Mechanism for Activation of Locomotor Centers in the Spinal Cord by Stimulation of the Mesencephalic Locomotor Region

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INTRODUCTION

During locomotion, hindlimb alpha-motoneurons undergo rhythmic depolarization and hyperpolarization, termed locomotor drive potentials or LDPs (Jordan 1983; Perret 1983). Motoneuron membrane conductance measurements (Jordan 1981; Shefchyk and Jordan 1985b), intracellular current or chloride-ion injection (Orsal et al. 1986; Perret 1983), and intravenous injections of strychnine (Pratt and Jordan 1987) during spontaneous or controlled fictive locomotion evoked by stimulation of the mesencephalic locomotor region or MLR (Shik et al. 1967) have demonstrated that these membrane oscillations are primarily due to alternating excitatory and inhibitory synaptic input during the step cycle.

Evidence for the existence of excitatory and inhibitory neurons in pathways that evoke locomotion come from the observation that stimulation of the MLR in sites maximally effective for the initiation of locomotion also produces short-latency excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in hindlimb motoneurons (Degtyarenko et al. 1998; Shefchyk and Jordan 1985a). The segmental latencies of these PSPs indicate that they are mediated by one or more spinal neurons. The amplitude of these PSPs strongly covary with the locomotor step cycle with the EPSPs largest during the phase of locomotion in which the motoneuron is depolarized and active and the IPSPs largest during the phase in which it is hyperpolarized and inactive (Shefchyk and Jordan 1985a). Because of these characteristics, it has been suggested that these neurons mediate short-latency PSPs contribute to the excitation and inhibition of the motoneurons during locomotor activity and therefore form part of the spinal neuronal network for locomotion (Shefchyk and Jordan 1985a). Evidence in favor of this hypothesis comes from the observation that these neurons are also activated by high-threshold muscle and skin afferents (Shefchyk and Jordan 1985a) previously implicated in the control of the locomotor rhythm in spinal animals treated with L-3,4-dihydroxyphenylalanine or L-DOPA (Jankowska et al. 1967a,b). However, not all populations of rhythmically active motoneurons show MLR-evoked EPSPs, and it has been suggested that other excitable last-order interneurons without MLR input must exist to account for the depolarization of these motoneurons (Burke et al. 2001; Degtyarenko et al. 1998).

Experiments were therefore conducted to further explore the relationship between the short-latency PSPs and locomotion as measured from intracellular recording (LDPs) or extracellular recording (electroneurograms or ENGs) of motoneuron activity during MLR stimulation. It was hypothesized that if the interneurons mediating these PSPs contribute to the generation of
Locomotor activity, the amplitude of the motoneuron PSPs will also vary during perturbations of MLR-evoked locomotion accordingly with the appearance or disappearance of locomotion. Perturbations were produced by reversibly blocking signal transmission through the descending locomotor pathway by localized cooling (Brooks 1983). Cooling probes were placed within the pontomedullary medial reticular formation (MedRF) to block synaptic transmission at the first relay site of the pathway originating in the MLR (Jordan 1991; Noga et al. 1988, 1991; Sheftchyk et al. 1984; Steeves and Jordan 1984) or against the ventral funiculus (VF) of the spinal cord to block fiber transmission through the descending reticulospinal pathway originating in the MedRF (Noga et al. 1991; Steeves and Jordan 1980). Cooling of the VF was done either ipsilateral or contralateral to the sites of MLR stimulation and motoneuron recording to distinguish the relative contribution of a bilaterally descending (Garcia-Rill and Skinner 1987; Orlovsky 1969, 1970a, b) and a possible crossed-spinal (Eidelberg 1981; Jankowska and Noga 1990; Kato et al. 1984; Sholomenko and Steeves 1987) component of the locomotor pathway. Preliminary results have been presented elsewhere (Jordan 1991; Noga 1988).

Methods

Animal preparation

Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 80-23; revised 1996). The number of animals used, and their pain and distress, were minimized.

Twenty-nine adult cats (2.0–3.8 kg) were anesthetized with halothane in a mixture of 70% nitrous oxide and 30% oxygen delivered using a face mask. The trachea was intubated for direct administration of the anesthetic. The left common carotid artery was cannulated for blood pressure monitoring. The right external jugular and the left brachial veins were cannulated for the administration of fluids. Each animal was given 2–4 mg of dexamethasone (hexadrol phosphate, Organon) intravenously to reduce tissue swelling. In some experiments, a bicarbonate solution (100 mM NaHCO3 with 5% glucose) was infused at 3–5 ml/h to replace fluid and blood loss and help maintain a normal pH balance. A number of hindlimb nerves were dissected free of the femoral nerve ipsilateral to the side of intracellular recording (Brooks 1983). Cooling probes were placed accordingly with the appearance or disappearance of locomotion. Perturbations were produced by reversibly blocking signal transmission through the descending locomotor pathway by localized cooling (Brooks 1983). Cooling probes were placed within the pontomedullary medial reticular formation (MedRF) to block synaptic transmission at the first relay site of the pathway originating in the MLR (Jordan 1991; Noga et al. 1988, 1991; Sheftchyk et al. 1984; Steeves and Jordan 1984) or against the ventral funiculus (VF) of the spinal cord to block fiber transmission through the descending reticulospinal pathway originating in the MedRF (Noga et al. 1991; Steeves and Jordan 1980). Cooling of the VF was done either ipsilateral or contralateral to the sites of MLR stimulation and motoneuron recording to distinguish the relative contribution of a bilaterally descending (Garcia-Rill and Skinner 1987; Orlovsky 1969, 1970a, b) and a possible crossed-spinal (Eidelberg 1981; Jankowska and Noga 1990; Kato et al. 1984; Sholomenko and Steeves 1987) component of the locomotor pathway. Preliminary results have been presented elsewhere (Jordan 1991; Noga 1988).

Stimulation and recording

After a recovery period of 1–2 h after the decerebration, bouts of locomotion were evoked by electrical stimulation of the MLR (typically 0.5–1.0 ms square wave pulses, 10–20 Hz, 30–200 μA; Fig. 1A) using a monopolar stimulating electrode (SNE-300; David Kopf Instruments). Rhythmic activity of the ENGs of hindlimb peripheral nerves was used as an indicator of “fictive” locomotion (Fig. 1D).

Intracellular recordings from motoneurons in the Lα and Lβ spinal cord segments (Fig. 1D) were obtained using single-barrelled glass microelectrodes filled with 2 or 3 M potassium citrate (1–7 MΩ resistance, 1–2 μm tip diameter). Motoneurons were selected for analysis providing they had stable resting membrane (Em) potentials more negative than ~50 mV (mean ± SD, ~60.4 ± 8.0), they did not spike during locomotion, or their spiking activity could be reduced by application of a constant hyperpolarizing current (1.3–33 nA) given throughout the trial of locomotion (n = 6). It was essential to limit our sample to motoneurons that did not spike, to detect the MLR-evoked PSPs. In all motoneurons, locomotion was sufficiently robust to produce both flexor and extensor nerve activity. Motoneurons were identified either by antidromic activation or by their pattern of synaptic input from stimulation of the peripheral nerves. The latter group of unidentified motoneurons were classified as extensor, flexor, or bifunctional by relating their activity during the fictive step cycle to that of identified peripheral nerves. Identified flexor motoneurons included TA and SA. Identified extensor motoneurons included VAST, SMAB, MG, and LGs. Identified bifunctional motoneurons included PBST, RF, and FDL. During each experiment, high-gain AC-coupled and low-gain DC-coupled intracellular signals were captured along with the cord dorsum potential (CDP), ENG activity, and a timing signal on a Vetter Model D, 8-track tape recorder (bandwidth: 0–2.25 kHz) or collected directly through a 1 MHz, 16 channel A/D converter (12 bit) and stored on computer disk. Digital sampling rates were 5 or 10 kHz for the DC trace and timing signal, 5 kHz for the AC trace, and 3.3 or 5 kHz for the CDP. The ENGs were first passed through a rectifier and then a low-pass filter (10- or 20-ms time constant) and subsequently digitized at 200 Hz. The DC trace and ENG activity was collected continuously throughout each bout of evoked locomotion. A timing unit was used to trigger MLR stimulation and synchronize the recording of 30- or 50-ms-duration sweeps of the AC and CDP signals. The monopolar CDP electrode was placed near the dorsal root entry zone slightly rostral to the intracellular electrode (Fig. 1D) and its indifferent reference electrode placed in the adjacent muscle.

