Propriospinal neurons are sufficient for bulbospinal transmission of the locomotor command signal in the neonatal rat spinal cord

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We recently showed that propriospinal neurons contribute to bulbospinal activation of locomotor networks in the *in vitro* neonatal rat brainstem–spinal cord preparation. In the present study, we examined whether propriospinal neurons alone, in the absence of long direct bulbospinal transmission to the lumbar cord, can successfully mediate brainstem activation of the locomotor network. In the presence of staggered bilateral spinal cord hemisections, the brainstem was stimulated electrically while recording from lumbar ventral roots. The rostral hemisection was located between C1 and T3 and the contralateral caudal hemisection was located between T5 and mid-L1. Locomotor-like activity was evoked in 27% of the preparations, which included experiments with staggered hemisections placed only two segments apart. There was no relation between the likelihood of developing locomotor-like activity and the distance separating the two hemisections or specific level of the hemisections. In some experiments, where brainstem stimulation alone was ineffective, neurochemical excitation of propriospinal neurons (using 5-HT and NMDA) at concentrations subthreshold for producing locomotor-like activity, promoted locomotor-like activity in conjunction with brainstem stimulation. In other experiments, involving neither brainstem stimulation nor cord hemisections, the excitability of propriospinal neurons in the cervical and/or thoracic region was selectively enhanced by bath application of 5-HT and NMDA or elevation of bath K^+ concentration. These manipulations produced locomotor-like activity in the lumbar region. In total, the results suggest that propriospinal neurons are sufficient for transmission of descending locomotor command signals. This observation has implications for regeneration strategies aimed at restoration of locomotor function after spinal cord injury.

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During locomotion, propriospinal neurons mediate forelimb–hindlimb coordination in quadrupeds (e.g. Miller *et al.* 1975; Halbertsma *et al.* 1976; Ballion *et al.* 2001; Juvin *et al.* 2005) and orchestrate intersegmental phase-lags in the lamprey (e.g. Matsushima & Grillner, 1990; Buchanan, 1992; Cohen *et al.* 1992; Miller & Sigvardt, 2000). Propriospinal neurons are also involved in selecting appropriate rhythmic motor patterns in the turtle (Berkowitz & Stein, 1994) and appear to have a role in coordinating arm–leg movements during walking in humans (for review see Dietz, 2002).

We recently showed that a propriospinal system of neurons also contributes to the descending activation of locomotor activity in response to brainstem stimulation (Zaporozhets *et al.* 2006*a*). This raises the possibility that regeneration of relatively short axonal connections belonging to this propriospinal system may restore propagation of the locomotor command signal across the site of spinal cord injury. Short propriospinal neurons are more vulnerable to contusion insults than long propriospinal projections; however, cell bodies of short propriospinal neurons are also relatively resistant to retrograde death after axotomy (Conta & Stelzner, 2004). Thus, if successful, re-establishing propriospinal connections across the lesion site may circumvent the need to develop potentially more difficult repair strategies aimed at regenerating direct bulbospinal projections over long distances.

However, in addition to propriospinal pathways, long direct pathways may have an essential role in transmitting the brainstem locomotor signal. For instance, long direct reticulospinal projections travelling in the

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ventral and ventrolateral fasciculi are thought to mediate bulbospinal activation of locomotion in the cat and rat (e.g. Steeves & Jordan, 1980, 1984; Eidelberg *et al.* 1981; Iwahara *et al.* 1991; Noga *et al.* 1991, 2003; Stelzner & Cullen, 1991; Magnuson & Trinder, 1997; Schucht *et al.* 2002; Matsuyama *et al.* 2004). Lloyd (1941) proposed a bulbospinal correlation system wherein brainstem motor impulses travel through long-projecting reticulospinal axons and mediate their influence on motoneurons via a propriospinal system. He suggested the propriospinal system was as an extension of the brainstem reticular nuclei, received input from vestibulospinal, corticospinal and primary afferent input, and set the state of the animal appropriate for initiation of voluntary movement. Thus, a critical issue with respect to functional recovery is whether re-establishing the propriospinal system alone, in the absence of regenerated long bulbospinal fibres, is sufficient to mediate descending activation of the locomotor network.

In order to examine whether the propriospinal pathway alone is capable of transmitting a functionally effective locomotor command signal, long direct pathways must be selectively blocked. Therefore, in the present study we tested the effect of staggered contralateral hemisections of the spinal cord on the capacity to induce locomotion in the lumbar region in response to electrical stimulation of the brainstem. In addition we examined whether neurochemical excitation of propriospinal neurons at the cervical and/or thoracic cord levels promoted locomotor activity in the lumbar region. Preliminary results were presented previously in abstract form (Zaporozhets *et al.* 2006*b*).

Methods

Experimental protocols used in this study were in compliance with the guidelines set by the Canadian Council on Animal Care and the University of Manitoba. Experiments were performed on 203 brainstem–spinal cord preparations isolated from Sprague–Dawley rats (1–5 days old). Isolation of the spinal cord, as well as methods of extracellular recording, were previously described (e.g. Cowley & Schmidt, 1995). In brief, animals were anaesthetized with isofluorane, decerebrated at the mid-collicular level, eviscerated, and placed in a bath chamber containing artificial cerebrospinal fluid (ACSF) composed as follows (mM): NaCl 128, KCl 4.0, NaH_2PO_4 0.5, $CaCl_2$ 1.5, $NaHCO_3$ 21, $MgSO_4$ 1.0 and glucose 30, equilibrated to pH 7.4 with 95% O_2–5% CO_2. Experiments were conducted at room temperature (ACSF approximately 22°C). In some experiments the spinal cord bath was partitioned using a barrier made of plastic strips sealed at cord contact edges with petroleum jelly.

Ventral root recordings were obtained using glass suction electrodes. The records were band-pass filtered (30–3000 Hz), digitized and captured using Axoscope (v. 9.0 Axon Instruments) software. Axoscope files were converted to an appropriate binary format for further analysis using special-purpose software (developed by the Spinal Cord Research Centre, University of Manitoba). Sigma-Stat was used for the logistic regression analysis.

