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# Monosynaptic and Dorsal Root Reflexes During Locomotion in Normal and Thalamic Cats

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## SUMMARY AND CONCLUSIONS

*1.* In normal and thalamic walking cats electrical stimulation of muscle nerves via chronically implanted electrodes produced electromyographic (EMG) and neurographic responses that were modulated in amplitude depending on the phase of the step cycle. These responses were examined for possible indications of effects of primary afferent depolarization (PAD) during stepping.

*2.* Monosynaptic reflexes (MSRs) produced by stimulating the lateral gastrocnemius (LG) and medial gastrocnemius (MG) nerves were recorded as EMGs in MG or LG muscles during treadmill locomotion in normal cats. These heteronymous MSR responses were greatest during the stance (extensor) phase.

*3.* In the same animals, after decerebration, similar modulation of the heteronymous ankle extensor MSRs occurred during spontaneous locomotion with the use of the same stimulus and recording sites.

*4.* In both normal and thalamic cats the amplitude of neurogram responses recorded from LG or MG nerve after stimulation of the other muscle nerve varied with phase of stepping but did not parallel the variations of the MSR measured as EMG amplitude in the same muscle. The nerve responses were largest during the flexion phase of the step cycle and had a calculated central latency of 0.6–1.0 ms. These are interpreted as arising from antidromic activity in large-caliber afferent nerve fibers (i.e., dorsal root reflexes).

*5.* Spontaneous antidromic activity in severed L7 dorsal rootlet fibers to triceps surae was observed in the thalamic cats during episodes of locomotion and was closely correlated with flexion phase EMG activity in semitendinosus, a bifunctional muscle.

*6.* In decerebrate cats, dorsal root reflexes (DRRs) in severed filaments of L4–L7 dorsal roots were produced by stimulation of saphenous and posterior tibial nerves. These DRRs were always smaller during locomotion than during rest and were smallest during the flexion phase.

*7.* The short-latency antidromic activity produced in muscle nerves by stimulating heteronymous muscle nerves thus appears to be a DRR produced in Group I terminal arborizations that are depolarized close to threshold during the flexion phase. Such PAD could account for changes in the MSR that do not always parallel the levels of recruitment of the motor pools as manifest by background EMG amplitude.

## INTRODUCTION

During locomotion, sensory inflow to the central nervous system that is necessary to regulate normal movement is itself regulated in two ways: *1*) at the receptor level, the mechanical sensitivity of Group Ia and II muscle-spindle afferents is under fusimotor control (reviewed by Loeb 1984), and *2*) at the terminal arborizations, transmitter release is modulated by presynaptic (axo-axonic) synapses onto these and other afferent terminals, activated by other afferents (Jankowska et al. 1981; Jimenez et al. 1984; Brooks and Koizumi 1956; Burke and Rudomin 1977) and by the central pattern generator (CPG) (Baev and Kostyuk 1982; Duenas and Rudomin 1988). During treadmill locomotion in decerebrate cats, the amplitude of the monosynaptic reflex (MSR) between the spindle afferents and the motoneurons of triceps surae muscles increases during activity of this motoneuronal pool during extension and decreases during the phase of flexor muscle activity (Akazawa et al. 1982). A similar modulation in the MSR has also been observed in decerebrate cats during fictive locomotion (Baev 1981; Duenas and Rudomin 1988; Jimenez et al. 1984; Lundberg 1982). During fictive locomotion, the change in MSR in triceps surae has been attributed in part to variations in primary afferent depolarization (PAD) (Duenas and Rudomin 1988).

If PAD becomes large enough, it can result in the local initiation of action potentials that are then conducted antidromically out along the dorsal roots and into the peripheral nerves as dorsal root reflexes (DRR). Dubuc et al. (1985) recorded such spontaneous and reflexly evoked antidromic activity in dorsal roots that varied with the phase of stepping in decerebrate cats. DRRs have been recorded in immobilized decerebrate cats after stimulation of cutaneous fibers (Brooks and Koizumi 1956) and muscle nerves (Eccles et al. 1961). Eccles et al. (1961) also interpreted the DRR as an effect of primary afferent depolarization and observed that the DRR decreased and perhaps ceased as body temperature increased from colder to normal temperatures.

It remains unclear which parts of this gating apparatus may be operating in normal cats as opposed to surgically reduced preparations. Therefore we have monitored the amplitude variations during stepping of the MSR and of muscle nerve activity in the medial (MG) and lateral (LG) gastrocnemius in normal cats and in the same cats after decerebration. We also recorded variations of the DRR at normal temperatures during stepping in decerebrate cats. A preliminary report of some of these findings appeared in Duenas et al. 1985.

