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Bilateral postsynaptic actions of pyramidal tract and reticulospinal neurons on feline erector spinae motoneurons

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Abstract

Trunk muscles are important for postural adjustments associated with voluntary movements but little has been done to analyse mechanisms of supraspinal control of these muscles at a cellular level. The present study therefore aimed to investigate the input from pyramidal tract (PT) neurons to motoneurons of the m. longissimus lumborum of the erector spinae and to analyse to what extent it is relayed by reticulospinal (RS) neurons. Intracellular records from motoneurons were used to evaluate effects of electrical stimulation of medullary pyramids and of axons of RS neurons descending in the medial longitudinal fasciculus (MLF). The results revealed that similar synaptic actions were evoked from the ipsilateral and contralateral PTs, including disynaptic and trisynaptic EPSPs and trisynaptic IPSPs. Stimulation of the MLF evoked monosynaptic and disynaptic EPSPs and disynaptic or trisynaptic IPSPs in the same motoneurons. All short latency PSPs of PT origin were abolished by transection of the MLF, while they remained after transection of PT fibers at a spinal level. Hence, RS neurons might serve as the main relay neurons of the most direct PT actions on m. longissimus. However, longer latency IPSPs remaining after MLF or PT spinal lesions and after ipsilateral or contralateral hemisection of spinal cord indicate that PT actions are also mediated by ipsilaterally and/or contralaterally located spinal interneurons. The bilateral effects of PT stimulation thereby provide an explanation why trunk movements after unilateral injuries of PT neurons (e.g. stroke) are impaired to a lesser degree than movements of the extremities.

Keywords

Spinal cord; motoneurons; back muscles; pyramidal tract; neurons; cat

Introduction

In spite of a large number of studies on postural adjustments, the neuronal networks underlying these adjustments remain to a great extent unknown. This is true in particular for supraspinal control of postural adjustments of back muscles, as most previous studies were concerned with adjustments of the whole body, or of proximal limb muscles rather than trunk muscles.

The importance of back muscles for stabilizing the trunk and enabling effective actions of the extremities is beyond doubt. Deficits in trunk control and postural asymmetry occur after stroke-related unilateral damage to pyramidal tract (PT) neurons [e.g. (Davies, 1990; van Nes et al., 2008)] as well as after more selective lesions of medullary pyramids in animals [(e.g.

(Lawrence and Kuypers, 1968)]. Deficits in postural control are also seen in patients with idiopathic low back pain (Zedka et al., 1999; Hodges et al., 2009). Studies in post-stroke hemiplegic patients have shown that the erector spinae muscles are particularly important for stabilizing the trunk and its movements, and that, in contrast to the abdominal muscles, their activity is relatively unimpaired (Dickstein et al., 1999). Residual function of the erector spinae muscles after unilateral stroke might indicate a stronger subcortical than cortical control of these muscles, or stronger ipsilateral actions of PT neurons in the non-injured hemisphere [for references see e.g. (Dum and Strick, 1996; Jankowska and Edgley, 2006)].

Both corticospinal and reticulospinal neurones have been shown to be involved in postural adjustments [(Massion, 1992, 1994); for recent references see (Deliagina et al., 2008; Schepens et al., 2008)]. However, activity of these neurons has primarily been related to limb movements or to anticipatory or corrective postural adjustments of the whole body (Alstermark et al., 1985; Luccarini et al., 1990; Chabran et al., 2001; Schepens and Drew, 2004; Deliagina et al., 2006; Schepens and Drew, 2006; Deliagina et al., 2008; Caronni and Cavallari, 2009a, b) and there are only scarce data on their actions on back muscles. For example, stimuli applied in the reticular formation were found to evoke short latency EMG responses in epiaxial muscles, including back muscles in the rat (Cottingham et al., 1988) and to facilitate likely monosynaptic reflexes from these muscles (Brink et al., 1981). The relative contribution of PT and reticulospinal neurons to postsynaptic excitation and inhibition of neck motoneurons (Wilson and Yoshida, 1968; Alstermark et al., 1985) and of reticulospinal neurones on back motoneurons in thoracic segments (Peterson et al., 1979) was examined in the cat, but no reports have been made on actions of these neurons on lumbar back motoneurons.

The main aim of the reported experiments was therefore to investigate the effects of pyramidal tract (PT) fibers on intracellularly recorded motoneurons innervating the *m. longissimus lumborum*, the most medial component of the erector spinae muscle group, and to compare effects of contralateral and ipsilateral PT neurons. We further aimed to investigate whether the corticospinal actions on the erector spinae motoneurons are relayed by reticulospinal neurons as found for PT actions on hindlimb motoneurons (Stecina and Jankowska, 2007).

Methods

Preparation

The experiments were performed on 8 deeply anaesthetised adult cats weighing 2.5-4.5 kg. All experimental procedures were approved by the University of Göteborg Ethics Committee and followed NIH and EU guidelines for animal care. Anaesthesia was induced with sodium pentobarbital (40-44 mg/kg, i.p.) and maintained with intermittent doses of α -chloralose (Rhône-Poulenc Santé, France; doses of 5 mg/kg administered every 1-3 hours, up to 50 mg/kg, i.v.). During recordings, neuromuscular transmission was blocked by pancuronium bromide (Pavulon, Organon, Sweden; about 0.2 mg/kg/h, i.v.) and the animals were artificially ventilated. Additional doses of α -chloralose were given when increases in blood pressure or heart rate, both of which were continuously monitored, were evoked by stimulation, or if the pupils dilated. Mean blood pressure was kept at 90-130 mm Hg (with the exception of 2 cats in which it dropped to 80-90 mm Hg towards the end of the experiments) and end-tidal concentration of CO₂ at about 4% by adjusting the parameters of artificial ventilation and the rate of a continuous infusion of a bicarbonate buffer solution with 5% glucose (1-2 ml/h/kg). The core body temperature was kept at about 37.5°C by servo-controlled infrared lamps. The experiments were terminated by a lethal dose of anaesthetic followed by formalin perfusion resulting in cardiac arrest.

A laminectomy exposed the first to fourth lumbar (L1-L4) and low thoracic (Th10-Th11) segments and a small area at the border between the third and fourth cervical (C3-C4) segments

of the spinal cord. The intermediate branches of the dorsal rami innervating m. longissimus lumborum were exposed at L2 and L3 levels bilaterally. The approach was made through a longitudinal split between the multifidus and longissimus muscles. The nerves were identified close to where they emerged in the intertransverse space and dissected from the surrounding tissue. They were ligated, transected and mounted on two pairs of stimulating electrodes in a paraffin oil pool.

The caudal part of the cerebellum was exposed by a craniotomy and tungsten electrodes (impedance 30-150 k Ω) were placed in the left and/or right pyramids (PT) in the caudal part of the medulla and in the left or right medial longitudinal fasciculus (MLF). The electrodes were inserted at an angle of 35 degrees (with the tip directed rostrally). The initial targets were at Horsley-Clarke co-ordinates P5, L and R 1.4, H -10 for the left and right PT and P10, L or R 0.5, H -5 for the MLF respectively. However, the final positions of the electrodes were adjusted on the basis of records of descending volleys from the surface of the lateral funiculus at the Th10 and the C3-C4 segments. The electrodes were left at sites from which distinct descending volleys were evoked at stimulus intensities of 10-20 μ A or less. At the end of the experiments these sites were marked with electrolytic lesions and their location subsequently verified on 100 μ m thick frontal sections of the brainstem cut in the plane of insertion of the electrodes using a freezing microtome. The MLF electrodes were usually placed at the level corresponding to the rostral border of the inferior olive and the PT electrodes at the level of the trapezoid body. In Fig. 1A and B the electrolytic lesions indicating stimulation sites in all 8 experiments are projected onto sections from one of these experiments.

In 2 experiments the MLF fibers were transected by a 3 mm wide blade mounted on a Horsley-Clarke manipulator and introduced in the midline a few mm rostral to the obex and a few mm caudal to the electrodes located in the MLF and the PTs. The blade was advanced until descending volleys evoked by 100 μ A stimuli applied in the MLF disappeared (in one experiment) or were reduced to about 90% (in the other experiment). Descending volleys from the PTs recorded at the C3 level were either unchanged or only moderately decreased (see results).

PT fibers descending to the spinal cord were transected by a lesion of the dorsal part of the lateral funiculus in the C3 segment (in one experiment), by lesions of the whole lateral funiculus in the Th 11 segment (in 2 experiments) and by hemisection of the spinal cord in the Th 11 segment (in 2 experiments). The C3 lesion was made under control of recording of descending volleys from the two PTs and from the MLF at the level of the C4 segment. The lesion was extended until the PT volleys disappeared while the MLF volleys remained unchanged (see results). The lesions of the lateral funiculus covered the whole area of the ventral part of this funiculus within which propriospinal neurons located in the C3-4 segments descend (Illert et al., 1978; Lundberg, 1979) but encroached on the lateral part of the ventral funiculus.