Induction of locomotor-like activity

Electrical stimulation of the brainstem was performed according to our previously reported method (Zaporozhets *et al.* 2004). In brief, an ACSF-filled glass electrode, with a tip diameter of 200–300 μm, was placed in contact with the ventral surface of the brainstem. Monophasic rectangular current pulses (4–20 ms, 0.5–10 mA, 0.8–2.0 Hz) were delivered using bipolar stimulation. Stimulation was applied for a maximum of 2–3 min. If initial attempts to induce locomotion were unsuccessful, brainstem stimulation was periodically administered over the course of several hours. Some preparations developed locomotor-like activity soon after completion of the hemisections and placement of the recording electrodes. Other preparations initially failed to respond to brainstem stimulation but became responsive after several hours.

Combinations of *N*-methyl-D-aspartate (NMDA), 5-hydroxytryptamine (5-HT) and bicuculline (BIC) are capable of inducing lumbar rhythmic activity when applied to the brainstem (e.g. Smith & Feldman, 1987; Zaporozhets *et al.* 2006*a*). Thus, in some experiments, these neurochemicals were applied to selected spinal cord regions in an effort to excite cervical and/or thoracic propriospinal neurons and thereby induce locomotor-like activity in the lumbar cord. Neurochemicals were applied from concentrated stock solutions (1–10 mM) and all concentrations refer to final bath concentrations which ranged as follows: NMDA, 2–10 μM; 5-HT, 15–50 μM; and BIC, 10–20 μM.

Criteria for locomotor-like activity

We recorded from L2 and L5 ventral roots, bilaterally, in all preparations. Phasic lumbar ventral root discharge in the *in vitro* neonatal rat varies in pattern and quality depending on the preparation and experimental conditions. Ventral root records were classified as *locomotor-like* if the activity was rhythmic, featured appropriate side-to-side and flexor–extensor alternation pattern, and contained at least five successive ventral root bursts. Phasic ventral root discharge was considered *rhythmic* if the coefficient of variation (expressed as CV ± standard deviation) of the cycle period was $\leq 25\%$. The latter criterion is based

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on previous observations of cycle period CV in the *in vitro* neonatal rat preparation during locomotor-like activity (Cowley *et al.* 2005). The pattern was deemed consistent with locomotion if (a) contralateral alternation was observed between the left and right sides at the L2 level and/or between left and right sides at the L5 level, and (b) ipsilateral alternating activity between the L2 (predominantly flexor-related activity) and L5 (predominantly extensor-related activity) roots was present on at least one side. Thus, for example, alternating activity between the right L2 and L5 ventral roots is observed in the bilaterally hemisected preparation shown in Fig. 1*A*; however, this recording was rejected because of the lack of left–right alternation. It is acknowledged that the presence of rhythmic alternation between L2 and L5 discharge, without rhythmic activity on the contralateral side, may reflect locomotor network operation recruited on one side of the cord. However, for the purpose of this series, we used stricter criteria requiring that lumbar discharge be present bilaterally. There was no evidence in this series that lumbar locomotor-like activity was consistently lost on one side or the other in relation to the side with the caudal hemisection. Figure 1*B* shows another example of a rejected record. In this case left–right alternation was present, but no evidence of L2–L5 alternation was seen.

Hemisections and histological processing

Hemisections of the spinal cord were made using iridectomy scissors. The completeness of hemisections was verified by separating the sectioned tissue such that an unobstructed view of the bottom of the bath chamber was seen, using a surgical dissection microscope. After the *in vitro* experiment, spinal cords were stored in 4% paraformaldehyde. The cord was transferred to 15% sucrose in 0.1 M phosphate buffer 48 h before slicing the tissue on a cryostat. The tissue was stabilized with agar before sectioning. Serial axial sections (30 μm thick) were prepared starting in the segment immediately rostral to the hemisection and continuing through the hemisected region until bilaterally intact spinal cord was again encountered caudal to the lesion. Sections were stained with cresyl violet and a digital image of each section was captured. In order to illustrate the lesion extent, the image of a section from the hemisected region was superimposed on an image of bilaterally intact spinal cord tissue obtained immediately rostral or caudal to the lesion. Thus, the dark overlap region illustrates the extent of contralaterally preserved tissue at the hemisection site (see Figs 3, 4, 5 and 7*A*).

Results

In order to examine whether descending propriospinal transmission, in the absence of conduction in long direct bulbospinal pathways, was capable of activating locomotor activity in the lumbar cord, two approaches were used. Either the brainstem was stimulated electrically (in preparations with staggered contralateral hemisections) or propriospinal neurons in the rostral spinal cord were

