Uncrossed actions of feline corticospinal tract neurones on lumbar interneurones evoked via ipsilaterally descending pathways

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Effects of stimulation of ipsilateral pyramidal tract (PT) fibres were analysed in interneurones in midlumbar segments of the cat spinal cord in search of interneurones mediating disynaptic actions of uncrossed PT fibres on hindlimb motoneurones. The sample included 44 intermediate zone and ventral horn interneurones, most with monosynaptic input from group I and/or group II muscle afferents and likely to be premotor interneurones. Monosynaptic EPSPs evoked by stimulation of the ipsilateral PT were found in 12 of the 44 (27%) interneurones, while disynaptic or trisynaptic EPSPs were evoked in more than 75%. Both appeared at latencies that were either longer or within the same range as those of disynaptic EPSPs and IPSPs evoked by PT stimuli in motoneurones, making it unlikely that premotor interneurones in pathways from group I and/or II afferents relay the earliest actions of uncrossed PT fibres on motoneurones. These interneurones might nevertheless contribute to PT actions at longer latencies. Uncrossed PT actions on interneurones were to a great extent relayed via reticulospinal neurones with axons in the ipsilateral medial longitudinal fascicle (MLF), as indicated by occlusion and mutual facilitation of actions evoked by PT and MLF stimulation. However, PT actions were also relayed by other supraspinal or spinal neurones, as some remained after MLF lesions. Mutual facilitation and occlusion of actions evoked from the ipsilateral and contralateral PTs lead to the conclusion that the same midlumbar interneurones in pathways from group I or II muscle afferents may relay uncrossed and crossed PT actions.

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Results presented in the accompanying paper (Stecina & Jankowska, 2007) indicate that uncrossed actions of pyramidal tract (PT) fibres on hindlimb motoneurones are relayed by ipsilaterally projecting reticulospinal (RS) neurones with axons running in the medial longitudinal fascicle (MLF) as well as by ipsilaterally descending PT fibres and other hitherto unidentified neurones. The aim of experiments reported in this paper was to examine the possibility that these other neurones include interneurones in reflex pathways between primary afferents and hindlimb motoneurones. The main questions addressed were whether premotor interneurones mediating disynaptic or trisynaptic actions of group I and II muscle afferents on motoneurones are activated by ipsilateral PT fibres and, if so, whether the timing of activation of these interneurones would allow them to contribute to the earliest actions of these PT fibres on motoneurones.

The reasons behind considering interneurones in reflex pathways from group I and II muscle afferents as mediating uncrossed PT actions were twofold. One of these was that some crossed and uncrossed PT actions were found to be relayed by the same neurones (see the accompanying paper) and that the crossed PT actions were previously shown to be relayed by interneurones mediating reflex actions of group I and II afferents on feline motoneurones (Lundberg *et al.* 1962; Lundberg & Voorhoeve, 1962; Harrison & Jankowska, 1985; Davies & Edgley, 1994). At least some of these interneurones might thus contribute to the uncrossed PT actions. Another reason was that the uncrossed PT actions were found to be to a great extent relayed by ipsilaterally descending RS neurones and by interneurones that mediate disynaptic RS actions on motoneurones (see the accompanying paper, Stecina & Jankowska, 2007) and these interneurones include Ib interneurones (Takakusaki *et al.* 1989, 2001, 2003), interneurones in pathways from group II afferents (Lundberg *et al.* 1962; Davies & Edgley, 1994) and possibly also Ia interneurones (Hultborn & Udo, 1972).

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The earliest disynaptic EPSPs and IPSPs following ipsilateral PT stimuli in motoneurones were evoked at latencies of 4.3–5.0 ms (Stecina & Jankowska, 2007). Interneurones mediating these PSPs should thus respond with action potentials at latencies not exceeding about 4.5 ms (taking into account some time for conduction along their axons and one synaptic delay) and EPSPs giving rise to these action potentials should be evoked at even shorter latencies. If only longer latency EPSPs were found in interneurones examined in this study, this would indicate that these interneurones might only contribute to later PT actions on motoneurones. In such a case other relay neurones would have to contribute to the earlier components.

Methods

The experiments were performed on eight deeply anaesthetized cats weighing 2.9–4.7 kg, most of which were also used for experiments reported in the accompanying paper (Stecina & Jankowska, 2007) where both the preparation and the main experimental procedures of stimulation, recording and data analysis are described. All these procedures were approved by the local ethics committee (Göteborgs djurförsöksetiska nämnd) and followed NIH and EU guidelines for animal care. Briefly, anaesthesia was induced with sodium pentobarbital (40–44 mg kg^{-1}, I.P.) and maintained with intermittent doses of α-chloralose (Rhône-Poulenc Santé, France; 5 mg kg^{-1}; administered I.V. every 1–2 h, up to about 25 mg kg^{-1}, and thereafter every 2–3 h up to about 55 mg kg^{-1}). Additional doses of α-chloralose were given when increases in continuously monitored blood pressure or heart rate were evoked by peripheral or central stimulation, or if the pupils dilated. 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 experiments were terminated by a lethal dose of anaesthetic followed by formalin perfusion resulting in cardiac arrest. The effectiveness of synaptic transmission was increased by intravenous administration of 4-aminopyridine (4-AP) in doses of 0.1–0.2 mg kg^{-1}, I.V. Atropine (0.05–0.2 mg kg^{-1} I.M.) and dexamethasone

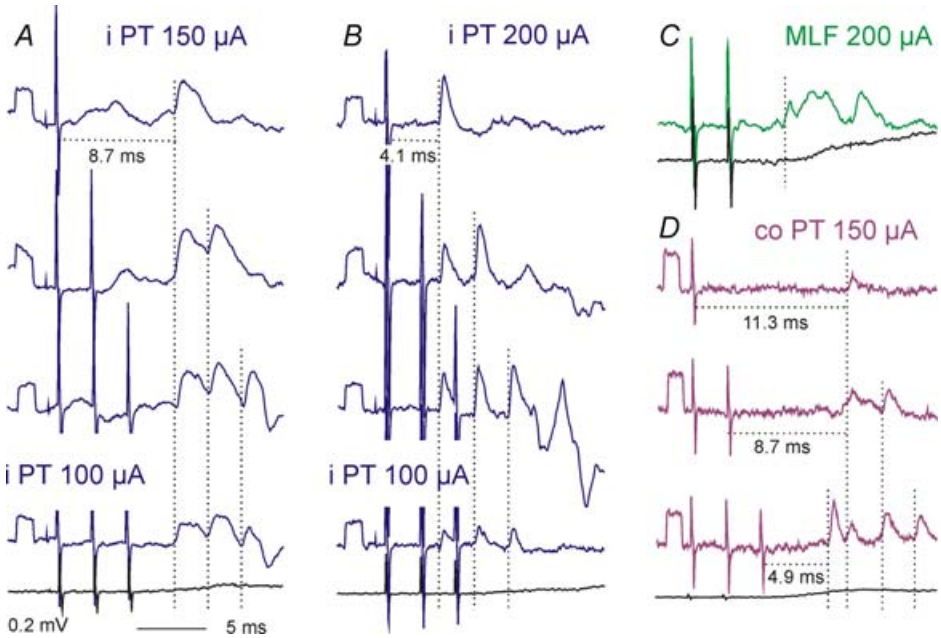

Figure 1. Examples of monosynaptic EPSPs evoked from the ipsilateral and contralateral pyramidal tracts (PTs)
Intracellular records from three interneurones and records from the cord dorsum (bottom traces). Averages of 10 single records. *A* and *B* show records from two interneurones in which EPSPs consistently followed successive ipsilateral PT stimuli. They are from a preparation with the medial longitudinal fascicle (MLF) intact. In the first neurone MLF stimuli evoked monosynaptic EPSPs (not illustrated) and in the second only longer latency PSPs shown in *C*. *D*, records from a preparation after transection of the MLF. Note that single and double stimuli applied to the contralateral PT evoked EPSPs at similarly long latencies and that only the 3rd stimulus evoked a disynaptic EPSP (at a shorter latency). Stimuli applied in the ipsilateral PT only evoked disynaptic EPSPs in this interneurone (at a latency of 4.93 ms; not illustrated). Dotted lines indicate onset of EPSPs. Square pulses at the beginning of the traces are calibration pulses, all of 0.2 mV. In this and the following figures the negativity is downward in intracellular records and upwards in cord dorsum records.