Data analysis

The onset of each step cycle was determined from the start of the intracellular depolarizing phase of the LDP by employing a threshold detection algorithm with trigger hysteresis. The step cycle was then time-normalized and the membrane potential values computed as averages over 100 equally spaced intervals.

The MLR stimulus-triggered AC and CDP recordings were grouped into bins and averaged (n = 15–30) according to their phase position during the step cycle. For the AC trace averages, the step cycle was divided into 5 or 10 equal intervals. AC trace frames containing spiking activity (both spontaneous and MLR-evoked) were excluded from the averages.
A variety of measurements and statistical analyses were made on the motoneuron responses to MLR stimulation. The average PSP latency was determined at points in the step cycle where the PSP was largest (see Locomotor modulation of MLR PSPs) and where a cyclically consistent amplitude response occurred. This latency usually appeared constant, with very slight variation noted in some infrequent cases. Latency measurements included: the descending latency, from the onset of the MLR stimulus to the peak of the first evoked positive wave (descending volley) (Noga et al. 1995) recorded from the CDP (lines a and b in Fig. 3D); the segmental latency, from the peak of descending volley to the onset of the PSP (lines b and c in Fig. 3D); and, the total latency, descending plus segmental latencies (lines a and c in Fig. 3D). These measurements were readily obtained for the first MLR-evoked PSPs (usually EPSPs) and for longer latency PSPs of the same polarity. The latency of a second PSP of the opposite polarity (IPSP) was determined from averages obtained from the phase of the step cycle when the IPSPs were largest and the EPSPs were absent. In cases when EPSPs were still present during this phase, the latency measurements were made from 15 to 25 individual traces that clearly lacked a preceding PSP. A similar method was used in cases where a second EPSP followed an IPSP. Differences between PSP latencies were assessed using independent t-test. In cases when EPSPs were still present during this phase, the latency measurements were made from 15 to 25 individual traces that clearly lacked a preceding PSP. A similar method was used in cases where a second EPSP followed an IPSP. Differences between PSP latencies were assessed using independent t-test. Differences in the variance between all compared populations were assessed using an F test. The level of significance was set to 0.05.

The amplitudes of the MLR-evoked PSPs were also plotted relative to the phase of the LDP and the $E_m$ of the motoneuron. Maximal PSP amplitudes were measured within a time “window” (usually 0.5–1 ms) surrounding their peak, previously determined from averaged records (line d in Figs. 3D, 4D, 5, D and H, and 6D and lines d and e in Fig. 4D) and relative to a baseline potential measured just prior to their onset. The amplitude modulation (AM) of the PSP was assessed from measurements of averages sorted into 5–10 bins spaced across the step cycle. The change in PSP amplitude observed at the level of greatest depolarization and hyperpolarization of the motoneuron membrane potential during the step cycle was assessed using an independent t-test. A linear regression between PSP amplitude and $E_m$ recorded during locomotion was also performed. The time-to-peak (TTP) was determined from the bin average showing the largest amplitude. Differences between PSP amplitude and TTP of different PSP types were assessed using independent t-test.

Cooling of brain stem and spinal cord

The relationship between MLR-evoked PSPs and locomotion, as determined from intracellular (LDPs) and extracellular (ENG) recordings of motoneuron activity, was examined by reversible cooling (Brooks 1983) of the brain stem or spinal cord to temperatures that block signal transmission through the descending locomotor pathway during locomotion.

To block synaptic transmission at the first relay site of the pathway originating in the MLR, a cooling probe was carefully inserted into the MedRF (Fig. 1B, Horsley-Clarke coordinates P7–10, L0–1) (Berman...
1968) at a site that could either evoke locomotion (Noga et al. 1988) or facilitate MLR-evoked locomotion when electrically stimulated (determined prior to placement of the cooling probe). The cooling probe consisted of a coaxial 18-gauge stainless steel needle, sealed at the tip, with an inner shaft through which cooled alcohol was introduced (Noga et al. 1991; Shefchyk et al. 1984). Cooling probe temperatures were measured with a tissue implantable thermocouple (Type IT-23, Physitemp Instruments, Clifton, NJ) glued to the outside surface of the probe tip and connected to a digital thermometer (Bailey Instruments, Saddle Brook, NJ). Tissue temperatures around the tip of the cooling probe were determined in separate experiments using a thermocouple glued to a needle placed at various distances from the probe. Brain stem isotherms produced during cooling of the MedRF are illustrated in Fig. 1B. A probe-tip temperature of 13–15°C was sufficient to block synaptic transmission (required tissue temperature of 18–20°C) (Brooks 1983) for distances of 1–2 mm from the sides of the cooling probe in the adjacent MedRF. Brain temperature was allowed to return to precooling levels by stopping the coolant flow.

To block fiber transmission through the descending reticulospinal locomotor pathway located in the VF (Noga et al. 1991; Steeves and Jordan 1980), cooled alcohol was circulated through a U-shaped cooling probe (Noga et al. 1991) placed against the ventral surface of the T12 or T13 spinal cord (Fig. 1C) on one side. Thermocouple measurements within the spinal cord during cooling of the VF (Fig. 1, C,E, and F) indicated that probe-tip temperatures of 0 to −1°C would block fiber transmission (required tissue temperature of 4–10°C (Brooks 1983) in the adjacent VF for distances of −1 mm and synaptic transmission in the spinal gray, bilaterally. Ipsilateral dorsolateral funiculus (DLF) surface temperatures were 10–12°C warmer than probe tip temperatures. Thus cooling of the VF produced as selective a blockade of fiber transmission in the spinal cord as that reported by Apskarian et al. (1989). This was verified in other experiments where probe-tip temperatures of 0 to −1°C blocked signal transmission through the ventral quadrant (Fig. 1G) and reduced but not abolished the transmission of a signal through the dorsal quadrant of the spinal cord (Fig. 1H). During cooling of the VF, probe-tip and ventral horn temperatures stabilized within 2 min (Fig. 1, E and F). Cooling of the dorsal half of the spinal cord to temperatures of 0 to −1°C (not shown) also blocked fiber transmission in the DLF and synaptic transmission in the adjacent spinal gray matter. However, fiber transmission through the VF was likely unaffected since this region is adjacent to the body of the animal which provides a conductive heat source.

In a few experiments, cooling of the VF of the spinal cord was done intradurally. Probe-tip temperatures of 4–8°C were sufficient for a similar complete blockage of fiber transmission in the adjacent spinal quadrant.

