Premotor interneurones contributing to actions of feline pyramidal tract neurones on ipsilateral hindlimb motoneurones

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The aim of the study was to analyse the potential contribution of excitatory and inhibitory premotor interneurones in reflex pathways from muscle afferents to actions of pyramidal tract (PT) neurones on ipsilateral hindlimb motoneurones. Disynaptic EPSPs and IPSPs evoked in motoneurones in deeply anaesthetized cats by group Ia, Ib and II muscle afferents were found to be facilitated by stimulation of the ipsilateral, as well as of contralateral, PT. The ipsilateral actions were evoked by either uncrossed or double-crossed pathways. The results show that interneurones mediating reflex actions of muscle afferents may be activated strongly enough by PT stimulation to contribute to movements initiated by ipsilateral PT neurones and that PT actions relayed by them might be enhanced by muscle stretches and/or contractions. However, in some motoneurones disynaptic IPSPs and EPSPs evoked from group Ib or II afferents were depressed by PT stimulation. In order to analyse the basis of this depression, the transmitter content in terminals of 11 intracellularly labelled interneurones excited by PT stimulation was defined immunohistochemically and their axonal projections were reconstructed. The interneurones included 9 glycinergic and 2 glutamatergic neurones. All but one of these neurones were mono- or disynaptically excited by group I and/or II afferents. Several projected to motor nuclei and formed contacts with motoneurones. However, all had terminal projections to areas outside the motor nuclei. Therefore both inhibitory and excitatory interneurones could modulate responses of other premotor interneurones in parallel with direct actions on motoneurones.

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Interneurones mediating reflex actions of group I and II muscle afferents and high threshold muscle, joint and skin afferents have been repeatedly shown to relay actions of PT neurones on *contralateral* feline (Lundberg & Voorhoeve, 1962; Lundberg *et al.* 1962; Harrison & Jankowska, 1985; Davies & Edgley, 1994; Leblond *et al.* 2001) and primate motoneurones (see, e.g. Jankowska *et al.* 1976; Iles & Pisini, 1992; Pauvert *et al.* 1998; Nicolas *et al.* 2001). However, much less is known about relay neurones that mediate PT actions on *ipsilateral* motoneurones that were demonstrated recently (Edgley *et al.* 2004; Jankowska & Edgley 2006). They appear to be relayed in two ways: firstly, by reticulospinal (RS) neurones, especially those with axons in the ipsilateral medial longitudinal fascicle (MLF), i.e. via uncrossed pathways indicated in Fig. 1*A* (Cabaj *et al.* 2006; Jankowska *et al.* 2006) and, secondly, by RS neurones descending contralaterally and commissural interneurones located contralaterally, i.e. via double-crossed pathways indicated in Fig. 1*D* (Edgley *et al.* 2004; Jankowska & Edgley, 2006; Jankowska *et al.* 2006). Commissural interneurones and reticulospinal neurones may affect hindlimb motoneurones directly by evoking monosynaptic EPSPs only (Grillner & Lund, 1968; Wilson & Yoshida, 1969; Peterson *et al.* 1979) or by evoking both EPSPs and IPSPs (Jankowska *et al.* 2003). However, these direct actions might be supplemented by actions relayed by premotor interneurones activated by reticulospinal neurones (represented by black cells in the diagrams in Fig. 1*A* and *D*; see, e.g. Takakusaki *et al.* 1989, 2001, 2003) and/or by commissural interneurones (Jankowska *et al.* 2005*c*; Cabaj *et al.* 2006). Uncrossed PT fibres have been shown to terminate within spinal cord regions (the intermediate zone and the ventral horn (Dum & Strick, 1991; Lacroix *et al.* 2004) where several populations of premotor interneurones are located and therefore the same, or other, premotor interneurones might be activated by ipsilaterally descending PT neurones.

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In order to determine the contribution of premotor interneurones mediating reflex actions of group I and II muscle afferents to actions of PT neurones on ipsilateral hindlimb motoneurones we undertook a two step analysis. In the previous study we showed that stimulation of the ipsilateral PT evoked monosynaptic and disynaptic EPSPs in a high proportion of interneurones with group I and II input (Jankowska & Stecina, 2007). However, by using this approach, we were unable, with only a few exceptions, to determine if these were premotor interneurones, or decide which of them was excitatory or inhibitory. Furthermore, in many interneurones only very small EPSPs were evoked by PT stimulation. This study therefore left open the question of whether actions of PT neurones on interneurones in feline spinal reflex pathways are only subthreshold and serve to modulate the activity of these interneurones, or could also discharge them, as would be required if the interneurones were to operate as relay neurones of centrally initiated movements.

The results of the present study represent the next step in our analysis. The aims were to verify: (1) that premotor interneurones in spinal reflex pathways from muscle afferents do indeed relay actions of PT neurones to ipsilateral motoneurones and (2) that they include both excitatory and inhibitory interneurones. We used spatial facilitation of disynaptic PSPs evoked by group Ia, Ib and/or II muscle afferents and from the PT as a measure of activation of interneurones (Lundberg, 1975). Enhancement of disynaptic IPSPs provided evidence for activation of inhibitory premotor interneurones and enhancement of EPSPs activation of excitatory interneurones.

As another means to investigate whether both excitatory and inhibitory actions of PT neurones on hindlimb motoneurones are relayed by premotor interneurones we compared terminal projection areas of excitatory and inhibitory interneurones that were monosynaptically or disynaptically activated by PT neurones. As reported

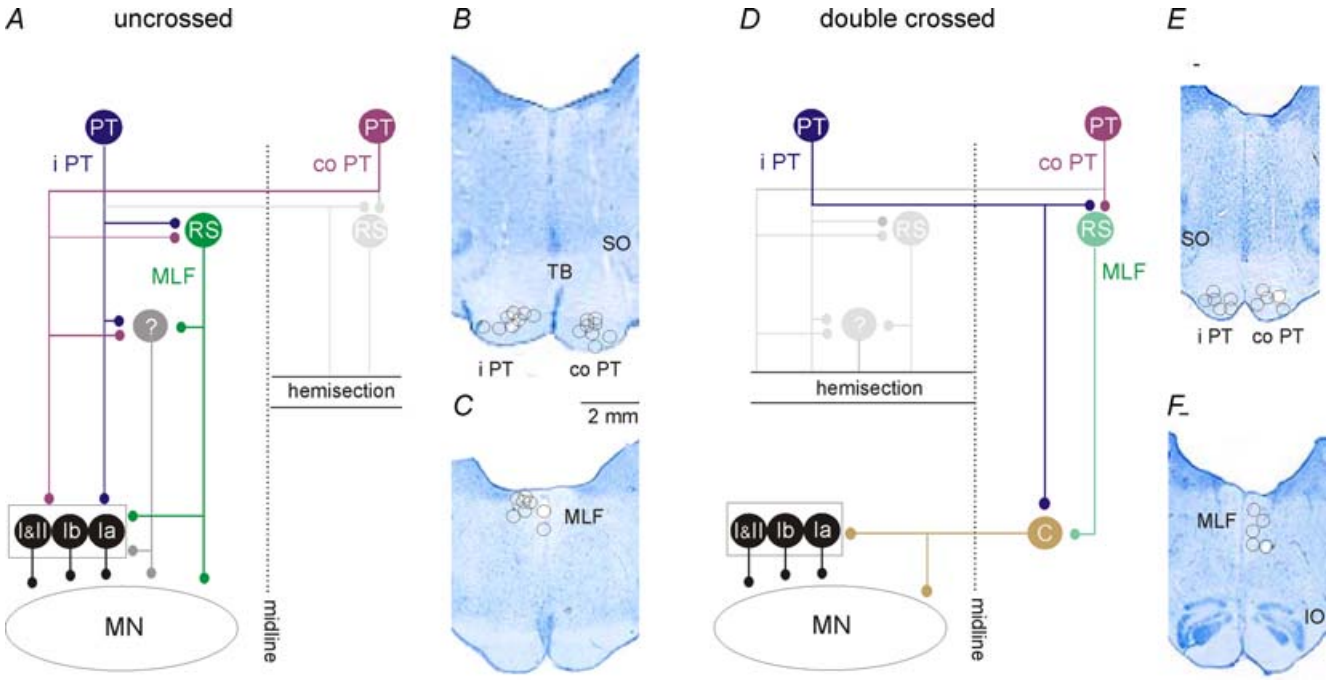

Figure 1. Diagrams of the neuronal pathways examined and the location of the stimulation sites
A, diagram of uncrossed pathways between pyramidal tract (PT) neurones and hindlimb motoneurones via reticulospinal (RS) neurones with axons descending within the ipsilateral medial longitudinal fascicle (MLF) and via interneurones in the lumbosacral enlargement with segmental input from group Ia, Ib and II muscle afferents (black circles in the box). The latter represent both excitatory and inhibitory interneurones. *B* and *C*, reconstruction of stimulation sites in the ipsilateral and the contralateral PT at the level of the superior olive (SO) and the trapezoid body (TB) and in the ipsilateral MLF rostral to the inferior olive in experiments examining neuronal relays in uncrossed pathways. They are displayed on representative brainstem sections in the plane of the inserted electrodes. *D*, diagram of the double-crossed pathways between descending PT neurones and hindlimb motoneurones via RS neurones with axons descending within the contralateral MLF and commissural interneurones (*C*) that target both motoneurones and interneurones. *E* and *F*, stimulation sites in the ipsilateral and contralateral PT at the level of the SO and TB and in the contralateral MLF at the level of the inferior olive (IO) in experiments examining neuronal relays in double-crossed pathways. Light grey elements of the diagrams in *A* and *D* are those not operating caudal to the hemisection.

previously, it has recently become possible to define transmitter content in axon terminals of intracellularly labelled interneurones and to examine axonal projections of those of different phenotypes (Bannatyne *et al.* 2003, 2006). This approach was of particular importance for the interpretation of depression of reflex actions of peripheral afferents by PT neurones because of two possible mechanisms of such depression. The depression could namely be due to inhibition of interneurones that mediate PSPs evoked by peripheral afferents, i.e. secondary to activation of inhibitory interneurones that target interneurones in pathways from peripheral afferents. However, it could also be secondary to occlusion between excitation of the same interneurones evoked in a quick succession first by PT stimuli and then by peripheral stimuli (see Cabaj *et al.* 2006), i.e. to excitatory rather than inhibitory actions of PT neurones on these interneurones.