## METHODS

The chronic recording techniques developed by Abraham and Loeb (Abraham et al. 1985) were used to stimulate and record from nerves and to record from muscles during normal locomo-

tion on a treadmill. The experiments were performed in five adult male and female cats weighing between 3.5 and 4.5 kg. The animals were trained to walk on a treadmill by the use of food reward. Bipolar epimysial "patch" electrodes (Loeb and Gans 1986) were used to record electromyograms (EMGs) in two flexors: sartorius medialis (SAm) and tibialis anterior (TA); two extensors: LG and MG; and two bifunctional muscles: posterior biceps (PB) and semitendinosus (ST). The contacts were oriented parallel to the axis of the muscle fibers and near to their presumed innervation point near the nerve entry zone. Tripolar cuff electrodes were used to record neurograms in sciatic (SCI), posterior biceps-semitendinosus (PBST), LG, MG, posterior tibialis (PT), and TA nerves (Fig. 1). The patch and cuff electrodes were implanted under aseptic surgery in cats deeply anesthetized with pentobarbital sodium (Nembutal; 35 mg/kg). Locomotor activity in intact animals was recorded several days after surgery, at which time the animals behaved normally, without limping or other indications of discomfort.

The cats were decerebrated under ketamine (20 mg/kg) and Brevital (20–40 mg/kg) anesthesia. High decerebration was performed by suction of all the cortical and striatal tissue rostral to the thalamus (thalamic cat). The lumbosacral spinal cord was exposed by laminectomy. Pools were formed with the skin and filled with warm mineral oil and maintained thermostatically near 37°C by radiant heat. Selected dorsal root filaments from segments L6 to S1 were mounted on floating bipolar platinum electrodes to avoid the transmission of movement into the recording system during locomotion. The following criteria were used to select the L6–S1 dorsal rootlets: *1*) stimulation of LG and/or MG (1–1.5 × T) but not PBST, ST, PT, or TA nerves evoked a response in the intact selected dorsal rootlet; and *2*) stimulation of the selected dorsal rootlets had to evoke an antidromic response recorded in LG or MG nerves. After testing for MSR or identification of LG and MG afferent fibers, the left L4–L7 dorsal roots were sectioned distally and mounted on bipolar hook electrodes to record any spontaneous or reflexly evoked antidromic activity. After 2 h, the decerebrate cats could again walk on the treadmill. Walking was episodic, typically in 2- to 10-min bursts and 1- to 3-min rests. Decerebrate cats were partially balanced from the skin around the incision and at the tail with elastic cords, but they supported most of the weight of their hindquarters during locomotion. The forelimbs were free to move but did so inconsistently.

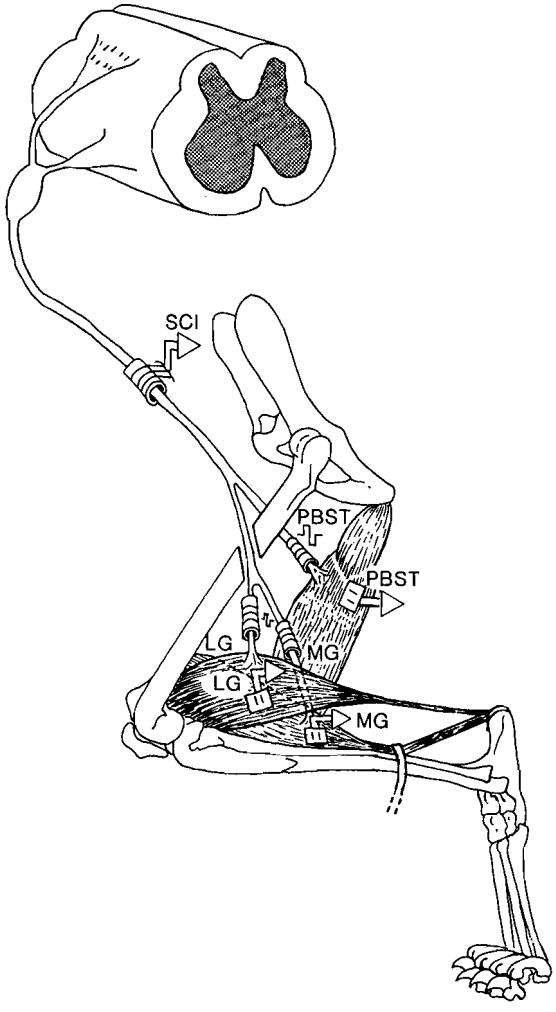


FIG. 1. Schematic experimental arrangement for MSR recording. Chronically implanted nerve cuffs were used either to stimulate or record from LG, MG, sciatic (SCI), and posterior biceps-semitendinosus (PBST) nerves. EMG activity was recorded by patch electrodes (not to scale) in 6 muscles, of which LG, MG, and PB are shown (MG retracted to reveal electrode site).