(1 mg kg^{-1} I.M.; Oradexon, Organon, Holland) were given at the beginning of the surgery in most of the experiments.

The interneurones were searched for in the lumbar 3rd to 6th (L3–L6) segments. Whenever possible interneurones were first analysed extracellularly, in particular to test their responses to stimuli applied to the lateral funiculi at the level of the thoracic 12–13 segments caudal to the contralateral hemisection. Neurones that were antidromically activated by such stimuli were eliminated from the analysis as being ascending tract cells, or long-axoned propriospinal neurones projecting outside the lumbosacral enlargement, rather than segmental interneurones. The interneurones were analysed for input from the ipsilateral and the contralateral medullary pyramidal tracts using $\leq 150\ \mu$A (at which intensity no spread of current was previously detected to the other PT) and from several hindlimb muscle nerves (up to 5 times threshold, 5*T*). For the procedures of the placement, control of the current spread, and histological control of the stimulation sites, see the accompanying paper. The interneurones were investigated in two preparations: with the MLF intact or with the MLF transected at the level of caudal medulla (see Methods in the accompanying paper), in both with the spinal cord hemisected contralaterally. When the MLF was intact, indirect effects of PT stimuli might be mediated either by RS neurones with axons in the MLF or by other neurones; after transection of the MLF, by other neurones. The peripheral input was investigated in about two-thirds of these interneurones and they were classified as belonging to

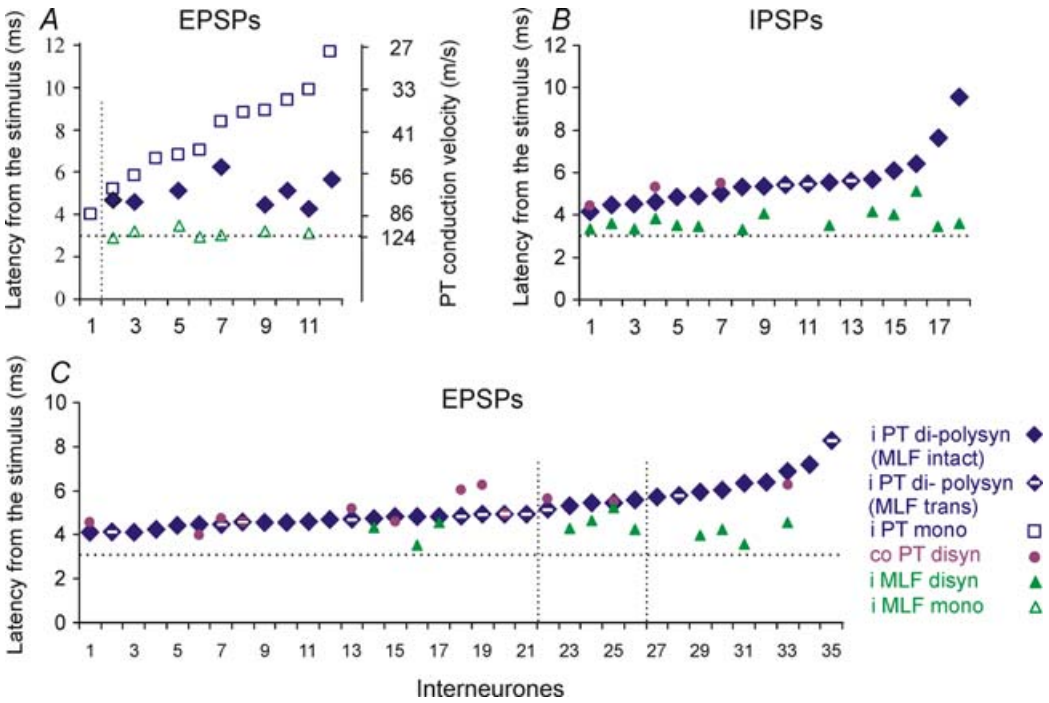

Figure 2. Comparison of latencies of EPSPs and IPSPs evoked from the ipsilateral PT, the contralateral PT and the MLF

A, comparison of latencies of EPSPs classified as evoked monosynaptically and disynaptically from the ipsilateral PT with latencies of EPSPs evoked monosynaptically from the MLF in the same neurones. The monosynaptic EPSPs of PT origin have been ranked in order of increasing latency. The data are for interneurones recorded in preparations with the MLF intact. Left ordinate, latencies from the 1st stimulus for the monosynaptic EPSPs and from the 2nd or 3rd stimulus for the disynaptic EPSPs. Right ordinate, equivalent conduction velocities of PT fibres evoking the monosynaptic EPSPs; their estimates were based on a conduction distance of 310 mm between the stimulation and recording sites, and 0.2 ms for utilization time (Jankowska & Roberts, 1972*b*) and 0.3 ms for one synaptic delay (Jankowska & Roberts, 1972*a*). For vertical lines see text. *B*, comparison of latencies of disynaptic IPSPs evoked from the ipsilateral PT (ranked in ascending order, the last two being probably evoked polysynaptically), the MLF and the contralateral PT, respectively. Data for 15 interneurones with the MLF intact and for 3 interneurones after transection of the MLF. In 14 of these interneurones monosynaptic and/or disynaptic EPSPs plotted in *A* and *C* preceded the IPSPs. *C*, comparison of latencies of disynaptic, trisynaptic and the last 1–3 most likely polysynaptic EPSPs from the ipsilateral PT (ranked in ascending order), the MLF and the contralateral PT, respectively. Data for 24 interneurones with the MLF intact (8 of which have been included in *A*) and 11 interneurones after transection of the MLF are plotted together because no statistically significant differences have been found between them. For vertical lines see text. The horizontal dotted lines in panels *A*, *B* and *C* indicate mean latency of monosynaptic EPSPs from the MLF.

one of three populations using the following criteria. Interneurones classified as Ia inhibitory interneurones were located just outside the quadriceps (Q) motor nuclei at the border between the L4 and L5 segments, with input from near-threshold Q afferents. They followed stimuli applied at 400 Hz in extracellular recordings and their responses were depressed when the test stimuli were preceded by a stronger stimulation of either Q or sartorius nerves at 5–10 ms conditioning–testing intervals, compatible with effects of recurrent inhibition (see Hultborn *et al.* 1971). Interneurones classified as mediating reflex actions of either Ib or both Ia and Ib afferents were located within the intermediate zone at the border between the L5 and L6 segments, with short segmental latency (1.5–1.8 ms) input from group I afferents stimulated at 1.5–2*T* and showing typical patterns of convergence from extensor group I afferents, e.g. gastrocnemius–soleus, plantaris, flexor digitorum and halucis longus and/or quadriceps (see Eccles *et al.* 1957; Harrison & Jankowska, 1985). Interneurones in pathways from group II afferents were classified according to their input from group II afferents of the quadriceps and/or sartorius nerves when stimulated at 3–5*T* or from both group I and group II afferents of these nerves. They were located within the areas where field potentials from both group I and II afferents were evoked in the L3, L4 and L5 segments (Edgley & Jankowska, 1987; Jankowska *et al.* 2005*b*).

Analysis

Both original data and averages of 10–20 single records (with the time resolution of 30 or 40 μs per address) were stored. The latencies of the postsynaptic potentials evoked by stimulation of the PTs and the MLF were measured from the stimuli, those from the MLF being also measured from the descending volleys. They are expressed as means ± S.E.M. Differences between data sets were assessed for statistical significance by using Student's *t* test for paired or unpaired samples.