Methods

Preparation

The experiments were performed on 16 deeply anaesthetized cats weighing 2.6–5.0 kg. All experimental procedures were approved by Göteborg Ethics Committee and followed NIH and EU guidelines for animal care. Anaesthesia was induced with sodium pentobarbital (40–44 mg kg^{-1}, I.P.) and maintained with α-chloralose (Rhône-Poulenc Santé, France; doses of 5 mg kg^{-1}, every 1–2 h, up to 55 mg kg^{-1}, I.V.). Additional doses of α-chloralose were given when increases in continuously monitored blood pressure or heart rate occurred, or if the pupils dilated. Mean blood pressure was 100–130 mmHg and the end-tidal concentration of CO_2 was about 4. During recordings, neuromuscular transmission was blocked by pancuronium bromide (Pavulon, Organon, Sweden; about 0.2 mg kg^{-1} h^{-1} I.V.) and the animals were artificially ventilated. The K^+ channel blocker 4-aminopyridine (4-AP; 0.2–04 mg kg^{-1} I.V.) was given in order to increase the effectiveness of synaptic transmission between PT and reticulospinal neurones and spinal interneurones (Jankowska *et al.* 2005*a*). The core body temperature was kept at about 37°C. The experiments were terminated by a lethal dose of pentobarbital.

Laminectomy exposed the fourth to seventh lumbar (L4–L7) segments, the border between the 3rd and 4th cervical (C2–C4) and low thoracic (Th11–Th13) segments of the spinal cord. In five cats, the spinal cord was hemisected on the left side and in eight cats on the right side at the Th12 level; it was not hemisected in three cats used for cell labelling. A number of peripheral hindlimb nerves were transected and mounted either on subcutaneous cuff electrodes (for ipsilateral quadriceps (Q) and sartorius (Sart)), or pairs of silver hook electrodes in paraffin oil pool (at 36–37°C) for the remaining nerves (posterior biceps/semitendinosus (PBST), anterior biceps/semimembranosus (ABSM), gastrocnemius/soleus (GS), plantaris, flexor digitorum and hallucis longus (FDL) and deep peroneal (DP)).

Tungsten electrodes (30–150 kΩ) were inserted through the cerebellum and placed in the left medial longitudinal fascicle (MLF) and the left and right pyramids (PT). The electrodes were inserted at an angle of 30 deg (tip directed rostrally). The initial targets were at Horsley–Clarke co-ordinates P7, H–0, L1.2 and R1.2 for the left and right PT, respectively, and P10, H–5 and L0.6, for the MLF. However, the final positions of the electrodes were adjusted on the basis of records of descending volleys. The electrodes were left at sites from which the volleys were evoked at thresholds of $\leq 20\,\mu$A. These sites were marked with electrolytic lesions and their location was subsequently verified on 100 μm thick frontal sections of the brainstem, cut in the plane of insertion of the electrodes. As shown in Fig. 1*B* and *C*, the PT electrodes at the level of the trapezoid body (TB) and superior olive (SO) and the MLF electrodes were placed at the level corresponding to the rostral border of the inferior olive (IO). All PT placements were within the pyramids or at the border with the trapezoid body and MLF electrode placements were within confines of the MLF.

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). Constant current cathodal stimuli (0.2 ms, 50–150 μA) were used to activate reticulospinal and corticospinal tract fibres. Precautions taken in this study to activate PT fibres only on one side and to avoid spread of current to the other side were the same as previously described (Jankowska *et al.* 2006; Stecina & Jankowska, 2007). Glass micropipettes filled with a 2 M solution of potassium citrate were used for intracellular recording. The motoneurones were identified by antidromic activation from muscle nerves with intact ventral roots. Descending volleys were recorded from the cord dorsum (at C3–C4 for PT volleys) or from the surface of the lateral funiculus (at Th11–Th12 for MLF volleys) over intact dura mater, in both cases monopolarly. Based on the latency of EPSPs evoked from the PTs in lumbar interneurones (see Fig. 2 in Jankowska & Stecina, 2007), the interval between test and conditioning stimuli was usually varied from 3 to 8 ms to find optimal conditions for facilitation of test PSPs.

Analysis

Both the original data and averages of 10–30 single records were stored online. Effects of pyramidal and

MLF conditioning stimulation on PSPs evoked by muscle afferents in hindlimb motoneurones were estimated by comparing the areas of test and conditioned potentials within selected time windows. The windows were within 0.8–2 ms from the onset of PSPs evoked by group I afferents and 2–3 ms for PSPs evoked by group II afferents, usually within their rising phase, as described by Cabaj *et al.* (2006). The effects found in individual motoneurones varied depending on the intensity of the test and conditioning stimuli and on intervals between them. The test PSPs were as a rule evoked by submaximal stimuli. The intensity of the conditioning stimuli did not exceed 150 μA even if it did not evoke maximal effects in order to avoid spread of current to the contralateral PT. When weaker conditioning stimuli were effective, those with minimal actions on the motoneurones were used for analysis in order to avoid complications of interactions between PSPs evoked by PT and peripheral stimuli at a motoneuronal level. Optimal intervals between the last conditioning and the testing stimuli were within the range of 3–9 ms. However, they were adjusted in order to minimize effects of any EPSPs and IPSPs evoked in the motoneurones tested. In general, changes of IPSPs were easier to analyse because they were more easily identified as being disynaptic and they were encountered in a greater number of motoneurones than EPSPs. Differences between the effects of conditioning stimulation in different samples of neurones were assessed for statistical significance using Student's *t* test for paired and unpaired samples with appropriate Bonferroni corrections due to multiple comparisons. Differences in the mean latencies were assessed by one-way ANOVA and when significant differences were found ($P < 0.05$) Tukey's *post hoc* test was applied.

Immunohistochemistry

In order to define transmitter content of premotor interneurones mediating ipsilateral PT actions, some of the intracellularly recorded interneurones analysed in the previous studies (Jankowska *et al.* 2006; Jankowska & Stecina, 2007) were injected with a mixture of Rhodamine–dextran and Neurobiotin and analysed as described by Bannatyne *et al.* (2003, 2006). Of particular interest were inhibitory commissural interneurones and ipsilaterally projecting interneurones with terminal projection areas both within motor nuclei (showing that they were last order interneurones) and in laminae V–VIII where they could affect activity of other premotor interneurones. Selected sections containing terminals from neurones were reacted with one of two combinations of antibodies: guinea pig anti-vesicular glutamate transporter 1 (VGLUT1, 1 : 5000, Chemicon International, Harrow, UK), sheep anti-glycine transporter 2 (GlyT2, 1 : 1000, Chemicon International) and/or mouse anti-gephyrin (1 : 100, Connex, Martinsried, Germany); or guinea pig anti-vesicular glutamate transporter 2 (VGLUT2, 1 : 5000, Chemicon International) and rabbit anti-glutamic acid decarboxylase (GAD: recognizes both 65 and 67 isoforms, 1 : 2000, Sigma-Aldrich, Poole, UK). The sections were inspected with a confocal microscope (Biorad, Hemmel Hempstead, UK). For details see Bannatyne *et al.* (2003, 2006).

Results

Facilitation of synaptic actions of inhibitory premotor interneurones on motoneurones by stimulation of the ipsilateral PT

Effects of conditioning stimulation of the ipsilateral PT were tested on IPSPs evoked in 103 motoneurones in eight preparations in which only the ipsilateral half of the spinal cord was intact and in 74 motoneurones in five preparations with only the contralateral half left intact. Most of these IPSPs fulfilled criteria of disynaptically evoked IPSPs, as indicated by segmental latencies of 1.3–1.8 ms for IPSPs evoked from group Ia and group Ib afferents and latencies of 2.5–3.5 or 3–4.5 ms from group I volleys in more proximal and more distal nerves for IPSPs evoked by group II afferents, which would correspond to latencies of about 1.8–2.8 ms from group II volleys in these nerves (see Jankowska *et al.* 2005*b*). Facilitation was demonstrated when IPSPs evoked following conditioning PT stimulation were much larger than the sum of IPSPs evoked by peripheral and PT stimuli alone. This is illustrated in Fig. 2 in two ways: by overlaying the sums of IPSPs evoked by peripheral and PT stimuli on conditioned IPSPs (records in the 3rd row) and by the differences between them (in the 4th row). The degree of facilitation was estimated by comparing areas of the early parts of conditioned and test IPSPs (see Methods) and is expressed in percentages of the latter. Conditioning stimulation of the ipsilateral PT was found to facilitate IPSPs evoked by group Ia (Fig. 2*A* and *D*) and group Ib (Fig. 2*B* and *E*) as well as group II muscle afferents (Fig. 2*C* and *F*), via uncrossed (Fig. 2*A*–*C*) as well as double-crossed (Fig. 2*D* and *E*) pathways. Depending on the size of the test IPSP, the parameters of the test and conditioning stimuli and the intervals between them, the facilitation was up to over 200%, but as shown in the lowest row of Fig. 2, the increases of the IPSPs were usually of only 0.2–0.5 mV.

For the whole sample of motoneurones the degree of facilitation of IPSPs evoked from different afferents is summarized in columns 4–7 in Table 1. The table shows that facilitation evoked via uncrossed and double-crossed pathways was generally similar and that no statistically significant differences were found between effects evoked via these pathways. Nor were differences found between PT actions on PSPs evoked from group Ia, Ib or II afferents.

