Locomotor Modulation of Disynaptic EPSPs From the Mesencephalic Locomotor Region in Cat Motoneurons

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INTRODUCTION

A variety of primary afferent and descending pathways produce short-latency synaptic potentials that exhibit phase-related modulation during fictive locomotion in cat hindlimb motoneurons (e.g., Andersson et al. 1978; Degtyarenko et al. 1996, 1998; Floeter et al. 1993; Gossard et al. 1996; Moschovakis et al. 1991; Schmidt et al. 1988; Schomburg and Behrend 1978). Such state-dependent changes in synaptic transmission can be used to infer the organization of oligosynaptic interneuronal pathways in the spinal cord, especially when applied to disynaptic connections between a given afferent system and α-motoneurons (see Burke and Fleshman 1986; Lundberg 1975). In particular, differential modulation of transmission in a given afferent system to multiple motoneuron pools provides evidence for the existence of distinct subgroups of last-order interneurons (Degtyarenko et al. 1996, 1998; Moschovakis et al. 1991).

Shefchyk and Jordan (1985) have demonstrated that stimulation of the mesencephalic locomotor region (MLR) in unanesthetized, de cerebrate cats not only evokes rhythmic alternating discharges in flexor and extensor hindlimb motor pools (fictive locomotion) but also generates short-latency excitatory postsynaptic potentials (EPSPs) in hindlimb motoneurons that exhibit phase-dependent modulation. The MLR EPSPs in flexor cells were enhanced during the flexion phase of fictive stepping, whereas those in extensor motoneurons were enhanced during extension. In the present work, we have reexamined this finding with particular attention to MLR effects in flexor digitorum longus (FDL) motoneurons because the FDL motor pool shows variable phase-related behavior during fictive locomotion (Fleshman et al. 1984; Moschovakis et al. 1991) as well as during walking in intact cats (O’Donovan et al. 1982; Trank and Smith 1996). We also compared the locomotor modulation patterns of MLR PSPs with those of disynaptic EPSPs produced by stimulation of reticulospinal axons activated in the medial longitudinal fasciculus (MLF) because these patterns are generally similar to those described for the MLR (Floeter et al. 1993; Gossard et al. 1996). Thus it seemed possible that the two descending systems might produce oligosynaptic EPSPs in hindlimb motoneurons by converging on common segmental interneurons. Some of this material has appeared in abstract form (Degtyarenko et al. 1997).

METHODS

The methods used for the present experiments were similar to those used in previous reports from this laboratory (Degtyarenko et al. 1996, 1998; Floeter et al. 1993; Gossard et al. 1996; Moschovakis et al. 1991). Data tapes from some of these previous
experiments were reexamined, and some previously unpublished observations from those experiments are included in the present report. The experiments were conducted in accordance with the ‘Principles of Laboratory Animal Care’ (National Institutes of Health Publication 86-23) and were approved by the National Institute of Neurological Disorders and Stroke Committee on Animal Care and Use.

Briefly, adult female cats (2.5–4.0 kg) were used. Halothane anesthesia was induced by mask and maintained (1–2% in air) via a tracheal cannula during surgery. One common carotid artery was catheterized for blood pressure monitoring and the other was ligated. Catheters were placed in both cephalic veins for administration of norepinephrine and fluids as necessary to maintain blood pressure within physiological limits. Rectal temperature was maintained near 38°C with a heating pad and lamp. The bladder was catheterized in most experiments.

After extensive denervation of the left hindlimb, the following muscle nerves were cut and mounted on bipolar platinum wire electrodes for stimulation and recording: posterior biceps-semitendinosus (PBST), lateral gastrocnemius-soleus (LGS), medial gastrocnemius (MG; sometimes together with LGS: GS), flexor digitorum longus (FDL), flexor hallucis longus (FHL), tibialis anterior (TA), and extensor digitorum longus (EDL). Cutaneous branches of the superficial peroneal (SP) and medial plantar (MPL) nerves were freed but not cut; all were mounted on bipolar stimulating electrodes. After a laminectomy exposing spinal segments L5–S1, the animals were transferred to a stereotaxic frame, and skin flaps surrounding the spinal cord and the hindlimb nerves were used to construct paraffin oil pools. After opening the skull bilaterally, precollicular postmammillary decerebration was performed with a spatula, suction, and cautery to minimize blood loss. Halothane anesthesia was then discontinued. A midline posterior fossa craniotomy was performed to expose the cerebellar vermis. The animal was paralyzed with gallamine triethiodide (Flaxedil; 10 mg/kg supplemented every 40 min) and artificially ventilated to maintain the expired CO₂ near 4%.