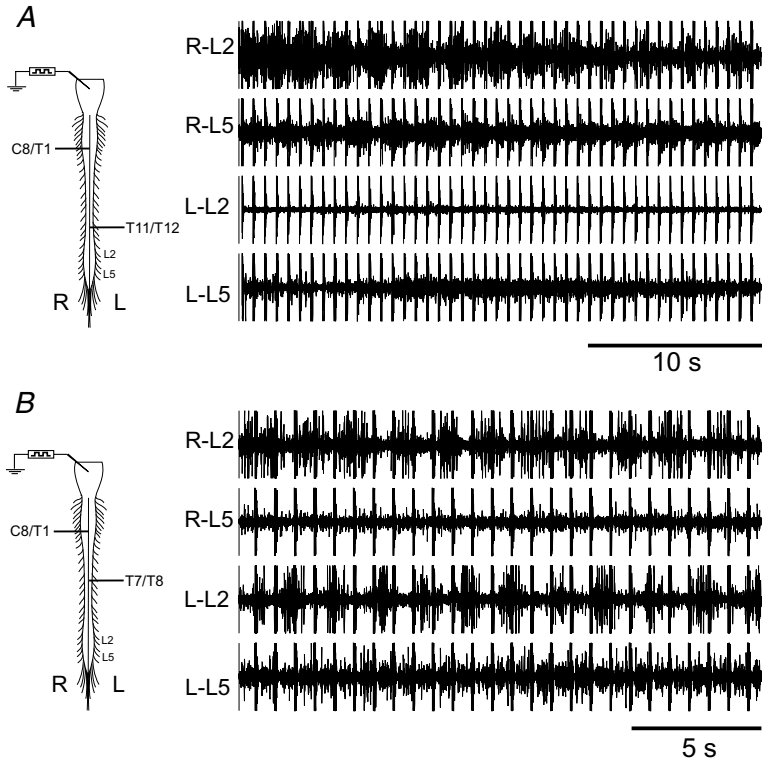

Figure 1. Two examples of lumbar ventral root rhythmic activity that did not meet criteria for locomotor-like activity
A, rhythmic alternating right (R) L2 and L5 was evoked in response to electrical stimulation of the brainstem, consistent with an alternating pattern of flexor and extensor activity, respectively. However, rhythmic activity was absent on the left (L) side and therefore side-to-side alternating activity could not be documented. *B*, in this example, left–right alternation is present at the L2 segmental level. However, the recording was not considered locomotor-like for the purposes of this series because of the absence of flexor (L2)–extensor (L5) alternation. Horizontal lines in spinal cord diagrams indicate hemisection levels. Regular occurring spikes in ventral root recordings are artifacts related to brainstem electrical stimulation.

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stimulated chemically (in unlesioned cords) in an attempt to induce locomotor-like activity in the lumbar region.

Staggered contralateral hemisections

Hemisection of the cervical or thoracic spinal cord disrupts bulbospinal axons projecting directly to the ipsilateral lumbar cord. In 174 preparations double hemisections, applied to opposite sides of the cord at various rostrocaudal levels, were used to disrupt all direct-projecting uncrossed bulbospinal projections. Using this protocol, ipsilaterally projecting propriospinal pathways were also disrupted. However, propriospinal axons that cross the midline in segments located between the two staggered contralateral hemisections should remain available for propagation of neural activity.

Stimulation of the brainstem evoked responses that met criteria for locomotor-like activity (see Methods) in 27% ($n = 47$) of the preparations. In contrast, electrical stimulation of the brainstem evokes locomotor-like activity in 93% of preparations without spinal cord lesions (Zaporozhets *et al.* 2004). The average number of ventral root bursts observed per brainstem stimulation episode was 13. Among successful attempts, the rostral hemisection was placed above the cervical enlargement (C1/C2, $n = 2$; C2/C3, $n = 21$; or C3/C4, $n = 4$), within the cervical enlargement (C5/6, $n = 1$), or below the cervical enlargement (C8/T1, $n = 13$; T1/2, $n = 2$; or T2/3, $n = 4$) as shown in Fig. 2*B*. The contralateral caudal

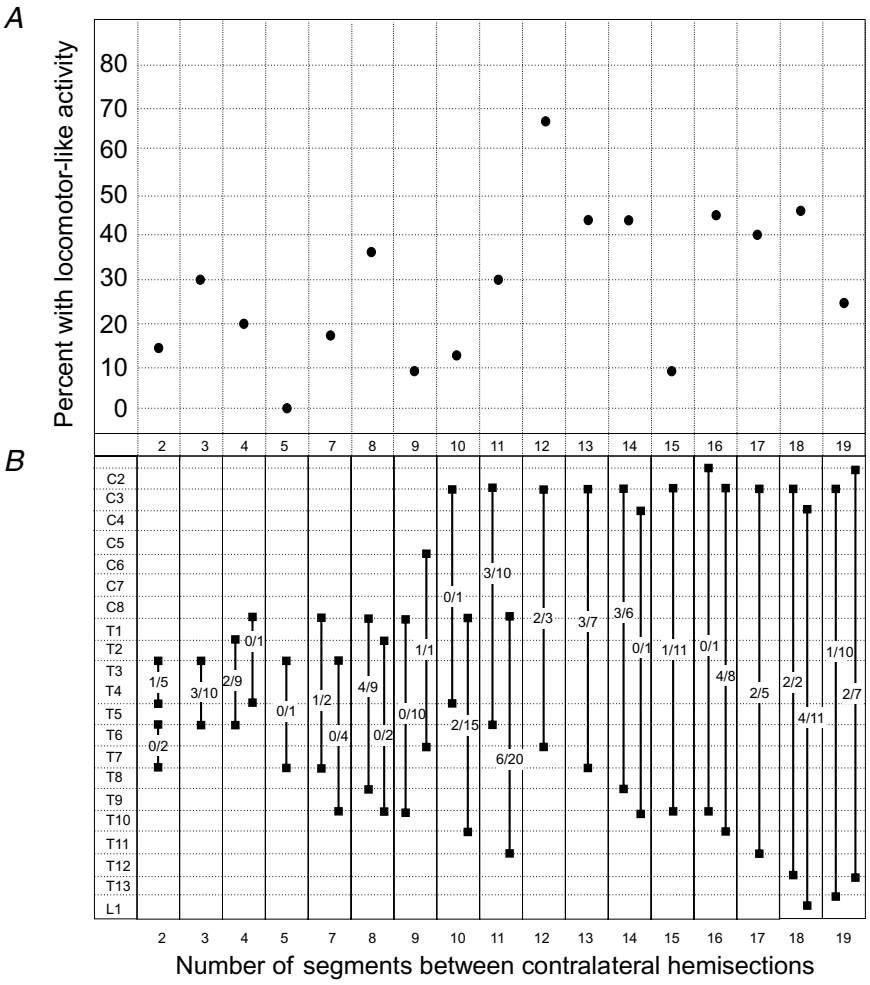

Figure 2. Segmental location of spinal cord hemisections
A, the percentage success rate of inducing locomotor-like activity is plotted against the number of segments located between staggered contralateral hemisections. Logistic regression analysis indicated no statistically significant correlation, $P = 0.261$. *B*, the segmental location of rostral and corresponding caudal hemisections for all preparations is illustrated. Fractions indicate the number of preparations displaying locomotor-like rhythm *versus* the total number of preparations tested with the specified lesion combination. A preparation with six segments between the two hemisections was not used.