Facilitation of synaptic actions of excitatory premotor interneurones on motoneurones by stimulation of the ipsilateral PT

To assess facilitation of EPSPs evoked by group Ib and II afferents was more difficult since there were fewer motoneurones with early EPSPs from these afferents (10 and 21, respectively) and the quantification of changes in these EPSPs met with several problems. Test EPSPs from group Ib afferents, as indicated by a threshold below that of group II afferents (usually $< 2T$, see Jack (1978), were most often evoked at segmental latencies 1.4–1.8 ms and EPSPs from group II afferents at latencies 2.5–3.5 ms from group I volleys. However, early components of EPSPs evoked by group II afferents may overlap with later components of IPSPs or EPSPs evoked from group Ib afferents, as in records in Fig. 3*B* and *C*, making it difficult to define the latency of group II EPSPs and to be confident that they were evoked disynaptically. Furthermore, test EPSPs were often mixed with IPSPs evoked by muscle afferents and conditioned EPSPs often overlapped with EPSPs or IPSPs evoked by PT stimuli, as in Fig. 3*A*–*C*. For these reasons, even when facilitation of the EPSPs was marked, its degree was usually unquantifiable and could be estimated only qualitatively. With these limitations, EPSPs from group Ib and group II afferents appeared to be facilitated in 20–45 of the motoneurones tested. However, the proportion of facilitated EPSPs is most likely to be underestimated because EPSPs not previously evident might have been induced in at least as many motoneurones when decrease of IPSPs by conditioning stimuli was noted; difference traces in Fig. 4 might for instance represent both decreases of the IPSPs and any emerging EPSPs.

EPSPs from group Ib afferents were found to be facilitated in three motoneurones via uncrossed pathways and in seven motoneurones via double-crossed pathways, two of which are illustrated in Fig. 3*B* and *C*. EPSPs from group II afferents were found to be facilitated in 13 motoneurones via uncrossed pathways with an example in Fig. 3*A* and in 4 motoneurones via double-crossed

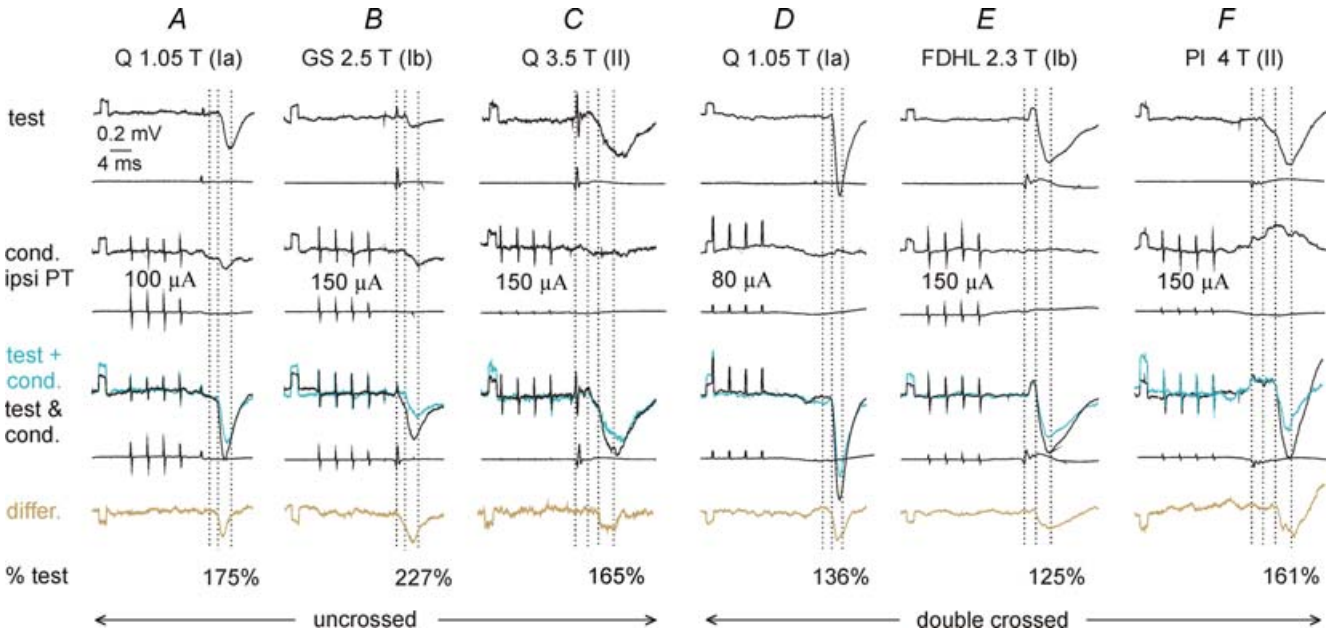

Figure 2. Examples of facilitation of IPSPs by conditioning stimulation of the ipsilateral pyramidal tract (PT) via uncrossed and double-crossed pathways
Averaged ($n = 10$–30) intracellular records from 6 motoneurones (upper traces in each panel) and records from the cord dorsum (lower traces), in preparations with intact ipsilateral (*A*–*C*) or contralateral (*D*–*F*) spinal halves, respectively. Records in each row were evoked from the top to the bottom by: (1) test stimuli alone (specified above the records, expressed in multiples of threshold, *T*, for the activation of lowest threshold muscle afferents); (2) conditioning stimuli alone (at intensities indicated between the records); (3) test stimuli following conditioning stimuli (test & cond.) superimposed on the sum of responses to test and conditioning applied alone (test + cond.); (4) the difference between the superimposed traces, with the relation between the conditioned and test IPSP area in percentage. Test responses were IPSPs evoked by Ia afferents in PBST motoneurones (*A*, *D*); by Ib afferents in ABSM and GS motoneurones (*B*, *E*), by Ib and group II afferents in GS and Q motoneurones (*C*, *F*). The 1st dotted lines indicate the arrival of the afferent volleys, the 2nd or both the 2nd and 3rd the onset of the IPSP and the last the widths of the time windows within which the areas were measured. Both in this and in the following figures negativity is down in the microelectrode records and up in the records from the cord dorsum. Rectangular pulses at the beginning of the intracellular records are calibration pulses.

Table 1. Comparison of effects of conditioning stimulation of the ipsilateral and contralateral PT and of the MLF on IPSPs evoked by peripheral afferents

			Increase		Decrease		
			Mean ± S.E.M. (%)	Proportion of Motoneurones (%)	Mean ± S.E.M. (%)	Proportion of Motoneurones (%)	Motoneurone sample *n*
1	2	3	4	5	6	7	8
Via uncrossed pathways	Group Ia IPSPs	ipsi PT	146 ± 9	75	68 ± 3*	10	20
		co PT	*155 ± 4	62	89 ± 3*	15	13
		MLF	153 ± 17	90		0	10
	Group Ib IPSPs	ipsi PT	*155 ± 15	28	*77 ± 5	31	29
		co PT	*145 ± 15	42	83 ± 11	8	12
		MLF	193 ± 31	73	89 ± 9	18	11
	Group II IPSPs	ipsi PT	144 ± 13	32	78 ± 6	32	22
		co PT	154 ± 17	70	80 ± 14	20	10
		MLF	158 ± 19	62	96 ± 3	7	13
Via double-crossed pathways	Group Ia IPSPs	ipsi PT	131 ± 4	89	—	0	20
		co PT	*125 ± 6	61	—	0	14
		MLF	152 ± 7	100	—	0	18
	Group Ib IPSPs	ipsi PT	*125 ± 4	56	*93 ± 2*	15	27
		co PT	*115 ± 3	52	76 ± 8*	14	21
		MLF	127 ± 6	74	97 ± 2	0	27
	Group II IPSPs	ipsi PT	133 ± 4	86	90 ± 3	14	21
		co PT	131 ± 6	77	94 ± 3	0	22
		MLF	140 ± 9	74	92 ± 4	4	27

Column 1, connections between PT fibres and motoneurones left intact; 2, origin of PSPs; 3, origin of PT actions; 4 and 6, mean changes evaluated from 'difference' traces (as illustrated in Figs 2–5) with standard errors of means (S.E.M.); 5 and 7, percentages of motoneurones in which changes of PSPs exceeded 10%; 8, total number of motoneurones examined. Asterisks in columns 4 and 6 indicate statistically significant differences between the degree of facilitation evoked by the ipsilateral and the contralateral PT (to the left of the mean ± S.E.M.) and between those evoked by the uncrossed and the double-crossed pathway (to the right of the mean ± S.E.M.).

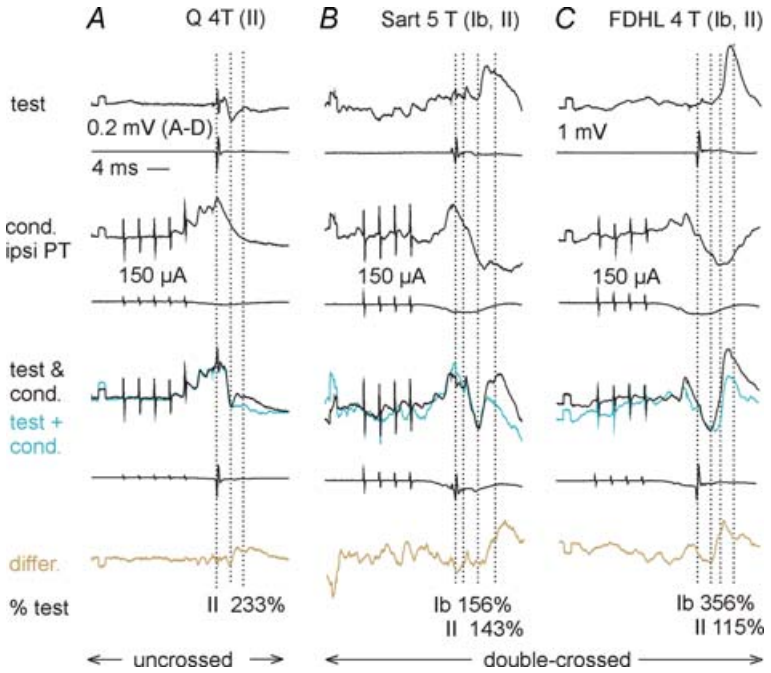

Figure 3. Examples of facilitation of EPSPs from group Ib and group II afferents evoked by conditioning stimulation of the ipsilateral PT
Averaged (n = 10–30) intracellular records from three posterior-biceps-semitendinosus (PBST) motoneurones (upper traces in each panel) and records from the cord dorsum (lower traces) from preparations with either intact uncrossed (*A*) or intact double-crossed (*B* and *C*) pathways from the ipsilateral PT. The format of the figure is as in Fig. 2, with differences between the test and conditioned PSPs shown in the third and fourth rows. The 1st dotted lines indicate incoming volleys from group I afferents following the test stimuli; the 2nd or both the 2nd and 3rd dotted lines the onset of EPSPs from group Ib and/or group II afferents and the last dotted lines the width of time windows within which the areas of the EPSPs were measured. *A*, facilitation of EPSPs evoked from group II afferents (onset at 2.2 ms). *B* and *C*, facilitation of EPSPs evoked by both group Ib afferents (onset at the level of the second dotted line) and group II afferents (at the third dotted line).

pathways, with two examples in Fig. 3*B* and *C*. Some facilitatory effects may have been overlooked when interneurones co-activated by PT fibres and group II afferents were refractory after their activation by PT stimuli. This might explain lack of facilitation of the earliest components of group II EPSPs (just after the 3rd dotted line in Fig. 3*C*), moderate facilitation of somewhat later ones and strongest facilitation of the latest components (those after the 4th dotted line). The possibility that only polysynaptic actions of group II afferents were facilitated in this case would nevertheless also be plausible.