Stimulation and recording

Nerves innervating m. longissimus lumborum were stimulated with constant voltage stimuli (0.2 ms duration, at intensities 2 to 5 times threshold intensities for the most sensitive fibers in the nerve). For activation of fibers of the reticulospinal and corticospinal tracts, constant current cathodal stimuli (0.2 ms, 25-100 μ A) were applied. The stimuli were applied as single stimuli (at about 3 Hz) or in trains of 2-6 stimuli at 300 or 400 Hz (delivered at about 3Hz). Near maximal stimuli applied in the MLF were expected to activate a large proportion of ponto- and medullary reticulospinal tract fibers [see (Jankowska et al., 2003)], as needed for purposes of this study, but also other descending fibers which might complicate the interpretation of the results. However, any co-activated tectospinal and vestibulospinal tract fibers arising from the medial vestibular nucleus would not project as far caudally as the lumbar segments and fibers from the lateral vestibular (Deiter's) nucleus would not be activated by stimuli of 100 μ A

(Nyberg-Hansen and Mascitti, 1964; Hongo et al., 1975). Hence effects evoked in the lumbar segments following MLF stimulation can be attributed to reticulospinal fibers.

Descending volleys were recorded from the surface of the dorsal columns at the C3-C4 segments for PT volleys, or from the surface of the left or right lateral funiculus at the Th 10 and L2 or L3 segments, for MLF volleys. The volleys were recorded monopolarly via intact dura mater.

Glass micropipettes filled with 2M solution of potassium citrate were used for intracellular recordings from motoneurons; the tips of the electrodes were broken to about 1.5 μm with an impedance of 3-5 MOhms. Motoneurons innervating m. longissimus were identified by antidromic activation following stimulation of one of the dissected nerves.

Sample

The reported results are based on the analysis of records from 141 motoneurons in the L2 and L3 segments. 120 of these were identified as innervating m. longissimus lumborum by antidromic activation following stimulation of muscle nerve branches of the L2 or L3 spinal nerves and 21 motoneurons by monosynaptic input from these nerve branches. These motoneurons were recorded in preparations in which connections between both the PT and RS neurons and spinal neurons were left intact ("intact preparations") and after various lesions of the spinal cord.

PSPs were attributed to either ipsilateral or contralateral PT fibers when they were evoked at intensities at which the collision tests illustrated in Fig. 1 [see also Fig. 2 in (Jankowska et al., 2006)] did not reveal any spread of current from the pyramid on one side of the midline to the other. In the 7 experiments in which such tests were made, no spread of current was found at intensities up to 100 μA (in 1 experiment), up to 110 μA (in 5 experiments) and up to 150 μA (in 1 experiment). The main reported effects are therefore based on effects of stimulation of the PTs at 100 μA or less; stronger stimuli (150 or 200 μA) were used primarily to verify lack of PSPs of PT origin when they failed to be evoked at 100 μA .

In contrast to effects evoked from PTs, those evoked from the MLF could not be attributed to RS neurons descending either to the left or to right of the midline because of unavoidable spread of current from one side to another when the electrodes were placed less than 0.6 mm from the midline [see (Gustafsson and Jankowska, 1976)]. The exceptions were effects of MLF stimuli applied after hemisection of the spinal cord when only fibers descending on one side were left intact.

Analysis

Both the original data and averages of 10-40 single records were stored on line. Peak amplitudes or the areas of the averaged potentials were measured off line using a software sampling and analysis system (designed by E. Eide, T. Holmström and N. Pihlgren, University of Göteborg). Differences between samples were assessed for statistical significance using Student's t-test (for two samples assuming equal variances). * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Mean values are given as means \pm SEM.

Results

The network of neurons that were investigated regarding their role in mediating actions of PT neurons on longissimus dorsi motoneurons and the location of the lesions performed in order to verify, or to exclude, their involvement are outlined in Fig 2. We will demonstrate that the most direct coupling between PT neurons and longissimus dorsi motoneurons is disynaptic or trisynaptic and hence as direct as the coupling between the PT neurons and proximal limb

muscles. It will also be shown that effects of contralateral and ipsilateral PT neurons on these motoneurons are comparable, allowing back motoneurons to be controlled by both ipsilaterally and contralaterally located corticospinal neurons and that reticulospinal neurons with axons descending within the medial longitudinal fascicle (MLF) are the main relay neurons of PT actions on axial motoneurons.

PSPs evoked from PTs in preparations with intact spinal cord

Short latency PSPs from the contralateral PT—Stimuli applied within the contralateral PT evoked short latency EPSPs and/or IPSPs in all but two of 50 motoneurons tested. EPSPs were recorded in 39 (78%) motoneurons. Fig. 3 H-J illustrates the main features of these EPSPs, in particular that EPSPs evoked by successive stimuli were distinct and showed marked temporal facilitation. In the illustrated motoneurons small EPSPs were evoked already by the 2nd stimuli and appeared with increasing amplitudes and steeper rising phases after the 3rd and 4th stimuli. However, plot A in Fig. 4 (black columns) shows that short latency EPSPs from the contralateral PT were most often evoked only after the 3rd or 4th PT stimuli, rarely appearing after the 2nd stimulus and never after the 1st stimulus. These features, together with latencies of 4.16 - 4.99 ms from the 3rd or 4th stimuli (mean latency 4.50 ± 0.03 ms, Table 1 column 3, row 1) characterise these EPSPs as being evoked either disynaptically or trisynaptically (see Discussion). They were evoked at stimulus intensities as low as 40-60 μ A, although longer trains of such weaker stimuli were required (cf. Fig. 3 J-L).

Short latency IPSPs followed stimulation of the contralateral PT in 9 motoneurons; they are illustrated in Fig. 5. However, in 12 motoneurons the decay phases of disynaptic EPSPs evoked by the 3rd or 4th stimuli were faster than of those evoked by the earlier stimuli, in particular when the motoneurons were depolarized by 10-20 nA. This was taken to indicate that IPSPs were evoked in parallel with these EPSPs but at slightly longer latencies and hence that IPSPs were evoked in a total of 21/50 (42%) motoneurons. As shown in Table 1 (column 3, rows 2 and 10), latencies of IPSPs were about 1 ms longer than those of EPSPs (5.50 ± 0.12 ms).

The incidence of short latency IPSPs following successive stimuli in a train (Fig.5D) resembled that of EPSPs. They appeared most often following the 3rd or 4th, rarely after the 2nd stimuli and were never evoked after single stimuli.

Short latency PSPs from the ipsilateral PT—Short latency EPSPs from the ipsilateral PT were recorded in a smaller proportion of motoneurons (30/50; 60%) than from the contralateral PT. Similar to EPSPs evoked from the contralateral PT, they were most often evoked only by the 3rd or 4th stimuli (see Fig. 3 B-D and the histogram in Fig. 4A). They were also temporally facilitated, showed similar time characteristics and amplitudes (Fig. 4B) and were evoked at similar latencies (Table 1, column 3, rows 3 and 10) and thresholds (see Fig. 3 D-F).

Short latency IPSPs were observed in 22/50 (44%) motoneurons. They were the sole effect in 7 motoneurons (with examples in Fig.5B, C) and followed EPSPs in an additional 15 motoneurons. The IPSPs were evoked at practically the same latencies as IPSPs from the contralateral PT (Table 1 column 3, rows 4 and 8) and were about 0.7 ms longer than latencies of EPSPs (row 11).

Long latency PSPs from the contralateral and ipsilateral PTs—In addition to short latency PSPs, longer latency EPSPs and IPSPs often followed the 2nd and sometimes the 1st stimuli. They are illustrated in Fig. 3G, H and in Fig. 5F respectively. Long latency EPSPs (evoked at 9.73 - 15.8 ms) were more commonly observed following stimulation of the contralateral PT than of the ipsilateral PT, and were most conspicuous when the short latency EPSPs or IPSPs were small or absent. However, the appearance of short latency PSPs induced

by the 3rd-5th stimuli made it difficult to distinguish or determine the presence of the longer latency PSPs evoked by earlier stimuli.

PSPs evoked from the MLF

Short latency EPSPs and/or IPSPs from the MLF were found in all motoneurons tested. In contrast to those evoked from PTs, EPSPs from the MLF were most frequently evoked by single stimuli (Fig. 4 A) and at much shorter latencies. EPSPs in 23/44 motoneurons were evoked at latencies 0.5 - 0.8 ms from the descending MLF volleys and the earliest components were evoked by successive stimuli and displayed fairly constant amplitudes, indicating that they were evoked monosynaptically. Such EPSPs were recorded in 6/12 motoneurons tested when the stimuli were applied in the ipsilateral MLF and in 17/32 motoneurons from the contralateral MLF, but under conditions when ipsilaterally descending fibers could be co-excited (see Methods). Later components of 10 of these EPSPs, with examples in Fig 6 A, B and E, were delayed with respect to the onset of the earliest components by 0.4-0.9 ms (see Table 1, column 3, row 12) and appeared at latencies 1.0 - 1.4 ms from the descending volleys. EPSPs at the same latencies were also evoked in additional 11 motoneurons. In contrast to the earliest EPSPs, all the longer latency EPSPs showed marked temporal facilitation and both these features indicated that they were evoked disynaptically.

IPSPs were evoked instead of EPSPs in 9 motoneurons. However in 25 motoneurons IPSPs were concluded to follow EPSPs, as judged from the faster decay phases of EPSPs evoked by the 2nd-4th than the 1st stimuli, thereby indicating the incidence of the IPSPs in up to 34/44 (72%) of the motoneurons. The segmental latencies of the 9 IPSPs not preceded by EPSPs (1.0 - 1.7 ms) were on average 0.61 ms longer than the latencies of monosynaptic components of EPSPs (Table 1, column 3, row 12) and only 0.09 ms longer than the latencies of EPSPs classified as evoked disynaptically (Table 1 column 3, row 13 and Fig. 6C and F). Latencies of IPSPs overlapping with EPSPs generally fell within the same range as latencies of IPSPs that were not preceded by EPSPs.

