Locomotor Rhythm Evoked by Ventrolateral Funiculus Stimulation in the Neonatal Rat Spinal Cord In Vitro

DAVID S. K. MAGNUSON AND TAMMY C. TRINDER
Department of Physiology, University of Manitoba, Winnipeg, R3E 0W3 Manitoba, Canada

Magnuson, David S. K. and Tammy C. Trinder. Locomotor rhythm evoked by ventrolateral funiculus stimulation in the neonatal rat spinal cord in vitro. J. Neurophysiol. 77: 200–206, 1997. Spinal cords from 2- to 8-day-old rats, maintained in vitro, were used to investigate the effects of discrete electrical stimuli applied to the ventrolateral funiculus (VLF) on motor neuron activity recorded from the lumbar ventral roots. Short trains of stimuli (1–3 s) delivered to one VLF in the low cervical region elicit rhythmic activity that persisted for up to 30 s. Responses consisted of short periods of activity (1–5 s) occurring simultaneously in the ipsilateral L₅ and contralateral L₃ ventral roots that alternated with similar bursts of activity in the ipsilateral L₁ ventral root, a pattern consistent with locomotion. The rhythmicity of the ventral root responses to VLF stimulation was not affected by midsagittal sectioning of the preparation rostral to T₁₀ and/or caudal to L₄. Midsagittal sectioning of the lower thoracic or upper lumbar segments, however, disrupted the rhythmicity of the ventral root responses, leaving only long-duration simultaneous activation of the ipsilateral roots following VLF stimulus trains. The minimum lesion that effectively abolished the rhythmicity was one that divided only the L₁ and L₃ segments. In preparations rendered arrhythmic to VLF stimulation by an L₂/L₃ midsagittal lesion, rhythmicity could still be induced by N-methyl-D-aspartate (NMDA; 2–5 μM) and serotonin (5-HT; 20–50 μM), a drug combination commonly used to induce locomotor-like rhythmicity and air-stepping in vitro. Field potentials recorded following single stimuli delivered to the VLF revealed short-latency, large-amplitude responses in the ventral horn and intermediate gray both ipsilateral and contralateral to the stimulus site at T₁₂ and L₂. These observations suggest that 1) the discrete pathway under study may be an important descending locomotor command pathway and 2) this pathway has a strong bilateral projection in the lower thoracic and upper lumbar segments that is crucial for the initiation of VLF-induced rhythmic motor output. The induction of rhythmicity by NMDA/5-HT in an L₂/L₃-lesioned preparation suggests that these two rhythmogenic mechanisms may represent different levels within the circuitry that comprises the central pattern generator for locomotion. The rhythmic activity resulting from VLF stimulation is dependent on a bilateral projection that can be bypassed by the generalized excitation and subsequent rhythmicity that results from bath application of the NMDA/5-HT combination.

INTRODUCTION

The initiation of locomotion in mammals is believed to involve the activation of specific brain stem and mesencephalic nuclei (see Jordan 1991 for review). The primary pathway is thought to originate in the mesencephalic locomotor region, passing through relays in the reticulospinal system of the lower brain stem and descending to the cervical and lumbar spinal cord in the ventrolateral funiculus (VLF) (Noga et al. 1991; Steeves and Jordan 1980; Yamaguchi 1986). Although a number of laboratories have described locomotor activity elicited by brain stem (Atsuta et al. 1988), mesencephalic (Skinner and Garcia-Rill 1984), or whole cord electrical stimulation (Iwahara et al. 1991), in vitro investigations into the central pattern generator for locomotion in the mammalian spinal cord have, to date, relied heavily on models utilizing N-methyl-D-aspartate (NMDA) and/or serotonin (5-HT) to induce locomotor-like rhythms (Cazalets et al. 1992; Kjerulff et al. 1994; Kudo and Yamada 1987).