### *Stimuli*

MSR responses were produced by stimulation of the LG and MG nerves through two leads of the implanted tripolar cuff electrodes. Single biphasic pulses (0.1 ms/phase) were delivered to the nerves at intervals of 1 s with an intensity adequate to stimulate Group I afferent fibers as monitored in the SCI cuff electrode. DRR were elicited by stimulation of PT and saphenous nerves (SAPH) through bipolar cuffs on these nerves. The intensity was adjusted to threshold for a just-noticeable twitch in flexor muscles, a response typically associated with cutaneous flexion-reflex afferents (FRA) (Eccles and Lundberg 1959).

### *Data analysis*

Data were collected during 2- to 5-min periods of regular walking on a motorized treadmill. EMG and neurograms were stored on FM tape (DC-10KHz bandwidth) together with a time code and stimulus markers for analysis by a PDP-11 computer. The EMG recordings in LG and MG were rectified, integrated, and digitized at 16-ms intervals as markers for the phases of the step cycle (Abraham and Loeb 1985). In a second pass selected EMGs and nerve recordings were digitized at 0.5-ms intervals in brief peristimulus bursts (5 ms before each stimulus to 10 ms after).

These peristimulus response records from one single episode of locomotion were displayed as a series of horizontal sweeps in rasters with the traces ordered vertically by the phase of the stimulus in the step cycle (e.g., Fig. 2). The stimulus phase was the fraction of the normalized step-cycle duration from the onset of LG activity to the time of the stimulus. The bar on the *left* side of Fig. 2, *A–C*, represents the extension phase ("E phase"), and the arrows denote onset and end of the flexion phase ("F phase"). These corresponded to the onset of LG or MG activity and TA activity, respectively. On the *right* edge of each raster is a bar graph showing the activity integrated over a period of time given by the horizontal bar on the peristimulus time scale, usually picked to include either the peak of the MSR or nerve volley (Fig. 2, *B* and *C*) or the smoothed sum of the prestimulus activity, used as the control EMG activity with which poststimulus activity should be compared (Fig. 2*A*).

### *Analysis of correlation*

In the experiments in which the MSRs were recorded, an analysis of covariance was performed. Samples of heteronymous MSR