IPSPs of MLF origin would have to be mediated by spinal premotor inhibitory interneurons as no evidence has been found for projections of inhibitory reticulospinal neurons to lumbar segments [(Grillner et al., 1968; Peterson et al., 1979; Stecina and Jankowska, 2007) see however (Baldissera and Roberts, 1975)]. In contrast, EPSPs evoked disynaptically from the MLF could be relayed via reticulospinal neurons activated by recurrent axon collaterals of fibers stimulated within the MLF, as indicated in Fig. 6G, as well as by spinal premotor interneurons. Records illustrated in Fig 6 show the rationale behind considering both of these possibilities. The appearance at the C3 level of second components about 1 ms after the earliest components of descending volleys following stimulation of the MLF (Fig. 6D, second dotted line) indicates that they reflected activation of more rostrally located neurons and would therefore be compatible with synaptic activation of additional RS neurons by the 2nd and 3rd stimuli. Although marginal after weak single stimuli, they increased with stronger stimuli and after successive stimuli. If the second components of EPSPs evoked by MLF stimuli (indicated by the 2nd dotted lines in Fig. 6B and E) were evoked by the second components of the descending volleys, both should appear at a similar delay with respect to the earlier components, as was indeed the case. Consequently, both the earlier and the later components of MLF evoked EPSPs could have been evoked by utilising direct connections between RS neurons and motoneurons indicated in the diagram of Fig. 2. However, the possibility that these disynaptic EPSPs are evoked via spinal interneurons interposed between RS neurons and motoneurons cannot be excluded on the basis of their latencies, as these fell within the same range as latencies of disynaptically evoked IPSPs (Fig. 6E and F; Table 1 column 3, row 15) which had to be relayed by spinal interneurons.

Which neurons relay PT actions?

Three main categories of neurons might relay disynaptically evoked excitation or inhibition of longissimus lumborum motoneurons by PT neurons. In Fig. 2 they are represented by green, grey and black relay neurons respectively. Green neurons denote reticulospinal (RS) neurons, while grey and black neurons represent two categories of spinal relay neurons. In order to differentiate between PT actions mediated via these neurons we used three different approaches. The first was to compare PSPs evoked from the two PTs after lesions eliminating direct contributions of either RS neurons (by transection of the MLF), or of PT neurons (by transection of PT fibers at a spinal level) with those evoked in preparations in which the spinal cord remained intact. The second was to investigate the convergence of PT and MLF actions on neurons hypothesised to relay these actions at spinal and supraspinal levels, and the third to compare latencies of PSPs of MLF and of PT origin in these preparations.

Effects of transection of the MLF—After transection of the MLF, PT stimuli failed to evoke EPSPs in any of the 27 motoneurons tested. As illustrated in Fig. 7, stimulation of either the contralateral or ipsilateral PT evoked only IPSPs. These were evoked by the 3rd, 4th or 5th PT stimuli at latencies of 6.00 ± 0.27 ms and 6.37 ± 0.24 from the contralateral and ipsilateral PT respectively and hence closer to the short-latency than the long-latency IPSPs described above. In the two experiments in which the MLF was lesioned, IPSPs were evoked in 7/16 and 10/11 motoneurons.

These results indicate that EPSPs evoked by PT stimuli are mediated predominantly, if not exclusively, via RS neurons. The same might be also true for the shortest latency IPSPs. The effects of MLF lesions could also, at least to some extent, be secondary to changes in the state of the animal. However there were no direct indications of this because the lesions did not result in changes in heart rate or blood pressure. In addition, control records from unidentified neurons located more dorsally revealed that the MLF lesions did not abolish all short latency effects of PT neurons. In these neurons EPSPs and IPSPs were evoked by PT stimulation at latencies as short as those in motoneurons recorded in intact preparations.

Effects of transection of ipsilaterally descending PT fibers—Effects of elimination of ipsilaterally descending PT fibers by transection of the dorsolateral part of the lateral funiculus or of the whole lateral funiculus at a cervical or thoracic level (see Methods) were strikingly minor, in particular as regards excitatory effects of PT stimulation. As shown in Table 1 (cf. column 4, 6 and 8, rows 1 and 3) the percentages of motoneurons in which EPSPs were evoked from either of the PTs after lesions to the lateral funiculus were similar to those of motoneurons recorded when the ipsilateral PT fibers were intact. Latencies of these potentials were likewise similar (any differences were not statistically significant) and amplitudes of EPSPs (Fig. 4D, F) were comparable to amplitudes of EPSPs recorded in preparations in which the spinal cord was intact (Fig. 4B) even though longer trains of stimuli were needed to evoke them after LF lesions (Fig. 4E). The only more conspicuous differences appeared to concern short latency IPSPs evoked from the contralateral PT. Fig. 8 illustrates the finding that weaker IPSPs were evoked from the contralateral PT than from the ipsilateral PT (cf. Fig. 8 K,L and N,O), until the length of the train of contralateral PT stimuli was increased (cf. L and M).

In order to investigate a potential contribution of ipsilaterally descending propriospinal neurons located between the C3 and Th11 levels and of ipsilaterally and contralaterally located lumbar interneurons to PT actions, effects of lesions restricted to the dorsal part of the lateral funiculus at the C3 level or to the entire lateral funiculus at the Th11 level or including both the lateral and ventral funiculus (hemisection) at the Th11 level were also compared.

Data in columns 3, 5 and 7 in Table 1 did not reveal any statistically significant differences that could indicate that ipsilaterally descending propriospinal neurons located in the C3-4 segments (Illert et al., 1978; Lundberg, 1979) were involved in relaying PT actions in addition to ipsilaterally descending reticulospinal tract fibers. The only more marked effect of lesions of the lateral funiculus was a reduced effectiveness of the 1st-3rd stimuli in the trains of PT stimuli, as most of the EPSPs appeared only following the 4th or even later stimuli (Fig. 4E), and smaller amplitudes of EPSPs from the ipsilateral than contralateral PT (statistically significant at $p < 0.02$) (Fig. 4F). However, this could be attributed to effects secondary to the transection of reticulospinal tract fibers descending within the lateral funiculus, as an additional decrease in the efficiency of PT stimuli occurred when all ipsilaterally descending reticulospinal tract fibers were transected (Fig. 4G,H) and amplitudes of EPSPs evoked from the MLF as well as from the PTs were significantly reduced.

Indications for common relay neurons of synaptic actions from the two PTs and the MLF—Two main observations indicate that synaptic actions evoked from the PTs and from the MLF were mediated by the same neurons: the qualitatively similar synaptic actions evoked in individual motoneurons from these three sources and the mutual facilitation of these actions. Opposite effects from the ipsilateral PT and contralateral PT were only encountered in one motoneuron.

Examples of the similar timing of EPSPs or IPSPs evoked from the two PTs are shown in Figs. 3, 5, and 8, while EPSPs or IPSPs evoked in parallel from the PTs and from the MLF are illustrated in Fig. 7. The most striking similarities between synaptic actions from both PTs and from the MLF occurred in the case of 9 motoneurons in which IPSPs were evoked instead of EPSPs. However, such qualitative similarities did not exclude quantitative differences. As shown in Table 1 (rows 1 and 3), stimulation of the contralateral PT evoked EPSPs in a larger proportion of motoneurons than stimulation of the ipsilateral PT, even though mean amplitudes of these EPSPs (Fig 4 BDFH; Figs 3, 8 and 9) were comparable. Differences were also found in the degree of inhibition evoked from the two PTs, but in this case with a tendency for IPSPs evoked from the ipsilateral PT to be larger, both when the spinal cord was intact (Fig. 3, Fig. 5) and after lesions of crossed PT fibers (Figs.8 and 9).

Joint application of subthreshold stimuli in the two PTs often rendered them effective, as has previously been demonstrated for effects of PT stimulation on hindlimb motoneurons (Jankowska et al., 2005). Both excitatory and inhibitory actions from the two PTs were facilitated in all 6 motoneurons tested, with examples in Fig. 9C-F (middle and right columns). Mutual facilitation also occurred between effects of subthreshold PT and MLF stimuli in all 4 motoneurons tested, with an example in Fig. 9C-F (left column), as previously found in hindlimb motoneurons (Edgley et al., 2004). Note that the facilitated PSPs (the onset indicated by dotted lines) appeared at the same latencies as EPSPs from the contralateral PT (A left) or ipsilateral PT (B middle) and as IPSPs from either the contralateral PT (C right) or ipsilateral PT (D right).

Such facilitatory effects provide evidence for converging input from the ipsilateral and contralateral PTs and from the PTs and the MLF on common relay neurons (Lundberg, 1964). However, it does not reveal where these common relay neurons are located. For example, disynaptic EPSPs appearing on joint stimulation of co PT and MLF (E in the left column in Fig. 9) could have been relayed by premotor interneurons as well as by RS neurons co-excited by PT fibers and recurrent collaterals of MLF fibers (see Fig. 6G). The latter possibility was in fact made particularly likely by the increase of the descending volleys illustrated in Fig. 9E (arrow). However, co-excitation of individual spinal interneurons by contralateral and ipsilateral PT fibers and by reticulospinal fibers running in the MLF has

previously been demonstrated [see (Jankowska et al., 2006; Jankowska and Stecina, 2007; Stecina et al., 2008b)].