Depression of synaptic actions of premotor interneurones on motoneurones by ipsilateral PT stimulation

In some motoneurones disynaptic IPSPs from group Ia, Ib and II afferents and EPSPs evoked from group Ib and II afferents were found to be depressed rather than facilitated following stimulation of the ipsilateral PT stimuli. Examples of decreases of IPSPs recorded in four motoneurones are shown in Fig. 4. In all of these motoneurones the sum of IPSPs evoked by the test and conditioning stimuli applied separately was larger than the IPSPs evoked by joint application of these stimuli. However, this effect was more difficult to interpret than the facilitation described in the previous sections because it could have been caused by several factors. When no IPSPs were evoked by conditioning stimuli, as in Fig. 4*A*, and the test IPSPs were evoked without any overlapping EPSPs, the depression would be likely to be caused by inhibition at a premotoneuronal level. However, in motoneurones in which PT stimuli evoked IPSPs by themselves, as in Fig. 4*B* and *C*, the decrease in the size of the test IPSP might also have been due to the occlusion between effects evoked by the test and conditioning stimuli at the level of premotor interneurones (Cabaj *et al.* 2006). The occlusion could occur when the test IPSPs coincided with IPSPs evoked by PT stimuli as well as when one of these PSPs followed another within a couple of milliseconds corresponding to the refractory period of the interneurones after their activation. Decreases in the size of the test IPSPs might also have been due to facilitation of EPSPs that either followed (Fig. 4*B*) or preceded (Fig. 4*D*) them and/or overlapped with them. Because of these reasons, the degree of the actual depression of the disynaptic IPSPs was difficult to evaluate in a reliable way and the overall effects summarized in Table 1 (columns 6–7) are most likely underestimates. On the basis of these overall effects it appears, however, that the degree of depression in inhibitory pathways from group Ia, Ib and II afferents evoked via uncrossed pathways was comparable, but

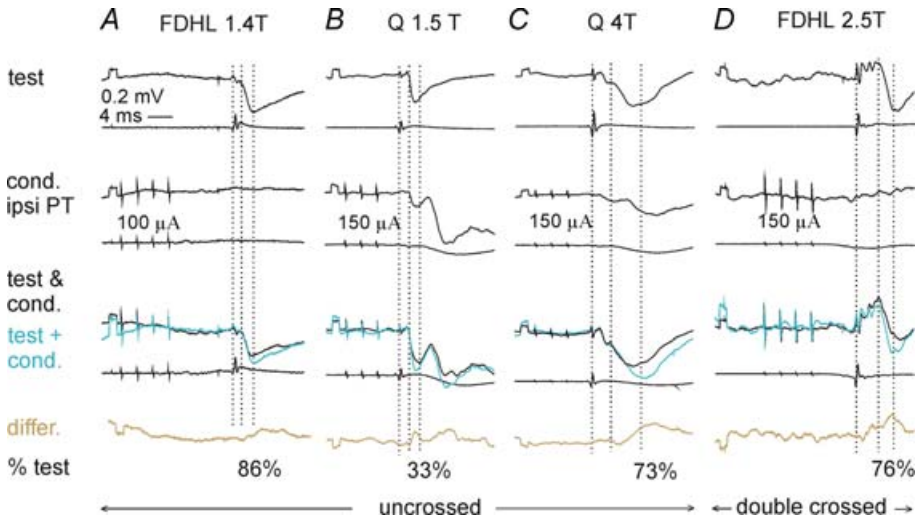

Figure 4. Examples of depression of IPSPs following conditioning stimulation of the ipsilateral PT
Averaged ($n = 10$–30) intracellular records (upper traces in each panel) from two GS motoneurones (*A* and *B*), an ABSM motoneurone (*C*) and a DP motoneurone (*D*), and records from the cord dorsum (lower traces) from preparations with either intact uncrossed (*A*–*C*) or intact double-crossed (*D*) pathways from the ipsilateral PT. The same format as in Figs 2 and 3. Note that the sums of responses evoked by the test and the conditioning stimuli (test + cond) applied separately are larger than the responses evoked by the joint application of these stimuli (test & cond); differences between them are shown beneath. Averaged ($n = 10$ and 20) intracellular records from four motoneurones (2 GS, ABSM and DP, upper traces) and records from the cord dorsum (lower traces) in preparations with intact uncrossed (*A*–*C*) or double-crossed (*D*) pathways. The test IPSPs were evoked by group Ib afferents (*A* and *B*) and group II afferents (*C* and *D*). The first dotted lines indicate incoming volleys from group I afferents following the test stimuli; the second lines show the onset of test IPSPs, the time windows between the second and third lines being used to estimate the level of the depression.

was observed in a higher proportion of motoneurones for IPSPs evoked from Ib and II afferents than from Ia afferents. The depression evoked via double-crossed pathways was similar for IPSPs evoked from group II afferents but weaker and in a smaller proportion of motoneurones for IPSPs from Ib afferents. In addition, no depression of Ia IPSPs was found.

Test EPSPs from group Ib and group II afferents were found to be depressed by conditioning stimulation applied to the ipsilateral PT in one motoneurone via uncrossed and in three motoneurones via double-crossed pathways, with an example in Fig. 5*A*. The depression of EPSPs occurred in motoneurones in which EPSPs were evoked by PT stimuli themselves and, as in the case of IPSPs, it may have been secondary to the occlusion between effects of excitation evoked by PT fibres and by peripheral afferents as well as to inhibitory PT actions.

Comparison of facilitatory actions evoked by ipsilateral and contralateral PT neurones and reticulospinal neurones

In view of bilateral projections of PT neurones to the reticular formation (Matsuyama & Drew, 1997; Rho *et al.* 1997) and co-excitation of individual reticulospinal neurones by ipsilateral and contralateral PT neurones (He & Wu, 1985; Canedo & Lamas, 1993), qualitatively similar effects were expected to be evoked from the ipsilateral and contralateral PT and from the MLF provided that those from the PTs were relayed by RS neurones.

The overall degree of facilitation or depression of IPSPs evoked from group Ia, Ib and II afferents (summarized in Table 1) did not show major differences between effects of stimuli applied in the ipsilateral PT and in the MLF. It should nevertheless be taken into account that facilitation from the MLF was most likely submaximal because it was evoked by only two or three stimuli and at longer than optimal conditioning testing intervals (to avoid the test IPSPs coinciding with large EPSPs or IPSPs evoked by the MLF stimuli). When effects of conditioning stimuli were compared in individual motoneurones, it was also a general rule that whenever facilitation was evoked from the ipsilateral PT, similar or stronger facilitation was evoked from the MLF and the same was the case for the depression.

The overall facilitatory and inhibitory effects from the ipsilateral and contralateral PT were likewise similar (see Table 1) but they were more differentiated in individual motoneurones. The degree of facilitation of Ia IPSPs and of EPSPs evoked from either group Ib or II afferents by stimulation of the ipsilateral and contralateral PT was similar in the majority of motoneurones. However, for IPSPs evoked from group Ib and II afferents this was the case in only about one third of motoneurones. In the remaining motoneurones facilitation was evoked from one PT while much weaker effects, no effects, or varying degrees of the apparent depression (illustrated in Fig. 5) were evoked from the other one. PT neurones from the ipsilateral and contralateral hemispheres might thus contribute to either similar or different patterns of movements via interneurones in pathways from peripheral afferents.

The variability of effects evoked from ipsi- and contralateral PT might depend on a number of factors. For a more systematic comparison we therefore selected IPSPs evoked by a narrower set of afferents (Ia afferents in the Q nerve) and a more restricted population of motoneurones (PB and ST motoneurones). The results of this comparison are summarized in Fig. 6. Plots in Fig. 6*A* show that despite similar overall effects from the ipsilateral and contralateral PTs (Table 1), the degree of facilitation evoked from them varied in individual motoneurones. In some of these, facilitation from the ipsilateral PT was weaker while in the other ones it was stronger, but it was generally stronger via uncrossed than via double-crossed pathways.