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hemisection in successful preparations was located at T4/5 ($n = 1$), T5/6 ($n = 8$), T6/7 ($n = 3$), T7/8 ($n = 4$), T8/9 ($n = 7$), T9/10 ($n = 1$), T10/11 ($n = 6$), T11/12 ($n = 8$), T12/13 ($n = 4$), T13/L1 ($n = 1$) and at the mid L1 level ($n = 4$). The average cycle period CV was $14 \pm 6\%$ (range 2–25%). The average side-to-side phase lag of the left *versus* right ventral root burst at the same segmental level was 0.46 ± 0.11. The number of segments between the rostral and caudal hemisections was not correlated with the rate of success inducing locomotor-like activity (Fig. 2*A*, logistic regression $P = 0.261$).

An example of locomotor-like activity, with ipsilateral flexor (L2) and extensor (L5) alternation, as well as left–right alternation at the L2 and L5 levels, despite the presence of over-hemisection at right C2/3 and left T5/6, is shown in Fig. 3*A*. The rostral hemisection interrupted direct brainstem projections travelling in the right half of the spinal cord, including projections to the cervical enlargement; the lesion at T5/T6 blocked direct descending projections in the left side of the cord. Thus, descending signal cross-over occurred in the cervical enlargement and/or upper thoracic region. If, in this example, brainstem projections from the left side recruited propriospinal neurons participating in forelimb rhythm-generating centres bilaterally, then the question is raised whether preservation of cervico-lumbar ipsilateral connections in particular, at least on one side, is critical for the propagation of the locomotor command signal. Additional observations suggest this is not the case. The rostral hemisection was placed at the caudal end of the cervical enlargement in some experiments (e.g. C8/T1, Fig. 3*B*). In combination with a contralateral thoracic hemisection (e.g. T8/9, Fig. 3*B*) all direct ipsilateral connections between the cervical and lumbar enlargements would be blocked. Despite these lesions, the locomotor command signal successfully crossed-over in the thoracic region.

Locomotor-like activity was recruited bilaterally in the lumbar region, even if one of the hemisections was located in the caudal thoracic or upper lumbar region (Fig. 4). Thus bilateral lumbar locomotor network activity can be recruited by descending excitatory signals travelling unilaterally throughout the length of the thoracic cord.

Propagation of the brainstem locomotor command signal was still possible when only two or three bilaterally intact segments remained available for signal cross-over between the rostral and caudal hemisections (Fig. 5).

Neurochemical stimulation of propriospinal neuron cell bodies

Neurochemical manipulations were used to selectively activate neuronal cell bodies and not axons of passage (i.e. long direct bulbospinal projections). Thus 5-HT and NMDA, with or without bicuculline, were added to the cervical and/or thoracic bath in order to excite neurons in these regions. Two types of experiment were performed.

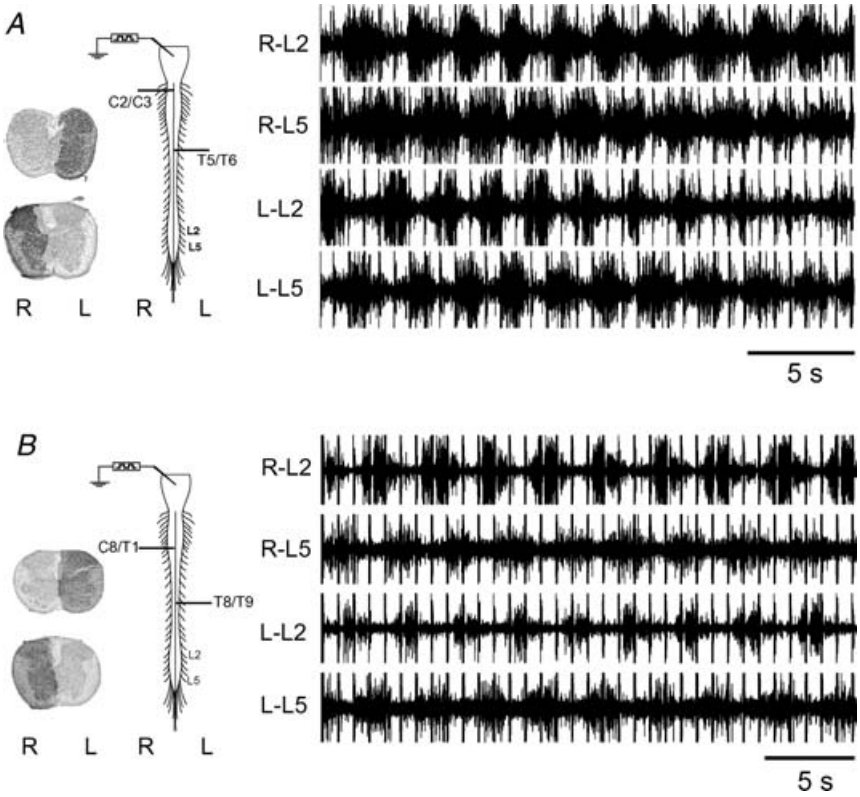

Figure 3. Locomotor-like activity evoked in response to electrical stimulation of the brainstem in the presence of staggered hemisections
A, locomotor-like activity, consisting of rhythmic alternation of ipsilateral L2 and L5 ventral roots and left–right alternation, was well developed (CV = 12%) in the presence of spinal cord hemisections located at right C2/3 and left T5/6. Note the hemisections extended beyond the midline ensuring interruption of all ipsilateral projections on the corresponding side. *B*, locomotor-like activity in this preparation was well developed (CV = 13%) despite hemisections located below the cervical enlargement (right C8/T1) and at left T8/9. Note, the dark region of the axial tissue sections indicates the extent of residual intact spinal cord at the lesion site, superimposed on the nearest section of non-lesioned bilaterally intact spinal cord.