RESULTS

Results are based on recordings made from 67 hindlimb motoneurons. Responses to stimulation of the MLR were analyzed from recordings made during fictive locomotion because stimulation of the MLR below the threshold for locomotion produced neither PSPs nor LDPs. Overall, LDPs averaged 6.3 ± 3.4 mV.

Patterns of MLR-evoked PSPs

The response pattern to stimulation of either the i- or coMLR (relative to side of motoneuron recording) was similar in the tested motoneurons (iMLR; n = 31; coMLR, n = 41) and therefore the data are combined. Three general PSP patterns were observed: pure excitatory (E,EE) (Figs. 3, B and D, 5, D and H, 6A, 8A, 9A, and 10A); mixed excitatory and inhibitory (EI, EIE, EII, EI and IEI; Fig. 4D); and pure inhibitory (I,II; not shown). The most common patterns were pure excitatory and mixed (38.9 and 48.6%, respectively). A pure inhibitory pattern was observed less frequently (12.5%). Pure excitatory PSPs were mostly single (E), occurring in 75% of the cases, with multiple excitatory (EE) PSPs observed in the remaining 25%. Mixed PSPs were usually of the E subtype (77%). Other subtypes were less frequently observed: EIE (14%) and EII, EIE, and IEI (3% each). Pure inhibitory PSPs were mostly single (I; 89%) and sometimes multiple (II: 11%).

Extensor motoneuron (n = 44) responses were mostly mixed (64%), with pure excitatory (23%) and pure inhibitory (13%) patterns making up the remainder. For flexor motoneurons (n = 15), the most common response was pure excitatory (81%), with the remainder being of the mixed type. PSP patterns observed in bifunctional motoneurons (n = 8) were pure excitatory (45%), mixed (22%), or pure inhibitory (33%).

Latency of MLR-evoked PSPs

The descending latency of CDPs evoked by stimulation of the iMLR (n = 17) and coMLR (n = 18; 1 measurement/experiment for each side) ranged from 3.0 to 5.7 ms with a mean of 4.3 ± 0.6 ms. These values were nearly identical to those (4.5 ± 0.6 ms) reported by Degtyarenko et al. (1998). No significant differences were found between the iMLR and coMLR, and in comparisons within the same animal (n = 5), descending latencies were within 0.3 ms in four of them. In one case, a 0.9-ms shorter descending latency was observed with iMLR stimulation. The fastest reticulospinal fibers would have a conduction velocity of ~145 m/s—assuming a 1-ms minimal delay for the activation of reticulospinal cells by impulses from the MLR (Garcia-Rill and Skinner 1987; Ikawirri et al. 1995; Orlovsky 1969,1970a) and a distance of 290 ± 20 mm (measured in 11 cats) from the MedRF to the lumbar cord dorsal electrode. The longest descending latencies (5.7 ms) can be attributed to fibers with a conduction velocity of 62 m/s. The average descending latency corresponds to a conduction velocity of 88 m/s.

The segmental latency (Table 1) of the first locomotor-related EPSP (all motoneurons) averaged 1.9 ± 0.5 ms (range: 1.2–3.4 ms). This was similar to that reported previously (Degtyarenko et al. 1998) (1.6 ± 0.4 ms). No significant differences were noted for segmental latencies of EPSPs evoked by either the i- or coMLR. The segmental latency histogram in Fig. 2B (top), showed three peaks, consistent with a di-, tri-, and polysynaptic linkage and therefore the involvement of one or more interposed spinal neurons in their production. There were no significant differences in segmental latencies between flexor, extensor or bifunctional motoneurons (data from combined stimulation sides; Table 1). Nor were there any differences between the latencies of EPSPs evoked in GS (1.7 ± 0.4 ms; n = 8) or SMAB motoneurons (1.8 ± 0.4 ms; n = 11). However, a significantly shorter segmental latency (P < 0.05) was noted for GS and SMAB compared with TA motoneurons (2.3 ± 0.7 ms; n = 9).

The total latency to the earliest locomotor-related EPSP (Table 1, Fig. 2A) had a mean of 6.4 ± 0.9 ms (n = 58; range: 4.4–8.2 ms). This was somewhat longer than that reported previously by Shefchyk and Jordan (1985a) (5.1 ms, range 3.0–7.0) but comparable to that of Degtyarenko et al. (1998) if
TABLE 1. Descending and segmental latencies of locomotor-related MLR-evoked PSPs in lumbar motoneurons

<table>
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<th>EPSP</th>
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<th>IPSP</th>
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<td></td>
<td>Segmental Latency</td>
<td>Total Latency</td>
<td>Segmental Latency</td>
<td>Total Latency</td>
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<tr>
<td>First</td>
<td>Flexor MNs</td>
<td>2.1 ± 0.6 (15)</td>
<td>6.5 ± 1.0 (15)</td>
<td>2.9 ± 0.1 (2)</td>
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<td></td>
<td>Extensor MNs</td>
<td>1.8 ± 0.5 (33)</td>
<td>6.4 ± 0.9 (37)</td>
<td>3.7 ± 0.8 (24)</td>
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<td></td>
<td>Bifunctional MNs</td>
<td>1.8 ± 0.2 (6)</td>
<td>6.9 ± 0.2 (6)</td>
<td>3.2 ± 1.6 (4)</td>
</tr>
<tr>
<td></td>
<td>All MNs</td>
<td>1.9 ± 0.5 (54)</td>
<td>6.4 ± 0.9 (58)</td>
<td>3.6 ± 1.0 (30)</td>
</tr>
<tr>
<td>Second</td>
<td>All MNs</td>
<td>5.7 ± 2.5 (11)</td>
<td>10.1 ± 2.3 (11)</td>
<td>3.1 ± 0.8 (2)</td>
</tr>
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</table>

Values are means ± SD with number of motoneurons (MN) in parentheses. MLR, mesencephalic locomotor region; EPSP and IPSP, excitatory and inhibitory postsynaptic potential, respectively.

the averaged descending and segmental latencies are added (4.5 and 1.9 ms, respectively). In comparisons between sides of MLR stimulation, the total latency to the earliest EPSP evoked by stimulation of the iMLR was significantly shorter (6.1 ± 0.9 ms) than that observed for coMLR stimulation (6.6 ± 0.8 ms).

Stimulation of the MLR also evoked a second locomotor-related EPSP in some motoneurons (n = 11). This EPSP had a mean segmental latency of 5.7 ± 2.5 ms (range: 3.1–10.9) and a total latency of 10.1 ± 2.3 ms (range: 7.2–14.9; Table 1, Fig. 2). These latencies were significantly longer (P < 0.001) than those observed for the first EPSP and IPSP. The segmental latencies are consistent with a polysynaptic linkage involving three or more interposed interneurons.

The total latency to the earliest locomotor-related IPSP measured from all motoneurons (Table 1) was 8.2 ± 1.2 ms and ranged from 5.4 to 10.4 ms. The segmental latency to this IPSP was 3.6 ± 1.0 ms and ranged from 1.6 to 5.8 ms. As seen in the histogram in Fig. 2B, the segmental latencies were consistent with tri and polysynaptic likages, likely involving two or more interposed interneurons in their production. The mean segmental and total latencies for these IPSPs were highly significantly longer (P < 0.001) than those observed for the earliest EPSPs. No significant differences were found for latencies of IPSPs evoked by stimulation of either side of the MLR. In two cases, a second locomotor-related IPSP was observed following MLR stimulation (Table 1). These IPSPs had average segmental and total latencies of 3.1 ± 0.8 and 7.2 ± 1.6 ms, respectively.