Since the coupling between ipsilateral PT fibres and Ia inhibitory interneurones is most probably indirect (disynaptic, trisynaptic or polysynaptic; Jankowska &

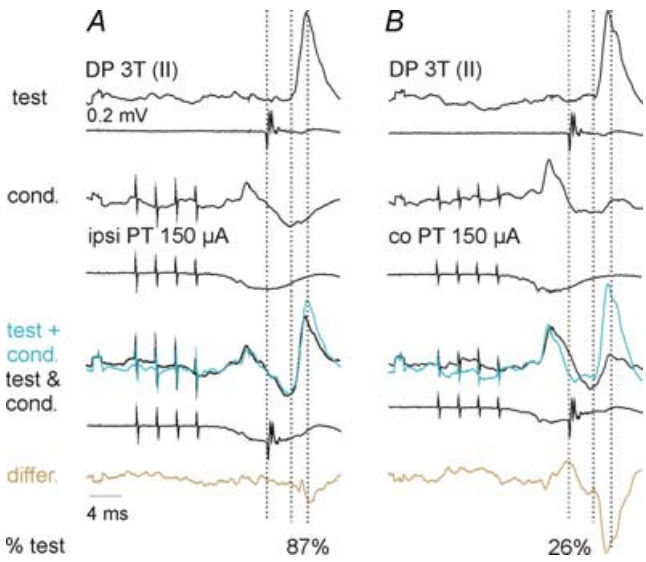

Figure 5. Examples of depression of EPSPs following conditioning stimulation of the ipsilateral and contralateral PT attributable to inhibition and/or occlusion at a premotoneuronal level
The same format as in Figs 2, 3 and 4, with differences between the test and conditioned PSPs shown in the bottom row. Averaged ($n = 30$) intracellular records from a PBST motoneurone (upper traces in each panel) and records from the cord dorsum (lower traces) in a preparation with intact double-crossed pathways from the ipsilateral PT. The first dotted lines indicate incoming volleys from group I afferents following the test stimuli; the second the onset of EPSPs from group II afferents, the third ones showing the end of time windows of the measurements.

Stecina, 2007), as originally shown for coupling between contralateral PT fibres and these interneurones in the cat (Lundberg, 1970; Hultborn *et al.* 1976*b*), RS neurones may operate as relay neurones of PT actions in parallel with other brainstem or spinal PT relay neurones. It was therefore of interest to find out whether facilitation of Ia IPSPs would persist or disappear after transection of axons of reticulospinal neurones within the MLF. Accordingly, we compared effects of PT stimulation under conditions when RS axons were intact and in two preparations in which the MLF was lesioned until no descending volleys were evoked by maximal stimuli applied rostral to the lesion (as shown in Fig. 5 of Stecina & Jankowska, 2007). After such lesions, conditioning stimulation of the ipsilateral PT continued to evoke facilitation of Ia IPSPs in 9/14 motoneurones tested and the degree of facilitation in these nine neurones ($171 \pm 20\%$) did not differ significantly from the degree of facilitation in preparations with the intact MLF. Also no significant differences were found between effects from the ipsilateral and contralateral PT in preparations with the MLF either transected or intact; compare their means represented by the continuous and dotted horizontal lines in Fig. 6*A*. Similar effects from the two PTs are illustrated in Fig. 7. This suggests that PT actions relayed by RS neurones with axons in the MLF and by other neurones may be similarly potent (see Discussion).

When short trains of stimuli applied within one PT were followed or overlapped with stimuli applied in the other PT, their effects were often stronger than effects of separate stimuli, with an example in Fig. 7*C*. The results of such a comparison in a sample of 18 motoneurones are summarized in Fig. 6*B* and *C*. Ia IPSPs evoked by joint stimulation of the two PTs were larger than IPSPs evoked by the ipsilateral or contralateral PT alone, via uncrossed pathways ($161 \pm 13\%$, $131 \pm 5\%$ and $119 \pm 4\%$ of control, respectively) as well as double-crossed pathways ($177 \pm 10\%$, $147 \pm 14\%$ and $155 \pm 19\%$ of control, respectively). However, the differences were statistically significant only for the latter, and effects of the joint stimulation exceeded sums of the separate stimuli in only two motoneurones while occlusion between these effects occurred in the remaining motoneurones. Similar tests on IPSPs from group Ib and group II afferents revealed facilitation in 5/6 and 6/8 motoneurones, respectively, and occlusion in the remaining one and two motoneurones. It appears, thus, that a high proportion of Ia inhibitory interneurones may be activated by either the ipsilateral or contralateral PT alone and that their activation does not depend on mutual facilitation of effects from the two PTs. As facilitation in pathways from group Ib or II afferents appeared more frequently to exceed occlusion, mutual facilitation of actions from the PTs might be more important for activation of interneurones in these pathways.

Anatomical evidence for PT input to both excitatory and inhibitory premotor interneurones that form synaptic connections with motoneurones and other interneurones

As discussed above, it was difficult to interpret the depression of PSPs evoked by primary afferents by conditioning stimulation of the PTs because this was compatible with inhibition of interneurones mediating these PSPs, or refractoriness following activation of premotor interneurones by PT stimuli but also with facilitation of PSPs in the opposite direction. By identifying transmitter content in axon terminals of intracellularly labelled interneurones with input from the ipsilateral

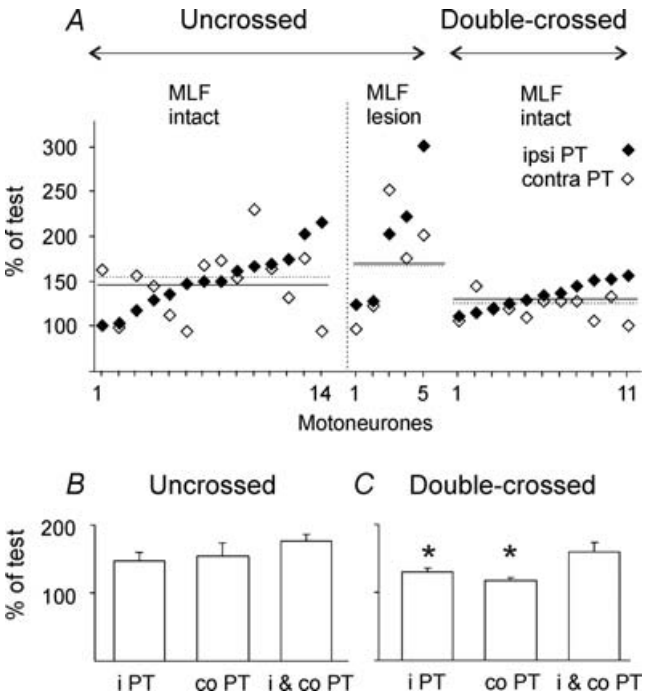

Figure 6. Comparison of effects of conditioning stimulation of the ipsilateral and the contralateral PT on Ia IPSPs evoked in the same motoneurones

A, facilitation of IPSPs evoked by group Ia afferents of the Q nerve stimulated at 1.05–2*T* in individual PBST motoneurones in preparations in which only uncrossed or only double-crossed pathways from the ipsilateral PT were left intact. Increases in the areas of the IPSPs are expressed as percentage of control and are ranked in increasing order of effects from the ipsilateral PT (♦). The data for effects evoked via uncrossed pathways are subdivided into those in preparations with intact or transected MLF (to the left and right of the vertical dotted line, respectively). Continuous and dotted horizontal lines indicate means of facilitation evoked from the ipsilateral and contralateral PT, respectively, in the three sets of the data. *B* and *C*, results of a paired comparison of a subset of data in *A* from motoneurones in which both separate and joint stimulation of the ipsilateral (i) and contralateral (co) PTs in preparations with the MLF intact. *B*, $n = 6$; *C*, $n = 11$. Asterisks indicate statistically significant differences between effects of joint and separate stimulation of the two PTs (ANOVA, Tukey's *post hoc* test, as well as paired *t* tests, $P = 0.03$, 0.005).

PT, we obtained evidence that these interneurones include inhibitory as well as excitatory interneurones and that they may mediate postsynaptic inhibition of both motoneurones and other premotor interneurones evoked by PT stimulation.

The interneurone illustrated in Fig. 8 is representative of inhibitory commissural interneurones relaying ispilateral PT actions via double-crossed pathways (Table 2B, interneurone 9). Its terminal projection area was found to be within contralateral laminae VI–IX. Collateral axons and terminals were present in lamina IX, but direct contacts onto motoneurones were not observed. Records in Fig. 8*B* show that this was an interneurone with monosynaptic input from group II afferents in the quadriceps nerve and disynaptic input from reticulospinal neurones with axons in the MLF (see Jankowska *et al.* 2005*b*). The top panel in Fig. 8*B* shows that it was in addition excited by PT fibres, and latencies of the main components of EPSPs from the ipsilateral PT from the 2nd and 3rd stimuli (indicated by the horizontal dotted lines) suggest that they might have been evoked di- or trisynaptically.

The interneurone illustrated in Fig. 9 is representative of ipsilaterally projecting inhibitory interneurones relaying uncrossed PT actions (Table 2A, interneurone 1). It had terminal projections within the ipsilateral motor nuclei and in laminae VII and VIII. Like the interneurone in Fig. 9, this neurone was characterized as glycinergic by the presence of gephyrin immunoreactivity at the junctions between the axon and motoneurones labelled with an anti-ChAT antibody (arrowheads in Fig. 9*D*) and the absence of GAD-immunoreactivity in axonal swellings (not shown). Records in Fig. 9*A* show that this interneurone was more directly excited by both PT fibres (disynaptically) and MLF fibres (monosynaptically). Its peripheral input also differed from the input to the commissural interneurone illustrated in Fig. 8, as the interneurone was excited by group I and II afferents. It could thus be an inhibitory premotor interneurone in reflex pathways from group Ib and II afferents.

Table 2 summarizes properties of all 11 interneurones with PT input in the lumbar 4th–6th segments which were sufficiently well labelled to allow reconstruction of projection areas of their initial axon collaterals and in which the transmitter content could be defined. As shown in column 3, glycinergic interneurones constituted the majority (9/11) of those successfully labelled. The remaining two interneurones were glutamatergic (Table 2C).