The rostrocaudal distribution of the central pattern generating network has been investigated with the use of a split-bath technique, in vitro, again with drug-induced air-stepping as the model for locomotion. Cazalets and colleagues (1992, 1995, 1996) used the entire brain stem and spinal cord for their in vitro studies. Rhythm was induced by the application of 5-HT and NMDA onto discrete regions of the cord that were isolated by vaseline barriers. This model of pattern generation suggests that an anatomically defined region, the L₁ and L₂ segments, is the primary site of pattern generation (Cazalets et al. 1995, 1996). Complementary to that observation are those of Kjerulff and Kiehn, who found that incomplete L₂/L₃ transections, sparing only small portions of the most ventral white and gray matter, permit coordination between the rostral and caudal lumbar segments to be retained during drug-induced locomotor-like activity. They further demonstrated that when completely separated from the more rostral cord, the segments L₁–L₃ are capable of producing a coherent alternating right-left locomotor-like pattern in the presence of 5-HT and NMDA (Kjerulff and Kiehn 1994, 1995). So, although the debate is ongoing, these studies suggest that the pattern-generating network for the hindlimbs, in the neonatal rat at least, is located throughout the lumbar enlargement but is dominated by neurons in the more rostral segments (L₁ and L₂) when a longitudinally contiguous preparation is used. The distribution of the locomotor pattern generated in the rostral segments to the rest of the lumbar enlargement may occur via the most ventral portion of the cord.

Studies in which “activity-dependent” labeling such as that with sulforhodamine uptake during NMDA/5-HT-induced air-stepping (in vitro) (Kjerulff et al. 1994) and cfos induction following treadmill activity (in vivo) (Dai et al. 1990) was used have suggested that neurons located in laminae VII and X of the lumbar segments are involved in those motor activities. We have recently described rhythmic fluctuations in membrane potential exhibited by lumbar lamina VII interneurons recorded intracellularly in a lumbar midsag-
METHODOLOGIES

Sprague-Dawley rats aged 2–8 days were decapitated and eviscerated. The forelimbs and ribs were removed to within 0.5 cm of the spinal column. The hindlimbs and spinal column were quickly chilled to 4°C in oxygenated (95% O₂–5% CO₂) artificial cerebral spinal fluid (ACSF) that contained (in mM) 124.5 NaCl, 5.0 KCl, 26.0 NaHCO₃, 1.0 NaH₂PO₄, 10.0 D-glucose, 2.0 MgSO₄, and 2.0 CaCl₂. The spinal cords were exposed ventrally, carefully dissected free, and placed in a 30°C recirculating incubating chamber filled with bubbled ACSF. Care was taken to retain, attached and undamaged, 0.5- to 1.0-cm lengths of lumbar ventral and dorsal roots. After ≥30 min, the cords were transferred to a Sylgard-bottomed recording chamber continuously perfused (1.5–2.0 ml/min) with bubbled ACSF at room temperature. The cords were pinned out, ventral surface uppermost, with the use of 0.1-mm-diam insect pins placed in the cervical, midthoracic, and sacral regions of the isolated cord.

Glass suction electrodes (150–300 μm) were placed on the right and left L₃ ventral roots and onto the L₃ ventral root ipsilateral to the VLF stimulation site. Population synaptic responses from ventral roots were filtered at 0.1 Hz and continuous recordings were filtered at 10 Hz. Filtering and amplification were accomplished with the Cyberamp 380 and A1 401 headstages (Axon Instruments).