Estimates of the relative contribution of ipsilaterally located interneurons and of commissural interneurons to PT actions were as difficult when the spinal cord remained intact as when it was hemisected. Theoretically, as shown the diagram in Fig. 2, commissural interneurons would be likely to mediate crossed actions after an ipsilateral hemisection (illustrated in the left column in Fig. 9) and ipsilaterally located interneurons to mediate uncrossed actions after a contralateral hemisection (illustrated in the middle and right columns in Fig. 9) However, the possible synaptic actions of crossed axon collaterals of reticulospinal tract fibers [see (Matsuyama et al., 1999) indicated by “?” in Fig. 2] make such interpretation less straightforward. Monosynaptic EPSPs evoked by contralaterally descending reticulospinal tract fibers after ipsilateral hemisection and thus attributable to such crossed collaterals were previously found in only 9% of hindlimb motoneurons (Jankowska et al., 2003) but they might have been evoked in as many as 19% of the present sample of motoneurons (Table 1 column 10, row 5), suggesting that they are more widespread in the rostral than in the caudal lumbar segments. Identifying these EPSPs as evoked monosynaptically was not unequivocal as their latencies (0.96 - 1.02 ms) fell at the border between latencies of monosynaptic and disynaptic EPSPs and they were tightly followed by later components that showed strong temporal facilitation. Nevertheless, if these EPSPs were evoked monosynaptically it would suggest that reticulospinal tract fibers not only affect motoneurons but also interneurons and via these, contribute to disynaptic EPSPs and IPSPs evoked in back motoneurons.

Discussion

The results of this study reveal (i) potent monosynaptic and disynaptic coupling between reticulospinal neurons and motoneurons innervating erector spinae muscles and (ii) di-, tri- and polysynaptic coupling between both contralateral and ipsilateral pyramidal tract neurons and these motoneurons. The results further provide evidence that pyramidal actions on erector spinae motoneurons may be relayed by both RS neurons and spinal interneurons, similar to their actions on hindlimb motoneurons (Lundberg, 1979; Edgley et al., 2004; Jankowska et al., 2006).

Coupling between corticospinal and reticulospinal neurons and erector spinae motoneurons

As shown in the Results section, stimulation of reticulospinal fibers in the MLF evoked monosynaptic EPSPs in the majority of motoneurons when ipsilaterally descending pathways were intact. However, EPSPs were also evoked at only marginally longer latencies when these pathways were transected at the Th 11 level of the spinal cord. Their presence would thus indicate a non-negligible contribution of crossed collaterals of reticulospinal neurons [see (Matsuyama et al., 1999)] to effects relayed via reticulospinal tract fibers. Disynaptically evoked EPSPs of MLF origin could thus be mediated by premotor interneurons located on either side of the spinal cord. However, disynaptic EPSPs could also be the result of re-excitation of RS neurons contacting motoneurons by axon collaterals of fibers stimulated within the MLF (Edgley et al., 2004).

EPSPs following PT stimuli were evoked at both relatively short and longer latencies. The latencies of the earliest EPSPs were close to latencies of disynaptic EPSPs evoked in hindlimb motoneurons [4.70 - 4.81 ms; (Jankowska and Stecina, 2007; Stecina and Jankowska, 2007)] but considerably shorter than latencies of trisynaptic EPSPs evoked via contralaterally descending pathways and commissural interneurons [6.07 - 6.72 ms; (Edgley et al., 2004; Jankowska et al., 2005)]. We may hence conclude that the shortest excitatory pathways between the PT neurons and back motoneurons are disynaptic. Latencies of IPSPs were consistently about 1 ms longer, indicating that the earliest IPSPs were evoked via trisynaptic pathways, as

would also be needed for the longer latency components of EPSPs. The results are thus fully consistent with the hypothesis that they are mediated via reticulospinal neurons (see Fig 2).

The most conclusive argument in favour of RS neurons mediating PT actions on longissimus lumborum motoneurons is the disappearance of disynaptic EPSPs after a lesion of the MLF and the persistence of these actions following lesions of the PT fibers at a spinal level. As RS neurons are co-activated by PT neurons from both hemispheres (He and Wu, 1985; Matsuyama and Drew, 1997; Kably and Drew, 1998), and descend ipsilaterally as well as contralaterally (Mitani et al., 1988), activation by PT neurons from one hemisphere could result in excitation of back motoneurons on both sides of the spinal cord. Furthermore, as activity of individual RS neurons is associated with activation of muscles of both ipsilateral and contralateral arm and shoulder muscles (Schepens and Drew, 2006; Davidson et al., 2007) they might also exert such bilateral actions on back muscles.

In individual motoneurons of our sample, EPSPs, IPSPs, or EPSPs followed by IPSPs were most often evoked in parallel by stimulation of the PTs and of the MLF, as expected for actions mediated by RS neurons. The only observations that might be considered at variance with this were the observations of only marginally longer latencies of PSPs evoked by PT stimulation after ipsilateral hemisection of the spinal cord, i.e. when axons of the ipsilaterally descending RS neurons that directly connect with motoneurons were transected and only contralaterally descending pathways remained intact. However, two possible explanations of these EPSPs might make them compatible with being mediated by RS neurons. One would be that disynaptic PT actions evoked after ipsilateral hemisection are mediated by crossed collaterals of RS axons descending contralaterally, provided that the number of such collaterals is much greater in the rostral than in the caudal lumbar segments (see above). Another possibility would be that these PSPs were relayed tri- rather than disynaptically, via very fast conducting RS neurons descending contralaterally and via exceptionally effectively activated and fast conducting commissural interneurons.

As the appearance of both short and long latency EPSPs of PT origin was prevented by transection of axons of RS neurones descending within the MLF these results are in contrast to effects of MLF lesions on synaptic actions evoked by PT stimulation on hindlimb motoneurons (Stecina et al., 2008a) in which disynaptic EPSPs appeared both when the MLF was intact and after MLF transection, indicating that PT excitatory actions on hindlimb motoneurons are relayed in parallel by spinal interneurons and by RS neurons. The results of the present study do not, however, allow the exclusion of such parallel actions on back motoneurons, because weak actions of PT fibers on their target interneurons might be subthreshold, unless combined with additional input from RS neurons, and co-excitation by PT and MLF fibers has been demonstrated for other premotor interneurons (Jankowska and Stecina, 2007).

The situation was different for IPSPs evoked by PT stimulation after MLF lesions, indicating that they might be mediated by spinal interneurons. Nevertheless, these IPSPs could also be mediated by RS neurons with axons joining the MLF within the most caudal part of the medulla below the lesion, or by neurons projecting via the lateral reticulospinal tract (Petras, 1967; Peterson et al., 1979). The latter were reported to affect thoracic back motoneurons (Peterson et al., 1979) and actions of these neurons might extend to interneurons inhibiting lumbar back motoneurons.

Pathways between RS neurons and motoneurons via which IPSPs are evoked should include an additional inhibitory interneuron in view of lack of evidence that inhibitory RS neurons project as far caudally as the lumbosacral segments (Wilson and Yoshida, 1968; Peterson et al., 1979). To account for IPSPs relayed by RS neurons the most direct inhibitory pathways

from RS neurons should thus be disynaptic. The interposed inhibitory interneurons could include interneurons located either ipsilaterally or contralaterally, including the lamina VII commissural interneurons with a previously demonstrated strong input from the MLF (Bannatyne et al., 2003) and bilaterally projecting inhibitory dorsal horn interneurons (Bannatyne et al., 2006; Bannatyne et al., 2009). However, inhibitory PT actions appeared to depend on reticulospinal neurons to a lesser extent than excitatory actions as they were present after MLF lesions and were somewhat more affected by lesions of the ipsilaterally descending PT fibers. A contribution of spinal interneurons activated more directly by PT fibers would thus be more likely for inhibitory than for excitatory interneurons.

Taken together, the reported observations provide evidence that disynaptic EPSPs of PT origin in *m. longissimus lumborum* motoneurons are mediated by RS neurons (although without excluding that they could be mediated in parallel by spinal interneurons) and leave open the possibility that trisynaptic EPSPs and IPSPs are mediated by both RS neurons and spinal interneurons.

Comparison with data on humans

Data from studies in humans using transcranial magnetic stimulation (TMS) to evoke contralateral and ipsilateral EMG responses in the erector spinae are broadly consistent with the observations of this study, as bilateral responses have frequently been observed, although not in all healthy subjects (Ferber et al., 1992; Taniguchi and Tani, 1999; Fujiwara et al., 2001; Kuppuswamy et al., 2008). Support for ipsilateral control of the erector spinae comes also from observations in patients following stroke. For instance, in the majority of stroke patients investigated by Fujiwara et al (2001) stimulation over the non-affected hemisphere evoked bilateral responses, whereas TMS of the affected hemisphere failed to evoke responses in either ipsilateral or contralateral erector spinae.

The activation or unmasking of ipsilateral pathways after unilateral damage to the corticospinal tract, as occurs after stroke, may explain the lack of impairment of erector spinae functions after stroke (Horak et al., 1984; Dickstein et al., 2004). Activation of erector spinae in advance of the moving limb could also be related to preferential activation of proximal rather than distal muscles during postural adjustments (Marsden et al., 1999) and the less severe paresis in proximal than in distal muscles. Sparing of uncrossed pathways after stroke may thus play a role in recovery of trunk muscle function and, by stabilizing the trunk and its movements, assist in the recovery of limb muscle activity, during rehabilitation programs.