**Locomotor modulation of MLR PSPs**

The majority of the MLR-evoked EPSPs (69/77) and IPSPs (35/46) showed significant (P < 0.001) AM during the step cycle. EPSPs were largest during the depolarized phase and IPSPs largest during the hyperpolarized phase of the LDP. These PSPs were classified as *locomotor-related* because their modulation is likely dependent on factors other than passive membrane properties, in which case the EPSPs would decrease with depolarization and the IPSPs would decrease with hyperpolarization.

Locomotor modulation of the EPSP is illustrated in Figs. 3–5. During the depolarized phase, EPSPs were observed after every MLR stimulus (see Figs. 3B and 4B). The amplitude of each EPSP varied slightly during this phase, even when evoked at similar E_m levels. This is also apparent from averages over a number of step cycles (Figs. 3D, 4D, and 5D and E). In 32% of cases, EPSPs were absent during the hyperpolarized phase. In the remainder, small EPSPs remained (e.g., Figs. 3D and 4D). The EPSP amplitude relative to the phase of the LDP is shown in Figs. 3, C and E, 4, C and E, and 5, B, C, F, and G. The EPSP amplitude changed most rapidly during the transition between depolarization and hyperpolarization of the LDP.

On average, the maximum amplitude of the EPSPs during the depolarized phase of the LDP was 2.0 ± 1.6 mV (range 0.2–11.4 mV). The first locomotor-related EPSP (n = 58) was significantly (P < 0.01) larger (2.2 ± 1.7 mV, range 0.3–11.4 mV) than the second (n = 11), longer-latency EPSP (1.2 ± 0.7 mV, range 0.2–2.3 mV). The EPSPs observed during the hyperpolarized phase were significantly (P < 0.001) smaller (0.5 ± 0.6 mV, range 0–3.2 mV). The mean modulation of the EPSP was 1.6 ± 1.3 mV (range: 0.2–8.6 mV).

The amplitude of the locomotor-related MLR-evoked EPSPs was also significantly linearly correlated to E_m depolarization (Figs. 3F and 4F) in 54 of the 69 EPSPs. The majority of these EPSPs (n = 48) had significance levels of P < 0.001, with 3 at P < 0.01 and 3 at P < 0.05. EPSPs that did not show a significant linear correlation to E_m (n = 15) were significantly (P < 0.01) smaller (1.3 ± 1.0 mV) than the correlated EPSPs (2.2 ± 1.7 mV). Six of these noncorrelated EPSPs were absent during the hyperpolarized phase, although six of them also had amplitudes exceeding 0.3 mV. They also were usually found in synaptically noisy cells or in cells with a short depolarized phase of the LDP. These conditions would tend to decrease the
The greatest changes in amplitude occurred during the transition $C_4$.

$E_m$ does not show any correlation.

The maximum amplitude during the hyperpolarized phase of the LDP was $1.7 \pm 1.0$ mV (range: $-0.6$ to $-5.0$ mV). IPSPs were smallest or absent during the depolarized phase of the LDP (mean: $-0.2 \pm 0.4$ mV, range 0 to $-1.4$ mV). As for EPSPs, the greatest changes in amplitude occurred during the transition between depolarization and hyperpolarization of the $E_m$ (Fig. 4, C and F). The IPSP amplitudes showed a significant linear relationship to membrane potential in 31 of the 35 examined IPSPs (Fig. 4H) with significance levels of $P < 0.001$ in 24 of them, $P < 0.01$ in 2 and $P < 0.05$ in the remainder.

Time-to-peak

MLR-evoked PSPs typically had a short TTP, but some differences were found between the respective types. The TTP and maximum amplitude of the EPSP of the E pattern ($3.7 \pm 1.6$ ms, $3.2 \pm 2.3$ mV, $n = 20$) was highly significantly ($P < 0.01$) greater than that observed for EPSPs from all other patterns ($2.1 \pm 1.1$ ms, $1.6 \pm 1.0$ mV, $n = 49$). This was true whether or not the EPSPs in this latter group were followed by other PSPs. The TTP of the IPSP observed in mixed patterns of MLR responses ($3.3 \pm 1.6$ ms, $n = 31$) was similarly long as that of the EPSP of the E type. However, the absolute amplitude of the IPSP was significantly ($P < 0.01$) smaller ($1.7 \pm 1.1$ mV) and similar to the amplitude of the other EPSPs. Pure IPSPs ($n = 4$) of the I pattern had shorter TTPs ($2.6 \pm 1.4$ ms) and absolute amplitudes ($1.4 \pm 0.6$ mV), similar to this latter EPSP group. No significant differences were seen in comparisons between IPSPs of differing patterns of response to MLR stimulation. In addition, no significant differences were found in the TTP of i- or coMLR-evoked PSPs or in comparisons between motoneuron cell types.

Relationship to strength of MLR stimulation

There were concomitant increases in the amplitude of LDPS and EPSPs with increased strength of stimulation of the MLR

FIG. 3. Relationship between MLR-evoked EPSPs and LDPS recorded from a tibialis anterior (TA) motoneuron (MN) during fictive locomotion. A: LDPS observed during an 8-s period of locomotion. B: expanded view of LDPS over a single step cycle (time of occurrence of MLR stimuli indicated by $\circ$). The MLR-evoked EPSPs are visible during the depolarized phase of the LDPS. Expanded AC traces for each stimulus are aligned above the DC record. C: averaged LDPS triggered at the onset of each rising phase of the LDPS from 11 step cycles (step cycle duration of 1,272 $\pm 128$ ms). D: averages of motoneuron AC traces sorted according to their occurrence during the step cycle (divided into 10 equal bins). Top: averaged CDP over the entire locomotor trial. Lines a–d indicate the timing of the MLR stimulus artifact, the peak of the 1st positive wave recorded from the cord dorsum and the onset and peak amplitude of the MLR-evoked EPSP, respectively. Descending and segmental latencies are 4.0 and 2.3 ms, respectively. Calibration pulses in B and D (and in all subsequent AC trace averages from all figures) are 2 mV, 1 ms. E: amplitude modulation (AM) (mean $\pm$ SD) of the MLR-evoked EPSP observed over the normalized step cycle. F: the amplitude of the MLR-evoked EPSP was linearly correlated to the $E_m$ ($r^2 = 0.61$, slope $m = 0.89$).

FIG. 4. Relationship between the MLR-evoked PSPs and LDPS recorded from a medial gastrocnemius (MG) motoneuron during fictive locomotion. A: LDPS observed over a 5-s period of locomotion. B: expanded portion of a LDPS from an 800-ms period showing the short-latency PSPs observed after each MLR stimulus (a). The EPSPs and IPSPs were largest during the depolarized and hyperpolarized phase of the LDPS, respectively. C: averaged LDPS from 18 step cycles (step cycle duration of 786 $\pm 46$ ms). D: averages of motoneuron AC traces sorted according to their occurrence during the step cycle. Lines a–d as in Fig. 3D. Line c, peak amplitude of the MLR-evoked IPSP. Descending volley and segmental latencies are 3.0 and 2.3 ms, respectively. E and G: averaged AM (mean $\pm$ SD) of the EPSP and IPSP, respectively, observed over the normalized step cycle. F and H: amplitude variation of the EPSP and IPSP (respectively) relative to $E_m$.
(Fig. 5). This effect was observed in 7/7 cells examined. Increasing the strength of stimulation also increased the occurrence of MLR-evoked spiking activity (Fig. 5E, spikes truncated). In recordings from ENGs, this stimulus-evoked spiking was evident as synchronous bursting of populations of motoneurons (not shown). Increasing the strength of stimulation also prolonged the depolarized phase of the LDP (compare A and B to E and F) and similarly extended the occurrence of the di-tri-synaptic MLR-evoked EPSP (compare C and D to G and H).