Table 2 shows that the two glycinergic commissural interneurones (nos 8 and 9) were located at the border between the laminae VII and VIII or in lamina VIII and had projection areas within the contralateral laminae VI–IX. One had monosynaptic input from the MLF and the other one from group II afferents. All of the ipsilaterally projecting glycinergic interneurones (nos 1–7) were located in lamina VII and all but one were monosynaptically excited from group II afferents, some being co-excited by group I afferents. Terminal projection areas of their initial axon collaterals were found within laminae VII–IX and in one case only VII–VIII. Three of these interneurones were found to form contacts with ChAT-positive motoneurones and three others had terminals within motor nuclei although we were unable to show definitively that contacts were formed with motoneurones. However, on the basis of previous evidence (Cavallari *et al.* 1987; Edgley & Jankowska, 1987), it would be expected that all interneurones co-excited by group I and II afferents would project to motor nuclei within one of the lumbar or sacral segments. The two glutamatergic interneurones were located within the border zone between

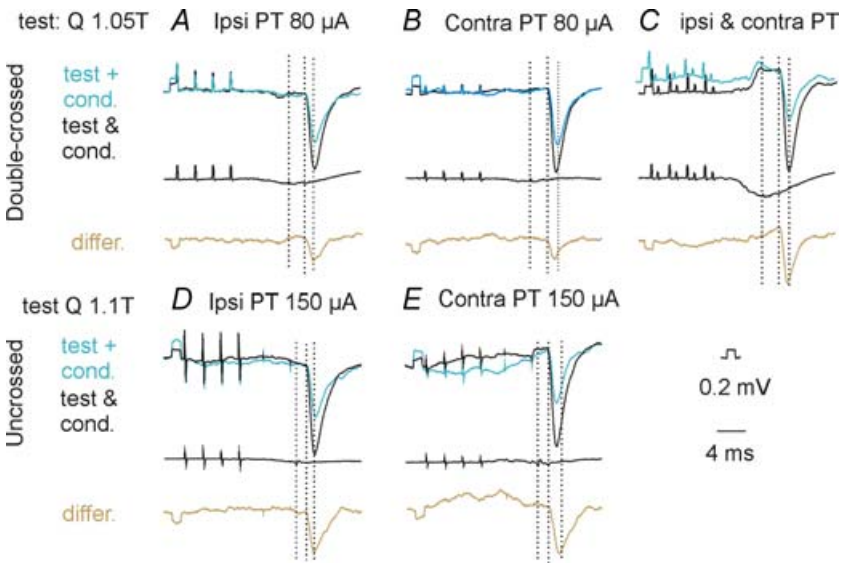

Figure 7. Effects of stimulation of the ipsilateral and contralateral PT on Ia IPSPs
A–C and *D–E*, records from two PBST motoneurones in preparations with only double-crossed or only uncrossed pathways intact, the latter after transection of the MLF (averages of 20 records). The records are like those in Figs 2–5, except that only superimposed records of PSPs evoked by test & conditioning stimulation and of sums of PSPs evoked by separately applied test and conditioning stimuli are shown together (top traces in each panel) with differences between them (bottom traces) and cord dorsum potentials (middle traces). Dotted lines indicate group I afferent volleys, the onset of the IPSPs and the width of the time window of the measurements. Note that the facilitatory effects evoked by the ipsilateral and the contralateral PT were similar and that the effects of their joint stimulation were stronger.

laminae VII and VIII. One projected ipsilaterally and the other bilaterally. No major differences were found in projection areas of the inhibitory and excitatory interneurones nor in their input, but the small sample of excitatory interneurones does not allow more definite conclusions in this respect.

The dominating PT input to both excitatory and inhibitory premotor interneurones was found to be excitatory, which indicates that PT neurones may facilitate both excitation and inhibition evoked by these interneurones, on either motoneurones or other interneurones. However, some of the interneurones were also subject to

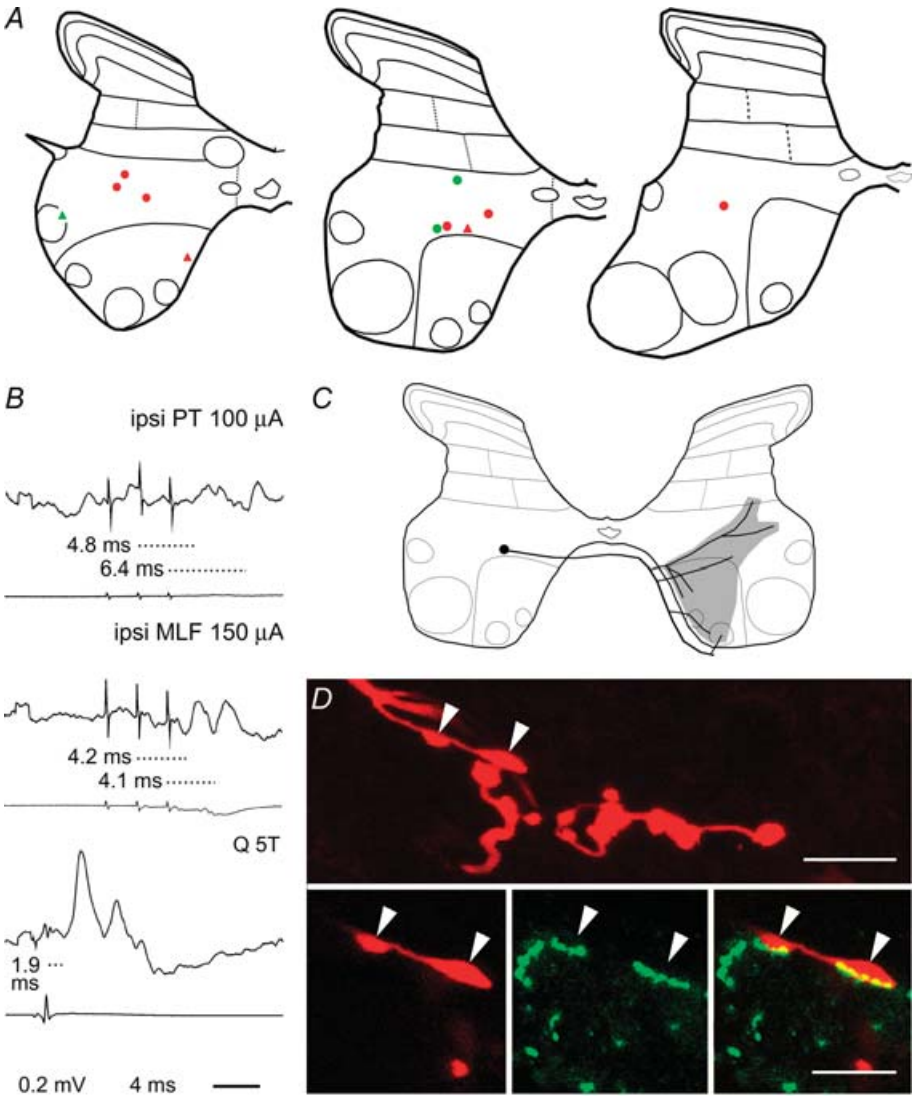

Figure 8. An example of a glycinergic commissural interneurone with input from the ipsilateral pyramidal tract
A, location of labelled cells. Circles represent ipsilaterally projection cells, triangles contralaterally projection cells; red glycinergic and green glutamatergic interneurones. *B*, intracellular records from this neurone obtained during injection of the marker (top traces in each panel) and records from the cord dorsum (lower traces). From the top to bottom: EPSPs following the 2nd and 3rd PT stimuli (at the indicated latencies from the stimuli), EPSPs following the 2nd and 3rd MLF stimuli (at the indicated latencies from the stimuli, corresponding to 1.3 and 1.2 ms from the first components of the descending volleys) and an EPSP from group II afferents in the Q nerve evoked at a latency of 1.9 ms from group I volley, corresponding to about 1 ms from group II volley. *C*, axonal projection areas of this interneurone with the location of the soma shown as a circle and stem axon as thick line. Areas in which terminals were found are shaded. *D*, upper panel: a projected series of images showing a group of labelled axon terminals in lamina VII; lower panels: single confocal images of the terminals indicated by arrows in the upper panel, demonstrating the association between labelled terminals (red) and gephyrin (green), confirming this cell is glycinergic. Scale bars: 10 μm upper panel, 5 μm lower panel.

Table 2. Characteristics of the labelled interneurones

Pathways from PT	Interneurone no.	Transmitter	Soma segment	Soma lamina	Axon ipsi laminae	Axon co laminae	Ipsi PT EPSP (ms)	Ipsi PT IPSP (ms)	MLF EPSP (ms)	Group I EPSP (ms)	Group II EPSP (ms)
1	2	3	4	5	6	7	8	9	10	11	12
A											
Uncrossed	1	gly	L4	VII	VII–IX*	—	4.2	—	3.0*	1.1*	2.4*
	2	gly	L5	VII	VII–IX	—	5.6	—	3.6*	—	2.3*
	3	gly	L4	VII	VII–IX*	—	5.5	—	3.5*	—	2.5*
	4	gly	L5	VII	VII–IX	—	5.5	5.1	4.6	—	4.5
	5	gly	L4	VII	VII–VIII	—	—	5.2	3.5*	—	2.5*
	6	gly	L5	VII	VII–IX	—	6.5	—	3.1*	0.9*	2.4*
	7	gly	L6	VII	VII–IX*	—	6.2	—	3.1*	0.8*	2.3*
B											
Double-crossed	8	gly	L4	VIII	VII–VIII	VIII–IX*	4.9	—	3.3*	—	—
	9	gly	L5	VII	—	VI–IX	6.4	—	4.1	1.9	2.1*
C											
Uncrossed	10	glut	L5	VII	VI–IX*	—	4.4*	—	3.2*	1.0*	3.7
	11	glut	L4	VII	VII–VIII	VII–VIII	5.3	—	3.3*	1.2*	—

Column 1, connections between PT fibres and motoneurones that were left intact; 2, interneurone number; 3, transmitter content indicated by the presence of gephyrin immunoreactivity at the junction between the axon terminals of the interneurones and their target cells, or by the presence of the glutamate transporter 2; 4, spinal segment of soma location; 5, Rexed's lamina of soma location; 6 and 7, axonal ipsilateral and contralateral terminal projection areas in the indicated laminae; asterisks mark cells for which monosynaptic contacts with motoneurones were identified; 8 and 9, latencies of EPSPs and/or IPSPs evoked by PT stimuli and of EPSPs from the MLF; asterisks mark monosynaptic EPSPs; 10 and 11, latencies of EPSPs evoked from peripheral afferents; asterisks mark most likely monosynaptic EPSPs. Note that latencies of EPSPs and IPSPs recorded in glycinergic and glutamatergic interneurones from any of these sources are within the same ranges and that no major differences in their location or projections are suggested by these data.

inhibitory PT actions which might have been evoked via other segmental interneurones.