The descending control of back muscles may also become of increased importance when reflex control of these muscles is impaired in idiopathic low back pain. Both acute and chronic back pain is associated with atrophy of the deep back muscles and deficits in postural control [see (Hodges et al., 2009)]. Patients with chronic low back pain have in particular been reported to have poor postural control in the absence of visual feedback (Radebold et al., 2001). It might thus be useful if these deficits were at least partly compensated by a more effective control of back muscles by PT and RS neurons activated in conjunction with planned voluntary movements.

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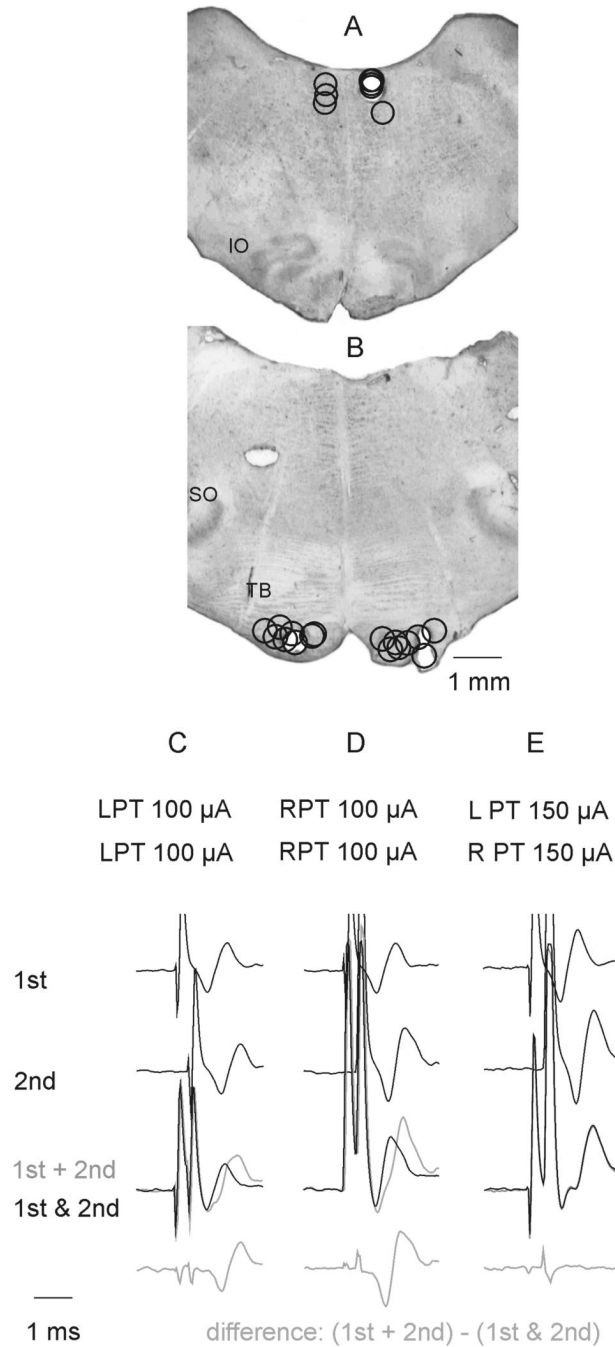


Figure 1. Stimulation sites and collision tests

A, Stimulation sites within the MLF and at its lateral borders as indicated by electrolytic lesions made at the end of the experiments. Stimulation sites to the left and right of the midline are for preparations in which the spinal cord was intact, or was hemisected on the left side respectively. IO, inferior olive. **B**, Stimulation sites within the left and right PTs. SO, superior olive; TB, trapezoid body. **C** and **D**, Examples of collision tests of effects of stimuli applied within the left or right PT respectively. They show that the 2nd stimulus applied about 0.3 ms after the 1st stimulus fell within the refractory period and failed to activate the same fibers. This is indicated by a difference between effects of the first & second stimuli applied together within this interval (black trace) and the sum of their effect when applied one at a time (grey trace)

shown at the bottom. **E**, Examples of records indicating that the stimuli applied within the left and right PT did not stimulate the same fibers because effects of the two stimuli were identical when applied jointly and when applied separately; note that that bottom trace did not show differences between them. In the illustrated case no spread of current was found from one PT to another at 150 μA but in other experiments this was the case only for stimuli ≤ 110 or 100 μA .

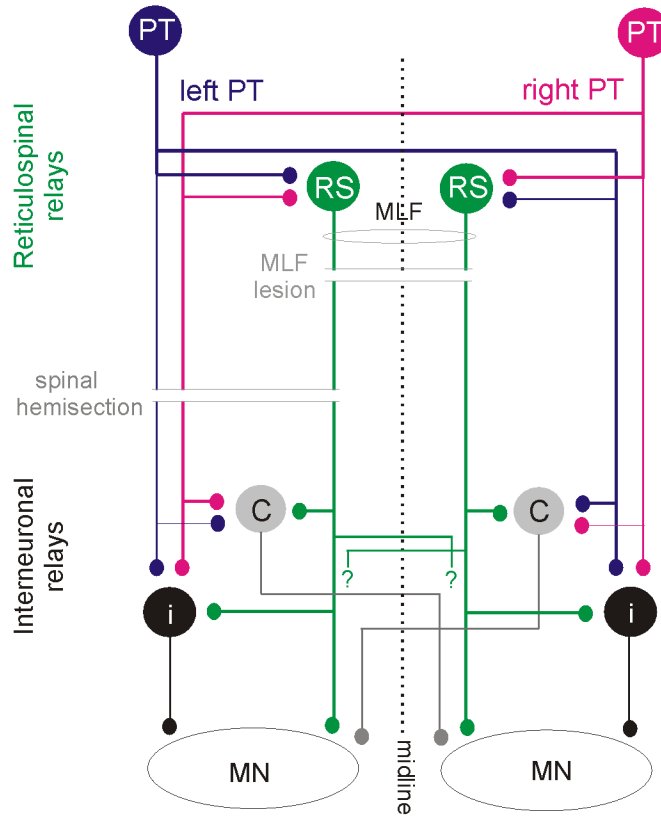


Figure 2. Diagram of hypothesized neuronal pathways from PT and RS neurons to back motoneurons and of two experimental approaches used to verify them
 Blue and red circles and lines represent left and right side pyramidal tract (PT) neurons and their projections. Green circles represent reticulospinal (RS) neurons with axons descending within the medial longitudinal fasciculus (MLF); crossed segmental axon collaterals with unspecified target cells are indicated with “?”. Grey and black circles represent spinal target neurons of the PT and RS neurons, black projecting ipsilaterally and grey contralaterally. Thinner lines denoting ipsilaterally descending PT fibers and axons of interneurons indicate weaker projections. Parallel horizontal lines indicate lesions of the MLF (made to test the relative contribution of RS neurons) and the spinal cord hemisection (made to test the relative contribution of all descending tract and propriospinal tract neurons on the left side as well as the relative contribution of ipsilaterally and contralaterally projecting spinal interneurons).