Recording and stimulation

The cord dorsum potential (CDP) was recorded with a monopolar platinum ball electrode placed near the dorsal root entry zone at the L₆–L₇ border, referred to a distant indifferent electrode. Stimulation intensity to peripheral nerves was expressed in multiples of the threshold for the most excitable fibers in the nerve, usually at twice threshold for the most excitable fibers. Intracellular recordings from motoneurons in the L₆–L₇ segments were made with glass micropipettes (1.0–2.5-m tip diam) filled with 2 M K⁺ acetate solution containing 26 mM QX314 (Alomone Laboratories, Jerusalem, Israel) to suppress sodium-dependent action potentials (Frazier et al. 1970). Motoneurons were identified by antidromic invasion from muscle nerve stimulation.

To produce fictive locomotion, a monopolar tungsten electrode insulated except at the tip was placed into the mesencephalic locomotor region (MLR; nominal coordinates: P₂, L₄, HC –1). Constant current (80–150 μA) biphasic, charge balanced pulses (pulse duration 0.2–0.5 ms separated by an equal interval) were delivered in trains (10–30 Hz), referenced to a wire in the neck muscles. The position of the MLR electrode was adjusted to produce rhythmic alternating activity in hindlimb muscle nerves (fictive locomotion), which was in most cases accompanied by distinctive CDP waves and short-latency synaptic potentials in the intracellular records (see Figs. 1B and 2A). The most effective stimulation site for producing fictive stepping was also best for producing the oligosynaptic PSPs, as reported by Shefchyk and Jordan (1985).

In some experiments, we also stimulated the MLF with electrodes that were similar to those used in the preceding paragraph. The MLF electrode was introduced through the cerebellar vermis within 0.5 mm of the midline while stimulating with biphasic
Data collection and analysis

Electroneurogram (ENG) activity in muscle nerves (usually LGS, FHL, FDL, TA or EDL, and PBST). CDP, and intracellular potentials was recorded with an eight-channel digital videotape recorder (Instrutech VR-100; band-pass DC-4 kHz for all channels or, for later experiments, an Instrutech VR-100B; band-pass DC-9 kHz for the intracellular channel). Separate digital signal channels were used to record pulses that were synchronized with stimuli delivered to peripheral nerves and to the MLR, MLF, and peripheral nerves.

Data from selected portions of bouts of fictive locomotion were digitized off-line (10 kHz) with a Macintosh 8100 computer equipped with a National Instruments NBIO-16 A/D board, and “virtual instrument” programs written with the LabView software package (LabView 3.0, National Instruments, Austin, TX). The ENG data streams were rectified digitally and smoothed using decremental look-ahead exponential weighting with a time constant of ~20 ms (Degtyarenko et al. 1998). The software package allowed burst onsets and offsets to be determined either automatically or manually, depending on signal-to-noise ratio. In most cases, the flexion phases were defined as beginning with the onset of ENG firing in the PBST and/or FDL nerves and ending with the termination of bursts in TA or EDL (see Figs. 3A, 5A, and 7A) or the

-Fig. 2. Parallel changes of MLR EPSPs and membrane potential in the same PB motoneuron shown in Fig. 1A: averaged records of MLR EPSPs as in Fig. 1C but accumulated during the early, middle, and late thirds of flexion (F1, F2, F3, respectively; number of sweeps: 56, 64, 57) and extension phases (E1, E2, E3; number of sweeps: 123, 122, 126; see METHODS). Note that time scale is plotted as segmental latency, with onset at the initial cord dorsum deflection (~ -- --). Disynaptic MLR EPSPs (segmental latency 1.2 ms) were much larger throughout flexion than during extension. B: graph of the intracellular potential (PB IC), amplitude of MLR EPSPs measured at 2.5 ms segmental latency (MLR EPSP at 2.5 ms; ~ -- -- line in A), and rectified, integrated FHL and PB ENGs, averaged in 10 equal duration time bins over 45 successive step cycles (see METHODS). Note the parallel trajectories of MLR EPSP amplitude and intracellular potential (~--).

FIG. 3. Modulation of MLR PSPs during fictive locomotion in an extensor digitorum longus (EDL) motoneuron. A: records of intracellular potential from an EDL cell (EDL IC) and electroneurograms (ENGs) from 5 muscle nerves during MLR-induced fictive locomotion. B: averaged records as in Fig. 2A of MLR PSPs during flexion and extension phases (number of sweeps in each average: 78–88). Small disynaptic EPSPs in E3 and F1 phases (segmental latency ~1.6 ms) were replaced by inhibitory postsynaptic potentials (IPSPs) with the same latency during F2 and F3, when the intracellular locomotor drive potentials (LDP) depolarizations were largest.
onset of extensor (MG, LGS, or FHL) bursts (Fig. 1A). The remaining time periods were defined as extension phases.