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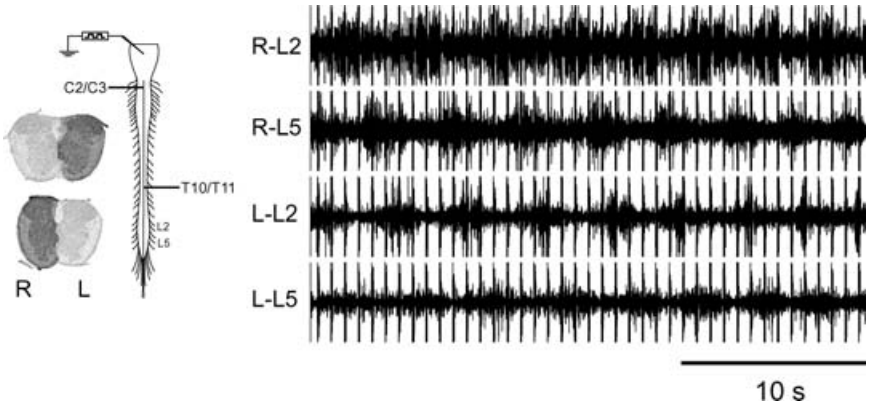

Figure 4. Locomotor-like activity (CV = 11%) evoked in response to electrical stimulation of the brainstem in the presence of staggered hemisections, one of which is located in the caudal thoracic cord (left T10/11)
The rostral right hemisection was made at C2/3. Thus, bilateral lumbar locomotor-like activity was recruited via descending excitation travelling in the right thoracic hemi-cord.

Bilaterally intact spinal cord preparations. In this group of experiments complete transections were made at the cervico-medullary junction and no cord hemisections were made. Application of 5-HT and NMDA to the C1–C4 bath compartment, with or without bicuculline, evoked rhythmic lumbar ventral root discharge in 27/27 preparations. The discharge pattern was considered locomotor-like in 8/27 preparations (Fig. 6, mean CV = 19 ± 4%). Similarly, application of neurochemicals to the C5–T5 or T4–T10 bath compartments produced locomotor-like activity in the lumbar region in 7/18 (mean CV = 17 ± 6%) and 3/6 (mean CV = 11 ± 2%) preparations, respectively. Locomotor-like activity was also evoked when neuron excitability was increased in 1/2 preparations by raising the K^+ ion concentration from 3.5 to 10 mM in the C5–T2 (CV = 15%) bath compartment. These results are compatible with previous studies reporting lumbar rhythmic activity in response to

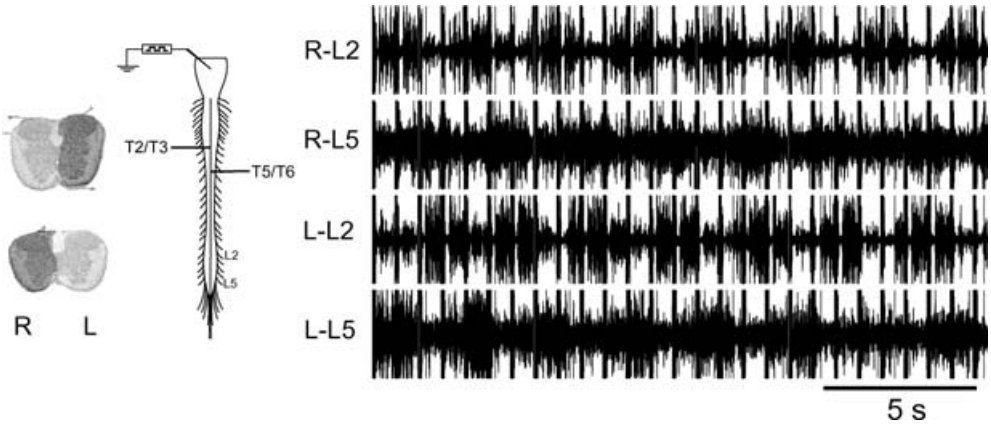

Figure 5. Locomotor-like activity (CV = 9%) evoked in response to electrical stimulation of the brainstem in the presence of closely spaced staggered hemisections
Only three segments were available, between the right hemisection at T2/3 and the left hemisection at T5/6, for left-to-right cross-over of descending transmission of locomotor excitation.

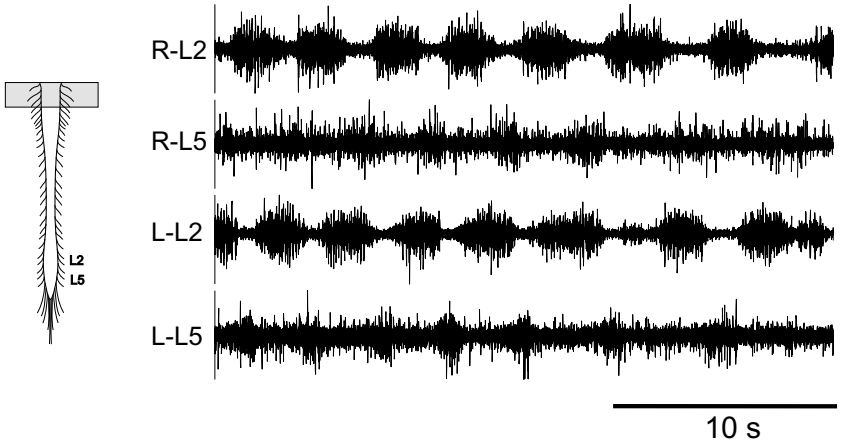

Figure 6. Locomotor-like activity (CV = 19%) was evoked in the lumbar region by chemical stimulation of propriospinal neurons in the rostral cervical region
Bath application of 5-HT (50 μM) and NMDA (15 μM) to spinal cord segments C1–C4 produced rhythmic alternating activity of left and right sides as well as ipsilateral L2 and L5 alternation.

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the application of 5-HT and NMDA to the cervico-thoracic cord (Cowley & Schmidt, 1997; Ballion *et al.* 2001).