VLF stimulation was delivered by a patch-clamp-style electrode with a resistance of 1–5 MΩ (tip diameter 2–5 μm; filled with ACSF) lowered onto the ventrolateral white matter with the use of a Newport 461 three-dimensional mechanical micromanipulator. No suction was applied to the stimulating electrode, and stimuli to laminar VII interneurons impaled with the use of sharp microelectrodes exhibited “drive potentials” in phase with 5-HT/NMDA-induced rhythmic ventral root activity. The amplitudes of these fluctuations in potential were modest, however, bearing little resemblance to drive potentials recorded from motorneurons in the cat (for example Shefchik and Jordan 1985) or rat (for example Schmidt 1994), or from interneurons in the mudpuppy (Wheatley et al. 1994). The possibility exists that a proportion of these neurons were activated unspecifically, and exhibited fluctuations in membrane potential through a process akin to common excitation. Because similar experiments performed in a drug-free model of locomotion would suffer less from this problem, we set out to develop a preparation that might allow pattern generation and descending locomotor command pathways to be studied without the need for drugs to induce rhythmic motor activity. Thus the present study examines the responses in lumbar ventral roots to stimulation of a specific descending pathway in VLF of the isolated neonatal rat spinal cord. The pathway is identified by location and by the high dependence the ventral root responses have on the stimulus frequency (Magnuson et al. 1995).

METHODS

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Midsagittal lesions of the cords were made in situ, while cords were still in the continuously perfused recording chamber and attached to the ventral root recording electrodes. The stimulating electrode was removed before creation of the lesion to ensure that no damage was done by the electrode rubbing against the stimulation site. Lesions were made manually with 0.1-mm-diam insect pins, with the aid of an Olympus 240 zoom dissecting microscope. The caudalmost exit points of each ventral root were easily visible under the dissecting microscope and were used to delineate the spinal cord segments. A lesion of segments L₂ and L₃ would permit an insect pin, held vertically, to be moved freely down the midline from the caudalmost exit point of L₁ to the caudalmost exit point of L₇; no further confirmation of lesion size was deemed necessary.

Field potential recordings were made via patch-clamp-style electrodes with a resistance of 1–5 MΩ (filled with ACSF). Averages of three to six sweeps were made following single 0.5-ms stimuli delivered at 0.1 Hz and signals were high-pass filtered at 0.1 Hz. The field potential electrodes were lowered into the cord almost vertically with the use of a Newport MX-510 hydraulic micromanipulator mounted on a Newport 461 three-dimensional mechanical micromanipulator. The depth of penetration of the field potential electrode tip was determined by the cylinder graduations on the MX-510 hydraulic manipulator. Care was taken to establish the depth of penetration when no dimpling of the cord was visible when viewed through the dissecting microscope. In some instances this required that the electrode be moved slowly through the cord beyond the desired depth and then be withdrawn slightly before making any records at a particular site. As a routine, records were made starting from the ventral surface.

RESULTS

In total, 51 preparations were included in the study. VLF-stimulation-induced rhythmic activity that alternated between ipsilateral and contralateral L₃ ventral roots (as a minimum criterion for inclusion) was elicited from 41 preparations. Six of these successful preparations were used for field potential recording and eight were used for lesioning experiments. The remaining 27 rhythmic preparations were studied for reproducibility of response and to examine stimulation parameters including the positioning of electrodes. The stimulating electrodes were routinely placed on the VLF in the lower cervical/upper thoracic region of the isolated spinal cords.

The dependence of the rhythmic responses on VLF stimulation parameters was found to be very similar to that of the long-duration responses recorded in the partially midsagittally sectioned preparation (Magnuson et al. 1995). Stimulus durations of 0.3–1.0 ms had ventral root thresholds of 5–10 μA. At stimulus durations of 0.5 ms, rhythmic activity could be elicited with as little as 10 μA when the stimulus trains were ~20 Hz in frequency and ~5 s in duration. Only rarely were we able to initiate and maintain some level of rhythmicity with the use of low-frequency continuous stimulation of 1–10 Hz; the rhythmic activity generally ceased.
FIG. 1. A: rhythmic responses to a short-train stimulation of the low cervical ventrolateral funiculus (VLF) involving the contralateral L3 ventral root in addition to the ipsilateral L3 and L5 ventral roots. B: another example of rhythmic activity in response to a short-train stimulation of the VLF, this time showing alternation between ipsilateral and contralateral L3 ventral roots. C: 2 rhythmic responses from the same preparation, 1 following a 3-s, 50-Hz train of stimuli delivered to the VLF (left) and the 2nd following mild abrasion of the stimulus site with a 0.1-mm-diam insect pin (right).