Results

Morphological studies have demonstrated terminals of uncrossed PT fibres within very large areas of the

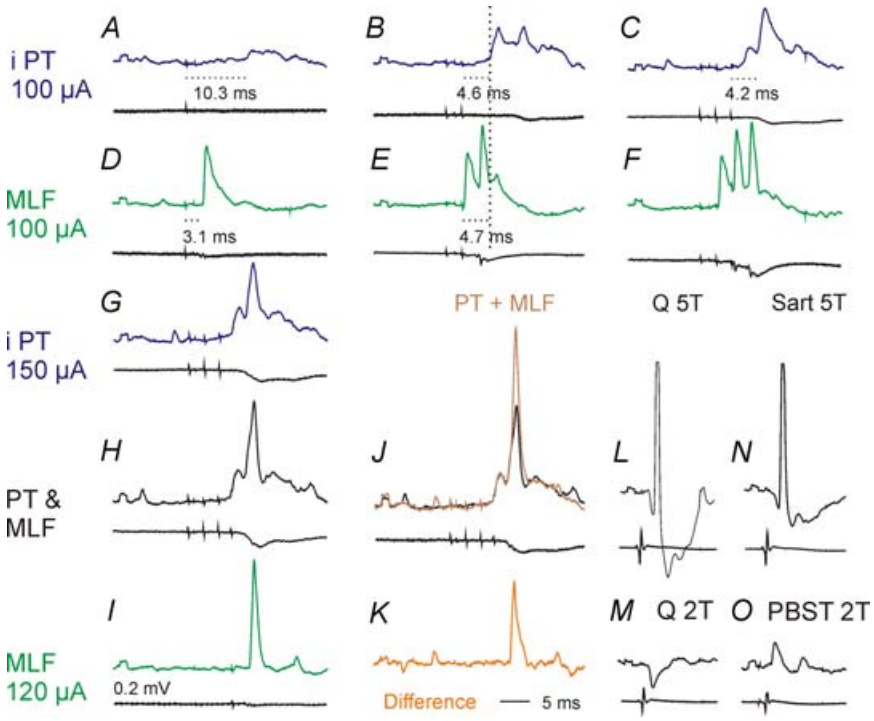

Figure 3. Disynaptic EPSPs from the ipsilateral PT and their occlusion with monosynaptic EPSPs from the MLF

Upper records are from an interneurone and lower records from the cord dorsum. Averages of 10 single records. *A–C* and *D–F*, effects of single, double and triple stimuli applied within the ipsilateral PT and ipsilateral MLF. Note that EPSPs from the MLF were evoked both monosynaptically and disynaptically (with the onset of disynaptic EPSPs at the level of the dotted line in *E*, showing the same latency as disynaptic EPSPs evoked by the 2nd PT stimulus in *B*). *G–K*, effects of PT and MLF stimuli, applied separately (*G* and *I*) and jointly (*H*), showing that the effects of the joint PT and MLF stimuli (*H*) were smaller than the sum of effects of these stimuli (*J*) when they were applied separately, with the difference in *K*. *L–O*, PSPs evoked from group I afferents (at 2*T*) and partly blocked spike potentials and PSPs from group II afferents (at 5*T*). Q, quadriceps; Sart, sartorius; PBST, posterior biceps–semitendinosus.

spinal grey matter, in the dorsal horn, intermediate zone and the ventral horn (in the cat, Casale *et al.* 1988; Casale & Light, 1991, as well as in the macaque, Dum & Strick, 1996; Lacroix *et al.* 2004), in particular in laminae V–VIII of Rexed (1954). Premotor interneurones contacted by uncrossed PT fibres might thus be widely spread throughout these laminae. However, if the density of such interneurones were higher at some locations, the probability of finding them would be greater within the areas where extracellular field potentials reflecting EPSPs in neighbouring neurones were evoked by uncrossed PT fibres. In preliminary experiments of this study we looked therefore for field potentials following stimulation of the ipsilateral PT throughout the L3–L6 segments, from the most lateral to the most medial and from the most dorsal to the most ventral parts of the grey matter. However, field potentials were only found in preparations in which the MLF was intact and they could be secondary to activation of RS neurones by PT stimuli. For these reasons the search for interneurones with input from uncrossed PT fibres was not restricted to any particular regions of the grey matter and any interneurones encountered in the intermediate zone and in the ventral horn were examined. The total sample of interneurones included 44 interneurones with input from the ipsilateral PT. As judged by field potentials from group I and/or group II afferents all of these were located within Rexed laminae VI–VIII. Evidence for monosynaptic input was found only in 27% of these interneurones while disynaptically or trisynaptically evoked EPSPs were present in more than 77%, some interneurones being excited both mono- and di- or trisynaptically.

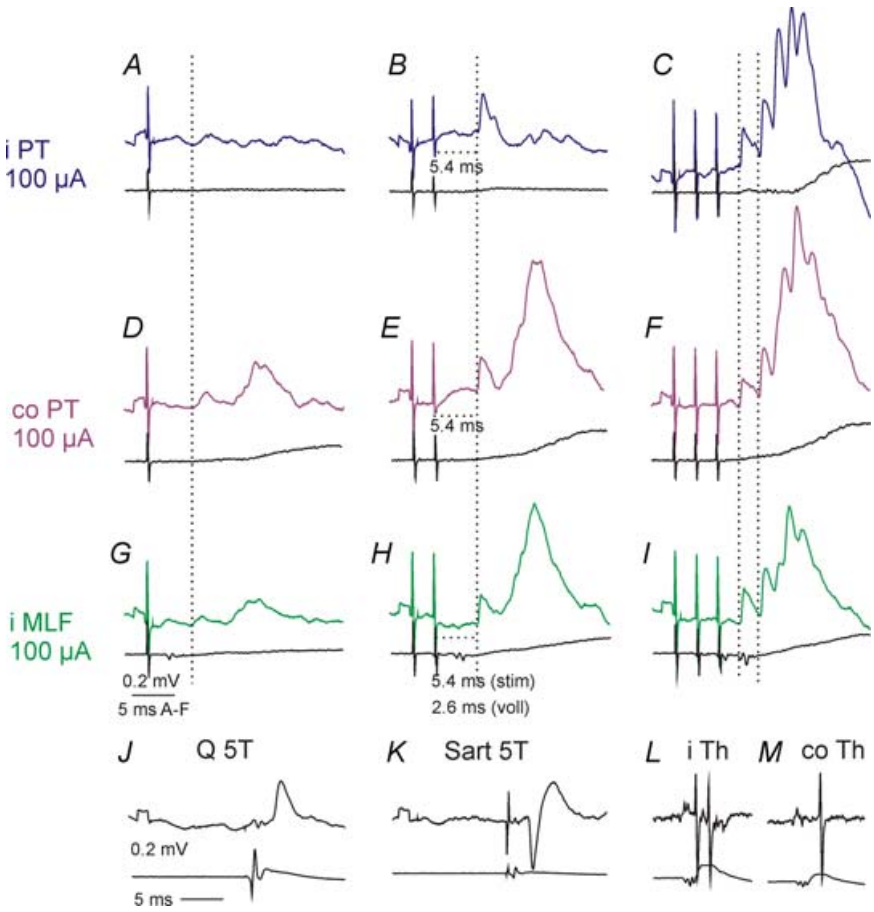

Figure 4. Disynaptic EPSPs evoked from the ipsilateral and contralateral PT and from the MLF
In each panel upper traces are intracellular records from an interneurone and the lower traces are records from the cord dorsum. Averages of 20 single records. *A–C*, *D–F* and *G–I*, effects of single, double and triple stimuli applied within the ipsilateral PT, contralateral PT and ipsilateral MLF. Note that if any EPSPs followed the 1st stimuli, they were much smaller than those evoked by the 2nd and 3rd stimuli. Note also that EPSPs evoked by the 2nd and 3rd i PT, co PT and MLF stimuli were evoked at similar latencies (with onsets indicated by vertical dotted lines). *J* and *K*, examples of PSPs evoked from peripheral afferents. *L* and *M*, records from the same interneurone before it was penetrated, showing its synaptic but not antidromic activation by stimulation (1 mA) of either the ipsilateral (i) or the contralateral (co) lateral funiculus at the thoracic (Th) 13th level.

Interneurones with monosynaptic input from the ipsilateral PT

The main requirements for monosynaptically evoked EPSPs were that they were induced by single stimuli and that effects of successive stimuli in a train did not involve temporal facilitation (see Jankowska *et al.* 2003). With these constraints, evidence for uncrossed monosynaptic PT actions was found in only 12 interneurones. The evidence was strongest for EPSPs evoked in 8 interneurones that followed single stimuli as well as the 2nd and 3rd stimuli in a train, all at the same latencies and without temporal facilitation, as illustrated in Fig. 1*A* and *B*. In the remaining interneurones, EPSPs induced by the 2nd or 3rd stimuli were superimposed on much larger disynaptic EPSPs that were evoked at shorter latencies than those evoked by the 1st stimuli which precluded tests for temporal facilitation.