Passively modulated or nonmodulated PSPs

A small number of MLR-evoked PSPs (8 EPSPs, 11 IPSPs) were classified as “non-locomotor.” Of the EPSPs, seven did not exhibit statistically significant AM during locomotion. These EPSPs were significantly smaller (0.7 ± 0.5 mV maximum amplitude) than locomotor-related EPSPs, but although their motoneuron’s LDPs tended to be smaller (4.7 ± 3.2 mV), they were not significantly different from cells with locomotor-modulated EPSPs (mean = 6.3 ± 3.3 mV). However, the locomotor-modulated EPSPs found in four of these seven cells were also significantly smaller than the total population of locomotor-modulated EPSPs (0.9 ± 0.4 mV). This, along with the fact that most (6 of 7) of the nonmodulated EPSPs had similar latencies as modulated EPSPs, indicates that they may be produced by the same population of spinal neurons, but that they were recorded during slightly lower amplitude locomotor activity. One EPSP also showed a “reverse” modulation during locomotion, being significantly larger during the hyperpolarized phase of the LDP and significantly (P < 0.001) correlated to membrane hyperpolarization.

Of the 11 IPSPs, 4 were not significantly modulated during fictive locomotion. The absolute amplitude (1.9 ± 1.4 mV) and segmental latency (2.9 ± 1.4 ms) of these nonmodulated IPSPs was not significantly different from those measured for modulated IPSPs. However, the amplitude of their motoneuron LDP (2.9 ± 0.8 mV) was significantly smaller than their locomotor-related counterparts, indicating that they too may have been produced by the same population of spinal neurons, but that they were recorded during lower amplitude locomotor activity. The remaining seven IPSPs were “reverse” modulated, being significantly (P < 0.01) larger during the depolarized phase and positively correlated to membrane depolarization. These reverse-modulated IPSPs were significantly (P < 0.01) larger (2.7 ± 1.8 mV maximal amplitude) than locomotor-related IPSPs. The segmental (2.6 ± 1.1 ms) and total (7.2 ± 1.4 ms) latencies of nonlocomotor IPSPs were also significantly shorter than those of locomotor-related IPSPs (compare with Table 1), indicating that they were likely produced by a different subset of spinal neurons.

Effects of brain stem cooling on MLR-evoked PSPs and LDPs during fictive locomotion

Cooling of the brain stem to temperatures that block synaptic transmission within the MedRF (bilaterally) was performed in three experiments. A total of six cooling trials were obtained while intracellularly recording from four lumbar motoneurons. Cooling of the MedRF to probe temperatures of 9–14°C could either abolish (2 trials from different cells) or significantly reduce (4 trials) MLR-evoked PSPs, LDPs, and motoneuron spiking activity as observed from intracellular or bilateral ENG recordings. Cooling of the MedRF also reduced the amplitude of the MLR-evoked positive (P) waves (Noga et al. 1995) recorded from the CDP: the P1, P2 and P3 waves were reduced to 55, 48, and 43% of their control values, respectively. The descending latency was increased in two trials (0.3 and 1.9 ms). These effects were reversed on rewarming. Step cycle lengths increased 124–181% during cooling, prior to blocking of the locomotor rhythm.

The effect of cooling the MedRF on ENG and intracellular FDL motoneuron activity is illustrated in Fig. 6. With rewarming of the MedRF, the amplitudes of the ENGs, LDPs, and PSPs returned to and eventually surpassed those observed in the control trial. No change was observed in the descending latency of the MLR-evoked CDP (Fig. 6, line b).

Effects of spinal cord cooling on MLR-evoked fictive locomotion

**Effect on ENG locomotor activity.** Extradural cooling of the VF (n = 25 trials) decreased the amplitude of ENG locomotor activity at cooling probe temperatures between 12 and 5°C. Locomotor activity was abolished on the side of cooling at temperatures between 10 and 0°C (mean: 4.3 ± 3.2°C). The mean temperature required to abolish locomotor activity during extradural cooling of the VF (4 trials) was...
Higher: 7.2 ± 1.6°C (range: 9–6°C). The greatest effects of cooling were observed on the side of cooling (ipsilateral), regardless of the side of stimulation (Fig. 7). In most cases ENG locomotor activity was abolished, but occasionally some rhythmic activity could be discerned in ipsilateral flexors (Fig. 7). The amplitude of flexor and extensor ENG locomotor activity was always reduced on the side contralateral to cooling (Fig. 7). The burst frequency of the remaining ENG activity was also affected (often increased). Occasionally, contralateral ENG activity was abolished. In these cases, extensors were the most vulnerable, and usually when the MLR stimulation and the VF cooling sites were on the same side. Cooling of the dorsal aspect of the spinal cord to temperatures blocking synaptic transmission in the adjacent gray matter rarely depressed MLR-evoked fictive locomotion.

**EFFECT ON LDPS AND MLR-EVOKED LOCOMOTOR-RELATED PSPS.** A total of 31 (23 VF and 8 dorsal cord) reversible spinal cord cooling trials were completed during intracellular recording from 17 lumbar motoneurons (3 flexor and 14 extensor motoneurons). Unless otherwise stated, the side of MLR stimulation and VF cooling in these trials is reported relative to the side of motoneuron recording. During cooling of the VF, a reduction or loss of ENG locomotor activity was accompanied by either a reduction or a deletion of the LDP and PSPs. Cooling probe-tip temperatures required for the complete block of LDPs with extradural or intradural cooling of the VF were 2.8–3.2°C (range: 9 to 2°C) and 7.0 ± 1.4°C (range: 8–6°C), respectively. These probe-tip temperatures were between those required to block rhythmic ENG activity (the warmest, see preceding text) and those needed to block transmission through fibers within 1 mm of the cooling probe (the coldest). The latency of the MLR-evoked CDP (volley) was often increased by 0.3 ms (maximum: 0.5 ms) during cooling of the VF, although no change was seen on some occasions.

In trials in which the LDPs were totally abolished by cooling of the VF (14 of 23 trials), EPSPs and IPSPs were reduced to 17 ± 21% (n = 14 trials) and 44 ± 42% (n = 10 trials) of their maximum control amplitudes, respectively. EPSPs were deleted in 7 of the 14 trials in which they were observed, and IPSPs were deleted in 3 of the 10 trials. PSP deletions were particularly common with iMLR stimulation and iVF cooling (5 of the 7 EPSP trials and 2 of the 3 IPSP trials). This is illustrated in Fig. 8. In this example, cooling of the iVF simultaneously abolished LDPs and EPSPs. The effect was reversible.

**FIG. 6.** Cooling of the medial reticular formation (MedRF; P10, L0) concurrently reduces the amplitude of the LDP and the short-latency EPSP recorded from a flexor digitorum longus (FDL) motoneuron and the ENG bursts recorded bilaterally. A–C, left: locomotion produced by stimulation of the right MLR. Top: intracellular recording of left FDL motoneuron; bottom 3 traces: left anterior biceps (AB), TA and right semimembranosus (SM) ENGs. All traces are at constant gain, throughout. Right: CDPs (top) and PSPs (bottom) sorted according to their occurrence during the normalized step cycle for precooL, cool, and rewarm trials, respectively.