Staggered hemisection preparations. In this group of experiments we examined whether brainstem stimulation of double hemisected cords, combined with enhanced excitation of propriospinal neurons, facilitated locomotor command signal propagation in preparations that otherwise failed to display locomotor activity during brainstem stimulation alone (e.g. Fig. 7*A*). Because locomotor-like activity can be directly induced by whole cord neurotransmitter application, 5-HT and NMDA were applied at concentrations subthreshold for rhythmogenesis (e.g. Fig. 7*B*). Bicuculline was not used in these experiments. In 10/42 such preparations the combination of 5-HT, NMDA and electrical stimulation of the brainstem elicited locomotor-like activity (CV $11 \pm 3\%$, e.g. Fig. 7*C*).

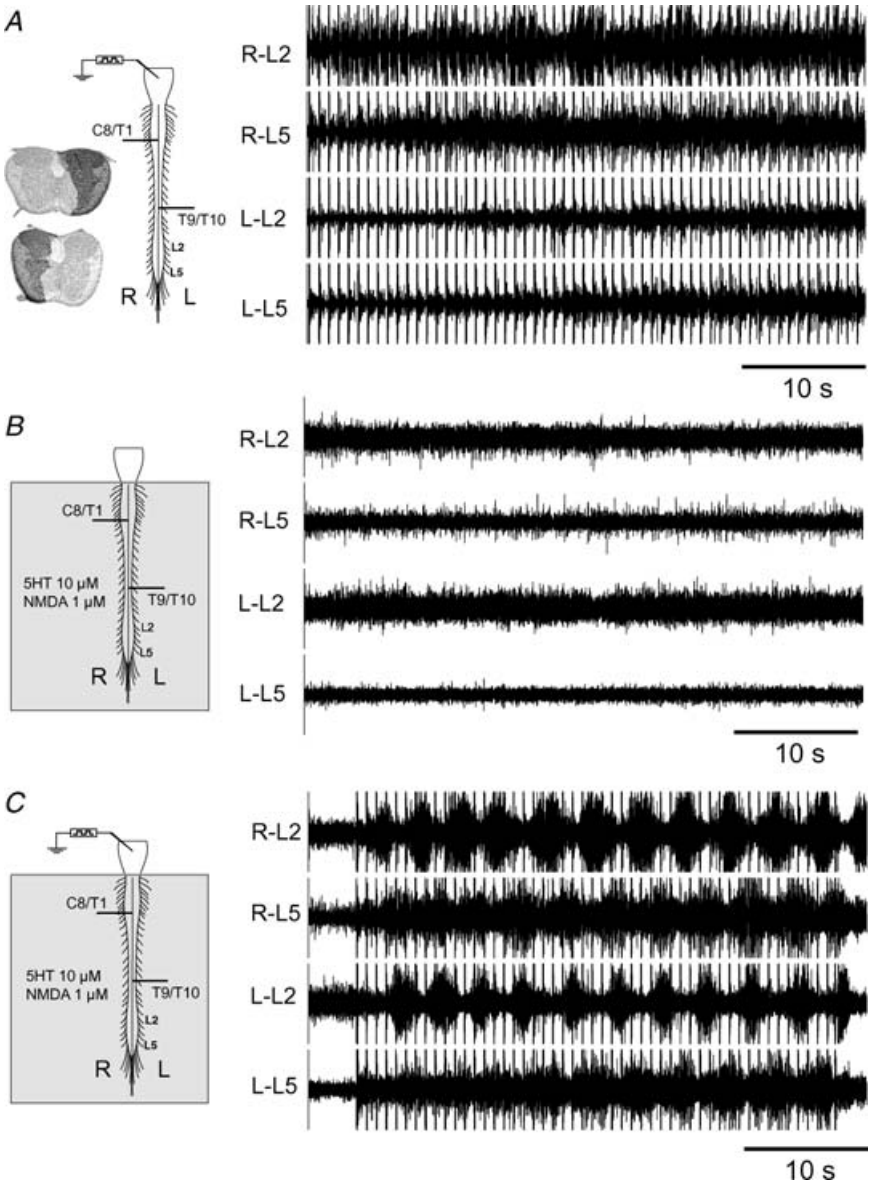

Figure 7. Neurochemical stimulation of the spinal cord facilitated propagation of the bulbospinal locomotor command signal
A, electrical stimulation of the brainstem failed to evoke a locomotor-like pattern in this preparation. *B*, whole cord application of 5-HT (10 μM) and NMDA (1 μM) was subthreshold for the induction of locomotor-like activity. *C*, electrical stimulation of the brainstem, in combination with subthreshold concentrations of 5-HT (10 μM) and NMDA (1 μM), evoked locomotor-like activity.

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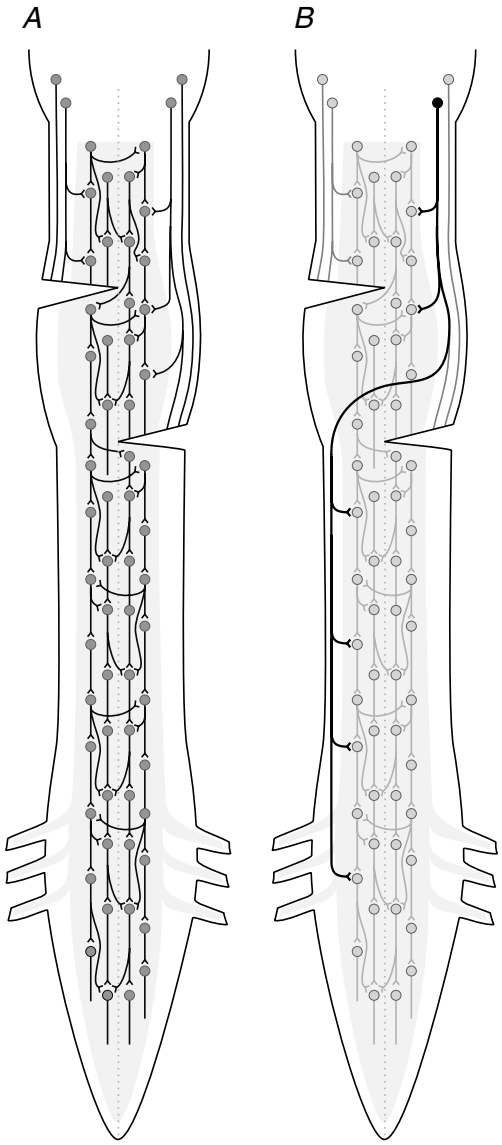

Figure 8. Hypothetical representation of propriospinal and direct bulbospinal systems involved in descending transmission of the locomotor command signal
A, the present data suggest that propriospinal neurons alone may be sufficient to mediate descending activation of locomotor networks. The range of projection length of individual locomotor-related propriospinal neurons is unknown. In addition to the propriospinal system, some bulbospinal axons or their collaterals may activate contralateral locomotor-related neurons via commissural projections. *B*, there is no direct evidence that locomotor-related bulbospinal projections can cross the midline in the spinal cord and continue descending long distances, without an intervening synapse, on the contralateral side; however, this possibility is not absolutely excluded.