Figure 2*A* shows that latencies of 12 EPSPs classified as evoked monosynaptically ranged between 4.1 and 11.7 ms. However, only the latency of one EPSP was < 4.5 ms (to the left of the 1st vertical dotted line) and latencies of five EPSPs were 1–2 ms longer and exceeded latencies of disynaptic EPSPs. This suggests that the fastest conducting uncrossed PT fibres (see right ordinate in Fig. 2*A*) might provide input to only a small minority of interneurones of populations examined in the present study. It also suggests that the most common actions mediated by monosynaptically activated interneurones on motoneurones or other neurones would be evoked at latencies much longer than the minimal latencies of disynaptic EPSPs or IPSPs of PT origin.

Interneurones with disynaptic input from the ipsilateral PT

EPSPs were classified as evoked disynaptically when they appeared only after the 2nd, 3rd or 4th stimulus in a train of ipsilateral PT stimuli and/or increased after successive stimuli (in contrast to monosynaptic EPSPs; see Jankowska *et al.* 2003). EPSPs fulfilling these criteria were more common than those classifiable as evoked monosynaptically. EPSPs evoked at 4.1–5 ms latencies (to the left of the 1st dotted line in Fig. 2*C*) were found in 13/30 and 8/14 of the interneurones in preparations in which the MLF was intact and transected, respectively. They are singled out because the range of their latencies was almost exactly that of latencies of EPSPs in motoneurones that were most reliably identified as evoked disynaptically (4.3–5 ms see Fig. 3*A* sample 'a' in Stecina & Jankowska, 2007). Longer latency EPSPs might have been evoked disynaptically or trisynaptically, although the longest ones were most likely evoked polysynaptically.

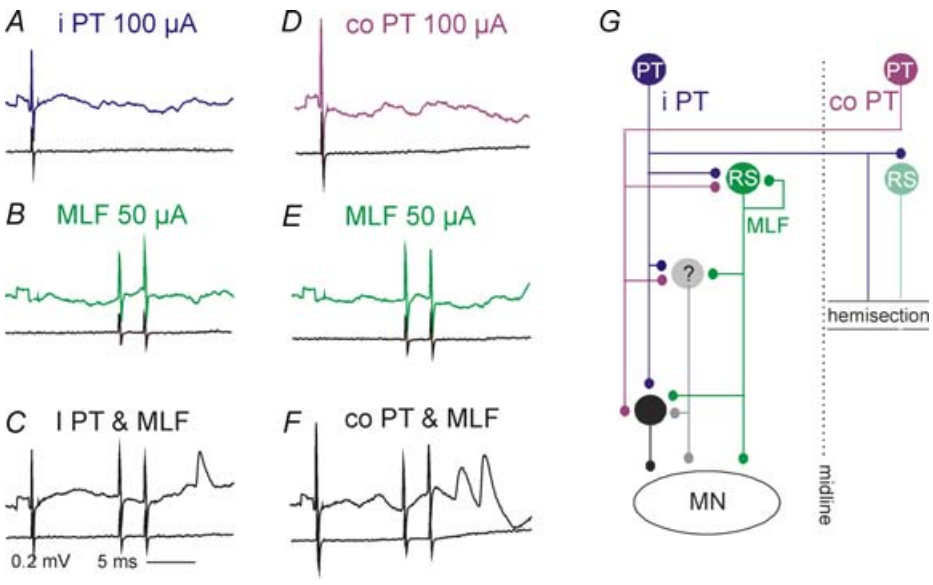

Figure 5. Mutual facilitation of disynaptic EPSPs evoked from the ipsilateral and contralateral PTs and from the MLF

In each panel upper traces are intracellular records from an interneurone (the same as in Fig. 4) and lower records from the cord dorsum. Averages of 20 single records. *A–C*, effects of stimuli applied to the ipsilateral PT or MLF alone and to both the PT and the MLF. *D–F*, as in *A–C* but for effects of the contralateral PT. Note hardly any effects of separate PT or MLF stimuli and potent facilitation of their effects when they were applied jointly. *G*, network of neurones likely to mediate both ipsilateral and contralateral actions of PT neurones on lumbar interneurones and motoneurones. Black circle represents premotor interneurones investigated in this study. The grey circle represents other spinal interneurones.

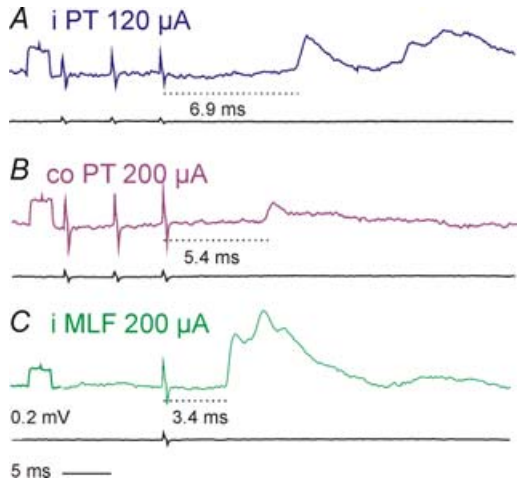

Figure 6. Examples of EPSPs from the ipsilateral and contralateral PT that were unlikely to be mediated by RS neurones
Averaged intracellular records from an interneurone and cord dorsum records. Note that the latency of the EPSP evoked from the ipsilateral PT that followed the 3rd stimulus (*A*) was longer than that of the EPSP evoked from the contralateral PT (*B*), and it was more than 3 ms longer than the latency of the monosynaptic EPSP from the MLF, and more than 2 ms longer than that of the disynaptic EPSP from the MLF (*C*).

One of the most striking features of these EPSPs was that their latencies were shorter than latencies of the majority of the monosynaptically evoked EPSPs. The probability that these disynaptic EPSPs were relayed by other interneurones of the populations examined in this study appeared therefore to be low. It was more likely that, as in motoneurones, they were relayed by RS neurones. When the MLF was intact, this possibility was tested in eight interneurones by verifying that occlusion and/or facilitation occurred between effects of PT and MLF stimuli.

Occlusion was demonstrated between near-maximal effects of PT and MLF stimuli. In the neurone illustrated in Fig. 3 and in two other interneurones it occurred between disynaptic EPSPs from the ipsilateral PT and monosynaptic EPSPs from the MLF because effects of the joint PT and MLF stimuli (*H*) were much smaller than the sum of effects of these stimuli (*J*) when applied separately (*G* and *I*). It showed thus that disynaptic PT actions on these neurones were to a great extent evoked via RS neurones. In five interneurones in which EPSPs were evoked by both PT and MLF stimuli, as in the interneurone illustrated in Fig. 4, the dominating effect of joint application of these stimuli was facilitation of their effects, especially when they were sub- or near-threshold. However, at higher stimulus intensities and at some critical intervals occlusion was also occurring. An example of facilitation of effects of subthreshold stimuli is shown in Fig. 5*A*–*C*. The site of the facilitation could not be decided with certainty, but the diagram in *G* shows that it could occur at the level of RS neurones as well as at the level of some so far unidentified spinal neurones. Facilitation at the level of RS

