying the reported cerebellar

effects. No EPSPs evoked from the cerebellum were matched by EPSPs from RN (either ipsilateral or contralateral) in eight motoneurons in which EPSPs following ipsilateral and contralateral cerebellar stimuli were evoked at 4.3–4.7 and 4.0–.7 ms latencies, respectively. Furthermore, IPSPs from either ipsilateral or contralateral side of the cerebellum were evoked at latencies (4.8–5.0 ms) only 0.1–1.0 ms longer than from those from the RN. Only in one motoneuron did the latency of an IPSP from the cerebellum (6.3 ms) exceed the latency of IPSPs from RN (4.4 ms) by 1.9 ms. However, the IPSP from the cerebellum was preceded by an EPSP, which might have obstructed detection of its earlier onset.

The effects of cerebellar stimuli were also examined in the cerebellar nuclei. Neurons in the ipsilateral nucleus interpositus were found to be activated at 1.6 ms latency. If neurons activated in this way in turn activated neurons in the RN (after an additional 1.5 ms), any effects relayed by first interposito-rubral and then rubro-spinal neurons would thus be delayed by at least 3 ms when compared with those from RN, resulting in the earliest PSPs at latencies of at least 7.0 ms from the stimuli. Taken together, these control tests would thus obviate the risk that any synaptic actions of cerebellar stimuli evoked at latencies $\leq$ 5.5 ms reported above would be relayed by rubro-spinal neurons.

Could some effects of cerebellar stimulation be relayed by vestibulospinal neurons?

Synaptic actions relayed by fastigial neurons and vestibulospinal neurons would be expected to be evoked at similar latencies as actions relayed by rubrospinal neurons. Even in the case of spread of current to fastigio-vestibular neurons, the earliest effects would be expected at latencies of earliest PSPs evoked by stimulation of axons of fastigial neurons, i.e. at least 5.3 ms (Matsuyama & Jankowska, 2004). However, if fastigial neurons were to be activated synaptically this would involve an additional delay of 1.6 ms, assuming the same latency of activation of fastigial as of interposital neurons (see previous section), i.e. total delays of about 7 ms.

Considering this possibility we compared effects of stimuli applied at cerebellar sites to effects of stimulation of the ipsilateral LVN. However, in 6/11 neurons PSPs evoked from the LVN appeared at latencies only 0.1–0.6 ms shorter than from i or co cerebellum) and in five neurons PSPs from the cerebellum were not matched by any PSPs from the LVN. The comparison did not exclude thus the possibility for some longer latency actions evoked by cerebellar stimuli to be relayed by vestibulospinal neurons, but a low probability for most actions from the cerebellum to be mediated by LVN neurons.

Discussion

Differentiation between synaptic actions of spinocerebellar neurons and potential actions of supraspinal neurons activated by stimuli applied within the cerebellum

The most decisive evidence for collateral actions of spinocerebellar neurons on spinal neurons found in this study have been monosynaptic EPSPs evoked in some spinal premotor interneurons and VSCT neurons and disynaptic IPSPs evoked in hindlimb motoneurons and VSCT neurons.

Monosynaptic EPSPs are the most obvious evidence for actions evoked by axons of spinocerebellar neurons stimulated within their cerebellar projection area but they were only found in small proportions of either Ia inhibitory interneurons (32%), intermediate zone premotor interneurons in reflex pathways from group I and II afferents (18%) or VSCT (17%) neurons. In addition, all these EPSPs were of small amplitudes and, while likely to modulate excitability of the neurons in which they were evoked, they would not alone be sufficient to excite them. Several factors might, however, contribute to the underestimation of these monosynaptic actions. First, stimuli applied at any of the stimulation sites in the anterior lobe might have activated only a small proportion of spinocerebellar neurons because even the strongest stimuli (100 μA) used would reach them within a distance not exceeding 0.5–1 mm (see Gustafsson & Jankowska, 1976) while axons of the majority of spinocerebellar neurons entering sub-lobules I–IV might run at distances of up to 2 or 3 mm from the stimulating electrode tip. Long latencies of antidromic activation of a considerable proportion of VSCT neurons stimulated in the anterior lobe (see Fig. 3) indicate that the stimulated branches might be not only slowly conducting but also require relatively strong electrical stimuli to be activated; the stimulus intensities used in the present study might thus be subthreshold for them but the use of stronger stimuli was avoided as it would increase the risk of spread of current to the fastigial and interposital nuclei. Another limiting factor might be that only actions of the fastest conducting neurons could be taken into account to allow differentiation between monosynaptic and disynaptic actions by their synaptic delays.