The flexion and extension phases were each subdivided into three equal time bins. The recorded stimulus timing pulses were used to trigger the computer to average together the intracellular potentials and CDPs resulting from selected stimuli falling into the appropriate bins (Moschovakis et al. 1991). Overlap between target PSPs (i.e., either MLR or MLF) and the responses to the other stimuli presented was avoided during averaging by using the stimulus timing pulses to include only those target PSPs falling within an acceptance window that contained no other responses. The programs also allowed exclusion of responses that produced action potentials.

We used an additional program that allowed comparison among averaged integrated ENG signals, baseline intracellular potentials, and peak amplitudes of reflex PSPs with finer resolution in normalized time (Degtyarenko et al. 1998). This program sampled rectified and integrated ENG signals from up to five muscle nerves, as well as the intracellular potential, each averaged during 1-ms windows excluding the evoked PSPs. The calculated average and standard deviation of these values were placed into 10–20 equal-increment time bins, chosen to provide continuous estimates with a reasonable number of data points per bin (Fig. 2B). The program also sampled the membrane potential immediately before and at 0.6 ms (n = 39 measurements; ) . The average segmental latency (±SD) from stimulus onset to the peak of the first volley at the cord dorsum (e.g., Fig. 1B) was 4.5 ± 0.6 ms (n = 39 measurements). The average segmental latency between this first CDP volley and EPSP onset (when detectable) was 1.6 ± 0.4 ms (n = 35 motoneurons). Three of these 35 cells exhibited MLR EPSPs with segmental latencies >2.0 ms (2.1, 2.6, and 2.8 ms for PBST, GS, and GS, respectively). The mean segmental latency for MLR EPSPs in the GS motoneurons (2.1 ± 0.4 ms, n = 7) was signifi-
Modulation of MLR EPSPs in flexor motoneurons

Oligosynaptic EPSPs produced by MLR volleys in the majority of PBST and TA motoneurons exhibited maximum amplitudes during the flexion phase of fictive locomotion, when the membrane potential of the cells showed large depolarizing locomotor drive potentials (LDPs) (see Shefchyk and Jordan 1985). Figure 1 shows an example from a posterior biceps (PB) cell that exhibited large depolarizing LDPs that were maximum during the first half of the flexion phase [Fig. 1A; PB intracellular (IC)]. The averaged MLR EPSPs (see METHODS; segmental latency 1.2 ms) were much larger throughout flexion (Figs. 1B and 2A; F1, F2, and F3) than during extension (Fig. 2B; E1, E2, E3). The average amplitude of these EPSPs, measured at segmental latency of 2.5 ms (see METHODS), showed a parallel modulation with the membrane potential (Fig. 2B). Observations in most other PBST and in TA motoneurons were similar, a shown in the summary diagrams in Fig. 4, A–C.

In contrast, stimulation of the MLR generated only small or undetectable PSPs in most EDL motoneurons, with little change during fictive locomotion. Only one EDL motoneuron exhibited enhancement of a small disynaptic MLR EPSP during the flexion phase, as shown in the summary diagrams in Fig. 4, D–F. In 5/10 EDL cells (e.g., Fig. 3), small short-latency MLR EPSPs were replaced by small disynaptic IPSPs during the flexion phase when the intracellular potential showed maximum depolarization. This might be explained by enhancement of existing IPSPs when superimposed on large depolarizing LDPs or by modulation of transmission through premotoneural pathways or possibly both (Degtyarenko et al. 1996). In any case, it was clear that the results in EDL cells were very different from those in the other flexor motoneuron pools.

Modulation of MLR EPSPs in extensor motoneurons

The MLR EPSPs recorded in GS and FHL motoneurons were larger during the extension phase of fictive stepping than in the flexor phase. An example is shown in Fig. 5B; Fig. 5 also illustrates the parallel change in MLR EPSPs and the depolarizing LDPs in this GS motoneuron (C). There was no difference between GS and FHL cells in this regard, as illustrated in the sample summary in Fig. 6. The MLR EPSPs were in this case measured at 4-ms segmental latency because of the relatively long onset latencies found in GS motoneurons as noted earlier in this section.
stimuli to the cutaneous SP peripheral nerves that also produced large PSPs in this cell (visible in the intracellular record in Fig. 7A and not synchronized with the low-frequency 30-Hz MLR tetanus; see METHODS). The minimum segmental latency for the MLR EPSP was \(~1.2\) ms, indicating that the first EPSP component (arrow) was disynaptic. This early EPSP, as well as later components, were enhanced markedly only during F1, coincident with the LDP depolarization and the short FDL burst in the first third of flexion. This parallel modulation is shown clearly in Fig. 7C (arrows).