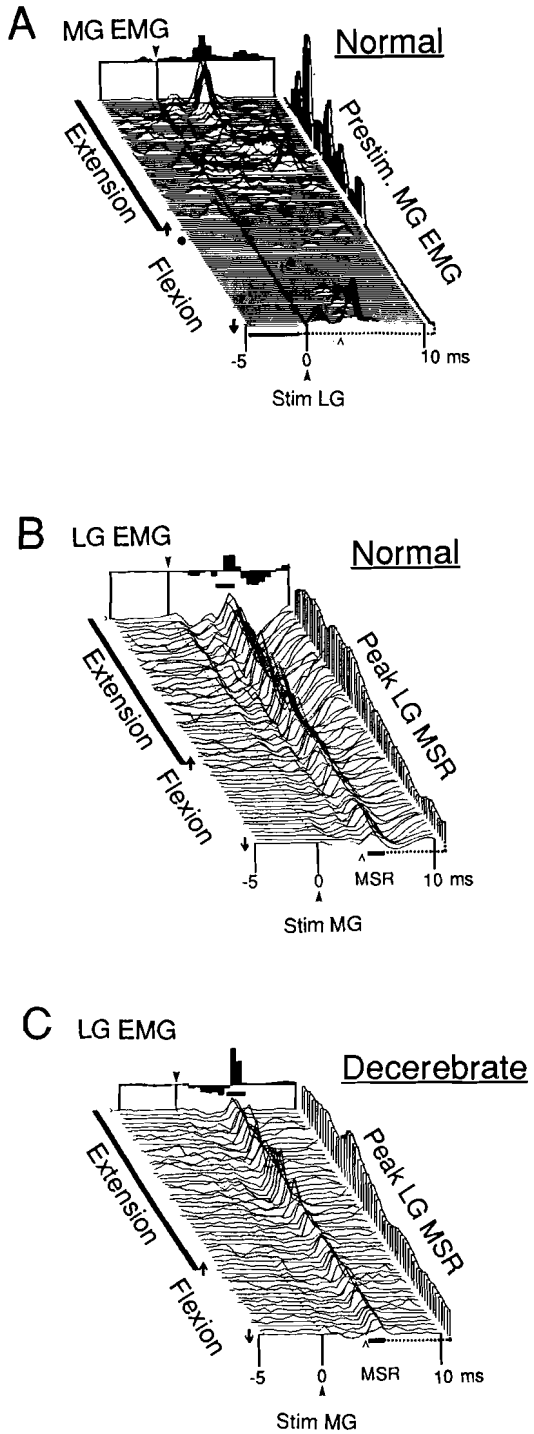


FIG. 2. *A*: heteronymous MSR recorded in MG muscle (rectified). *B*: heteronymous MSR recorded in LG muscle (unrectified). *C*: heteronymous MSR after decerebration. (*B* and *C*: same animal, different from *A*). In *B* and *C*, peristimulus activity traces (5 ms before stimuli to 10 ms after) are arranged vertically in a raster of step-cycle phase based on the time between the stimulus and the start of the most recent Extension (E) phase. Bar graph on the *right* of *A* shows the EMG activity before stimuli. Bar graph on the *right* of *B* and *C* shows the average amplitude of the MSR during a 1-ms interval that includes its peak. Summing interval for *A–C* is shown by a bar at the *bottom*; a carat marks the onset of the reflex response. Average EMG amplitude for all of the traces is shown in the bar graph at the *top* of the raster. Thick diagonal bar at *left* indicates the E phase (time of triceps surae activity); the F phase (time of TA activity) is indicated between arrows.

and nerve volley were integrated as described above to generate bar graphs along the *right* edge of the rasters. Any pair of such bar graphs from the same set of stimuli could be plotted against one another in *X-Y* format (Fig. 4). The step cycle was divided into as many as four discreet segments that were plotted separately if it appeared that the relationship between the two variables might be different in different phases of the step cycle. To interpret these plots, we assumed that the nerve volley was dominated by the activity of large fibers in the nerve, the $\alpha$-efferents and the Group I afferents (see DISCUSSION). If we assume that efferent activity must covary with EMG amplitude, then changes in nerve activity that are reciprocal to EMG amplitude and out-of-phase with normal EMG activity must reflect changes in afferent activity.

## RESULTS

### *MSR in normal and decerebrate locomotion*

In these experiments homonymous and heteronymous reflexes always refer to EMGs in LG or MG muscle evoked by stimuli to either the LG or MG nerve. Reflex responses were considered to be MSR because the stimulus was adjusted for Group I afferents and because the calculated latency implied a central delay of <1 ms (Boyd and Davey 1968; Burke and Rudomin 1977). For the heteronymous LG or MG in the normal walking cat, the MSR had a latency of 4–5 ms and a duration of 2–3 ms. In four animals it reached maximum amplitude during the E phase, decreased in late stance, and reached a minimum during flexion. Figures 2*B* and 3*B* show these cases with the MSR amplitude at 4.5- to 5.5-ms latency plotted against the phase of the stimulus along the *right* edge of the raster. In another animal (Fig. 2*A*) the MSR decreased suddenly and early in the extension phase. Note that in this raster the prestimulus MG EMG activity is plotted in bar graph along the *right* edge of the raster; it had a normal modulation over the step cycle. In the decerebrate walking cat the amplitude of the homonymous and heteronymous MSR waxed and waned in a similar way to that during normal locomotion (Fig. 2, *C* vs. *B*).

In three preparations the M-wave amplitude itself remained relatively constant, but in two cases there was significant variation with the phase of the step example (Fig. 3*A*). Presumably this resulted from movement of the nerve within the stimulating cuff, changing the potential gradient acting on the nerve fibers. If this phase-dependent stimulus variability was producing an artifactual dependency of the MSR on phase, it could be detected as a correlation between the amplitudes of the M-wave and MSR. Figure 3*C* shows that this effect was not important in this particular experiment. Figure 3*D* shows the expected positive correlation between the homonymous (ordinate) and heteronymous (abscissa) MSRs. Similar results were obtained in the other preparation exhibiting M-wave fluctuations. This analysis is important because the M-wave and MSRs overlap in time, and there is some cross talk of the large, synchronous M-wave to the heteronymous EMG recordings (note short-latency negative potentials in Fig. 3*B*).

### *Opposite variation of nerve potential and EMG*

In three experiments we stimulated MG or LG nerve and recorded the responses in the LG or MG muscles and their