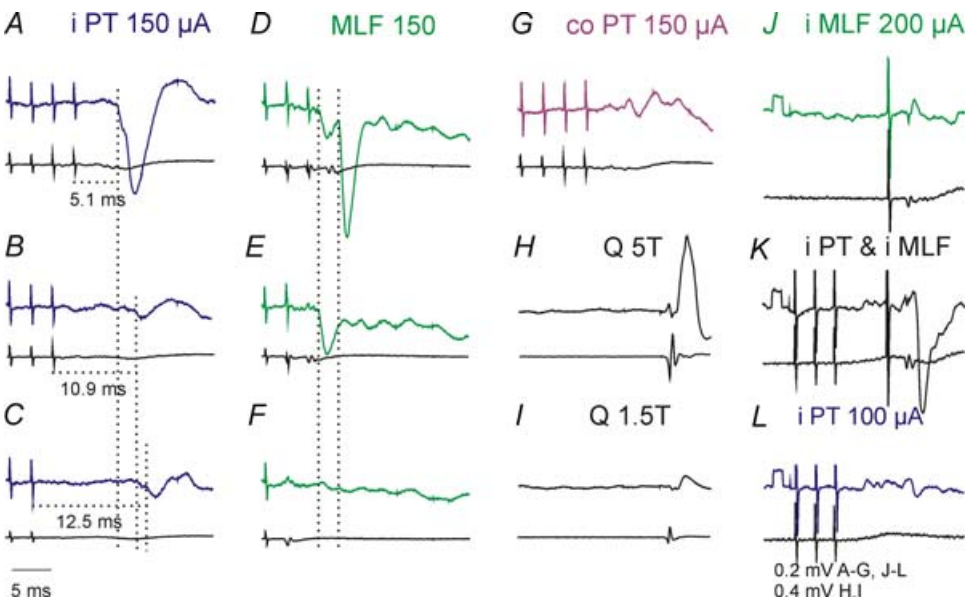

Figure 7. Examples of IPSPs evoked by PT and MLF stimuli and mutual facilitation of effects of these stimuli
Intracellular records from two interneurones (*A*–*I* and *J*–*L*) and cord dorsum potentials (lower records in each panel). *A*–*C*, *D*–*F*, effects of decreasing numbers of stimuli applied to the ipsilateral PT and MLF. Vertical dotted lines indicate onset of IPSPs evoked by the 4th, 3rd and 2nd ipsilateral PT and the 3rd and 2nd MLF stimuli (with the indicated latencies). *G*, effects of stimulation of the contralateral PT. *H* and *I*, records showing short latency and low threshold input from group I afferents in the Q nerve. *J*–*L*, appearance of IPSPs when near-threshold ipsilateral PT stimuli preceded similarly near-threshold MLF stimuli which were ineffective when applied alone.

Table 1. Proportions of interneurones with excitatory input from the ipsilateral (i PT) and contralateral (co PT) pyramidal tracts in samples of interneurones with different types of peripheral input

		PT input (number)				
Peripheral input		i PT (monosyn)	i PT (di- and trisyn)	i PT and co PT	co PT (monosyn)	co PT (di- and trisyn)
Group Ia	$n = 5$	—	5	1	—	1
Group Ia and b	$n = 5$	—	2	1	1	3
Group I and II	$n = 10$	1	2	1	—	2
Group II excited	$n = 9$	—	2	7	1	6
Group II inhibited	$n = 5$	1	2	1	—	1
Latency mean ± S.E.M. (ms)		7.25 ± 0.75	5.19 ± 0.16	—	8.12 ± 3.19	5.45 ± 0.31
Latency range (ms)		4.1–9.9	4.1–8.3	—	4.9–11.3	3.9–8.9

Data for neurones in which both peripheral and descending input could be analysed. The bottom row shows latencies for all interneurones with monosynaptic and/or disynaptic input from the ipsilateral and contralateral PTs (including those in which the peripheral input was not defined).

neurones would be likely in view of indications that MLF stimuli evoke not only descending volleys and antidromic activation of RS neurones, but also synaptic excitation of either the same or other RS neurones via their medullary axon collaterals (Edgley *et al.* 2004; Jankowska *et al.* 2006).

Facilitation and/or occlusion of PT and MLF actions was found in interneurones in which the latencies of EPSPs evoked by PT stimuli were considered to be compatible with their mediation by RS neurones. Those represented by the data points to the left of the 1st vertical dotted line in Fig. 2*C* were compatible because they were < 2 ms longer than latencies of monosynaptic EPSPs evoked by MLF stimuli (indicated by the horizontal dotted line), the RS neurones being activated by PT stimuli at 1–2 ms

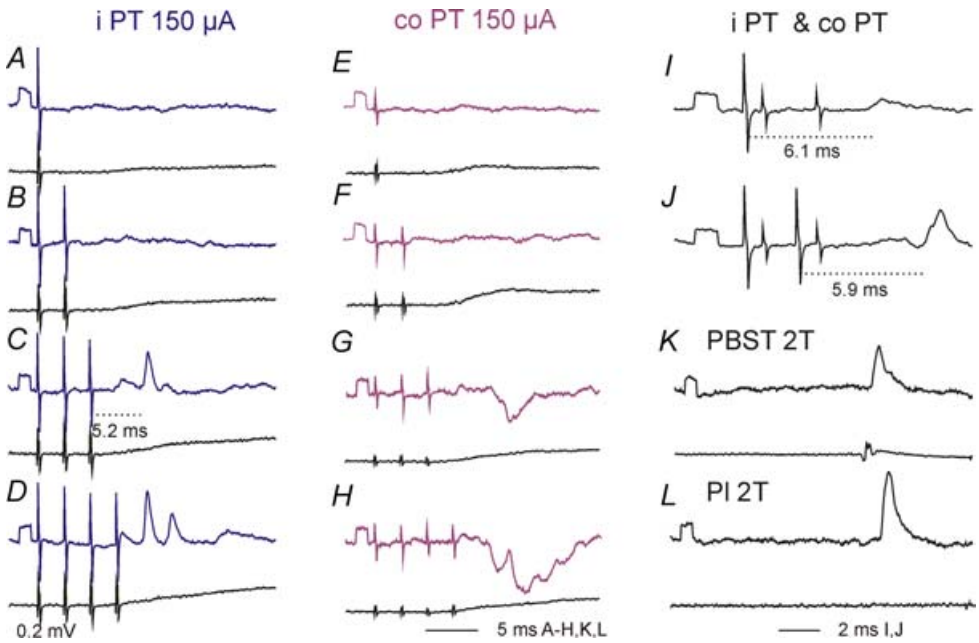

Figure 8. Mutual facilitation between effects of stimuli applied within the ipsilateral and the contralateral PT

Intracellular records from an interneurone and from the cord dorsum after transection of the MLF. Averages of 20 single records. *A–D* and *E–H*, effects of increasing numbers of stimuli, showing mixed excitatory–inhibitory effects from both PTs when at least 3 or 4 stimuli were applied and no effects of single and double stimuli. *I*, weak facilitation when one ipsilateral PT stimulus preceded two contralateral PT stimuli. *J*, stronger facilitation when two ipsilateral PT stimuli were combined with two contralateral PT stimuli. Dotted lines in *I* and *J* indicate latencies of the resulting EPSPs with respect to the first and second ipsilateral PT stimuli; note that they would be shorter with respect to co PT stimuli. *K* and *L*, monosynaptic EPSPs evoked by stimulation of the posterior biceps–semitendinosus (PBST) and plantaris (Pl) muscle afferents at 2*T*.

latencies (Jankowska *et al.* 2006). Latencies of five EPSPs between the two vertical dotted lines in Fig. 2*C* would also be compatible, given that the EPSPs were mediated by RS neurones that were activated by either PT or MLF stimuli disynaptically rather than monosynaptically. However, they would also be compatible with trisynaptically evoked actions.

EPSPs evoked in the nine remaining interneurones were evoked at latencies that were much longer than latencies of EPSPs that were either monosynaptically or disynaptically evoked from the MLF. They are exemplified in Fig. 6 and represented by the data points to the right of the 2nd vertical dotted line in Fig. 2*C*. They might be compatible with EPSPs mediated via RS neurones that were fairly inefficiently activated by PT neurones or, more plausibly, via other neurones. However, since the latencies of disynaptic EPSPs evoked in preparations with the MLF intact and after its transection were similar (see two sets of diamonds in Fig. 2*C*), our results indicate that RS neurones with axons in the MLF are not the only source of even the shortest latency EPSPs (see Discussion for other possibilities).

IPSPs evoked from the ipsilateral PT

Given that both the corticospinal and reticulospinal tract fibres reaching lumbar segments are excitatory, any IPSPs evoked by these descending neuronal systems should be mediated by spinal inhibitory interneurones. IPSPs due to direct actions of ipsilaterally descending PT fibres on premotor inhibitory interneurones should thus be evoked disynaptically and, if they were mediated by the fastest conducting PT fibres, they might be evoked at similar latencies to disynaptic EPSPs evoked by stimulation of the MLF. In contrast, IPSPs relayed by RS neurones and inhibitory interneurones activated by them, should be evoked at least trisynaptically.