Figure 8, A–C, illustrates the same phenomena in another FDL motoneuron. When the MLR and the SP nerve were stimulated with independent trains, the FDL ENG exhibited early flexion bursting (although not as well developed as in Fig. 7), plus associated depolarizing LDPs (A). Disynaptic MLR EPSPs were enhanced markedly during F1 (B) in parallel with the membrane potential depolarization (C). However, when the same low-frequency MLR tetanization was continued but with unsynchronized 10-Hz stimulation of the medial plantar nerve (MPL, Fig. 8, D–F), the FDL ENG activity during flexion disappeared, and the intracellular record showed depolarization coincident with firing in the LGS nerve (Fig. 8D). The MLR EPSPs that were averaged during this episode were smaller than in the first condition (note change in amplitude scale), but the largest EPSPs now occurred throughout the extension phase (E), closely parallel with the intracellular depolarization (F).

Figure 9 shows another example of extensor phase modulation of the MLR EPSP in a different FDL motoneuron. In this case, fictive locomotion occurred without MLR stimulation but during 10-Hz stimulation of the SP nerve in which the pattern of FDL activity and intracellular depolarization resembled that in Fig. 7A. This locomotion ceased when the SP stimulation was replaced by 10-Hz stimulation of the MPL nerve, but regular stepping reappeared when MLR stimulation at \(~25\) Hz was superimposed (Fig. 9A). However, the activity of the FDL nerve was now entirely during the extension phase, coincident with FHL activity and without any FDL activity in flexion (open arrow). The intracellular record now showed only a small, plateau-like depolarization confined to the extension phase with no F1 depolarization (open arrow). The averaged MLR EPSPs obtained during extension showed marked increases in late EPSPs, but close examination also showed no early component in the F1 response (oblique arrow).

Modulation of MLR EPSPs in FDL motoneurons

It was of particular interest to examine the pattern of modulation of MLR PSPs in FDL motoneurons during fictive locomotion because this motor pool can exhibit variable phase-related activity during fictive locomotion (Fleshman et al. 1984; Moschovakis et al. 1991), as it does during normal walking in intact cats (O’Donovan et al. 1982; Trank and Smith 1996). In both situations, the FDL usually fires a short burst during the first third to half of the flexion phase but sometimes also exhibits activity during the extension phase.

An example of the usual pattern is shown in Fig. 7A, in which brief bursts of FDL ENG activity (oblique arrows) coincided with equally brief intracellular depolarizations in an FDL motoneuron during early flexion (vertical arrows). Averaged records of the MLR EPSPs illustrated in Fig. 7B were synchronized with the MLR stimulation pulses after omitting those stimulus pulses that were within 10 ms of stimuli to the cutaneous SP peripheral nerves that also produced large PSPs in this cell (visible in the intracellular record in Fig. 7A and not synchronized with the low-frequency 30-Hz MLR tetanus; see METHODS). The minimum segmental latency for the MLR EPSP was \(~1.2\) ms, indicating that the first EPSP component (arrow) was disynaptic. This early EPSP, as well as later components, were enhanced markedly only during F1, coincident with the LDP depolarization and the short FDL burst in the first third of flexion. This parallel modulation is shown clearly in Fig. 7C (arrows).

Modulation of MLF EPSPs: comparison with MLR

Earlier reports from this laboratory described locomotor modulation of disynaptic EPSPs produced in a variety of cat lumbosacral motoneurons by stimulation of reticulospinal fibers in the MLF (Floeter et al. 1993; Gossard et al. 1996). In general, this reticulospinal system produces disynaptic EPSPs in both extensor and flexor motoneurons that exhibit patterns of locomotor modulation that resemble the observations with MLR EPSPs; MLF disynaptic EPSPs are enhanced during extension in extensor cells and during flexion in flexor motoneurons. Thus it seemed possible that the MLR pathway, which relays at least in part within the reticular...
Fig. 7. Enhancement of disynaptic MLR EPSPs during early flexion in an FDL motoneuron. A: record of intracellular potential in the FDL cell (FDL IC) and ENG records in FHL, FDL, TA, and PBST nerves. Brief bursts in the FDL nerve in the 1st 3rd of flexion (oblique arrows) were synchronous with LDP depolarizations in the motoneuron (upward arrows). Synaptic potentials elicited by stimulation of the cutaneous SP nerve at 10 Hz are evident in the intracellular record. Stimulation of the MLR was at ∼30 Hz and not synchronized with the superficial peroneal (SP) stimulation. B: averaged records of the MLR PSPs (40–60 sweeps each) after omitting responses within ±20 ms of any SP nerve stimulus (see METHODS). Di- and trisynaptic MLR EPSPs (minimum segmental latency 1.2 ms) clearly were enhanced during F1 and virtually disappeared during E1 and E2. C: averaged amplitude of the MLR EPSP measured at 3.0-ms segmental latency (dashed line in B) showed a parallel modulation with the intracellular potential and the bursts of FDL ENG (arrows). Averages accumulated during 14 steps; format as in Fig. 2B.

formation (Jordan 1991), might converge onto segmental interneurons that also receive reticulospinal input from the MLF. The earlier work also suggested that disynaptic MLF EPSPs in FDL motoneurons were facilitated only during the extension phase of fictive locomotion, even when the FDL motor pool was active in early flexion (Floeter et al. 1993; Gossard et al. 1996). However, the sample of FDL cells studied was small and was not completely documented in those publications.