Disynaptic IPSPs evoked in motoneurons, or in any other spinal neurons, provide further strong evidence of monosynaptic actions of the stimulated fibres – on interneurons mediating these IPSPs. As illustrated in Figs 4 and 8, activation of the interneurons was not very efficient and depended on temporal facilitation of effects of a few cerebellar stimuli. Nevertheless, as disynaptic IPSPs followed cerebellar stimuli in the great majority of hindlimb motoneurons, at least some premotor interneurons acting on individual motoneurons would be likely to be activated after the second or third stimuli. Mutual facilitation between Ia reciprocal inhibition or inhibitory actions from group Ib and II afferents and disynaptic inhibitory actions following cerebellar stimuli is also compatible with direct coupling between stimulated spinocerebellar tract fibres and the same premotor interneurons.

In contrast, mediation of disynaptic IPSPs evoked by cerebellar stimuli via supraspinal neurons could be practically discarded, as no evidence has been found that inhibitory supraspinal neurons in the cat project as far caudally as midlumbar segments, at least to hindlimb motoneurons (Wilson & Yoshida, 1969; Peterson *et al.*, 1979). In particular, even though fastigial neurons have some spinal actions, according to Takahashi *et al.* (1987) they do not project in the cat caudal to cervical segments. Any cerebellar actions on motoneurons evoked via fastigial neurons should thus be relayed by supraspinal or propriospinal neurons with further inhibitory relays at a lumbar level, i.e. at least trisynaptically. Even if stimuli applied rostral to nucleus fastigii activated some of these neurons directly, any synaptic actions evoked via them would be expected at latencies > 5.3 ms, as effects evoked by stimulation of axons of fastigial neurons (Matsuyama & Jankowska, 2004). Any actions evoked via rubrospinal neurons activated by interposital neurons would likewise be evoked at least trisynaptically in view of a disynaptic coupling in even the most direct pathways between rubrospinal neurons and hindlimb motoneurons (Hongo *et al.*, 1969). As latencies of IPSPs evoked from the RN were about 4.5 ms (Hongo *et al.*, 1969) the total latencies of IPSPs evoked by interposital neurons via rubrospinal neurons would thus be expected to exceed 5.5–6 ms from the effective stimuli. Any actions of cerebellar stimuli mediated by directly activated fastigial or interposital neurons would thus exceed

latencies of the majority of indirectly evoked EPSPs and IPSPs in this study. Effects relayed by transynaptically activated fastigial or interposital neurons would appear even later. Only if the longest latency IPSPs or EPSPs evoked by cerebellar stimuli were relayed trisynaptically, they might have been evoked via collateral actions of VSCT neurons as well as via fastigio-vestibulospinal or interposito-rubrospinal pathways.

However, even taking into account cerebellar actions at longer or undefined latencies, facilitation of Ib IPSPs in motoneurons would not be likely to be mediated by fastigio-vestibular and vestibulospinal neurons in view of only marginal, if any, actions of vestibulospinal neurons on interneurons mediating IPSPs evoked from group Ib and II afferents (Grillner & Hongo, 1972). Cerebellar actions evoked via interposito-rubral and rubrospinal neurons would fit better with facilitation of Ib inhibition but would be expected to involve excitation to a similar extent as inhibition as both disynaptic EPSPs and IPSPs are evoked via rubrospinal neurons (Hongo *et al.*, 1969; Burke *et al.*, 1970) while cerebellar stimuli evoked EPSPs in a much smaller proportion of motoneurons than IPSPs.