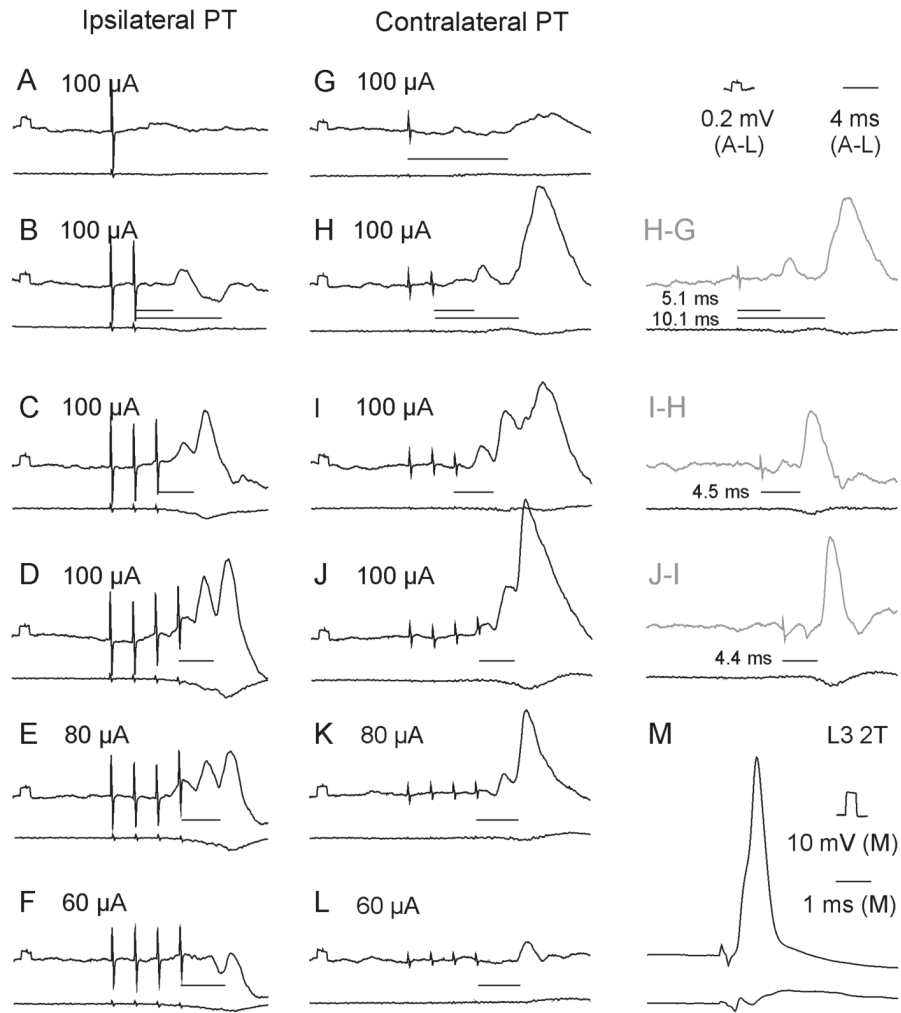


Figure 3. Examples of EPSPs evoked from the ipsilateral and contralateral PTs

Upper traces in **A-M** are intracellular records from a motoneuron in a preparation with the spinal cord intact. Lower traces are simultaneously obtained cord dorsum potentials. Averages of 20 records. **A-D** and **G-I**, EPSPs evoked by increasing numbers of stimuli at the same intensity (100 μ A). **D-F** and **J-L**, EPSPs evoked by decreasing intensities of a train of four stimuli. **M**, record of an action potential antidromically evoked by stimulation of nerve branches of the 3rd lumbar (L3) nerve. Records to the right of **H**, **I** and **J** show differences between effects of two and one, of three and two, and of four and three stimuli respectively. In this and the following figures the negativity is down in intracellular records and up in records from the cord dorsum. Rectangular pulses at the beginning of the intracellular records are calibration pulses. Horizontal lines indicate latencies of potentials evoked by the last stimuli.

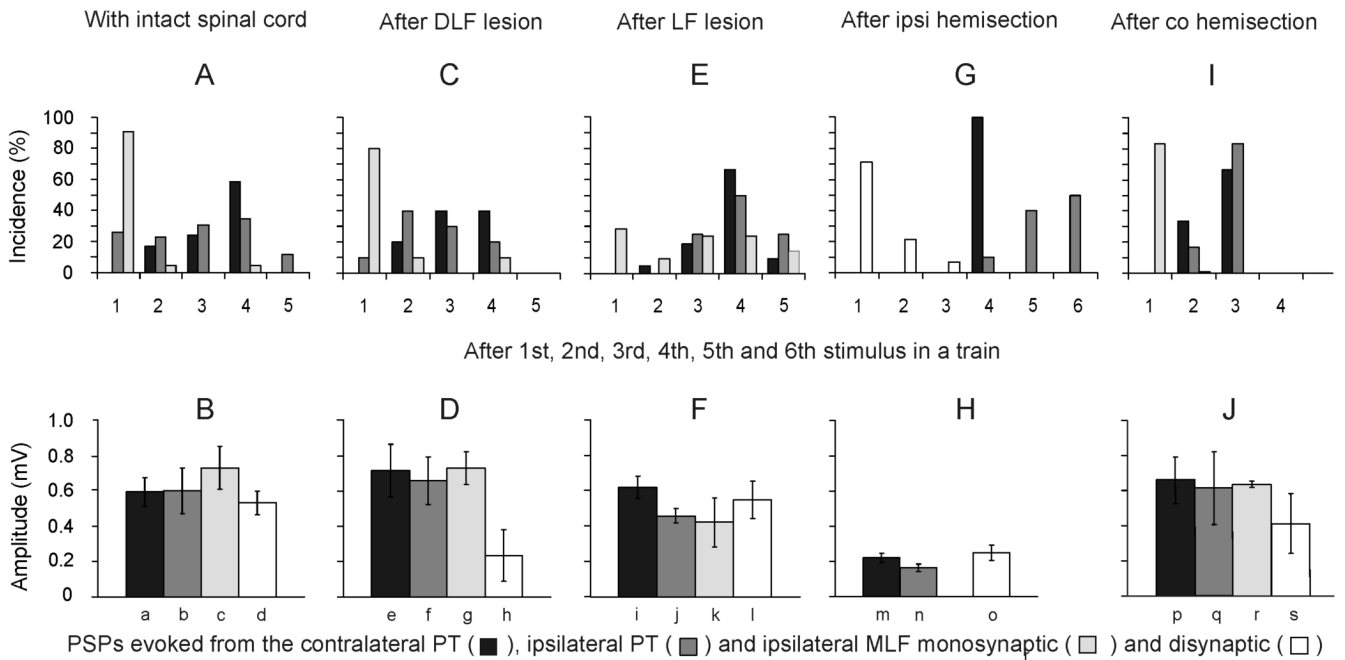


Figure 4. Comparison of stimulus related incidence and amplitudes of short latency EPSPs evoked from the contralateral and ipsilateral PTs and from the MLF

A, Proportions of motoneurons recorded in preparations with the spinal cord intact in which the first EPSPs were evoked by the 1st - 5th stimuli (100 μ A). The plots are for EPSPs with latencies not exceeding 5 ms for the contralateral PT (n = 29) and ipsilateral PT (n = 26), and for monosynaptic EPSPs from the MLF (n = 22). In the remaining motoneurons no EPSPs were induced by such stimuli or no comparison was made of effects of the varying numbers of stimuli. **C**, **E**, **G** and **I**, as in **A** but for preparations in which descending tract fibers were transected, as indicated, and for a mixture of monosynaptic and disynaptic rather than only monosynaptic EPSPs from the MLF in **G**. The lesions were of the dorsal part of the lateral funiculus (DLF; n = 12), the whole lateral funiculus (LF; n = 22), ipsilateral hemisection of the spinal cord (n = 14) and contralateral hemisection (n = 12). **B**, **D**, **F**, **H** and **J**, amplitudes of EPSPs evoked in the same samples of motoneurons. Amplitudes of EPSPs with characteristics of disynaptic EPSPs from the PTs were measured for those evoked by the second effective stimuli after having subtracted PSPs evoked by previous stimuli but without including EPSPs preceded by IPSPs evoked by earlier stimuli, or cut short by IPSPs that followed them. Amplitudes of monosynaptic EPSPs from the MLF were measured for those evoked by single stimuli in **B**, **D**, **F** and **J** but for combined mono- and disynaptically evoked EPSPs in **H** (bar o) because of difficulties in differentiating between them (see text). Amplitude of disynaptic EPSPs from the MLF were measured only in motoneurons in which no monosynaptically evoked EPSPs were evoked. No statistically significant differences were found between amplitudes of EPSPs evoked from the two PTs in the same preparation (except in **F** i-j *), or between those from PTs and monosynaptic EPSPs from the MLF in the same preparation. In contrast highly statistically significant differences were found between EPSPs evoked after ipsilateral hemisection and in other preparations (a-m**, b-n*, d-o**).

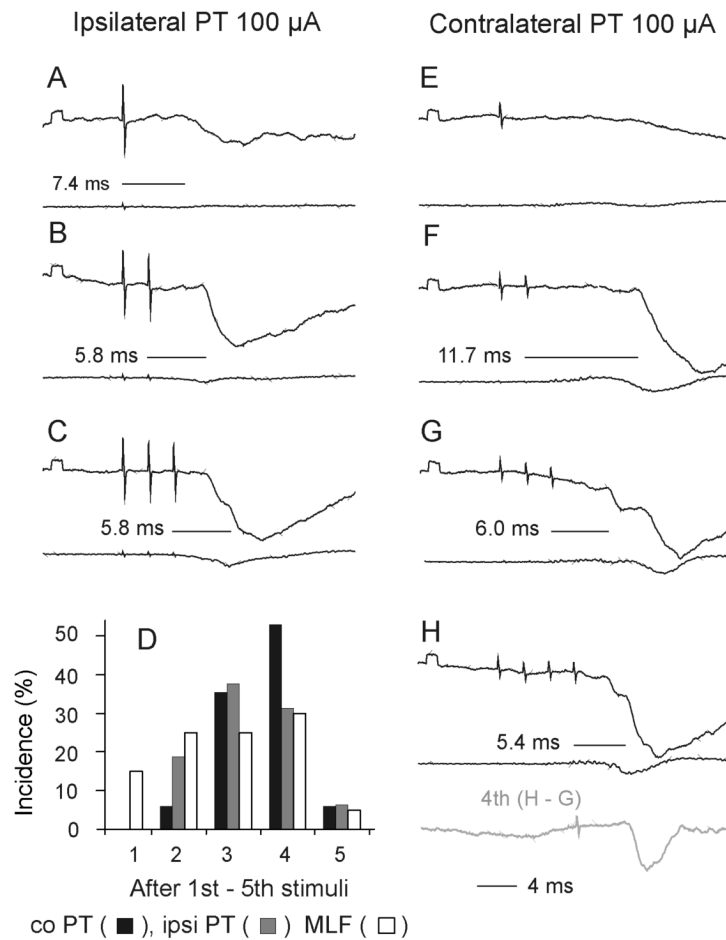


Figure 5. Examples of IPSPs evoked from the ipsilateral and contralateral PTs and their incidence Upper and lower traces in A-H are intracellular records from a motoneuron and cord dorsum potentials, respectively, in a preparation with the spinal cord intact. Averages of 20 records. A-C and E-H, IPSPs evoked by increasing numbers of stimuli applied within the ipsilateral and contralateral PT. D, Other indications as in Fig. 3. D, Proportions of motoneurons in which IPSPs with latencies not exceeding 6 ms appeared following successive stimuli (100 μ A) applied within the ipsilateral and contralateral PT and within the MLF when the previous stimuli were not effective in preparations with spinal cord intact, as in Fig. 4A for EPSPs.