Figure 10 shows an example of modulation of MLF EPSPs in an FDL motoneuron during spontaneous fictive locomotion with a “normal” pattern of FDL activity and LDP depolarization confined to the F1 period (A, →). Stimulation of the MLF at ∼3 Hz during this period of locomotion produced di- and trisynaptic EPSPs in this cell (central latencies 1.3 and 2.2 ms, respectively; note inflection in Fig. 10B). Both MLF EPSPs showed enhancement throughout the extension phase of stepping (B), entirely out of phase with the membrane depolarization evident in F1, as illustrated in the graph in C (→). Unfortunately no MLR PSPs were available for direct comparison because fictive locomotion was spontaneous.

Figure 11 shows an example in which locomotor modulation of MLF and MLR EPSPs could be compared in the same motoneuron, during fictive locomotion in which FDL activity was confined to the early flexion phase (not shown but similar to Fig. 10A). Di- and trisynaptic MLF components were enhanced during F1 and F2 (Fig. 11A), whereas the disynaptic MLF EPSPs were enhanced only during the extension phase (Fig. 11B). Double-pulse stimulation was used to reveal the disynaptic component in this case (see Floeter et al. 1993). Enhancement of the disynaptic MLF EPSP during E1 and E2 was clear in Fig. 11B, inset, which shows the responses to the second pulse. The monosynaptic component (→) did not change with locomotion phase.

Figure 12 presents a summary of results in FDL motoneurons, comparing the locomotor modulation of MLR (A–C) and MLF (D–F) disynaptic EPSPs in response to single-pulse stimulation. The MLF sample does not include the cell illustrated in Fig. 11, where double-pulse stimulation was necessary. As in Figs. 4 and 6, the top panels show representative PSPs superimposed to permit shape and amplitude comparisons during early flexion (A and D) and extension (B and E) phases of fictive locomotion. The heavy traces in A and B are responses from the FDL cell shown in Fig. 9, where FDL activity was confined to the extension phase. The amplitudes of MLR PSPs in 10 FDL motoneurons, measured at segmental latency of 2.5 ms, are shown in C, ranked according to the amplitude during extension. The two cases marked with asterisks were from bouts of locomotion in...
FIG. 8. Records from an FDL motoneuron showing 2 patterns of FDL activity and associated modulation of MLR EPSPs during fictive stepping. A: stimulation of the cutaneous SP nerve (10 Hz, 2 times threshold) superimposed on a low-frequency tetanus to the MLR (~25 Hz) produced fictive stepping with the FDL nerve active during early flexion, accompanied by depolarizing LDPs. B: averaged MLR EPSPs (flexion phase: 32–40 sweeps; extension phase: 64–67 sweeps) after eliminating responses in proximity to SP stimulations show marked facilitation of di- and trisynaptic components in F1. C: average amplitude (16 steps; format as in Fig. 2B) of MLR PSPs measured at 2.3-ms segmental latency (± ± ± in B) exhibit modulation that closely paralleled the membrane potential in the FDL motoneuron (FDL IC). D: when the peripheral nerve stimulation was changed to the cutaneous medial plantar (MPL) nerve, patterned FDL muscle nerve activity disappeared but the intracellular potential exhibited depolarizations in parallel with the extensor LGS activity. E: averaged MLR EPSPs (flexion phase: 44–48 sweeps; extension phase: 58–62 sweeps) during this period were largest throughout the extension phase and virtually disappeared during flexion. F: average amplitude of MLR PSPs measured at 2.3-ms segmental latency were now minimal during flexion and showed a ramp-like increase to maximum during extension (27 steps). As in C, the changes were parallel to the membrane potential variation but the timing was very different.

which FDL activity was confined to the extension phase; in the others, FDL activity and/or FDL depolarizing LDPs were found exclusively in early flexion. In these eight cases, the disynaptic MLR EPSPs were enhanced during early flexion in five and showed little change in three.

The behavior of MLF EPSPs in FDL motoneurons was more consistent in that the disynaptic EPSP measured at segmental latency of 2.0 ms were absent during the early flexion period in all seven FDL cells when a single MLF stimulation pulse was used. One cell showed a monosynaptic MLF EPSP (heavy trace in Fig. 12D), with no detectable disynaptic component (open arrow). However, during the extension phase (Fig. 12E), all of the motoneurons exhibited disynaptic EPSPs, including the cell with the monosynaptic component (heavy trace, arrow; the monosynaptic EPSP from D is indicated by the thin dashed trace). This case is denoted by the asterisk in F.