After only one or two cycles. In apparent contrast, short trains of higher-frequency stimulation (0.5–3.0 s; 50 Hz, 0.5 ms, 10–80 μA) could be used to elicit fairly uniform responses every 5–10 min for 2 h or more in some preparations. Although there was an obvious relationship between stimulus parameters and quantitative aspects of the rhythmic response (amplitude of burst, frequency of firing within a burst, and number of rhythm cycles), this relationship held only up to a certain threshold; exceeding that threshold yielded no further increases in the rhythmic responses and increases in background activity were common. For the majority of preparations (~20 of 27) this threshold was ~50 Hz and 50 μA.

Figure 1A contains extracellular recordings from the L3 of the ipsilateral ventral roots being recorded. Similarly, if the midsagittal lesion of the lumbar region was extended rostrally to include the L3 segment, the response to VLF stimulation degraded into a tonic coexcitation in the ipsilateral L3 and contralateral L5 ventral roots. Finally, if only the L2 and L3 lumbar segments were midsagittally lesioned, the responses to VLF stimulation were found to degrade into tonic coexcitations (Fig. 3A). Lesions that involved only one lumbar segment (L2 or L3) allowed the responses to VLF stimulation to retain varying degrees of rhythmicity. Single midsagittal lesions in any of the segments from T10 to L3 were effective in attenuating the VLF-induced rhythmicity, however, the minimum midsagittal lesion that consistently and completely abolished VLF-stimulation-induced rhythmic activity was one that included the segments L2 and L3. Any midsagittal lesion of spinal cord segments T10–L3 affected the response to VLF stimulation in two ways: first of all, it attenuated the rhythmic responses to VFL stimulation, and second, it greatly reduced the responses of the contralateral L3 ventral root, even if the lesion did not directly involve the L3 spinal cord segment.

In three separate preparations following a minimum lesion...
VLF-INDUCED LOCOMOTOR RHYTHM

FIG. 2. Diagrams representative of lesions created manually with the use of 0.1-mm insect pins. Following the lesions shown on the left, low-cervical-VLF-stimulation-elicited rhythmic responses were unaffected. The lesions shown on the right completely abolished VLF-elicited rhythmic activity. Intermediate lesions involving the lower thoracic or upper lumbar segments leaving intact L2 or L3 resulted in a disruption but not an abolishment of rhythmic responses.

(L2 and L3) in which VLF stimulation elicited only tonic coexcitation of ipsilateral ventral roots, application of 5-HT and NMDA elicited rhythmic alternating activity in the L1 and L4 ventral roots (Fig. 3B). This was also the case in numerous other preparations with more extensive midsagittal lesions (MacLean et al. 1995; Magnuson et al. 1995).

In Fig. 4, representative field potentials are shown for T12, L2, and L4 spinal cord segments from a 5-day-old rat. These records demonstrate that field potentials in response to single stimuli delivered to the VLF were substantial at T12 and L2 ipsilateral to the stimulating electrode, whereas contralateral responses were somewhat smaller in amplitude but still short in latency at those levels (see Table 1).

During the recording of field potentials we would occa-
sionally record from neuronal cell bodies activated by the VLF stimulation. When the recording electrodes were optimally located, extracellular action potentials could be recorded that were >0.5 mV in amplitude that did not degrade during six sweeps at 0.1-Hz stimulation. These action potentials could not follow high-frequency (>10 Hz) stimulus frequencies (data not shown). Five of these neurons showed response latencies identical to those of the ipsilateral L3 ventral roots. All of these neurons were located in L1 and L2, four of them contralateral and one ipsilateral to the stimulus site. An example of one such neuron is shown in Fig. 5A. Three other neurons located contralaterally in L1 and L2 had short-latency responses of 1.8, 2.8 and 4.2 ms longer than those of the ipsilateral L3 ventral root; the responses of one of these neurons is shown in Fig. 5B. Finally, three neurons located ipsilaterally in L1 and one located contralaterally in L4 had latencies that ranged from 8.6 to 18.8 ms longer than those of the ipsilateral L3 ventral root. The responses of one of these neurons is shown in Fig. 5C. In total, 12 neurons were recorded extracellularly, 8 of these were located contralateral and 4 were ipsilateral to the stimulus site.