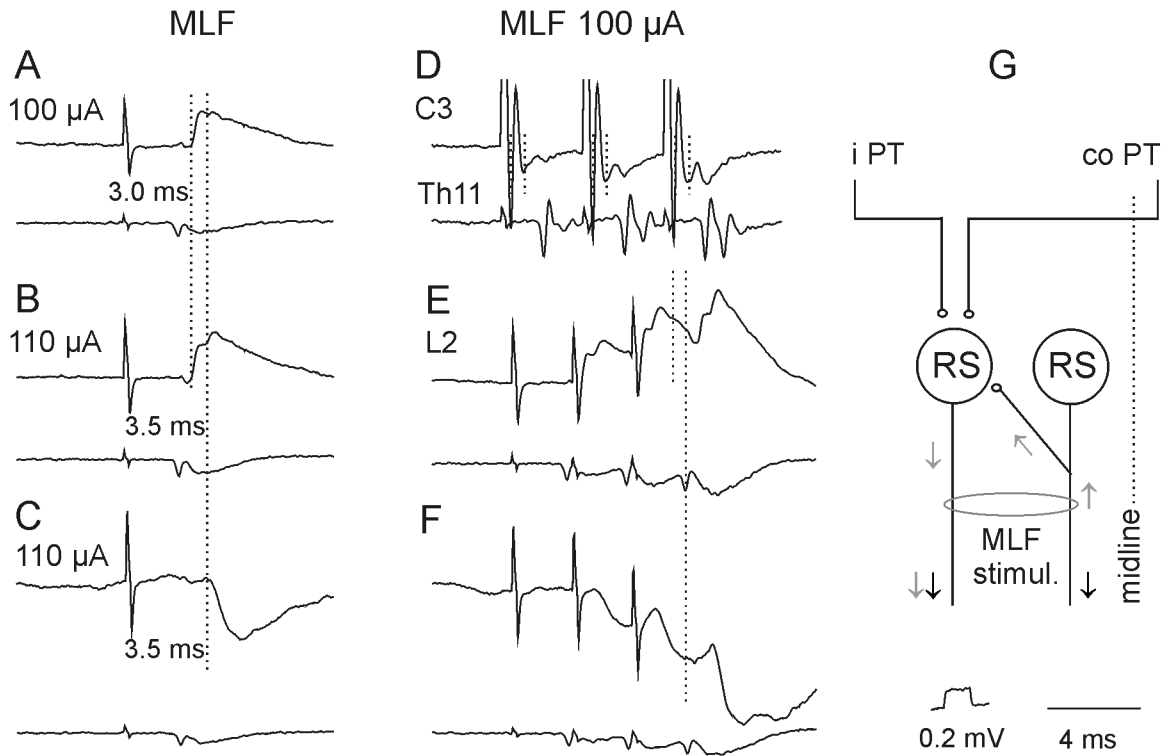


Figure 6. Examples of descending volleys and PSPs evoked from the MLF

D and lower traces in the remaining panels show descending volleys recorded at cervical, thoracic and lumbar segmental levels, respectively. Note that the early volleys in **D** were followed by later volleys, indicated by two vertical dotted lines, and that the second volleys were larger after each successive stimulus. Upper traces in **A**, **B** and **E** and in **C** and **F**, intracellular records from two motoneurons in the same preparation (with the spinal cord intact). Note that the early components of EPSPs (indicated by the first dotted line in **A**, **B** and **E**) were followed by later components (indicated by the second dotted lines). Note also similar latencies of the later components of the EPSPs and of the IPSPs with respect to the last stimuli. **G**, Diagram of a neural network allowing direct actions of descending volleys (black arrows) to be followed by recurrent actions evoked via axon collaterals of fibers stimulated within the MLF on RS neurons (grey arrows) and activation of the same neurons by axon collaterals of PT fibers [modified from Fig. 1 in (Edgley et al., 2004)]. Other indications are as in F.

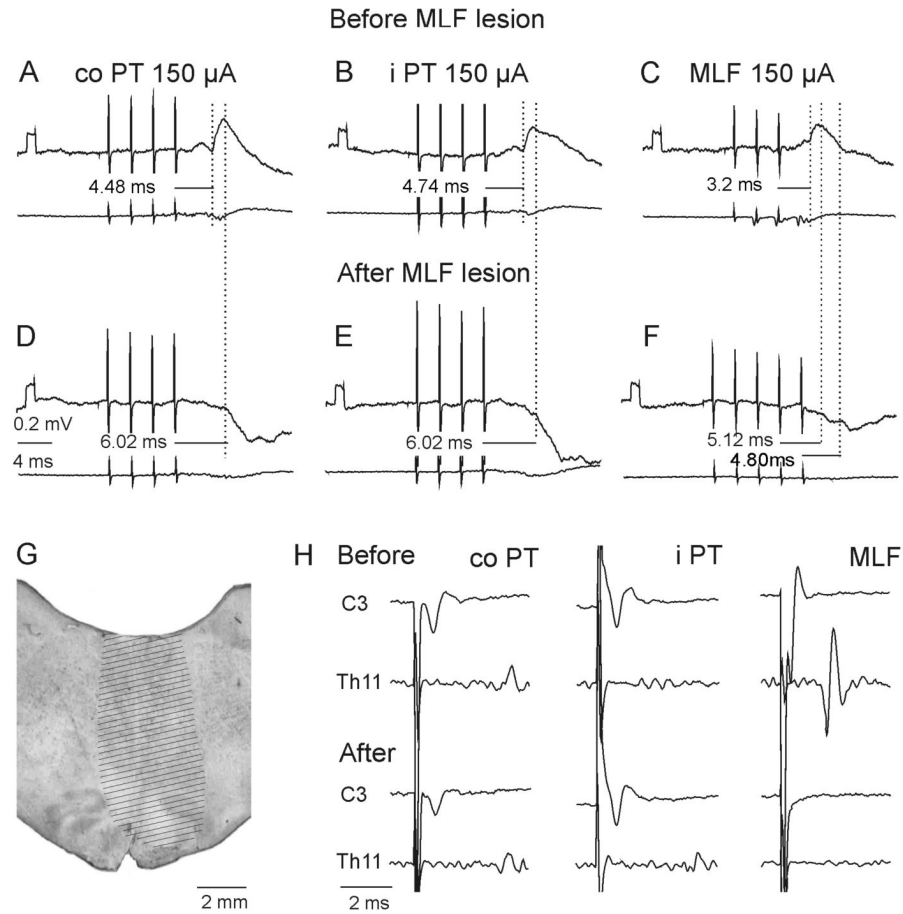


Figure 7. Effects of transection of the MLF on synaptic actions evoked by stimuli applied in the PTs and in the MLF

A-C and **D-F**, Intracellular records from two motoneurons and records from the cord dorsum in the same experiment. They were obtained before and after transection of the MLF about 5 mm caudal to the MLF stimulation site. **G**, Maximal extent of the lesion. **H**, records of descending volleys from the left lateral funiculus before and after the lesion (extended until the volleys following MLF stimulation disappeared). Note that the lesion abolished EPSPs but not IPSPs evoked by contralateral (co) PT, ipsilateral (i) and MLF stimuli. Note also that it abolished early volleys from the MLF but not, or only partially, cord dorsum potentials following PT stimuli.

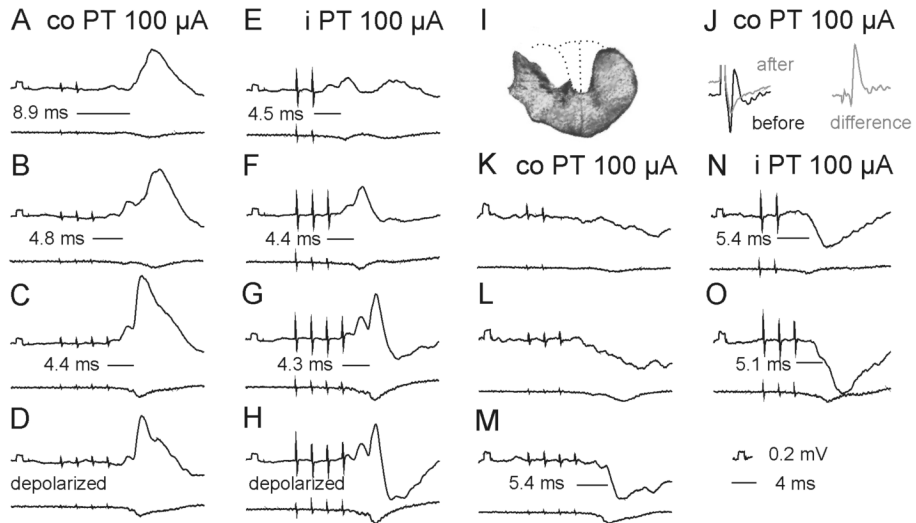


Figure 8. PSPs evoked by PT stimulation after a DLF lesion

In all panels, except J, upper traces are intracellular records from two motoneurons (A-H and K, L, M, N, O respectively) and lower traces are from the cord dorsum in the L2 segment, with the same format as in Figs. 3 and 5. All these records were obtained after a lesion of the dorsolateral part of the left lateral funiculus (DLF) in the rostral half of the C3 segment. A reconstruction of this lesion is shown in **I**. **J**, Cord dorsum potentials recorded in the C4 segment, evoked by stimuli applied in the co PT before (black) and after (grey) the lesion. The difference between these potentials is shown to the right. Note that EPSPs were evoked as efficiently from the contralateral (co) PT as from the ipsilateral (i) PT (and at the same latencies) but IPSPs required a longer train of stimuli (and were evoked at longer latencies, i.e. less efficiently).