DISCUSSION

The principal finding of this work is that MLR stimulation, when effective in generating fictive locomotion in decerebrate cats, produced EPSPs with disynaptic segmental latencies in lumbosacral motoneurons. In a majority of cells, the amplitudes of these MLR EPSPs were enhanced concurrent with depolarizing LDPs, during the ON phase of stepping for the motoneuron in question. These observations confirm and extend the earlier report by Shefchyk and Jordan (1985) in which short-latency EPSPs appeared in all 22 motoneurons tested (MG, LG, TA, PBST, and FDL) during the phase of fictive locomotion when the cells were depolarized. These authors reported latencies from MLR stimulus to EPSP onset between 3.0 and 7.0 ms (mean 5.1 ms); these are somewhat shorter than the present observations (range 4.7–7.2 ms; mean 6.1 ± 0.7 ms).

Although Shefchyk and Jordan did not report CDP or segmental latency data, a later study from the same laboratory (Noga et al. 1995) described short-latency CDP responses to MLR stimulation that closely resemble the present observations. It seems clear that the same fast-conducting descending pathway was responsible for the PSPs in these studies. Except for EDL, Shefchyk and Jordan studied mostly the same motor pools as in the present work, although
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were relatively hyperpolarized. These IPSP had latencies >0.6 ms longer than the EPSPs. The IPSPs we observed in EDL motoneurons are clearly different because they had disynaptic segmental latencies and appeared during the flexion ON phase. We have no explanation for this difference in observations, especially because the behavior of MLR EPSPs in the present work was for the most part similar to that described by Shefchyk and Jordan.

Nature of oligosynaptic MLR EPSPs

As noted in the results, each MLR pulse produced a characteristic multiphasic negative CDP in the lumbosacral segments of decerebrate cats when the stimulation was successful in eliciting fictive locomotion (Fig. 1B), as described earlier by Noga et al. (1995). The relatively short delay between the MLR stimulation pulses and the arrival of the well-defined early CDP wave (the P1 wave of Noga et al. 1995) in the lumbosacral segments (4.5 ms in the present sample) suggests that descending fibers with uniform fast conduction velocities transmit the descending volley. There was often a delay of several seconds between the onset of MLR stimulus trains and the coincident appearance of the CDP waves and rhythmic ENGs in the muscle nerves. This delay was reminiscent of the delayed appearance of discharges in reticulospinal neurons after the beginning of MLR tetani described by Orlovsky (1970). The late positive waves in the MLR-evoked CDP are modulated during locomotion (Noga et al. 1995) and are suppressed during fictive scratching (see Fig. 11 in Degtyarenko et al. 1998), implying that these waves signal activity in segmental interneurons involved in the locomotor central pattern generator (CPG).

The delay between the arrival of this early volley and the onset of EPSPs in motoneurons was as short as 1.2 ms in some cells, and was >2.0 ms in all but 3 of the 35 cells with detectable EPSPs. Not surprisingly (see Degtyarenko et al. 1998), the shortest segmental latencies were found during the ON phase for the recorded motoneurons (Figs. 4, A and B, 8B, 9B, and 12, A and B). We have discussed elsewhere evidence suggesting that segmental latencies >2.0 ms indicate disynaptic connectivity in a variety of reflex pathways (Degtyarenko et al. 1996, 1998). Accordingly, we infer that the descending axons activated by MLR stimulation monosynaptically excite last-order segmental interneurons that make direct excitatory contacts on alpha-motoneurons (i.e., the minimal segmental pathway is disynaptic) (see also Jordan 1991; Shefchyk and Jordan 1985). Longer-latency MLR EPSPs also were observed during the motoneuron ON phase, suggesting that multisynaptic chains also exist, very likely located in the lumbosacral spinal cord and possibly involving the same last-order excitatory interneurons. These conclusions are entirely compatible with the timing and spatial distribution of intraspinal field potentials in the lumbosacral cord after MLR stimulation (Noga et al. 1995).

Nature of locomotor modulation of MLR EPSPs

For reasons discussed in detail elsewhere (Degtyarenko et al. 1996, 1998; Floeter et al. 1993; Gossard et al. 1996; it is likely that they included the FHL muscle nerve in identifying FDL motoneurons (L. M. Jordan, personal communication).

The only systematic exception to the general linkage between enhancement of oligosynaptic MLR EPSPs during the motoneuron ON phase of fictive stepping occurred in the sample of EDL motoneurons, in which only 1 of 10 cells exhibited this behavior. The EDL sample was also exceptional in that 5 of the 10 motoneurons exhibited disynaptic IPSPs during the flexion phase when EDL motoneurons were strongly depolarized (Fig. 3) (see also Degtyarenko et al. 1996). Oligosynaptic IPSPs were infrequent in the other motor pools during fictive locomotion (see Figs. 4, 6, and 12). The lack of oligosynaptic MLR IPSPs in most motoneurons during their OFF phase is different from the report of Shefchyk and Jordan (1985), who found short-latency MLR IPSPs in 17/22 motoneurons when the target motoneurons were relatively hyperpolarized. These IPSP had latencies >0.6 ms longer than the EPSPs. The IPSPs we observed in EDL motoneurons are clearly different because they had disynaptic segmental latencies and appeared during the flexion ON phase. We have no explanation for this difference in observations, especially because the behavior of MLR EPSPs in the present work was for the most part similar to that described by Shefchyk and Jordan.