The plot of latencies of IPSPs evoked by PT stimuli in Fig. 2*B* shows that most of the IPSPs were evoked at the same latencies as disynaptic EPSPs and may thus represent disynaptic IPSPs relayed by direct actions of fast uncrossed PT fibres and inhibitory interneurones. However, individual data points in Fig. 2*B* show a continuum rather than distinct groupings, thus not allowing the separation of those evoked disynaptically and trisynaptically. In addition, in some interneurones both short and long latency IPSPs were evoked, depending on the stimulus parameters. This is illustrated in Fig. 7*A*–*C*, where longer latency IPSPs were evoked by two or three stimuli while shorter latency IPSPs appeared after the 4th stimulus. As the shortest latencies were only 1.2 ms longer than those of IPSPs evoked from the MLF, they would be compatible with trisynaptic coupling via RS neurones while the longer latency IPSPs evoked in this particular interneurone could be attributed to disynaptic actions relayed by interneurones directly activated by PT fibres.

In order to examine whether IPSPs from the ipsilateral PT and IPSPs from the MLF were evoked by the same interneurones, facilitation or occlusion between these IPSPs was tested in five interneurones. In all of these

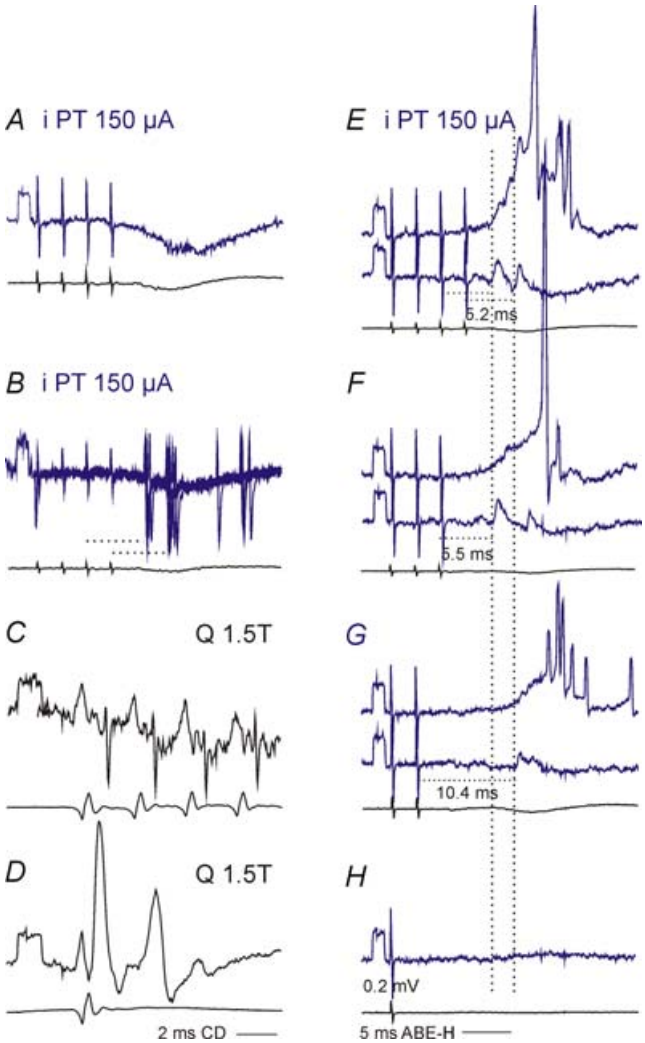

Figure 9. Examples of extracellular spikes and EPSPs evoked from the ipsilateral PT in a group Ia inhibitory interneurone in a preparation with the MLF intact
Top and middle traces, microelectrode records. Lower traces, records from the cord dorsum. *A*, extracellular field potential close to the interneurone; averaged records ($n = 10$). *B*, extracellular records from the interneurone; 5 traces superimposed. *C*, extracellular records from the interneurone; single record of responses to stimulation of the Q nerve at 400 Hz. *D*, monosynaptic EPSPs from Q after penetration of the neurone. *E*–*H*, intracellular records showing the effects of decreasing numbers of ipsilateral PT stimuli. Top, just after penetration of the interneurone. Middle, a few minutes after the penetration. Dotted vertical and horizontal lines indicate latencies of responses evoked by the indicated stimuli. Note the longer latency of EPSPs following the 2nd stimulus in *F* and *G* and shorter latencies of spikes (*B*) and EPSPs in *E* and *F* following the 3rd and 4th stimuli.

interneurones facilitation (illustrated in Fig. 7*J–L*), occlusion, or both were found, depending on the intervals between the PT and MLF stimuli.

Comparison of synaptic actions evoked from the ipsilateral and contralateral PTs

Postsynaptic actions evoked by stimulation of the ipsilateral PT were compared with those evoked from the contralateral PT in 11 interneurones in preparations with the MLF intact and in fourteen interneurones after transection of the MLF. The comparison was made using the same number of stimuli and at the same intensities (100–150 μA). In both preparations the effects of stimulation of the contralateral PT were similar to those described above. The dominant actions were disynaptic EPSPs (Fig. 4*D–F*). They were found in 11 interneurones in parallel with disynaptic EPSPs from the ipsilateral PT (e.g. Fig. 2*C*). Monosynaptic EPSPs, like those illustrated in Fig. 1*D* have been found in only three interneurones.

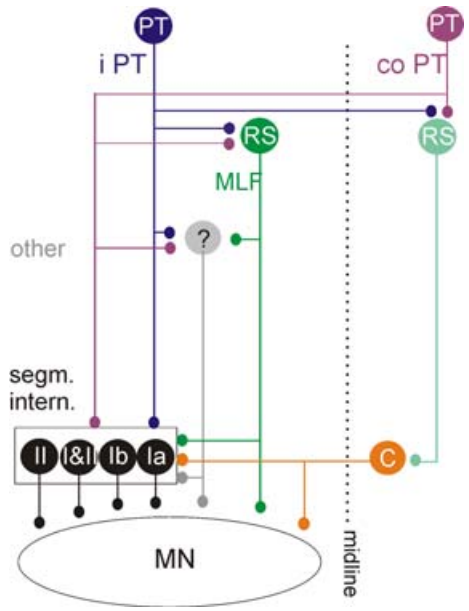

Figure 10. Diagram of neuronal organization in four so far investigated parallel pathways between PT neurones and ipsilateral motoneurones
These pathways are represented by disynaptic pathways via segmental interneurones (black neurones in the box), so far unidentified other neurones (grey neurone) and ipsilaterally descending RS neurones with axons in the MLF and by trisynaptic pathways via contralaterally descending RS neurones and commissural interneurones (labelled C; Edgley *et al.* 2004; Jankowska *et al.* 2006). Relay neurones in all these pathways appear to be used to mediate synaptic actions of both ipsilateral and contralateral PT neurones. For the sake of simplicity, somata of ipsilaterally and contralaterally descending PT and RS neurones are indicated to be located only on the left or the right sides, even though they are located on both sides. For further comments see text.

Latencies of both the monosynaptic EPSPs and the di- or trisynaptic PSPs evoked from the contralateral PT were within the same range as PSPs from the ipsilateral PT and no statistically significant differences were found between them. The ranges were 4.9–11.3 ms for monosynaptic EPSPs and 3.9–8.9 ms for di- or trisynaptic EPSPs, as compared to 4.1–9.9 ms and 4.1–8.3 ms for monosynaptic and di- or trisynaptic EPSPs from the ipsilateral PT. For IPSPs they were 4.4–5.5 ms (mean 5.08 ± 0.33 ms) as compared to 4.2–9.6 ms (mean 5.59 ± 0.31 ms) for those evoked from the ipsilateral PT.

Mutual facilitation was as effective between actions of contralateral PT and MLF stimuli as of ipsilateral PT and MLF stimuli (see above), with an example in Fig. 5*D–F*. Mutual facilitation has also been found between the effects of stimuli applied within the ipsilateral and the contralateral PTs, both when the MLF was intact (in 2 neurones tested) and when it was transected (in 3 neurones tested), as exemplified in Fig. 8.

Which interneurones are affected by ipsilateral PT neurones?

Peripheral input was investigated in 34 of the 44 interneurones with ipsilateral PT input. These have been subdivided according to whether they were excited by the lowest or higher threshold group I afferents and/or group II afferents, or inhibited by group II afferents (Table 1). They were considered as Ia inhibitory interneurones, as interneurones mediating either inhibitory or excitatory actions of group Ia and Ib afferents, or interneurones in excitatory or inhibitory pathways from group II afferents using criteria described in the last section of Methods. Records from one of the facultative Ia interneurones are shown in Fig. 9, from Ib interneurones in Figs 7 and 8 and from group II interneurones in Figs 3 and 5.