DISCUSSION

Until recently, the terms ‘‘locomotor,’’ ‘‘locomotor-like,’’ and ‘‘rhythmic’’ activity have not been well defined for the neonatal rat spinal cord in vitro preparation. Observations by Cowley and Schmidt (1994a,b), using simultaneously recorded ventral root and ankle flexor and extensor electro-

FIG. 3. A: 1 example where a minimum effective lesion (L2 and L3) abolished rhythmic response to train stimulation of the VLF. B: in same preparation, the L2/L3-lesioned cord still responded rhythmically to the application of serotonin (5-HT)/N-methyl-D-aspartate (NMDA) in the superfusate.
neurograms, suggest that drug-induced rhythmic activity recorded from ventral roots cannot always be interpreted as locomotor or locomotor-like activity. However, these authors do not give examples where alternating rhythmic activity recorded in either L₂ or L₃ and L₅ ventral roots was seen as something other than a locomotor-like pattern of activity in flexor and extensor electroneurograms, respectively (Cowley and Schmidt 1994a,b). In addition, the recent report by Kiehn and Kjærulff (1996) demonstrates that L₅ ventral root bursts correspond to extensor electromyogram activity during drug-induced locomotor patterns. In the present study we chose to record from L₂ and L₅ ventral roots, which contain predominantly flexor and extensor motor axons, respectively. Although the absence of identified flexor and extensor electroneurograms makes it impossible to positively assign stepping phases to the recorded ventral root activity, the findings of Cowley and Schmidt and Kiehn and Kjærulff outlined above suggest that the particular pattern of alternating activity in L₂ and L₅ ventral roots is unlikely to reflect something other than a locomotor pattern of the postnatal day 2–8 rat pup.

We recently demonstrated that the lumbar midsagittally sectioned neonatal rat spinal cord in vitro, with one lumbar hemiscord removed but with thoracic segments intact, is capable of producing rhythmic locomotor-like activity in the presence of 5-HT and NMDA (MacLean et al. 1995). In this preparation, ~80% of intermediate laminae interneurons in L₂–L₅ were found to exhibit rhythmic fluctuations in membrane potential that were phase linked to rhythmic activity in nearby ventral roots. We also demonstrated recently that relatively low-intensity, high-frequency stimulation of the cervical VLF in the spinal cord–hindlimb preparation (intact spinal cord) can elicit long bouts of rhythmic hindlimb movements that resemble locomotion (Magnuson et al. 1995). When we attempted to induce rhythmic activity by VLF stimulation in the lumbar midsagittally sectioned spinal cord, however, we were unsuccessful, observing only long-duration tonic coactivation of lumbar ventral roots on the intact hemiscord. Thus we faced a conundrum whereby both VLF stimulation and 5-HT/NMDA could elicit rhythmic

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**FIG. 4.** Representative field potentials from the T₁₂, L₂, and L₄ spinal cord segments following single VLF stimuli. Records are shown in relation to a diagram showing the approximate relative location of the motor nuclei in the various spinal cord segments represented. These records suggest that VLF inputs are greatest at T₁₂ and L₂, with strong bilateral projections at L₂ and, in some preparations, at T₁₂ and L₄ (see Table 1 for averaged responses).