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Nature of locomotor modulation of MLR EPSPs

For reasons discussed in detail elsewhere (Degtyarenko et al. 1996, 1998; Floeter et al. 1993; Gossard et al. 1996;
FIG. 10. Example of extensor phase facilitation of di- and trisynaptic EPSPs produced in an FDL motoneuron during stimulation of the medial longitudinal fasciculus (MLF; 80 μA, 3 Hz) during spontaneous fictive locomotion (no MLR stimulation). A: FDL motor pool showed the usual pattern of early flexion phase bursting, accompanied by a sharp depolarizing LDP in the intracellular record (FDL IC; r). B: averaged MLF EPSPs showed enhanced di- and trisynaptic components throughout the extension phase of stepping (flexion phase: 31 ± 33 sweeps; extension phase: 45 ± 46 sweeps). C: amplitude of the MLF EPSPs measured at 3.4-ms segmental latency (± in B) showed enhancement throughout extension, entirely out of phase with membrane potential changes and FDL ENG bursting (±; 19 step cycles).

Moschovakis et al. 1991), we assume that the observed modulation of disynaptic MLR (and MLF) EPSPs results primarily from convergence of synaptic drive from the CPG for locomotion onto the segmental last-order interneurons in those disynaptic pathways. Whether that CPG control is excitatory, inhibitory, or both is unclear, although we favor active excitation of pathway interneurons during the ON phase of the target interneurons. In the case of these descending systems, the main alternative explanation is nonlinear interaction with other postsynaptic conductances. These would not be expected to produce the alterations in PSP shapes and segmental latencies that are observed during EPSP enhancement (e.g., Figs. 4, A and B, 5B, 6, A and B, 7B, 8B, 9B, and 12, D and E).

There is considerable evidence that MLR stimulation monosynaptically activates neurons in the medial reticular formation that give rise to descending reticulospinal axons traveling in the ventrolateral funiculus (Edgley et al. 1988; Noga et al. 1991; Orlovsky 1970; reviewed in Jordan 1991). Edgley and coworkers (1988) have described interneurons the upper lumbar (L4 and L5) segments that receive monosynaptic EPSPs after stimulation of the cuneiform nucleus (the region of the MLR), as well as from knee extensor group II afferents. The delays between MLR stimulation and EPSPs in the interneurons were <6 ms. The appearance of MLR-evoked EPSPs required two to three pulses, suggesting that temporal facilitation was needed to activate the reticulospinal relay in these chloralose-anesthetized cats. Some of these cells could be activated antidromically from more caudal motor nuclei, and they have been shown to produce monosynaptic EPSPs and IPSPs in the S1 ventral root (Cavallari et al. 1987), with latencies between 0.8 and 3.0 ms. Shefchyk and coworkers (Shefchyk et al. 1990) demonstrated that L4 interneurons fitting this description are activated during fictive locomotion, mostly during the flexion phase in the ipsilateral hindlimb. Finally, Noga and coworkers (1995) found field potentials in the L4–L7 gray matter beginning at 0.8 ms after the P1 wave that signals the arrival of the fast descending MLR volley. All of this evidence is consistent with the conclusions drawn above. It is quite possible that the midlumbar interneurons may be responsible for some of the observations in the present paper, particularly in extensor motoneurons. The longer central latencies we observed in GS cells in the L2 and S1 segments (e.g., Fig. 6) may have resulted from axonal transit delay from midlumbar interneurons. The shorter central latencies seen in PBST, TA, FHL, and FDL cells suggest that more caudal interneurons may be involved in the disynaptic pathway from MLR to those motoneurons (see Jankowska and Riddell 1994; Noga et al. 1995).

**MLR EPSPs in FDL motoneurons**

The behavior of MLR EPSPs in FDL motoneurons was of particular interest to us because the FDL muscle exhibits variable firing patterns during treadmill walking in intact cats (O’Donovan et al. 1982) and during fictive locomotion in immobile, decerebrate preparations (Figs. 7–9) (Flesh-
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In the disynaptic pathway from MLR to hindlimb motoneurons, including FDL, participate in producing the depolarizing LDPs observed in these motoneurons during their ON phase. The suggested circuit diagram in Fig. 13 denotes this by reddish interneurons that receive excitatory drive from the locomotor CPG and from the MLR. Only the EDL nucleus does not receive this convergent input (see next section).