Only small samples of interneurones of each type were analysed and therefore the observed PT actions on them may not be representative of their whole populations. Nevertheless, Table 1 shows that disynaptic actions from both ipsilateral and contralateral PTs were evoked in all types of interneurones examined and suggests that the probability of monosynaptic input from the ipsilateral PT is higher in interneurones with group II input than in Ia or Ib interneurones.

Discussion

The results of this study lead to the conclusion that lumbar interneurones in pathways from groups Ia and Ib as well as from group II muscle afferents may mediate uncrossed PT actions but would contribute more substantially to the later than to the earliest components of disynaptic uncrossed

PT actions on motoneurones and to trisynaptic rather than to disynaptic.

On timing of ipsilateral PT actions relayed by spinal interneurones

PT actions on motoneurones relayed by ipsilaterally descending RS and by spinal neurones could be evoked at similar latencies only if conduction velocities of PT neurones and RS neurones were similar. Maximal conduction velocity of RS neurones was estimated to be up to 110–130 m s^{-1} (see, e.g. Peterson *et al.* 1979; Floeter *et al.* 1993) while PT fibres were most frequently reported to conduct at up to 60–70 m s^{-1} (see Porter & Lemon, 1993). However, the estimates for PT fibres were often made from conduction times over very short conduction distances (between the cortex and the medulla) and apparently without having subtracted a utilization time for the generation of action potentials by electrical stimuli (which amounts to about 0.2 ms; see Jankowska & Roberts, 1972*b*) from the latencies of effects of these stimuli. More reliable, therefore, are estimates of maximal conduction velocities of crossed PT fibres of about 100 m s^{-1}, or more, that were made for distances of at least 200–300 mm (Woolsey & Chang, 1947; Brookhart & Morris, 1948; Casale & Light, 1991; Edgley *et al.* 1997). With respect to uncrossed PT fibres, neither their conduction velocities nor the distribution of their diameters have been reported, although examples of these fibres in the macaque spinal cord (e.g. in Fig. 4 of Lacroix *et al.* 2004) suggest that they may be of similar sizes, and therefore have similar maximal conduction velocity as crossed PT fibres. Our estimates of conduction velocities of uncrossed PT fibres responsible for the earliest monosynaptic EPSPs were 75–85 m s^{-1} (see Fig. 2*A*); they were lower than those of RS fibres in the MLF, but overlapped with the range of 80–88 m s^{-1} for crossed PT neurones targeting the same area of the spinal grey matter as determined by Casale & Light (1991).

Latencies of monosynaptic EPSPs evoked in the majority of interneurones of our sample were as long, or longer, than latencies of the earliest disynaptic EPSPs evoked in motoneurones (4.3–5 ms; see Fig. 3 in the accompanying paper). A major contribution of these interneurones to the earliest components of EPSPs evoked in motoneurones would thus seem unlikely. The ranges of latencies of monosynaptic EPSPs evoked from the ipsilateral PT (4.1–11.7 ms) and from the contralateral PT (6–11.3 ms, as found in the present study and by others) were similar. Of these, latencies of 6–8 ms of the earliest monosynaptic EPSPs evoked by stimulation of the contralateral motor cortex (Lundberg *et al.* 1962) would be equivalent to 5–7 ms latencies of EPSPs evoked from the medullary pyramids, given the conduction time longer by about 1 ms. They were found in interneurones with input from group II muscle afferents located in lower lumbar segments. Latencies of monosynaptic EPSPs evoked from the contralateral medullary PT in two other samples of interneurones were 4.7–6.6 ms in midlumbar interneurones (Davies & Edgley, 1994; S.A. Edgley, personal communication) and about 5.1–9.8 ms in lower lumbar interneurones (Harrison & Jankowska, 1985; see their Fig. 11). These were interneurones with input from group II and from group Ib afferents, respectively.

Latencies of disynaptic EPSPs evoked in interneurones were shorter (4.1–5.0 ms and 3.9–5.0 ms from the ipsilateral PT and the contralateral PT, respectively) but were similarly too long to allow the interneurones to mediate the earliest PT actions on motoneurones.

Which interneurones are likely to mediate the earliest actions of ipsilateral PT neurones on motoneurones?

Monosynaptic EPSPs from the ipsilateral PT were found in interneurones with input from group II muscle afferents, i.e. in the same subpopulation of interneurones in which monosynaptic EPSPs were found to be evoked from the contralateral PT (Lundberg *et al.* 1962; Davies & Edgley, 1994) but not in Ib interneurones in which monosynaptic input from the contralateral PT was found both previously (Harrison & Jankowska, 1985) and in one interneurone of the present study. In contrast, disynaptic EPSPs were evoked in all kinds of interneurones, indicating that all of these interneurones might mediate trisynaptic uncrossed PT actions to motoneurones. Some of these interneurones were coexcited by stimuli applied to the ipsilateral and the contralateral PT. Since the excitation from both the ipsilateral and the contralateral PT was usually disynaptic, this would be in keeping with its mediation by RS neurones coexcited by PT neurones from both hemispheres (He & Wu, 1985; Canedo & Lamas, 1993; Matsuyama & Drew, 1997; Kably & Drew, 1998). However, our data leave open the question as to which of these interneurones, other than Ia interneurones, actually acted on motoneurones as last-order premotor interneurones and whether uncrossed PT actions on inhibitory and excitatory interneurones are similar. Experiments aimed at answering this question will be reported separately.

The contribution of interneurones in reflex pathways from low threshold cutaneous afferents and from high threshold muscle, skin and joint afferents (flexor reflex afferents, FRA; Eccles & Lundberg, 1959) to the uncrossed PT actions remains another open question. It is well established that various populations of interneurones in reflex pathways from cutaneous afferents are targeted by contralateral PT neurones and they might be affected by ipsilateral PT neurones as well. However, interneurones monosynaptically excited by skin afferents are only exceptionally premotor interneurones (Fleshman *et al.* 1988; Hongo *et al.* 1989*b*); if they are not, they could

mediate PT actions via a variety of other interneurones. Another complication is that premotor interneurones with input from cutaneous afferents are usually coexcited by muscle afferents and belong to their various categories (e.g. Ib interneurones, Harrison & Jankowska, 1985; or group II interneurones, Edgley & Jankowska, 1987). This may also be true for last-order FRA interneurones and for interneurones in which only cutaneous peripheral input was investigated (Hongo *et al.* 1989*a*,*b*).

With respect to other spinal neurones that might mediate uncrossed PT actions on motoneurones, of particular interest are two populations of propriospinal neurones. One of these are propriospinal neurones located between the C4 and Th2 segments. They were found to evoke monosynaptic EPSPs in hindlimb motoneurones and in view of their high conduction velocity, estimated to be about 100 m s^{-1} (Jankowska *et al.* 1973, 1974), they could mediate disynaptic PT actions as effectively and as quickly as RS neurones. However, sources of input to these neurones, in particular from uncrossed PT fibres, have not been investigated. Another population to consider are propriospinal neurones located in the C3–C5 segments, with potent input from a number of descending tract neurones and axons traced as far caudally as the lumbar segments (Illert *et al.* 1978; Alstermark *et al.* 1991). These were found to conduct at 62–124 m s^{-1} (Alstermark *et al.* 1991), i.e. within the required range of conduction velocities, but uncrossed PT actions on these neurones were not investigated and monosynaptic actions from the contralateral PT were reported to be fairly weak and were found in only a small proportion of these cells (Alstermark *et al.* 1987). Some of these neurones might belong to the population of the fast conducting propriospinal neurones described by Jankowska *et al.* (1973, 1974) but it has not been established whether they project more caudally than the L3–L5 segments, nor whether they have direct actions on motoneurones or premotor interneurones.