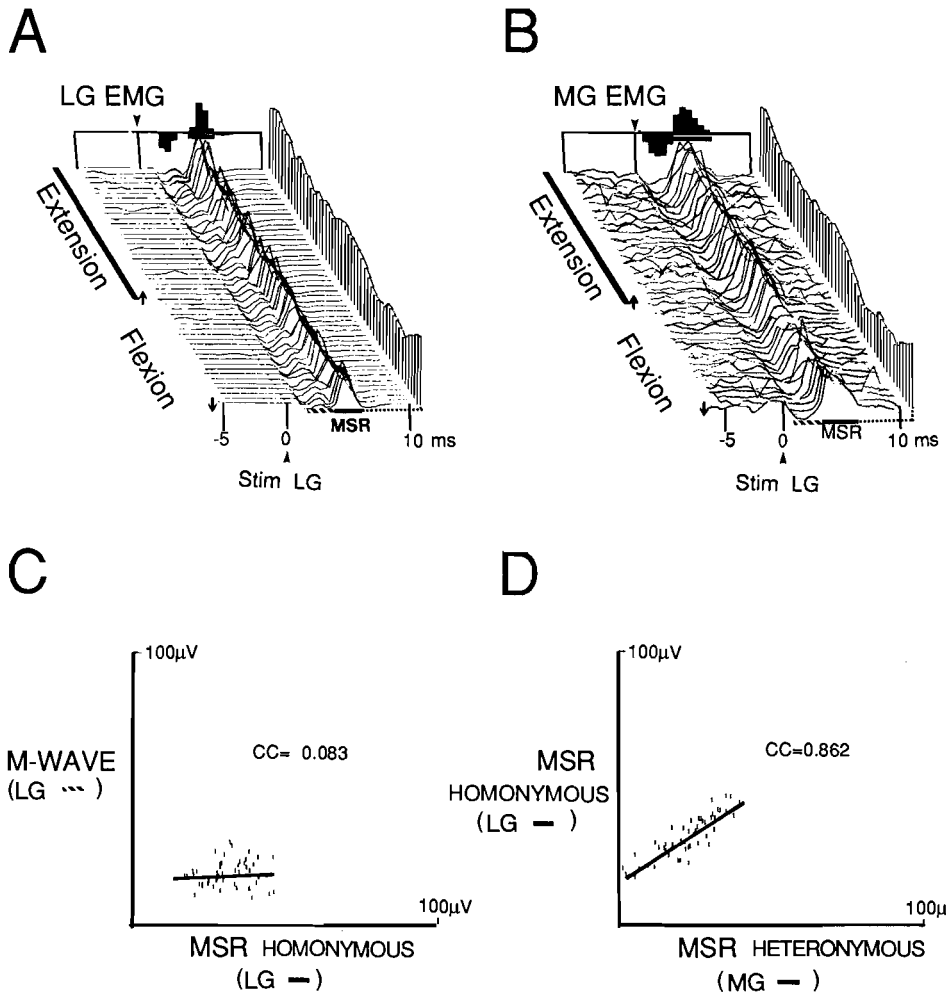


FIG. 3. Rasters showing unrectified EMGs in response to stimulating LG nerve during normal walking. *A*: homonymous EMG activity in LG muscle. *B*: heteronymous activity in MG muscle. Bar graph to the *right* of *A* and *B* shows the integrated amplitude in each trace at 4.5- to 5.5-ms poststimulus, as denoted by the sold bar under the raster (the homonymous and heteronymous MSR, respectively). Dashed bar under the raster denotes the integration period (1.5- to 2.5-ms poststimulus) for the M-wave. *C*: correlation between the directly recorded M-wave in LG against the homonymous MSR (data from raster in *A*). *D*: correlation between homonymous and heteronymous MSRs (data from raster in *A* and *B*).