Discussion

Both excitatory and inhibitory premotor interneurones in reflex pathways from muscle afferents relay ipsilateral actions of PT neurones

The results of this study provide three lines of evidence that premotor interneurones in reflex pathways from muscle afferents may operate as relay neurones of ipsilateral actions of PT neurones. They show also that this is the case for both excitatory and inhibitory interneurones.

Firstly, IPSPs and EPSPs evoked in hindlimb motoneurones from group Ia, Ib and II afferents are facilitated by conditioning stimulation of the ipsilateral PT. Latencies of Ia IPSPs and of IPSPs and EPSPs evoked from group Ib afferents were within the ranges of previously defined segmental latencies of PSPs mediated by single interneurones (1.2–1.6 ms and only occasionally up to 1.8 ms). Synaptic actions of group I afferents would thus be facilitated at the level of premotor interneurones. However, IPSPs and EPSPs from group II afferents were evoked within a wider range of latencies (2.5–4.8 ms) and might have been evoked di-, tri- or even polysynaptically. PT fibres might thus have facilitated synaptic actions of these afferents at the level of both premotor interneurones and of earlier order interneurones. As shown by intracellular records from another sample of spinal interneurones (not identified as premotor interneurones) the facilitation could indeed be evoked by either monosynaptic, disynaptic or polysynaptic actions of PT neurones (Jankowska & Stecina, 2007). The second line of evidence is more indirect and is based on depressive rather than facilitatory effects of PT stimulation. As shown in the Results section, the depression of test IPSPs and EPSPs in a number of motoneurones could be due to genuine inhibition of premotor interneurones, i.e. secondary to activation of some inhibitory interneurones by PT stimuli. However, when the depression occurred in motoneurones in which IPSPs were evoked by stimulation of both PTs and peripheral afferents, occlusion between effects of these stimuli could be an alternative explanation. Occlusion would be particularly likely when these IPSPs overlapped or followed each other within a period corresponding to the refractory period after activation of the interneurones by the preceding stimuli. Occlusion between IPSPs evoked by ipsilateral PT stimuli and IPSPs evoked by the afferents would in turn indicate that both were mediated by the same interneurones, i.e. that some ipsilateral inhibitory PT actions are relayed by interneurones mediating IPSPs from group Ib and II afferents. The same would be true

for excitatory actions relayed by interneurones mediating EPSPs from group Ib and/or II afferents in view of the likely collision between EPSPs from these afferents and from the PTs.

Finally, the majority of interneurones with input from group Ib and II afferents and from the ipsilateral PT were found to project to motor nuclei and contacts between axon terminals of at least some of these interneurones and motoneurones could be demonstrated. Such connections were found for both excitatory and inhibitory interneurones.

The reported results thus extend the evidence that interneurones in reflex pathways from muscle afferents may relay PT actions previously found for contralateral PT neurones to ipsilateral PT neurones (Lundberg & Voorhoeve, 1961; Lundberg, 1975; Jankowska *et al.* 1976).

No major differences were found between overall PT actions on disynaptic IPSPs evoked by group Ia, Ib and II afferents, or between actions on interneurones in inhibitory and excitatory pathways to motoneurones, even though PT actions on the excitatory premotor interneurones could not be quantified. However, in view of the somewhat stronger and more frequent facilitation of Ia IPSPs than of IPSPs evoked from other afferents, Ia inhibitory interneurones might be utilized to a greater extent to mediate ipsilateral PT actions than other premotor interneurones. The higher proportion of labelled inhibitory *versus* excitatory interneurones with oligosynaptic input from the ipsilateral PT might also suggest that shared inhibitory interneurones are more numerous than excitatory ones and perhaps play a more important role in the modulatory PT actions.

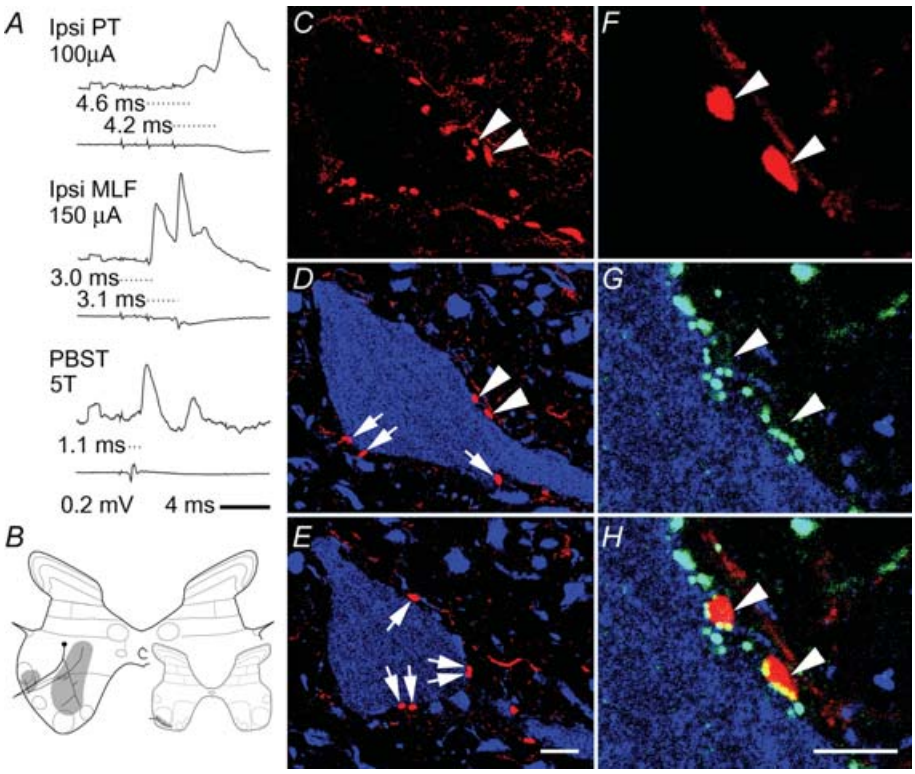

Figure 9. An example of an ipsilaterally projecting glycinergic intermediate zone interneurone with input from the ipsilateral pyramidal tract

A, intracellular records from this neurone (upper traces in each panel) and records from the cord dorsum. From top to bottom are disynaptic EPSPs following the 2nd and 3rd PT stimuli (at the indicated latencies from the stimuli), monosynaptic EPSPs following both the 1st and 2nd MLF stimuli (at the indicated latencies from the stimuli, corresponding to 0.5 ms from the first components of the descending volleys) and an EPSP from group I afferents in the PBST nerve evoked at a latency of 1.1 ms from group I volley. *B*, reconstruction of axon projection areas in laminae VII–IX extending through L4 and L5 segments. *C*, a projected image showing a number of labelled interneuronal terminals (red) associated with a cell body within the motor nucleus. Two terminals (arrowheads) are shown at higher magnification in *F–H*. *D* and *E*, two single optical sections through the cell body (labelled with an anti-ChAT antibody in blue) illustrating contacts (arrows) from labelled interneuronal terminals (red). *F–H*, a single optical section illustrating two of the boutons (red, arrowheads) in contact with the motoneurone (blue) with gephyrin located at the appositions (green). Scale bars: 10 μm for *C–E* and 5 μm for *F–H*.

Similar effects via uncrossed and double crossed pathways from the ipsilateral PT

The reported results show that ipsilateral PT actions evoked via uncrossed and double-crossed pathways are generally similar, whether facilitatory or depressant, and that any quantitative differences are only minor. In addition, even though facilitation of the tested IPSPs appeared to have been stronger via uncrossed than via double-crossed pathways, it was evoked via the double-crossed pathways in a higher proportion of motoneurones which might compensate for their weaker actions. However, as effects mediated via the uncrossed and double-crossed pathways had to be investigated in different preparations (after either contralateral or ipsilateral spinal hemisection) it is an open question whether these actions are similar in individual motoneurones or whether facilitation would be evoked via uncrossed and depression via double-crossed pathways or vice versa.

If similar PT actions were evoked also in individual motoneurones, this would secure their effectiveness because they would strengthen each other. However, one could only speculate on how activity in the two distinct networks of neurones in the uncrossed and double-crossed pathways might be integrated. Of the neurones indicated in the diagrams of Fig. 1*A* and *D* only the PT neurones appear to be the common link, because reticulospinal neurones descending ipsilaterally or contralaterally, commissural interneurones and any propriospinal neurones involved would be separate. One possibility might therefore be that PT neurones provide common input to all of these neurones. PT neurones might also activate particular subsets of ipsilaterally and contralaterally descending RS neurones with equivalent actions, or some other neurones coordinating activity of functionally related subsets of commissural neurones and ipsilaterally located segmental interneurones (see below). One might also consider that integration of PT actions mediated by uncrossed and double-crossed pathways is related to bilateral rather than unilateral actions of neurones descending on one side of the spinal cord. For instance, ipsilaterally descending reticulospinal and propriospinal neurones could have crossed axon collaterals below the level of the contralateral hemisection, or above the level of the ipsilateral hemisection, and activate not only ipsilateral segmental interneurones but also commissural interneurones. Alternatively, collaterals of reticulospinal neurones descending contralaterally might cross below the level of the ipsilateral hemisection and act on ipsilateral premotor interneurones both directly and via commissural interneurones. Crossed ipsilateral PT neurones might likewise re-cross below the level of the ipsilateral hemisection (as found in young and newborn macaques; Galea & Darian-Smith, 1997; Martin, 2005; Martin *et al.* 2006) and target the same interneurones as the uncrossed PT fibres. However, all of these possibilities are only hypothetical so far (see discussion in Edgley *et al.* 2004 and in Jankowska *et al.* 2003).