Are MLR and MLF EPSPs produced by common last-order segmental interneurons?

An earlier paper from this laboratory showed that disynaptic EPSPs in FDL motoneurons produced by reticulospinal axons in the MLF were enhanced only during the extension phase of fictive stepping (Floeter et al. 1993, their Fig. 11). This is confirmed and documented more extensively in the present paper in Figs. 10, 11, and 12, D–F, regardless of the stepping phase in which the FDL pool was active. In contrast to this consistent behavior of MLF EPSPs, the largest disynaptic MLR EPSPs were found during the early flexion phase in most FDL cells. Extensor phase enhancement of MLR EPSPs occurred in only one of the two examples in which FDL activity was active exclusively during the extension phase (Figs. 9 and 12, A–C). This differential control of the MLF and MLR pathways strongly suggests that the two sets of disynaptic EPSPs in FDL motoneurons are produced by entirely different sets of last-order segmental interneurons, as indicated on the proposed circuit diagram in Fig. 13. An analogous conclusion can be drawn from observations in EDL motoneurons, which receive little or no evident MLR excitation (at least under the conditions of the present experiments) but which do receive disynaptic MLF EPSPs that are powerfully enhanced during the flexion phase of fictive stepping (Gossard et al. 1996, their Fig. 7).

On the other hand, disynaptic MLF EPSPs are enhanced during the ON phase of fictive locomotion in other motoneuron pools (Floeter et al. 1993; Gossard et al. 1996), exactly as found for disynaptic MLR EPSPs. This evidence is compatible with the idea that descending MLF and MLR axons converge onto common last-order interneurons that project to lumbar spinal motoneuron pools other than FDL in the cat. This suggested convergence is denoted in Fig. 13 by bicolored interneurons carrying convergent MLR, MLF and CPG input to “flexor” and “extensor” motoneuron groups. However, such patterns of common modulation during locomotion are necessary but not sufficient to establish that common interneurons are involved. It is quite possible that separate sets of last-order interneurons carry MLR and MLF input to all of these motoneuron groups. For this reason, the bicolored interneurons in Fig. 13 have question marks appended.

Are last-order MLR interneurons part of the CPG for locomotion?

Because MLR EPSPs appeared during depolarizing LDPs (the ON phase of fictive stepping) in flexor and extensor motoneurons and were replaced by IPSPs during relative membrane hyperpolarization (the OFF phase), Shefchyk and
Jordan (1985) suggested that the last-order interneurons in the oligosynaptic pathways from MLR to motoneurons were "... the same as those involved in the production of the LDP in motoneurons ..." (p. 1353). With respect to MLR EPSPs, we have confirmed their results in all lumbosacral motoneuron groups examined, except for the majority (9/10) of EDL cells (Figs. 3 and 4), which had not been specifically examined in the earlier work. Our results do not speak to the issue of the involvement of inhibitory last-order interneurons because we observed only occasional IPSPs in the OFF phases during locomotion (e.g., Figs. 4B, 6A, and 12B) and made no specific search for them. However, it should be noted that the clear disynaptic IPSPs found in some EDL motoneurons (Figs. 3 and 4) were evident only during LDP depolarization, entirely out of phase with the observations of Shefchyk and Jordan (1985) in other motor nuclei.

The available evidence is compatible with the conclusion that many extensor and flexor motoneuron pools receive disynaptic excitation from excitatory last-order interneurons that in turn receive convergent excitatory drive from MLR-activated reticulospinal neurons and the segmental CPG for...
locomotion. There must be at least two sets of such cells, one projecting to many (but not all) flexor and the other to extensor motor pools. However, it is necessary to postulate an additional group that projects to FDL motoneurons, which receives CPG drive that can switch from the flexion to the extension phase (Fig. 13). Such last-order interneurons very likely contribute to depolarizing LDPs during fictive locomotion and might be responsible for a major fraction of these CPG-driven depolarizations (Fig. 13). There is insufficient evidence to answer whether such last-order interneurons are simply driven by the CPG or could be considered as elements within the pattern generating network itself.

On the other hand, the absence of MLR EPSP enhancement in EDL motoneurons suggests that excitatory last-order interneurons without MLR input must exist (Fig. 13) to drive the large flexor-phase depolarizations found in these cells (Fig. 3A) (see also Degtyarenko et al. 1996). It seems unlikely that the small disynaptic IPSPs found in some EDL cells during flexion phases (Fig. 4D) could mask the existence of facilitated EPSPs with the same brief latency, although we cannot rule out that possibility with the available data. It seems safest to conclude that excitatory last-order interneurons that receive MLR drive could well contribute to LDP depolarization during fictive locomotion, but that other groups of excitatory interneurons yet to be identified are also likely to participate.

REFERENCES