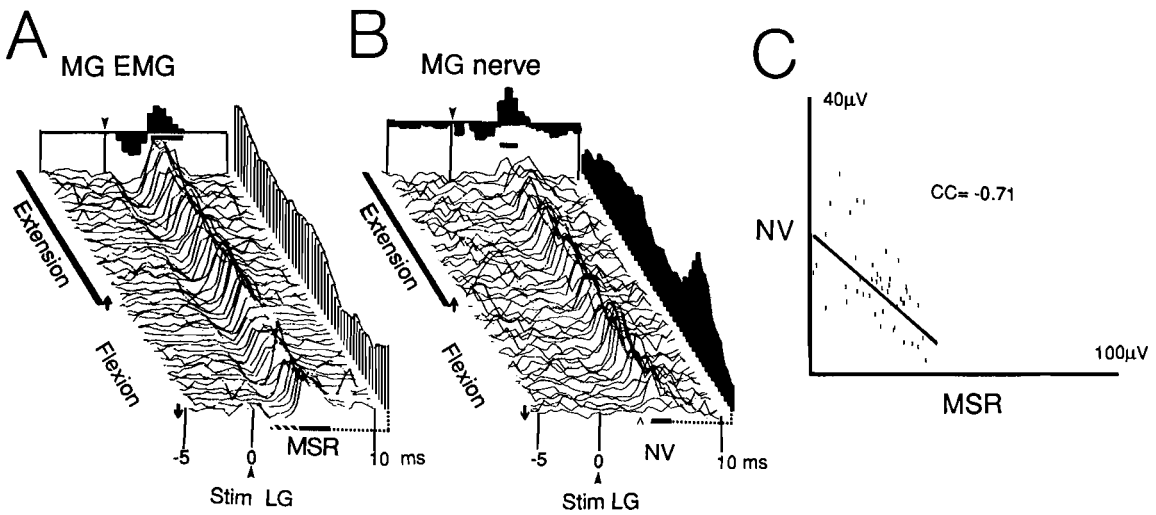


FIG. 4. MG muscle and nerve activity in response to LG stimulation during normal walking. *A*: MG EMG reflects a combination of cross talk from the LG M-wave at short latency (dashed bar under raster) and heteronymous MSR at longer latency (solid bar under raster) whose amplitude fluctuations during the step cycle are summarized in the bar graph along the *right* edge of the raster. *B*: nerve volley in MG nerve. *Right* bar graph shows the summed MG nerve activity from 4.5 to 5.5 ms, indicated by bar NV under raster. *C*: correlation between the heteronymous MSR and nerve volley.

nerves. Both the nerve and muscle responses varied with the phase of the step, but they varied oppositely. The MSR in MG was largest at the beginning or middle E phase of the step cycle and was smallest during the F phase (Fig. 4*A*), whereas the neurogram recorded in MG nerve reached a maximum at the end of the F phase (Fig. 4*B*). This opposite fluctuation in amplitude during the step was quantified by plotting the *right* bar graphs of 4, *A* and *B*, against one another (Fig. 4*C*). The correlation coefficient (CC) was −0.71 for the experiment shown in Fig. 4, *A–C*; in a second experiment the CC was −0.72. In a third, it was negative only during the last one-half of the extension phase (this was the animal whose MSR was completely inhibited throughout late stance and swing as shown in Fig. 2*A*).

*Unstimulated activity in cut dorsal rootlets*

In two experiments during decerebrate locomotion, rhythmic discharges were recorded from the proximal stumps of cut L7 dorsal rootlets that, before cutting, had been demonstrated to carry afferents from the triceps surae muscles. This antidromic activity occurred spontaneously through the episode of locomotion. The antidromic activity bursts were modulated in phase with the flexion-phase EMG activity (Fig. 5, sequence *A–C*) recorded from ST muscle. Note that the episode started and ended with phasic EMG activity in ST and not in LG. The low-amplitude antidromic activity recorded during the extensor phase (increases in background asynchronous activity) could have originated in another modality of afferents or in a small subpopulation of afferent fibers from other muscles (see DISCUSSION).

*Stimulated antidromic activity in cut dorsal rootlets*

In three decerebrate cats we studied the dorsal root reflexes produced by stimulating the SAPH or PT nerves. The DRRs were recorded from cut dorsal root filaments without identifying the sources of their afferent fibers (i.e., unselected rootlets). Stimulating the SAPH nerve evoked a short-latency (2.9 ms), small DRR followed by a larger, long-latency reflex (5–6 ms). Both responses required a stimulus strength adequate to evoke just-noticeable flexor twitches. To calculate the central delays, we therefore assumed that the afferent conduction velocity was that of FRA fibers, 40–70 m/s (Boyd and Davey 1968). The conduction distance was 13 cm, so the delay from the SAPH nerve was 1.8–3.3 ms. Thus the central delay for the early response was 1 ms or less and for the later response, 2–4 ms. Figure 6*A* shows the DRRs in L4 and L7 dorsal rootlets evoked by stimulation of the SAPH nerve, which enters the cord primarily via L4 and L5 dorsal roots. The DRR was not recorded in the L7 rootlet, suggesting that the antidromic response evoked by SAPH may be localized in the more rostral, perhaps homonymous, segments of the spinal cord. The short- and long-latency DRRs were modulated during stepping; the short-latency reflex occurred only occasionally throughout the step cycle without clear preference of phase, whereas the long-latency response was observed more frequently during extension (Fig. 6*B*).

Stimulation of PT nerve also evoked short- and long-latency DRRs. We again assumed the FRA conduction velocity in PT nerve because of the similar thresholds for both DRRs and flexor muscle twitches. The two DRR latencies were similar to SAPH stimulation, so that the central delays for PT responses were similar to SAPH. Figure 6*C, top trace*, shows LG DRRs produced by stimulation of PT nerve (a mixed muscle and cutaneous nerve that is supplied by segmental levels L6–S1). Figure 6*C* also shows that during episodes of decerebrate locomotion, both responses were reduced from the amplitudes recorded with the animal standing at rest. The short-latency DRR disappeared completely during stepping. The amplitude of the long-latency DRR was generally reduced but depended on the phase of the step; this particular DRR response was smallest during flexion (Fig. 6*D*).