The same premotor interneurones may contribute to actions of the ipsilateral and the contralateral PT

One of the striking features of actions of ipsilateral and contralateral PT neurones on directly recorded interneurones was that they greatly resembled each other (Jankowska & Stecina, 2007; Stecina & Jankowska, 2007). No marked differences were found in amplitudes of PSPs evoked from the two PTs, or in the frequency of their occurrence in intracellularly recorded interneurones (Jankowska & Stecina, 2007). Nor have major differences been found in the overall facilitatory effects of stimulation of the ipsilateral and contralateral PT on synaptic actions of premotor interneurones in pathways from group Ia, Ib or II afferents in the present study. Furthermore, facilitation of PSPs evoked in individual motoneurones by group I and II afferents by conditioning stimulation of one PT was often replicated by stimulation of the other PT and submaximal actions from one PT were often facilitated by actions from the other PT. All these observations are therefore consistent with the mediation of the ipsilateral and contralateral actions of PT neurones by the same relay neurones.

Effects of stimulation of the two PTs relayed by either contralaterally or ipsilaterally descending RS neurones could be predicted to be similar in view of the co-excitation of RS neurones by the left and right PT neurones (He & Wu, 1985; Canedo & Lamas, 1993). Since effects of stimulation of the two PTs were found to be similar before and after transection of the MLF (Jankowska & Stecina, 2007; Stecina & Jankowska, 2007 and the present study; see Fig. 7*A*), PT actions on motoneurones mediated by RS neurones should be evoked in parallel with actions evoked via spinal interneurones and propriospinal neurones monosynaptically excited by crossed PT fibres (Lundberg *et al.* 1962; Alstermark *et al.* 1987; Davies & Edgley, 1994) or by uncrossed PT fibres (Jankowska & Stecina, 2007). This leads to two non-exclusive conclusions.

The first conclusion is that PT actions mediated by reticulospinal neurones that descend outside the MLF and/or by spinal neurones are as potent as PT actions mediated by reticulospinal neurones with axons in the MLF and that either may be sufficient for activation of premotor interneurones. This might indicate a kind of redundancy but also the possibility of activation of premotor interneurones by PT neurones via subsets of relay neurones operating under different behavioural condition. For instance, actions mediated by RS relay neurones might be particularly important when voluntary movements are associated with postural reactions and

actions mediated by extra-MLF relay neurones in other centrally initiated movements.

The second conclusion is that any neurones that mediate PT actions after MLF transection are as effectively activated by uncrossed ipsilateral as by crossed contralateral PT neurones. However, verification of these conclusions would require experiments that lie outside the scope of this study.

Depression of actions of premotor interneurones by ipsilateral PT neurones

Both excitatory and inhibitory PT actions on interneurones have been reported previously, with IPSPs frequently following EPSPs or preceding them. IPSPs were seen in commissural interneurones co-excited by PT and RS neurones (Figs 8 and 9 in Jankowska *et al.* 2006) and in interneurones co-excited by group I and II afferents, and by PT and RS neurones descending ipsilaterally (Figs 1 and 7 in Jankowska & Stecina, 2007). However, these experiments could not establish whether interneurones in which the IPSPs were evoked by PT stimulation were premotor interneurones, or whether they were excitatory or inhibitory. The depression of IPSPs and EPSPs evoked in motoneurones found in this study could be linked to premotor excitatory or inhibitory interneurones but without resolving whether it reflected genuine inhibition of the interneurones. One of the reasons might have been that the decreases in the amplitude of the IPSPs were caused by facilitation of EPSPs that overlapped them and that the depression of the IPSPs was only apparent. Another reason might have been that the conditioning PT stimuli activated rather than inhibited interneurones mediating the test stimuli. If these interneurones were to be re-excited at only a few milliseconds short intervals, effects of the test stimuli might fall within the refractory period after the conditioning stimuli, rather than be counteracted by inhibitory actions of the conditioning stimuli as discussed above. We therefore used another approach to investigate how PT neurones might counteract actions of muscle afferents on motoneurones. To this end two questions were addressed: (1) whether PT neurones provide input to only excitatory or to both excitatory and inhibitory interneurones with input from these afferents and (2) whether inhibitory interneurones activated by PT neurones might be used to modulate activation of premotor interneurones.

We labelled a sample of interneurones with input from the ipsilateral PT, defined their transmitter content and compared input and projections of glutamatergic and glycinergic interneurones as described in the last section of the Results. Unexpectedly, more inhibitory than excitatory interneurones were found in this sample but both types projected to motor nuclei and the grey matter outside these nuclei where motoneurone dendrites extend and premotor interneurones in pathways from group Ia afferents in lamina VII are located (Hultborn *et al.* 1971; Jankowska & Lindstrom, 1972), group Ia and Ib afferents in laminae V, VI and VII (Czarkowska *et al.* 1981; Jankowska *et al.* 1981) and group II afferents in laminae VI, VII and VIII (Edgley & Jankowska, 1987; Lundberg *et al.* 1987; Bras *et al.* 1989; Bannatyne *et al.* 2003). Hence inhibitory interneurones may inhibit other interneurones in addition to motoneurones. Of these, Ia inhibitory interneurones might be used to adjust the degree of activation of other Ia interneurones (Hultborn *et al.* 1976*a*), inhibitory interneurones in pathways from group Ib and/or group II afferents of other group Ib or II excited interneurones (Brink *et al.* 1983; Edgley & Jankowska, 1987) and inhibitory commissural interneurones might modulate actions of contralaterally located interneurones with input from group Ib and group II afferents (Arya *et al.* 1991; Bajwa *et al.* 1992; Davies & Edgley, 1994; Cabaj *et al.* 2006).

Functional consequences

One of the ultimate aims of studies of ipsilateral actions of PT neurones has been to analyse mechanisms that might assist in the recovery of motor functions after injuries to contralateral corticospinal neurones. In clinical studies, more and more attention has been recently paid to ipsilateral PT actions and to improvements in the control of the paretic limb during bimanual movements (see, e.g. Mudie & Matyas, 2000; Thuret *et al.* 2006). Experiments on animals may only partly reproduce effects of injuries in humans, both because of the species differences and conditions of these experiments, especially if they are carried out under deep anaesthesia in animals without chronic lesions and in reduced preparations in which only some neuronal systems are operating. However, under conditions of our experiments, we have found weak but fairly regularly evoked actions of PT neurones on ipsilateral hindlimb motoneurones (Edgley *et al.* 2004; Jankowska *et al.* 2005*a*; Stecina & Jankowska, 2007) which is in keeping with the weak actions of the ipsilateral PT neurones reported in humans (for references see, e.g. Turton *et al.* 1996; Hallett, 2001). In this study, we have found that PT stimuli regularly facilitated PSPs evoked in motoneurones from muscle afferents, albeit the facilitation was relatively weak, which is in keeping with rare monosynaptic but frequent di- and trisynaptic ipsilateral PT actions on interneurones in the lumbosacral enlargement (Jankowska & Stecina, 2007). However, it should be kept in mind that the extent to which spinal and supraspinal neuronal systems could contribute to PT actions was reduced not only by anaesthesia but also by the hemisection of the spinal cord and by a considerable reduction of the background input to spinal neurones by peripheral nerve dissection and blockade of neuromuscular

transmission. Under these conditions, PT actions on premotor interneurones would thus be likely to be greatly underestimated. The relative contribution of these interneurones to PT actions should thus be more potent and might be enhanced in different ways.

Based on the finding that ipsilateral PT actions are to a great extent mediated by RS neurones and commissural interneurones located contralaterally (Edgley *et al.* 2004), one might for instance consider the possibilities of strengthening these actions by raising the level of activity of commissural interneurones by increasing input to them from peripheral afferents and descending neuronal systems (Jankowska *et al.* 2005*b*) and by using knowledge of their pharmacology (Hammar *et al.* 2004; Jankowska *et al.* 2005*a*). Involvement of last order interneurones of uncrossed spinal reflex pathways as relay neurones of ipsilateral PT actions (Cabaj *et al.* 2006; Jankowska & Stecina, 2007; Stecina & Jankowska, 2007 and the present study) would also open other possibilities. For instance, excitability of premotor interneurones in pathways from group Ia, Ib and II afferents that mediate PT actions might be raised by strengthening input to these neurones from muscle spindles and tendon organs by muscle stretches and contractions. On the basis of our data we would expect that increasing input from the healthy extremity to commissural interneurones by muscle stretches should be as effective as increasing input from the hemiplegic extremity to ipsilaterally located interneurones by either muscle stretches or contractions. These might be evoked either in a reflex way, voluntarily, or by electrical or mechanical stimulation. Excitability of ipsilaterally located interneurones and of commissural interneurones could also be increased in parallel by proper postural reactions in keeping with strong linkage between neuronal systems mediating phasic voluntary movements and those involved in postural adjustments (see, e.g. Massion, 1992). Furthermore, as a high proportion of spinal interneurones appear to be co-excited by ipsilateral and contralateral PT and RS neurones, they might be more effectively activated during attempts to perform bilateral movements. In clinical practice several of these possibilities have been successfully utilized (for review see Harkema, 2001; Carson, 2005) but better knowledge of mechanisms behind them might help in making the rehabilitation both faster and more effective by widening the range of procedures to be used as optimal for individual patients.

We have already argued that synaptic actions mediated by interneurones in pathways between PT neurones and motoneurones would not only strengthen but also prolong PT actions (Jankowska & Stecina, 2007) and that an increase in duration of PT actions might in turn be important for induction of persistent inward current and tonic discharges of motoneurones (Schwindt & Crill, 1980; Hounsgaard *et al.* 1988; Hultborn, 1999; Hultborn *et al.* 2003). Another consequence of the involvement of interneurones of spinal reflex arcs as relays of both ipsilateral and contralateral PT actions would be that reorganization of spinal neuronal networks after injuries of the contralateral PT neurones would not depend on formation of new synaptic connections between the ipsilateral PT neurones and interneurones (see, e.g. Martin, 2005) but also, or even primarily, on strengthening of the pre-existing connections and on their more effective activation by commands from the ipsilateral PT neurones. Strengthening of the pre-existing connections could in turn be assisted by increasing peripheral input to the interneurones.

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