**FIG. 7.** Effect of unilateral cooling of the VF (T12) on MLR-evoked locomotion. Fictive locomotion (A) was produced by stimulation of the right MLR and was monitored by right (R) and left (L) ENGs during extradural cooling (B) and rewarming (C) of the right VF. All ENG waveforms are at constant gain throughout. Locomotion was blocked on the side of cooling (right) and decreased on the opposite side of cooling (left) to the extent that extensor nerve activity was abolished.

**FIG. 8.** Cooling of the iVF reversibly abolishes iMLR-evoked LDPs and PSPs recorded from a TA motoneuron. A–C: control, cooling, and rewarm trials, respectively. Left: LDPs observed during stimulation of the MLR. Right: averaged signals obtained from triggered CDPs (top) and from motoneuron AC traces sorted according to their occurrence during the normalized step cycle. Loss of MLR-evoked EPSP and CDPs during cooling was concomitant with the loss of the LDP.
was also effective in depressing LDPs and EPSPs (to 17% and 71% respectively. Cooling of the iVF during coMLR stimulation reduced LDPs and EPSPs (78 and 71% of the trials, respectively). Of these trials, LDPs and EPSPs were effective in deleting LDPs and EPSPs (78 and 71% of the trials, respectively).

A is illustrated in Fig. 9 for coMLR stimulation before (and 30°C) and during extradural cooling of the iVF. Here, cooling significantly reduced the amplitude of the LDP and revealed a longer latency EPSP (compare lines c and d), even though cooling depressed the MLR-evoked CDP and did not change the descending volley’s latency. LDPs and EPSPs were only deleted in 60 and 40% of such trials, and overall fell to 17 ± 24 and 25 ± 25% of their control amplitudes, respectively. This overall reduction in amplitude was not statistically significant.

In trials in which the LDPs were not totally abolished by cooling of the VF (9 of 23 trials), LDPs were reduced to 38 ± 12% of their control amplitude. Five of the nine trials with residual LDPs were observed with coMLR stimulation and iVF cooling. PSPs were observed in all but one of these trials. On average, EPSPs and IPSPs were reduced to 31 ± 18% (n = 8 trials) and 53 ± 29% (n = 5 trials) of their control peaks, respectively.

Cooling of the iVF during iMLR stimulation was most effective in deleting LDPs and EPSPs (78 and 71% of the trials, respectively). Of these trials, LDPs and EPSPs were reduced to 11 ± 23 and 9 ± 16% of their control amplitudes, respectively. Cooling of the iVF during coMLR stimulation was also effective in depressing LDPs and EPSPs (to 17 ± 17 and 30 ± 20% of their control amplitudes, respectively). This is illustrated in Fig. 9 for coMLR stimulation before (A) and during (B) cooling of the iVF. Here, cooling significantly reduced the amplitude of the LDP and revealed a 1 ms longer latency EPSP (compare lines c and d), even though cooling depressed the MLR-evoked CDP and did not change the descending volley’s latency. LDPs and EPSPs were only deleted in 44 and 10% of trials in which they were observed, respectively. In comparisons of data obtained with stimulation of the i- and coMLR during cooling of the iVF, significant differences were observed in the frequency of EPSP deletions and the EPSP amplitude (P < 0.05).

Surprisingly, cooling the coVF during iMLR-evoked locomotion (n = 5) was quite effective in reducing the activity of the ipsilateral locomotor network as measured by responses of five extensor motoneurons. This, however, would be consistent with the observation (see Effect on ENG locomotor activity) that contralateral extensors were more vulnerable than contralateral flexors to cooling of the VF. LDPs and EPSPs were deleted in 60 and 40% of such trials, and overall fell to 17 ± 24 and 25 ± 25% of their control amplitudes, respectively. This overall reduction in amplitude was not significantly different from the effects of cooling the iVF. Figure 10 (A–D) illustrates this concomitant depression of LDPs and EPSPs during cooling of the coVF. In this trial, the late polysynaptic EPSP was also abolished. In contrast, cooling of the dorsal spinal cord (Fig. 10D) slightly enhanced the amplitude of the LDP and the EPSPs. Cooling of the coVF during coMLR-evoked locomotion was not successfully completed.

MLR-evoked IPSPs were deleted in a small percentage of trials (iMLR-iVF: 29%; coMLR-iVF: 25%; iMLR-coVF: 0%). The overall effect of cooling different sides of the cord on the amplitude of iMLR-evoked IPSPs was similar (reduced to 56 ± 42 and 60 ± 32% of their control amplitude with cooling of the iVF and coVF, respectively). Although, cooling the iVF strongly reduced coMLR-evoked IPSPs (17 ± 14% of control amplitude), the difference from iMLR stimulation was not statistically significant.

**DISCUSSION**

**Relationship of MLR-evoked PSPs to locomotion and LDPs**

The results of the present study demonstrate that MLR-evoked fictive locomotion, as measured from intracellular (LDP) or extracellular (ENG) recordings of motoneuron activity, is mediated by a reticulospinal pathway descending bilaterally through the ventral funiculi. The results also demonstrate that this pathway activates spinal neurons that evoke PSPs in motoneurons during locomotion. These PSPs are strongly modulated during the step cycle in a manner consistent with...
that reported by Shefchyk and Jordan (1985a) and Degtyarenko et al. (1998). Furthermore, these PSPs show a highly significant correlation to \( E_m \). The spinal neurons are thus highly likely to contribute to the excitation or inhibition of motoneurons during locomotion and possibly to the excitatory and inhibitory phases of the LDP, respectively. This conclusion is supported by the fact that the amplitude of the LDPs and PSPs covary with each other as activity conveyed through the descending locomotor pathway is reversibly blocked by localized cooling and as the strength of the MLR stimulation is changed.

The results of the present study are consistent with the concept that spinal neurons mediating the production of MLR-evoked PSPs in motoneurons form part of the circuitry involved in the initiation and control of locomotion (Shefchyk and Jordan 1985a). According to this hypothesis, the excitability of the population of interneurons generating the excitatory drive to motoneurons is greatest during the depolarized phase of the LDP when they are most easily activated by and synchronized to the stimulation of the MLR, to produce the largest EPSPs. Conversely, interneurons generating inhibitory drive to motoneurons are most excitable during the hyperpolarized phase of the LDP. Thus they are most easily activated by and synchronized to the stimulation of the MLR, to produce the largest IPSPs. The relative amplitude of the PSPs thus reflects the synaptic input of the interneuronal population onto motoneurons during that phase of the step cycle with excitation predominating during the depolarized phase and inhibition predominating during the hyperpolarized phase of the LDP.

It is unlikely that voltage dependence of the EPSPs (Brownstone et al. 1994) is responsible for the AM of the MLR-evoked PSPs during locomotion. To study these EPSPs, it was necessary to either study nonspiking motoneurons or to hyperpolarize the motoneurons, which would not only eliminate the firing but also take the membrane out of the range of activation for voltage-gated channels. Furthermore, individual EPSPs vary in amplitude at similar \( E_m \) levels throughout the depolarized phase of the LDP (Figs. 3B and 4B), implying that the increase in size is not related to voltage-dependency alone. In addition, the amplitudes of the MLR-evoked EPSP and LDP show a linear relationship (Figs. 3F and 4F) and not one indicative of a negative slope conductance. It would be predicted that these EPSPs would in fact also show some voltage dependence once the motoneuron is sufficiently depolarized, as input from locomotor centers to motoneurons is indeed voltage dependent (Brownstone et al. 1994). At such membrane potentials, the EPSP amplitude would likely increase fairly suddenly rather than linearly. MLR-evoked IPSPs also show a similar (but opposite) relationship to membrane hyperpolarization and are largest during the hyperpolarized phase of the LDP (Fig. 4, G and H). Therefore it can safely be said that premotoneuronal mechanisms are responsible for this modulation.