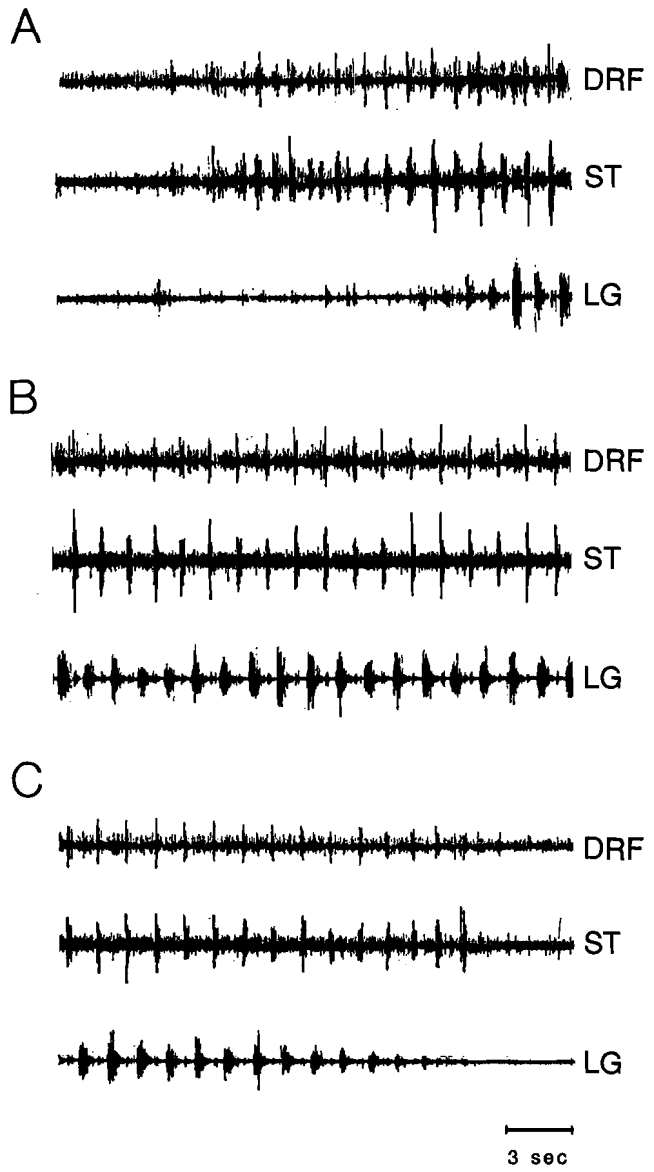


FIG. 5. Spontaneous antidromic activity during an episode of decerebrate locomotion. *Top trace*: electroneurogram in a cut L7 dorsal rootlet. *Center* and *bottom traces*: EMG activity in ST and LG muscles. *A*, *B*, and *C* each show several steps at the onset (*A*), middle (*B*), and end (*C*) of an episode of locomotion.