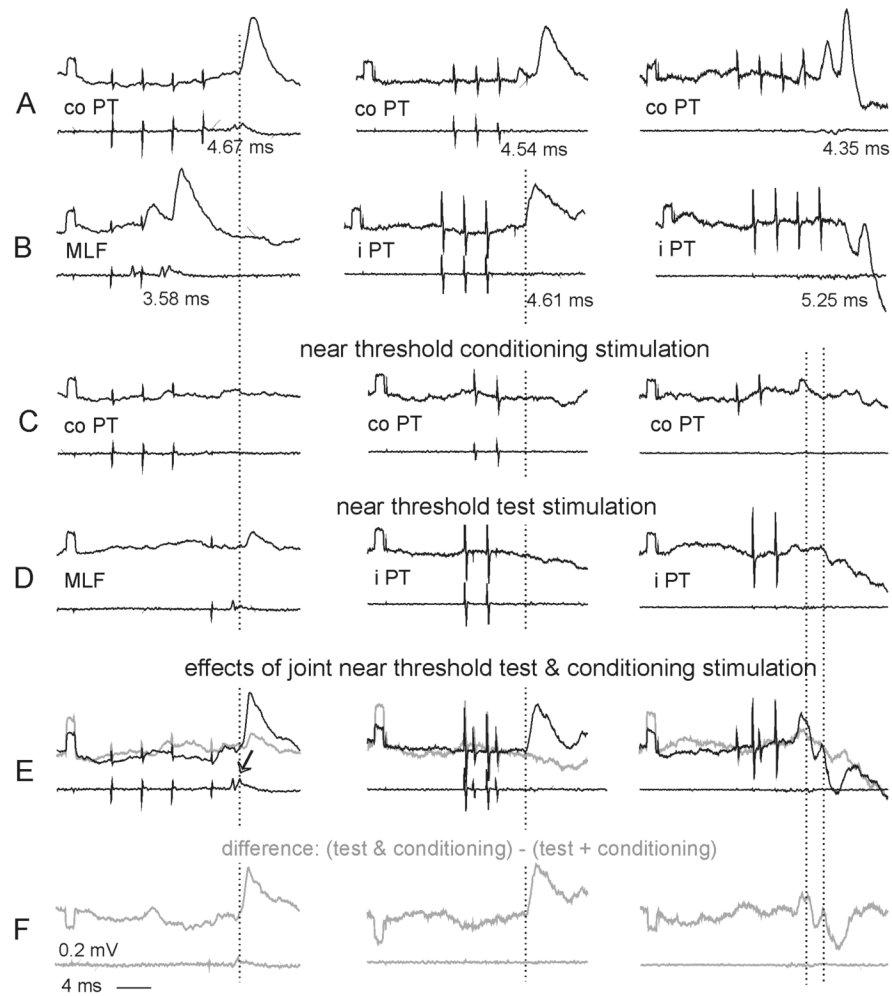


Figure 9. PSPs evoked by PT stimulation after a hemisection of the spinal cord

In all panels upper traces are intracellular records (20 averages) from three motoneurons recorded after ipsilateral (left column) or contralateral (middle and right columns) hemisection of the spinal cord at the Th11 segment. Lower traces are records from the cord dorsum. All of the potentials were evoked by stimuli of 100 μ A. Rows **A** and **B**, effects of 3 or 4 stimuli applied to the contralateral or ipsilateral PT or the MLF. Rows **C** and **D**, effects of shorter trains of these stimuli, that were either subthreshold or just at threshold for evoking EPSPs or IPSPs. Row **E**, effects of joint application of stimuli in rows C and D (black traces) and sums of effects in C and D (blue traces). Row **F**, differences between black and grey traces in row E, i.e. net effects of facilitation between effects of the two stimuli. The arrow in E indicates a larger 2nd component of descending volleys from the MLF after a preceding PT stimulation [see Fig. 4 in (Edgley et al., 2004)]. The records are timed such that the weaker stimuli coincide with either first or last stronger stimuli. Dotted lines indicate onset of the facilitated PSPs.

Table 1
Latencies and incidence of short latency EPSPs and IPSPs evoked in the analysed motoneurons

Vertical columns 1-10. Origin and category of the analysed PSPs and effects of stimulation the contralateral and ipsilateral PT and of the MLF upon them in preparations with the spinal cord intact, after lesions of the dorsolateral part of the ipsilateral lateral funiculus (DLF), the whole lateral funiculus (LF) and both the lateral and ventral funiculi (hemisection). **Horizontal rows 1-7, 9 and 11.** Latencies (means and SEM) and incidence of EPSPs and IPSPs evoked from the contralateral and ipsilateral PT and from the MLF. **Horizontal rows 8-19 in columns 3, 5, 7, 9 and 11.** Comparisons of latencies and incidence of EPSPs and IPSPs and of those evoked from the coPT, iPT and MLF. Statistically significant differences are indicated by * (p<0.05), ** (p 0.001-0.01) and *** (p<0.001), non statistically significant differences by ns; t-tests for paired two samples (when the number of pairs allowed such test) by p. **Horizontal rows 8-19 in columns 4, 6, 8, 10 and 12.** Differences in incidence (% of motoneurons) in the same combinations.

	origin of PSPs	PSP	intact (N=50)		DLF lesion (N=8)		LF lesion (N=35)		i hemisection (N=21)		co hemisection (N=11)	
			latency	%	latency	%	latency	%	latency	%	latency	%
1	1	2	3	4	5	6	7	8	9 vs intact	10	11 vs intact	12
	contralateral PT	EPSPs	4.50 ± 0.03	78	4.39 ± 0.04	75	4.57 ± 0.05	86	4.59 ± 0.08 ns	76	4.18 ± 0.07 ****	100
2		IPSPs	5.57 ± 0.12	42	6.23 ± 0.47	50	5.70 ± 0.06	60	5.62 ± 0.07 ns	62	5.52 ± 0.48 ns	27
3	ipsilateral PT	EPSPs	4.65 ± 0.05	60	4.30 ± 0.03	75	4.72 ± 0.04	66	4.76 ± 0.08 ns	42	4.32 ± 0.11 **	100
4		IPSPs	5.33 ± 0.29	44	5.49 ± 0.14	50	5.72 ± 0.10	35	5.55 ± 0.10 ns	62	5.70 ± 0.19 ns	27
5	MLF mono	EPSPs	2.99 ± 0.03	52	2.93 ± 0.03	50	3.21 ± 0.06	6	3.31 ± 0.59	19?	2.87 ± 0.03 *	73
6	MLF dis	EPSPs	3.54 ± 0.07	42	3.46 ± 0.1	13	3.44 ± 0.03	71	3.36 ± 0.04 ns	66	3.37 ± 0.07 ns	45
7		IPSPs	3.63 ± 0.04	72	3.88 ± 0.42	25	3.60 ± 0.13	9	3.60 ± 0.13 ns	14	3.61 ± 0.44 ns	27
8	co PT - i PT	EPSPs	-0.15 *** p	18	0.09 * p	0	-0.15 *** p	20	-0.17 * p	34	-0.1 ns	0
9	co PT - i PT	IPSPs	0.23 ns p	-2	0.74 ns	0	-0.02 ns p	25	0.07 ns p	0	-0.2 *	0
10	co PT	IPSPs-EPSPs	1.07 ***	-36	1.84	-25	1.12	-26	1.03 ***	-14	0.92 ***	-73
11	i PT	IPSPs-EPSPs	0.68 **	-16	1.19	-25	1.00	-31	0.79 ***	20	1.21 ***	-73
12	MLF d - m	EPSPs	0.55 ****	-10	0.54	-37	0.23	65	0.5 ****	0.5 ****	0.5 ****	-28
13	MLF d	IPSPs-EPSPs	0.09 ns	30	0.42	12	0.17	-62	0.24 ***	-52	0.27 *	-18
14	co PT - MLF m	EPSPs	1.51 ****	26	1.46	25	1.36	80			1.37 ***	27

	origin of PSPs	PSP	intact (N=50)		DLF lesion (N=8)		LF lesion (N=35)		i hemisection (N=21)		co hemisection (N=11)	
			latency	%	latency	%	latency	%	latency	%	latency	%
	1	2	3	4	5	6	7	8	9 vs intact	10	11 vs intact	12
15	co PT - MLF d	EPSPs	0.96 ***	36	0.93	62	1.14	15	1.23 ***	10	0.87 ***	-55
16	i PT - MLF m	EPSPs	1.67 ***	8	1.37	25	1.51	60			1.61 ***	27
17	i PT - MLF d	EPSPs	1.11 ***	18	0.84	62	1.28	-5	1.40 ***	-24	1.12 ***	-55
18	co PT - MLF d	IPSPs	1.94 ***	-30	2.35	25	2.09	51	2.02 ***	48	1.55 *	0
19	i PT - MLF d	IPSPs	1.70 ***	-28	1.61	25	2.11	26	1.95 ***	48	2.09 *	0