On networks of neurones mediating actions of ipsilateral PT neurones and their contribution to centrally initiated movements

Generally weak actions of stimuli applied in the ipsilateral PT on interneurones investigated in this study raise the question of whether these interneurones could contribute to ipsilateral PT actions in any essential way. The question is difficult to answer but estimating the role played by these interneurones, one should take into account that the effectiveness of their activation by uncrossed PT fibres ought to be higher under natural conditions than in reduced, anaesthetized preparations in which a great part of the input to the interneurones is eliminated. By leaving intact only the ipsilateral half of the spinal cord we deprived the interneurones of a considerable amount of background descending excitatory actions, including any evoked via collaterals of contralaterally descending corticospinal tract fibres (for review see Martin, 2005), or reticulospinal fibres (Matsuyama *et al.* 1993, 1999) and commissural interneurones activated by these fibres (Jankowska *et al.* 2005*c*; Cabaj *et al.* 2006). Additional lesions of the ipsilateral MLF further aggravated this situation. On the other hand, partial denervation of the hindlimbs and immobilization reduced input from the periphery. One should also consider that the state of the interneurones has deteriorated soon after their penetration (with an example in Fig. 9) and that both monosynaptically and disynaptically evoked EPSPs were originally larger than those illustrated. Thus even if the actions of uncrossed PT fibres on premotor interneurones of our sample were weaker than other synaptic actions, nerve impulses in PT fibres might activate these interneurones by acting on the top of other synaptic actions.

With respect to the disynaptic actions of ipsilateral PT neurones on interneurones, our results suggest that they are evoked similarly on motoneurones and, as indicated in Fig. 10, are collateral to actions on motoneurones, including actions of RS neurones and of currently unidentified ipsilaterally located spinal neurones. However, disynaptic collateral actions of interneurones of the populations analysed in this study would be unlikely because latencies of both monosynaptic and disynaptic excitation of these neurones were generally longer than those of the earliest actions of PT neurones on motoneurones.

The organization of networks of neurones in pathways between PT neurones and their ipsilateral target neurones thus far investigated is outlined in Fig. 10. It includes several parallel pathways represented by uncrossed disynaptic pathways via black and grey interneurones, by uncrossed disynaptic pathways via ipsilateral RS neurones and by double crossed trisynaptic pathways via contralateral RS neurones and commissural interneurones. This diagram is of course far from being complete. We have in particular included in it only one variant of double crossed pathways although several might be involved. For instance commissural interneurones might be activated not only via RS neurones (as shown to the right) but also via some spinal neurones relaying actions of crossed PT (see discussion of this possibility by Jankowska *et al.* 2006). Crossed PT fibres might also target commissural interneurones within the cervical, thoracic and lumbar segments which in turn would either directly or indirectly affect motoneurones on the other side of the spinal cord. However, there are so far only two kinds of preliminary observations to this end. One is the occasionally found monosynaptic input from PT to commissural interneurones with monosynaptic input from group II muscle afferents (B. A. Bannatyne,

D. J. Maxwell, I. Hammar, K. Stecina & E. Jankowska, unpublished observations). Another is the demonstration of long-axoned cervical commissural neurones (long C3–C5 contralaterally projecting propriospinal neurones with PT input; Alstermark *et al.* 1987). Crossed PT fibres and contralaterally descending RS fibres might also contact ipsilaterally located interneurones and propriospinal neurones via their re-crossing axon collaterals (for references see Matsuyama *et al.* 1993, 1999; Martin, 2005) which would in turn affect motoneurones.

Mediation of PT actions via several parallel channels appears to be well suited for securing these actions by their joint activation. Because of different conduction velocities and transfer delays in the various pathways, nerve impulses initiated in even the fastest conducting PT neurones will reach motoneurones over a time period of several milliseconds rather than synchronously. As indicated in Table 1, the latencies of monosynaptic and di- or trisynaptic uncrossed PT actions span between 4 and more than 9 ms and the range of latencies of double crossed trisynaptic PT actions evoked via contralateral RS neurones and commissural interneurones is similar (4–7 ms; Jankowska *et al.* 2005*a*). Actions mediated by these four pathways may be further strengthened by neurones providing positive feedback and/or feed-forward input to their constituent neurones. For instance, activation of RS neurones by direct actions of PT fibres is followed by their re-activation via other medullary neurones excited by axon collaterals of RS neurones or other neurones, as found previously both under our experimental conditions (Fig. 1*C* in Edgley *et al.* 2004; Fig. 6 in Jankowska *et al.* 2006) and in other studies. Delayed activation of RS neurones would result in even more delayed activation of commissural interneurones and additional spinal interneurones which are in turn activated by them (Jankowska *et al.* 2005*c*; Cabaj *et al.* 2006). It may thus further strengthen and prolong their effects on motoneurones.

Strengthening and prolonging the earliest PT actions on motoneurones by even as little as 5–10 ms might play a role in triggering voltage-dependent persistent inward current and plateau potentials (Schwindt & Crill, 1980; Hounsgaard *et al.* 1988; Hultborn, 1999, 2003) and thereby for inducing sustained discharges required for tonic voluntary movements by relatively short lasting corticospinal commands. The critical level of depolarization needed for the persistent inward current has been defined but the critical duration of synaptic actions needed to reach such level of depolarization has apparently not. On the basis of published records it appears to be at least some 10 ms (see Fig. 6*C* and *E* in Schwindt & Crill, 1980). A delay of 20–40 ms appears to have been involved when plateau potentials and tonic discharges of a motoneurone were evoked by stimulation of corticospinal tract fibres (Fig. 4 in Hultborn *et al.* 2003), but it exceeded 100 ms (Fig. 1 in Lee & Heckman, 1996) when they were induced by muscle vibration. A similar range of delays in the appearance of plateau potentials was found in extensor motoneurones by stimulation of group I afferents during the extension phase of fictive locomotor activity in decerebrate cats (D. McCrea, personal communication). Doubling or tripling of the period of depolarization evoked in motoneurones by PT actions via several pathways with different overall conduction times might thus add to other mechanisms ensuring sustained motoneuronal responses (for references see Lundberg *et al.* 1987; Li *et al.* 2006).

Despite their importance, not all of the available parallel pathways would be needed to allow PT neurones to act on ipsilateral motoneurones. It has been demonstrated that under favourable conditions, ipsilateral PT actions may be mediated by RS relays alone. For double crossed pathways this was shown by a negligible effect of lesions of both the right and left corticospinal tract fibres running within the dorsolateral funiculus (DLF) on the trisynaptic actions of PT neurones on motoneurones evoked via commissural interneurones (see Fig. 4 in Edgley *et al.* 2004). Since the lesions were made at a C2–C3 level, they eliminated the effects of PT fibres on both segmental interneurones and propriospinal neurones located caudal to the C3 segment. These lesions also had negligible effects on uncrossed disynaptic PT actions on ipsilateral motoneurones evoked via RS fibres descending within the ipsilateral MLF (see Fig. 4 in Edgley *et al.* 2004 and Fig. 3*C* and *D* in Jankowska & Edgley, 2006), especially when synaptic transmission was enhanced by 4-AP (Jankowska *et al.* 2005*a*). Another case of actions evoked by stimulation of the motor cortex after transection of medullary pyramids was reported by Hongo & Jankowska (1967). They found that monosynaptic reflexes were then facilitated under chloralose anaesthesia but not after an additional small dose of pentobarbital that weakens the activation of RS neurones by PT neurones, nor after lesions of deeper parts of the lateral funiculus including reticulospinal tract fibres. The facilitation was evoked from both the contralateral and the ipsilateral motor cortex (see Fig. 4 in Hongo & Jankowska, 1967).

Corticospinal actions might also be evoked without involving RS relays. This was indicated by the disappearance of facilitation of Ia reciprocal inhibition by crossed PT fibres after transection of the contralateral pyramid in preparations under pentobarbital anaesthesia (Lundberg & Voorhoeve, 1962), showing that the facilitation depended critically on PT fibres running within the caudal part of the medulla.

The neuronal organization of pathways between PT neurones and ipsilateral motoneurones summarized in Fig. 10 would be of importance not only for strengthening and prolonging PT actions on motoneurones, but also for gating actions evoked by PT neurones at the level of relay neurones in these various pathways. As shown repeatedly, e.g. by Drew and colleagues (Drew & Rossignol, 1990;

Drew, 1991; Drew *et al.* 2002) and Fetz and colleagues (Fetz *et al.* 2000, 2002) the actions of both corticospinal and reticulospinal neurones are intricately linked to the patterned activity of spinal neuronal networks, in particular during movements requiring their proper coordination. Shaping of various patterns of movements may thus require a highly plastic use of spinal networks, involving both excitation and inhibition of relay neurones in these networks.

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