**TABLE 1.** Amplitudes and latencies of field potentials

<table>
<thead>
<tr>
<th>Level</th>
<th>Latency, ms</th>
<th>Amplitude, mV</th>
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<tbody>
<tr>
<td>cT₁₂-100</td>
<td>9.4 ± 3.0</td>
<td>0.36 ± 0.06 (2)</td>
</tr>
<tr>
<td>cT₁₂-200</td>
<td>10.7 ± 1.3</td>
<td>0.35 ± 0.05 (4)</td>
</tr>
<tr>
<td>cT₁₂-300</td>
<td>10.8 ± 0.8</td>
<td>0.28 ± 0.03 (2)</td>
</tr>
<tr>
<td>cL₂-100</td>
<td>12.4 ± 1.5</td>
<td>0.91 ± 0.04 (3)</td>
</tr>
<tr>
<td>cL₂-200</td>
<td>10.8 ± 0.3</td>
<td>0.61 ± 0.21 (4)</td>
</tr>
<tr>
<td>cL₂-300</td>
<td>16.3 ± 6.0</td>
<td>0.40 ± 0.11 (3)</td>
</tr>
<tr>
<td>cL₂-400</td>
<td>13.9 ± 3.5</td>
<td>0.59 ± 0.07 (4)</td>
</tr>
<tr>
<td>L₃ VR</td>
<td>13.9 ± 0.9 (6)</td>
<td></td>
</tr>
<tr>
<td>cL₄-200</td>
<td>17.6 ± 2.1</td>
<td>0.35 ± 0.05 (5)</td>
</tr>
<tr>
<td>cL₄-300</td>
<td>14.6 ± 1.0</td>
<td>0.44 ± 0.12 (2)</td>
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<tr>
<td>cL₄-400</td>
<td>14.5 ± 3.5</td>
<td>0.59 ± 0.14 (4)</td>
</tr>
<tr>
<td>iT₁₂-100</td>
<td>9.1 ± 0.7</td>
<td>0.65 ± 0.17 (3)</td>
</tr>
<tr>
<td>iT₁₂-200</td>
<td>9.8 ± 2.0</td>
<td>0.88 ± 0.30 (4)</td>
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<tr>
<td>iT₁₂-300</td>
<td>8.5 ± 1.4</td>
<td>1.26 ± 0.39 (3)</td>
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<tr>
<td>iT₁₂-400</td>
<td>11.1 ± 2.5</td>
<td>0.37 ± 0.04 (3)</td>
</tr>
<tr>
<td>iL₂-100</td>
<td>12.3 ± 2.1</td>
<td>0.56 ± 0.38 (4)</td>
</tr>
<tr>
<td>iL₂-200</td>
<td>9.7 ± 0.8</td>
<td>1.07 ± 0.17 (5)</td>
</tr>
<tr>
<td>iL₂-300</td>
<td>10.2 ± 1.1</td>
<td>1.04 ± 0.37 (4)</td>
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<tr>
<td>iL₂-400</td>
<td>11.2 ± 1.1</td>
<td>0.70 ± 0.26 (4)</td>
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<tr>
<td>iL₃ VR</td>
<td>10.9 ± 0.7 (6)</td>
<td></td>
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<tr>
<td>iL₄-100</td>
<td>13.7 ± 0.3</td>
<td>0.31 ± 0.07 (2)</td>
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<tr>
<td>iL₄-200</td>
<td>12.1 ± 0.7</td>
<td>0.49 ± 0.06 (5)</td>
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<td>iL₄-300</td>
<td>12.9 ± 1.0</td>
<td>0.57 ± 0.18 (3)</td>
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<tr>
<td>iL₄-400</td>
<td>13.5 ± 1.7</td>
<td>0.32 ± 0.09 (3)</td>
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Values are means ± SE, with number of preparations in brackets. Mean latencies for L₂ ventral root responses are shown in bold. cT₁₂-100 indicates the records were made at the T₁₂ level, contralateral to the stimulus site, at a depth of 100 μm from the ventral surface. * Null values are not included.
locomotor-like activity in the intact cord, but only the 5-HT/NMDA was successful in the lumbar midsagittally sectioned preparation. This conundrum was solved, however, when we midsagittally divided the upper lumbar segments in spinal cord–hindlimb preparations that had already demonstrated locomotor-like activity in response to stimulation of the VLF. After the midsagittal sectioning of the L₂ and L₃ segments, VLF stimulation elicited only cocontractions in the flexor and extensor muscles of the ipsilateral hindlimb; these responses appeared disorganized and uncoordinated with occasional rapid shaking and twitching (small spasmlike movements; unpublished observations). These same preparations displayed robust locomotor-like activity when treated with 5-HT/NMDA. These observations indicated that the initiation of rhythmic activity by VLF stimulation was dependent on fibers that crossed the midline in the upper lumbar segments. Drug-induced rhythmicity is not dependent on this pathway, but can be influenced by it; this we demonstrated by stimulating the VLF during drug-induced rhythmic activity, observing that VLF stimulation could perturb the ongoing rhythm (Magnuson et al. 1995).