Discussion

The results demonstrate that a locomotor command signal originating in the brainstem propagates caudally despite disruption of long direct axonal projections to the lumbar cord. In addition, because fibres lesioned by staggered hemisections also include part of the propriospinal system, the observations show that even partial preservation of propriospinal pathways is sufficient for transmission of the locomotor signal in the neonatal rat preparation.

Do bulbospinal projections cross the midline in the spinal cord?

Our interpretation of the results assumes propriospinal, but not long direct bulbospinal, projections underlie the propagation of the locomotor command signal that crosses the midline to the opposite side of the cord and continues caudally to the lumbar region (Fig. 8*A*). It is well established that propriospinal neurons may be ascending or descending, short or long, and project ispilaterally or contralaterally (e.g. Sherrington & Laslett, 1903; Lloyd, 1941; Barilari & Kuypers, 1969; Jankowska *et al.* 1973; Vasilenko, 1975; Matsushita *et al.* 1979; Skinner *et al.* 1979; Menetry *et al.* 1985; Alstermark *et al.* 1987; Sherriff & Henderson, 1994; Eide *et al.* 1999; Butt & Kiehn, 2003; Strauss & Lev-Tov, 2003; Conta & Stelzner, 2004; Reed *et al.* 2006). It is also known that bulbospinal projections can mediate contralateral spinal effects via ipsilateral synaptic contact with spinal neurons, which then project across the midline (e.g. Scheibel & Scheibel, 1966). However, less is known about whether any long bulbospinal axons are capable of decussating in the cervical or thoracic region and then descending on the contralateral side (without an intervening synapse) as direct projections to the lumbar region (Fig. 8*B*). If such a decussating bulbospinal system exists, it could in theory contribute to descending propagation of the locomotor signal, in addition to crossed propriospinal pathways, in the presence of staggered contralateral hemisections.

Stelzner & Cullen (1991) injected [^{3}H]proline and horseradish peroxidase into the lumbar cord of newborn rats subjected to bilateral staggered hemisections in the mid-thoracic region. Retrogradely labelled cells were found in the intermediate grey matter of the interlesion zone bilaterally, consistent with propriospinal neurons, and no evidence of cell labelling was found in the brainstem (Stelzner & Cullen, 1991). However, these authors point out several limitations of the labelling technique. In addition, the short interlesion length of thoracic cord (1–3 segments) may have contributed to a failure to detect any bulbospinal fibres that traversed the interlesion zone.

Considerable evidence implicates an important role for reticulospinal projections in the control of locomotion in mammals (e.g. Orlovsky, 1970; Mori *et al.* 1983; Shefchyk *et al.* 1984; Garcia-Rill & Skinner, 1987; Noga *et al.* 1988; Rossignol *et al.* 2006) and other vertebrates (e.g. Deliagina *et al.* 2000). For instance some descending reticulospinal axons terminate in the ventromedial grey

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region, which contains neurons that are rhythmically active during locomotion, including commissural neurons (Harrison *et al.* 1986; Huang *et al.* 2000; Antonino-Green *et al.* 2002; Butt *et al.* 2002; Lanuza *et al.* 2004; Hinckley *et al.* 2005; Wilson *et al.* 2005; Matsuyama *et al.* 2006). Electrophysiological studies (Jankowska *et al.* 2003) have shown that reticulospinal axons originating on one side of the cat brainstem terminate bilaterally in the mid to lower lumbar and sacral regions. Of note, however, reticulospinal axon collaterals which cross the midline in the lumbosacral region terminate at the same segmental level (Kausz, 1991). Thus, in the present study, these particular long direct projections are not candidates to mediate the descending locomotor command because they would have been severed by the staggered hemisections made in the cervical and contralateral thoracic regions. Peterson *et al.* (1975) used microelectrode stimulation and recording techniques to show that some reticulospinal axon collaterals cross the midline in the cervical cord. More recently, Matsuyama *et al.* (1997, 2004) employed *Phaseolus vulgaris* leucoagglutinin to determine the termination patterns of reticulospinal axon collaterals originating from gigantocellular tegmental field (FTG) neurons in the cat. Approximately 20% of FTG collaterals innervated the contralateral (as well as ipsilateral) grey matter in the cervico-thoracic region. However, similar to the observations of Kausz (1991), the rostro-caudal extent of the collateral terminations was less than 1 mm. Therefore, if these bulbospinal axons transmit excitation to the contralateral lumbar region an interposed propriospinal neuron in the contralateral cervico-thoracic grey matter would be required.

Some corticospinal tract fibres re-cross the midline within the spinal cord of dogs and monkeys (e.g. Sherrington, 1889; Liu & Chambers, 1964; Kuypers & Brinkman, 1970) and cats (Satomi *et al.* 1988: Li & Martin, 2002). However, the corticospinal tract is not a likely critical pathway for the activation of locomotor activity (Steeves & Jordan, 1980; Loy *et al.* 2002; Schucht *et al.* 2002), although it does have a role in skilled limb movements during locomotion (e.g. Metz *et al.* 1998; Muir & Whishaw, 1999; Drew *et al.* 2002; Kanagal & Muir, 2007). Moreover, with respect to the present study, although most supraspinal inputs to the lower spinal cord are present at birth (Leong *et al.* 1984; Kudo *et al.* 1993), corticospinal tract axons are not found in the lumbar grey matter until postnatal day 9 (Donatelle, 1977).