It is likely that the majority of the neurons of the locomotor network (locomotor pattern generator or LPG) were activated by the MLR stimulation at the strengths used. Whether these neurons are activated by reticulospinal neurons directly or indirectly and the degree to which they influence motoneuron activity will depend on the anatomical details of the circuit and the level of excitability of the neurons involved. The locomotor network is highly complex and multitiered with components generating rhythm and others shaping this into a spatiotemporal pattern (see discussion by Burke et al. 2001). Providing that interneurons are recruited during locomotion, they are in a position to directly or indirectly influence the timing (frequency) and/or the degree of depolarization and hyperpolarization (pattern) of the motoneuron membrane potential and to thus shape motoneuron output. The contribution of these neurons to the locomotor output will ultimately depend on the moment-to-moment regulation of their excitability and may therefore be “state-dependent.”

The hypothesis that the spinal interneurons mediating the production of MLR-evoked PSPs in motoneurons form part of the circuitry involved in the initiation and control of locomotion does not necessarily mean that only they are responsible for the generation of locomotion. We and others (Degtyarenko et al. 1998) have found that those motoneurons lacking MLR-evoked EPSPs or IPSPs are nevertheless rhythmically modulated during fictive locomotion. Obviously, these motoneurons receive rhythmic excitation or inhibition from some source even though pulsed activity through MLR activated interneuronal circuits fails to evoke excitatory or inhibitory PSPs. Different interneurons could be activated by other descending pathways that become modulated during locomotion and they could contribute to the formation of the LDP (Burke et al. 2001; Degtyarenko et al. 1998; Gossard et al. 1996; Leblond and Gossard 1997; Leblond et al. 2000). The ability to activate the excitatory premotor neurons well enough to synchronize their activity with stimulation of the MLR is likely dependent on a number of factors, and failure to do so in some cases may be an experimental shortcoming dependent on the precise state of the animal. It may also depend on the organization of the circuitry involved in the activation and inhibition of these motoneurons (e.g., see Burke et al. 2001). This may be the case for extensor digitorum longus motoneurons, which primarily receive rhythmic excitation or inhibition from some source.

Premotoneurons generating inhibitory drive to motoneurons are most excitable during the hyperpolarized phase of the LDP when they are most easily activated by and synchronized to the stimulation of the MLR, to produce the largest IPSPs. Conversely, interneurons activated by and synchronized to the stimulation of the MLR, to produce the largest EPSPs. The relative amplitude of the PSPs thus reflects the synaptic input of the interneuronal population onto motoneurons during that phase of the step cycle with excitation predominating during the depolarized phase and inhibition predominating during the hyperpolarized phase of the LDP.

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Descending and segmental pathway for MLR-evoked locomotion, LDPs, and PSPs

The present study demonstrates that electrical stimulation of the MLR produces locomotion by activating a reticulospinal pathway originating in the MedRF and that this pathway projects bilaterally through the VF of the spinal cord to activate spinal networks for locomotion as originally demonstrated by Steeves and Jordan (1980, 1984). Cooling of the MedRF or VF to temperatures that block synaptic or fiber transmission, respectively, reversely abolishes MLR-evoked fictive locomotion, extending observations that cooling of these sites is effective in abolishing MLR-evoked treadmill locomotion (Noga et al. 1991; Shefchyk et al. 1984). Bilateral projections from the MLR to the MedRF have been described previously (Garcia-Rill et al. 1983b; Shefchyk and Jordan 1984), and, as confirmed in the present study, unilateral stimulation of the MLR may activate reticulospinal neurons that descend ipsilaterally and/or contralaterally in the VF (Garcia-Rill and Skinner 1987; Orlovsky 1969, 1970a,b; Perreault et al. 1993). Our results, however, are inconsistent with the proposal that stimulation of the MLR evokes locomotion via a polysynaptic pathway projecting via the lateral tegmentum of the brain stem and the DLF of the spinal cord (Kazennikov et al. 1985; Mori et al. 1977; Shik 1983) because cooling of the spinal cord at the level of the T13 segment to block synaptic transmission in the adjacent gray matter and fiber transmission in the dorsal half of the cord never abolished locomotion. This is compatible with our previous report of the effects of cervical lesions on MLR-evoked treadmill locomotion (Noga et al. 1991; Steeves and Jordan 1980).

As observed in this and other studies, stimulation of the MLR activates a fast conducting pathway (Degtyarenko et al. 1998; Shefchyk et al. 1985a) originating in the MedRF (Noga et al. 1988, 1991; Shefchyk et al. 1984) that projects to the spinal cord to ultimately influence hindlimb motoneurons. The maximum conduction velocity of this pathway ranges between 62 and 145 m/s (average: 88 m/s), after taking into account the ∼1-ms minimal delay for activation of reticulospinal cells by the MLR (Garcia-Rill and Skinner 1987; Iwakiri et al. 1995; Orlovsky 1969, 1970a). These results are comparable with conduction velocities previously reported for locomotor-related reticulospinal cells (Drew et al. 1986; Iwakiri et al. 1995; Orlovsky 1969, 1970a; Perreault et al. 1993; see, however, Garcia-Rill and Skinner 1987). Short-latency excitatory and inhibitory responses have also been observed bilaterally from hindlimb electromyographic (EMG) and ENG recordings after stimulation of the MedRF (Drew and Rossignol 1984; Perreault et al. 1994), from lumbar intraspinal field potential recordings after stimulation of the MLR (Noga et al. 1995) or the cervical ventrolateral funiculus (Magnuson and Trinder 1997), and in forelimb motoneurons during stimulation of the lateral funiculus (Kinoshita and Yamaguchi 2001). The short-latency excitatory responses have also been correlated to the activity of cells in the MLR (Garcia-Rill et al. 1983a) and MedRF (Drew et al. 1986; Shimamura et al. 1982).

Results obtained from unilateral cooling of the VF during MLR-evoked locomotion show that both ipsilateral and contralateral reticulospinal projections and their spinal targets contribute to the excitation and inhibition of motoneurons on both sides of the spinal cord and thus to locomotor activity bilaterally. Even though bilateral descending/segmental activity is not obligatory for the formation of locomotor activity on a single side of the cord (see following text), the normal operation of the locomotor circuit likely involves integrated activity from both sides of the cord in such a way that it functions as a single unit. In this sense, our data supports the concept of a “bilateral shared core” proposed by Stein et al. (1995) for fictive rostral scratching. According to this hypothesis, contralateral circuitry contributes to the production of ipsilateral extensor rhythmicity. A similar finding was observed in the present study for MLR-evoked fictive locomotion. In this case, unilateral cooling of the VF, in addition to blocking locomotor activity on the same side (Figs. 7 and 8), was especially effective in reducing or abolishing the ENG and LDP activity of extensor motoneurons on the opposite side (Figs. 7 and 10). This was associated with the amplitude reduction or loss of the second or longer-latency MLR-evoked EPSP (Fig. 10, A–C).