The bilaterally occurring field potentials we recorded following single unilateral stimuli are suggestive of a direct crossing or bifurcation of the descending VLF fibers. The largest field potentials were consistently recorded in L₂ and L₃ on both sides of the cord; however, the rostrocaudal distribution of the field potentials appeared to be greatest ipsilaterally (see Fig. 4 and Table 1). The field potentials observed are grossly similar (relative latency and duration) to those recorded in the ventral parts of the lumbar enlargement of the cat by Noga et al. (1995) with the use of similar recording and filtering paradigms. Their field potentials were elicited by stimulation of the mesencephalic locomotor region located just dorsal to the brachium conjunctivum in the vicinity of the cuneiform nucleus. Although the locations of the cell bodies giving rise to the VLF fibers we stimulated are not known, the fibers themselves are located in a region previously shown to carry the descending output from the mesencephalic locomotor region in the cat (Noga et al. 1991). The occasional recording from a presumed neuronal cell body following single stimuli (Fig. 5) further supports the contention that the stimulated fibers descend to the lower thoracic and upper lumbar segments in the VLF and enter the ventral horn both ipsilaterally and contralaterally. The short-duration, large-amplitude extracellular potentials did not follow high-frequency (>10 Hz) stimulation, but were consistently elicited by low-frequency stimuli delivered at 5 times the ventral root threshold. The majority of these responses were found contralateral to the stimulus site, had short latencies, and may represent monosynaptic inputs onto intermediate laminae neurons. This type of response was perhaps not reported by Noga et al. (1995), because they consistently recorded their field potentials ipsilateral to the mesencephalic locomotor region stimulus site, and they made no recordings rostral to L₃.

In 1984, Drew and Rossignol reported that hindlimb electromyograms displayed either excitatory or inhibitory responses to medial reticular formation (MRF) stimulation during spontaneous locomotion in the decerebrate cat, and that these responses were modulated during the step cycle. They further reported that response latencies fell between 8 and 20 ms, and that the latencies were similar for muscles contralateral and ipsilateral to the mesencephalic locomotor region stimulus site. They proposed, therefore, that the short-latency bilateral representation of the mesencephalic locomotor region input onto motoneurons indicated that “the responses were synchronously organized on both sides of the body rather than one being a consequence of the other.” The present findings strongly support this proposal and further suggest that locomotor command output from the mesencephalic locomotor region and MRF may be bilaterally represented in the lumbar enlargement via fibers that travel in the VLF, cross in the most rostral lumbar segments, and terminate in the ventral and intermediate laminae. Evidence of the presence and accessibility of this pathway in the VLF of the cat, for the forelimbs at least, has been adequately provided by Yamaguchi (1986). Functionally, one could speculate that the bilateral symmetry of output from one mesencephalic locomotor region might assist in making the transition from standing to walking, which requires simultaneous changes in motor nuclei on both sides of the spinal cord, a smooth one.

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Present address and address for reprint requests: D. S. K. Magnuson, Dept. of Neurological Surgery, 210 East Gray St., Suite 1102, University of Louisville, Louisville, KY 40292.

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