Inhibitory postsynaptic potentials relayed by inadvertedly stimulated corticospinal fibres and reticulospinal neurons would likewise be evoked tri- rather than disynaptically. If they were relayed by corticospinal fibres and spinal interneurons the coupling would be even more likely to be polysynaptic in view of primarily polysynaptic actions of corticospinal neurons on cat motoneurons. In addition, stimuli applied at the entry to lobules II–IV may not be likely to reach axons of corticospinal neurons connecting motor cortex to the lobules V–VIII of the vermis according to Coffman *et al.* (2011).

It thus appears that the most plausible explanation of the reported observations is that not only monosynaptic excitation and disynaptic inhibition but also disynaptic excitation and some longer latency synaptic actions of stimuli applied in the anterior lobe might be mediated by collateral actions of spinocerebellar neurons and spinal interneurons.

Differentiation between spinal collateral actions of VSCT and of other SCT neurons

The first two sections of the results show that latencies of PSPs evoked by cerebellar stimuli are compatible with actions evoked by axon collaterals of VSCT neurons and the regions of location of neurons in which they were evoked are in keeping with the terminal projection areas of VSCT neurons. However, we have too few data on descending projections of cervical spinocerebellar neurons and ascending projections of sacral spinocerebellar neurons to draw any conclusions on their contribution to the reported actions in parallel with VSCT neurons, except for considering that such a possibility exists. In particular, there is no information as to whether the cervical or sacral neurons might terminate within the ventral horn of midlumbar segments, as the only information at hand is that both were antidromically activated from lower thoracic segments (Hirai *et al.*, 1978; Grottel *et al.*, 1998; Krutki *et al.*, 1999a; Mrowczynski *et al.*, 2001). Neither do the similar ranges of conduction velocities of these neurons and of VSCT neurons provide the basis for differentiation of the timing of their actions. However, two pieces of information might be of use in future attempts for such differentiation. One is that axons of at least some of these neurons with descending projections (Mrowczynski *et al.*, 2001) as well as of sacral neurons with ascending spinal projections (Grottel *et al.*, 1998; Krutki *et al.*, 1999a) run in the inferior cerebellar peduncle, rather than the superior cerebellar peduncle together with axons of VSCT neurons (Grant & Xu, 1988), and that a considerable proportion of these neurons could be activated not only from the inferior cerebellar peduncle but also from the lateral reticular nucleus. One might therefore compare effects of stimuli applied within the cerebellar regions of termination of VSCT neurons and of stimuli applied in the inferior cerebellar peduncle and the lateral reticular nucleus, with careful tests to exclude co-activation of VSCT neurons by these stimuli.

In this context it would be particularly important to verify the relationships between VSCT neurons and a population of spinoreticular neurons often referred to as bilateral ventral flexion reflex tract (bVFRT) neurons terminating within the lateral reticular nucleus and the possibility that the same spinal neurons give rise to the VSCT and bVFRT tracts (Matsushita & Hosoya, 1979; Matsushita *et al.*, 1979). For instance, axons of some spinoreticular neurons of the bVFRT subpopulation terminating not only within the lateral reticular nucleus and LVN (Ekerot *et al.*, 1979) but also in the cerebellum might have similar actions as VSCT neurons at both supraspinal and spinal levels. However, much more must be known about bVFRT neurons before being able to compare spinal actions of these neurons and of VSCT neurons.

Possible functional meaning of spinal collateral actions of SCT neurons

The results of this study show that in addition to forwarding information to the cerebellum, VSCT and other spinocerebellar neurons may operate as spinal interneurons and affect spinal motoneurons. These neurons have not been found to have any direct actions on motoneurons. The earliest PSPs evoked by stimulation of their axons in the cerebellum had features of disynaptically evoked PSPs, indicating that they were relayed by single interposed interneurons and EPSPs were evoked much less frequently. Spinocerebellar neurons should therefore in the first place act on motoneurons via premotor inhibitory interneurons, including interneurons mediating disynaptic inhibition of motoneurons from Ia afferents or joint actions of group Ia, Ib and II afferents. The excitatory actions of motoneurons appeared to be not as direct, but also these might be evoked via premotor interneurons. The only indications on the identity of these interneurons have so far come from the observations that the most effective facilitation of excitation of motoneurons following cerebellar stimuli involved excitation by group II muscle afferents and by reticulospinal neurons.