Lateral pontine noradrenergic projections to the spinal cord have also been studied. Fluorescent and biochemical examination of rat coerulospinal projections indicate these fibres decussate throughout the spinal cord; more specifically, approximately 50% of the coerulospinal noradrenaline content on one side of the cord derives from the contralateral locus coeruleus (Karoum *et al.* 1980; Commissiong, 1981; also see Davies *et al.* 1983). However, these studies do not indicate whether coerulospinal axons continue to course caudally after crossing the midline. In the monkey, most descending projections from the lateral pontine region travel through the ipsilateral spinal cord, although a small number of subcoeruleus/parabrachial axons descend in the contralateral cervical, thoracic and lumbosacral lateral funiculus (Westlund & Coulter, 1980). The level at which subcoeruleus and parabrachial projections cross (i.e. brainstem *versus* spinal cord) is unclear, as is the extent to which these axons travel caudally after crossing the midline (Westlund & Coulter, 1980). Locus coeruleus projections cross in the lower lumbar and sacral regions (Westlund & Coulter, 1980). A study of cat lateral pontine neurons (locus coeruleus, subcoeruleus, Kolliker-Fuse, and lateral parabrachial) showed that axons project ipsilaterally to thoracic segments, decussate starting in the lower lumbar segments, and display extensive bilateral representation in the sacral cord (Kausz, 1986). These long descending projections, which cross in lumbosacral spinal cord regions, would have been lesioned by the staggered hemisections used in the present series. Experiments involving unilateral Evans blue injection into the cat lumbar cord suggested that lateral pontine neurons innervating the lumbar cord crossed above and below mid-thoracic hemisections (Stevens *et al.* 1985). Thus, the literature on descending projections from the lateral pontine region suggests that long direct crossed pathways reaching the lumbar cord may exist. If this is the case in the neonatal rat, the staggered contralateral hemisections used in the present experiments may not have abolished all direct bulbospinal projections to the lumbar cord. Nevertheless, even if such pathways exist our observation that neurochemical stimulation of the rostral cervical cord, which does not activate bulbospinal axons of passage, induced locomotor-like rhythm in the lumbar segments supports the idea that a propriospinal system, in isolation, has the capacity to mediate descending activation of the locomotor network.

Implications for functional recovery

A consistent observation in rodents (Feringa *et al.* 1976; Malmsten, 1983; Little *et al.* 1988; Ballerman & Fouad, 2006), cats (Jane *et al.* 1964; Kato *et al.* 1985; Eidelberg *et al.* 1986), monkeys (Mettler & Liss, 1959; Lassek & Anderson, 1961) and even humans (Nathan & Smith, 1973) is that spinal cord hemisection produces ipsilateral motor deficits which can spontaneously recover, starting after several days and continuing for several weeks (or months, in the case of humans). Bilateral staggered hemisection, on the other hand, is associated with permanent paraplegia in monkeys (Lassek & Anderson, 1961) and humans (Nathan & Smith, 1973). Adult rats display bilateral lower limb paralysis after a second (contralateral) hemisection and

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subsequent recovery was not observed; however, animals in this study were kept alive for only 2 days after the second lesion (Harris *et al.* 1994). In the presence of contralateral cord hemisections cats recover locomotor ability over the course of several weeks, after an initial phase of paraplegia, if the ventral column is spared on at least one side (Jane *et al.* 1964). Recovery was postulated to be due to a propriospinal system travelling in the preserved ventral column(s) (Jane *et al.* 1964). In contrast, the present results demonstrate that the *in vitro* neonatal rat preparation retains the capacity to generate lumbar locomotor-like activity in response to brainstem stimulation, even in the acutely lesioned state and in the presence of complete hemisections. Whether or not the propriospinal system demonstrated in the present acute neonatal rat preparation is recruited by long-term recovery processes after lesions in adult animals remains to be determined.

Stelzner & Cullen reported that one-month-old rats subjected to bilateral staggered mid-thoracic hemisections show little or no recovery of lower limb motor function over the course of 6 months (1991). They also showed that locomotor responses did recover in similarly lesioned newborn rats. Subsequent complete cord transection rostral to the region of double hemisections had no effect on the locomotor responses, leading to the conclusion that intrinsic cord mechanisms, rather than propriospinal connections, mediated the recovery (Stelzner & Cullen, 1991). However, the small interlesion region (1–3 segments) in these experiments may have limited the capacity of descending systems to influence the lumbar region. Whether locomotor recovery would occur in adult rats after staggered hemisections with larger interlesion regions (5–10 segments), remains to be tested.

The present results, in combination with our previous observations (Zaporozhets *et al.* 2006*a*), provide strong evidence that a propriospinal relay system is an important and probably sufficient conduit for descending activation of the locomotor network in the neonatal rat. In addition, one might speculate that the propriospinal system may be more than a passive conduit for descending transmission. It may serve as an active component of locomotor circuitry, consistent with the concept that locomotor networks are distributed rostro-caudally throughout the spinal cord in rats (Cowley & Schmidt, 1997) and humans (Dietz *et al.* 1999).

Most spinal cord injuries in humans are partial rather than complete and it is highly improbable that partial spinal cord injury would completely abolish all long-projecting bulbospinal fibres while selectively sparing propriospinal fibres. Thus, even if spinal relay connections alone should prove insufficient for transmission of the locomotor command signal in adult mammals, the propriospinal system remains a logical target for functional recovery strategies involving regeneration and direct pharmacological and/or electrical excitation (e.g. Yakovenko *et al.* 2007). In addition, descending propriospinal neurons may be able to serve as an 'alternative route' for transmitting information normally carried by long bulbospinal projections. Indeed there is good evidence that this type of re-routing occurs spontaneously during recovery of locomotor activity in the lamprey (for review see McClellan, 1998) and embryonic chick (Sholomenko & Delaney, 1998) and during the re-establishment of cortical influence on lumbar neurons (Bareyre *et al.* 2004; Vavrek *et al.* 2006).

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