Figure 11 illustrates a proposed model of the organization of the descending and segmental pathway for the production of MLR-evoked fictive locomotion in the decerebrate cat. The model summarizes the relationships between the reticulospinal pathways, the flexor and extensor components of the LPG and their respective motoneurons for bilateral hindlimb locomotion after unilateral MLR stimulation. Details of the LPG flexor and extensor components are omitted to emphasize the possible interconnections between them and their target neurons. Relative strengths of activation for the reticulospinal pathways are indicated by the degree of shading. The figure also shows the effects of interrupting signal transmission through the various components of the descending locomotor pathway.

According to this model, one-sided MLR stimulation results in a relatively greater reticulospinal output to the same side of the cord. This asymmetry in the relative strength of the reticulospinal output is indicated by the observation that cooling of the iVF during iMLR stimulation was the most effective combination for deleting LDPs and EPSPs. This conclusion is also supported by the observation that the majority of activated reticulospinal neurons project through the VF on the same side as the stimulated MLR (Garcia-Rill and Skinner 1987). Although unilateral stimulation of the MLR may produce a slight asymmetry in reticulospinal output, it is assumed that spontaneous locomotion would provide a balanced descending input to the spinal locomotor centers.

The LPG neurons (flexor and extensor related) on each side of the spinal cord are predominantly activated by reticulospinal neurons descending on the same side (Fig. 11). This conclusion is supported by a number of observations. 1) Unilateral cooling of the VF almost always abolished locomotor ENG activity on the same side, regardless of the side of MLR stimulation. 2) Stimulation of the MLR will evoke treadmill locomotion, preferentially on the side contralateral to an acute spinal cord hemisection (Steeves and Jordan 1980) or unilateral ventrolateral funiculus transection (Noga et al. 1991; Steeves and Jordan 1980). 3) A surgically isolated single side of the spinal cord will generate locomotor activity similar to that seen in intact animals if it is appropriately activated (Cowley and Schmidt 1997; Kato 1988, 1990; Kudo and Yamada 1987). 4) Stimulation of the MLR or its anatomical equivalent evokes ipsilateral, segmentally monosynaptic, field potentials (Noga et al. 1995) and directly activates spinal interneurons (Carr et al. 1995; Huang et al. 2000; Jankowska and Noga 1990; Jordan
The anatomical details of this crossed spinal projection are unclear. There are two possibilities that may occur alone or in combination (Fig. 11). Reticulospinal axons may cross to innervate contralateral spinal neurons directly (Holstege and Kuypers 1982; Kausz 1991; Magnuson and Trinder 1997; Nyberg-Hansen 1965). Reticulospinal axons may also activate commissural neurons (Jankowska et al. 2003) that are either components of the LPG or relay interneurons. Crossed segmental pathways from the contralateral rhythm generator have been described (Kjaerulff and Kiehn 1997; Kremer and Lev-Tov 1997; Stokke et al. 2002) possibly forming the substrate for interlimb coordination during locomotion (Butt et al. 2002). The targets of these pathways are likely to involve both motoneurons (Butt and Kiehn 2002; Jankowska and Noga 1990; Jankowska et al. 2003; Kjaerulff and Kiehn 1997) as well as the flexor and extensor components of the LPG (this study; Butt et al. 2002). Preliminary evidence also indicates that their actions may be excitatory and inhibitory (Butt and Kiehn 2002). The details of this interaction could be explored using localized cooling of the spinal cord to reversibly block synaptic or fiber transmission (present study) or using irreversible spinal cord lesions (Kjaerulff and Kiehn 1996; Stein et al. 1995) in specific lumbar segments during MLR-evoked fictive locomotion.

**Spinal neurons mediating MLR-evoked PSPs**

In the present study, the shortest latency locomotor-related EPSPs were evoked at di- and tri-synaptic segmental latencies (Fig. 2B, top) consistent with their being mediated by one or two interposed spinal neurons. Longer-latency EPSPs likely involve three or more intervening neurons. IPSPs were evoked at latencies consistent with trisynaptic and polysynaptic linkages and therefore require at least two or more intervening interneurons for their production (Fig. 2B, bottom). These observations are consistent with previous reports for MLR-evoked PSPs (Degtyarenko et al. 1998; Shefchyk and Jordan 1985a) and intraspinal field potentials (Noga et al. 1995).
evoked fictive locomotion nor PSPs, we predict that neurons within these segments do not contain elements crucial for mediating MLR-evoked PSPs. This is consistent with data on the activity-dependent expression of c-fos protein in spinal neurons following MLR stimulation. In the cat, locomotor-activated c-fos labeled neurons have been found in the base of the dorsal horn, the intermediate zone and in the ventral horn of the lumbar-sacral cord from T12 to S1, with greatest numbers in the mid-lumbar segments (Carr et al. 1995; Huang et al. 2000; Johnson et al. 2002). A distributed thoraco-lumbar locomotor network has also been described for the neonatal rat and mouse (Cowley and Schmidt 1997; Kjaerulf and Kiehn 1996; Kjaerulf et al. 1994; Kremer and Lev-Tov 1997; Nishimaru and Kudo 2000), although there is a tendency for more rostral segments to have greater rhythmogenic potential (Kjaerulf and Kiehn 1996; Kremer and Lev-Tov 1997).

A growing number of studies have examined the responses of identified spinal neurons to stimulation of the MLR or its anatomical equivalent. The first group of interneurons are monosynaptically excited by group II muscle spindle afferents and produce either excitatory or inhibitory PSPs in ipsilateral motoneurons of lower lumbar segments (Cavallari et al. 1987; Edgley and Jankowska 1987). These last-order neurons are activated by the MLR at latencies of 4–7.5 ms and are rhythmically active during locomotion (Shefchyk et al. 1990). These neurons could produce PSPs in motoneurons at latencies between 5.5 and 9.5 ms. The second group are contralaterally-projecting mid-lumbar laminae VIII interneurons. These neurons, which project to contralateral motor nuclei in more caudal segments, are disinaptically activated by stimulation of the cuneiform (MLR) nucleus at latencies of 4.4–4.6 ms (Jankowska and Noga 1990) and are monosynaptically activated by stimulation of the ipsilateral reticular formation (Jankowska et al. 2003). These cells would produce PSPs in motoneurons at latencies between 5.2 and 6.1 ms and could mediate effects of the crossed segmental pathway. The third group are cholinergic commissural lamina VII partition cells. These cells are activated by stimulation of the MLR at di- to oligosynaptic segmental latencies of 1.2–3.5 ms (Huang et al. 2000). They contain choline acetyltransferase and project contralaterally, ascending to at least the T1 level (Huang et al. 2000). The spinal sites of terminations of these cells are unknown.

Other unidentified spinal neurons within the intermediate zone and ventral horn of the L4–L7 segments are rhythmically activated by stimulation of the MLR at latencies appropriate for a role in mediating MLR-evoked PSPs (Carr et al. 1994; Jordan and Noga 1991). Their location coincides with the anatomical projections of reticulospinal pathways (Holstege and Kuypers 1982; Kaus 1991; Nyberg-Hansen 1965; see also Peterson et al. 1975) and with the site of action of pathways descending in the VF (Magnuson and Trinder 1997; Skinner et al. 1970).

Conclusions

In conclusion, we present evidence that stimulation of the MLR activates a pathway that relays in the MedRF and descends bilaterally in the VF to contact spinal interneurons that project to motoneurons. Local segmental pathways that activate or inhibit motoneurons during MLR-evoked fictive locomotion appear to be both ipsilateral and contralateral.

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REFERENCES