If crossed collateral actions of VSCT neurons are excitatory, one would expect that activation of VSCT neurons by group I afferents would be reflected by crossed excitation of motoneurons. However, crossed excitatory actions of group I afferents on motoneurons were reported to be rare and weak. They were considered to require a train of stimuli and to be evoked at fairly long segmental latencies; the shortest were of about 3 ms and were considered as compatible with a trisynaptic coupling (Harrison & Zytnicki, 1984) in good agreement with the first synapse on VSCT neurons, the second synapse between VSCT neurons and contralateral interneurons and the third one between interneurons and contralateral motoneurons. Whether procedures which revealed crossed excitatory actions of group I afferents would be suitable for finding crossed inhibitory actions of these afferents on motoneurons should be verified. Regardless, they would be expected, provided that segmental axon collaterals are given by a sufficient proportion of VSCT neurons which are activated by group I afferents.

Inhibition of contralateral motoneurons by axon collaterals of VSCT neurons could be considered within the frames of the reciprocal control of neuronal networks on both sides of the spinal cord

and of the coordination of activity of these networks. Nevertheless, the functional meaning of these actions is difficult to predict. One of the consequences might be that inhibition of motoneurons by Ia and Ib premotor interneurons (or other interneurons) associated with inhibition of VSCT neurons on one side of the body (see Hammar *et al.*, 2011) would result in weaker actions of VSCT neurons not only on cerebellar neurons but also on their target neurons on the opposite side. Weaker actions of VSCT on contralateral inhibitory interneurons will in turn result in disinhibition of contralateral motoneurons as well as VSCT neurons, allowing changes in the degree of inhibition on both sides of the body to be monitored at the same time. Another consequence might be in the actual adjustments of contralateral reflex actions of group I and II afferents in advance of those relayed by the cerebellar control systems and in parallel with them.

Spinal collateral actions of VSCT neurons might also be considered as adding to collateral actions of other ascending tract neurons including spinoreticular neurons (Rosen & Scheid, 1973; Ekerot *et al.*, 1979; Grottel *et al.*, 1998; Krutki *et al.*, 1999b), spinocervical neurons (Jankowska *et al.*, 1976; Brown *et al.*, 1977; Rastad *et al.*, 1977), spinothalamic and spinomesencephalic neurons (Light *et al.*, 1979; Hylden *et al.*, 1986; Verburgh *et al.*, 1990) and postsynaptic dorsal column neurons (Brown & Fyffe, 1981). Even if only a few per cent of these neurons were found to give off axon collaterals at a spinal level, together they might indicate functionally meaningful and not merely aberrant actions worthy of further analysis.

Acknowledgements

The study was supported by the grant from NINDS/NIH (R01 NS040863). We thank Mrs Jytte Grännsjö for excellent technical assistance during experiments and for histological control and Drs I. Hammar and H. Jörntell for comments on the preliminary version of this paper.

Abbreviations

4-AP, 4-aminopyridine; bVFRT, bilateral ventral flexor reflex tract; DSCT, dorsal spinocerebellar tract; EPSP, excitatory postsynaptic potential; GS, gastrocnemius – soleus; FDL, flexor digitorum longus; ipsi, ipsilateral; IPSP, inhibitory postsynaptic potential; L, lumbar; LF, lateral funiculus; LVN, lateral vestibular nucleus; MN, motoneuron; MLF, medial longitudinal fascicle; PBST, posterior biceps – semitendinosus; Per, peroneus; Pl, plantaris; PSP, postsynaptic potential; Q, quadriceps; RN, red nucleus; SCT, spinocerebellar tract; T, threshold; Th, thoracic; VSCT, ventral spinocerebellar tract.

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