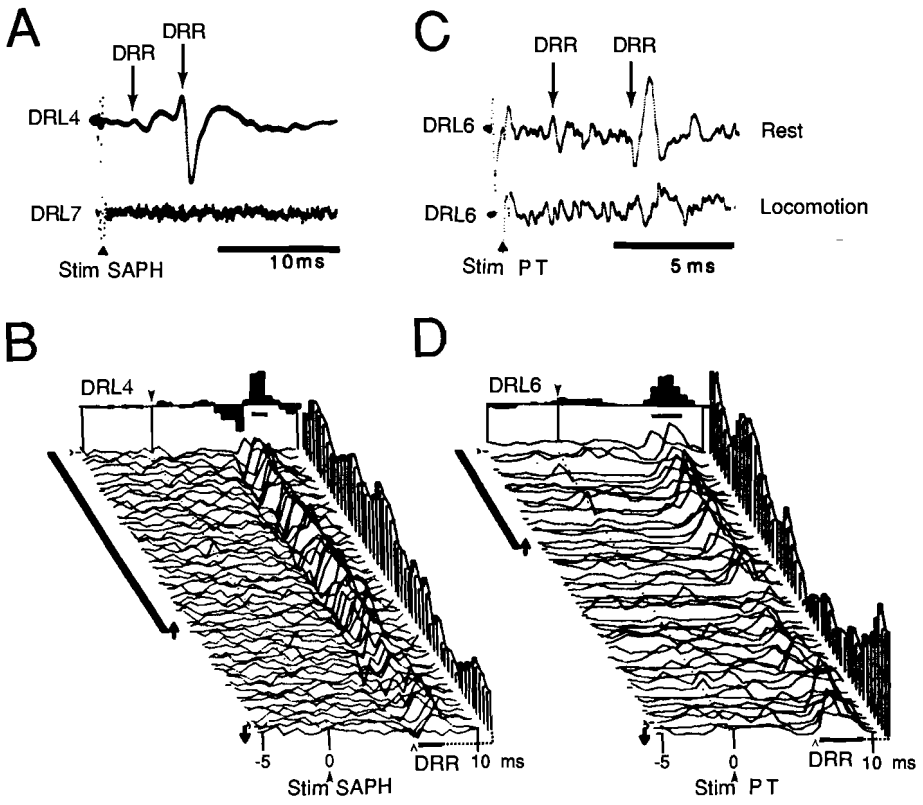


FIG. 6. Dorsal root reflexes in central dorsal rootlets produced by stimulation of SAPH and PT nerves. *A*: average of 68 dorsal root reflexes produced by stimulation to SAPH nerve. *Top trace*: dorsal root reflexes of short (2.9 ms) and long (5 ms) latencies recorded in an L4 dorsal rootlet. *Bottom trace*: absence of dorsal root reflexes in an L7 dorsal rootlet. *B*: peristimulus rasters showing the modulation of the amplitude of L4 dorsal root reflexes during step cycle. Bar graph to the *right* of *B* shows traces summed from 5 to 6 ms (long latency DRR indicated by solid bar under raster) *C*: average of 68 dorsal root reflexes produced by stimulation in PT nerve. Reflexes were recorded in an L6 dorsal rootlet. *Top trace*: DRR before an episode of locomotion. *Bottom trace*: during locomotion. *D*: peristimulus raster showing phasic variations in amplitude of PT dorsal root reflexes during the step cycle.

## DISCUSSION

Most of the muscle and nerve responses described here appear to be regularly modulated during locomotion. First, the MSRs were strongest during extension in both the normal and the decerebrate preparations. Second, the presumed DRRs produced by muscle-nerve stimulation represented by the neurograms that did not covary with the MSR were strongest during flexion phase in both normal and decerebrate preparations. Third, the DRRs produced by stimulating SAPH or PT consisted of two waves, the first of which was reduced or eliminated during locomotion and the second of which was reduced phasically during locomotion, especially in midflexion (Fig. 6).

What are the possible mechanisms responsible for these reflexes and their modulation? Does the variation of the MSR during locomotion arise solely through the cycling of the level of polarization of the motoneurons, or does PAD contribute a modulated level of presynaptic inhibition? What processes contribute to DRRs, and how might they relate to such modulations of PAD?

### *MSR*

Previous observation of partially immobilized decerebrate cats walking with three limbs on a treadmill (Akazawa et al. 1982) also found the MSR amplitude in the ankle extensor muscles to be greatest during extension. A similar increase in the MSR has also been observed in decerebrate cats during fictive locomotion (Baev 1981; Perret and Cabelguen 1980; Schomburg et al. 1977) and in human subjects (Stein and Capaday 1988) where the reflex is better identified as the H-reflex because of uncertainties in central delay (Burke 1983). Perret (1983) and Jordan (1983) found an increase in excitability of triceps surae motoneurons during the extension phase of fictive locomotion, when they are maximally depolarized. This might be enough to explain the relative increase in amplitude of the MSR during the extension phase observed in our experiments, because the maximum amplitude coincides with the greatest EMG activity in LG and MG muscles. If this were the case, the MSR amplitude should always parallel the prestimulus EMG amplitude in our rasters of reflex waveform versus step phase. In two of the present experiments the amplitude of the MSR did decrease slowly throughout extension and disappear during flexion. However, in one animal with a similar pattern of locomotor EMG, the MSR decreased suddenly and disappeared during middle and late extension, whereas the prestimulus EMG amplitude in LG remained high (Fig. 2*A*), suggesting that a presynaptic mechanism reduced the efficacy of transmission without affecting motoneuron excitability. Similarly, Stein and Capaday (1988) compared background EMG with H-reflex amplitude in human subjects and concluded that presynaptic inhibition was required to account for changes in reflex efficacy in different parts of the step cycle.

However, arguments for presynaptic gating of the MSR in intact cats and humans depend on the assumption that the background EMG level is a reliable indicator of motoneuron excitability throughout the motor pool. The EMG amplitude reflects the aggregate activity of only those motoneurons that are above threshold for generating action potentials. Similar levels of EMG can be generated by different numbers of motoneurons firing at different rates.

There is evidence that the motoneurons may have nonlinear, perhaps bistable, membrane properties (Hounsgaard et al. 1988) that would affect their sensitivity to an added input and that these membrane properties may be under the independent control of pathways that have yet to be identified. Furthermore, a substantial part of the MSR is likely to be produced by motoneurons that are subthreshold for recruitment by the background synaptic input. The size of the MSR thus depends on how many such motoneurons are close enough to threshold to be recruited by the composite excitatory postsynaptic potential (EPSP) of the Ia volley. This variable can be modulated independently of locomotor recruitment either through inputs that violate the size principle (e.g., cutaneous) (Kanda et al. 1977) or through inputs that are size-ordered but more or less steeply graded in their distribution to motoneurons of different sizes. Because of these complexities, a more direct manifestation of PAD in behaving, intact preparations seems necessary before concluding that it constitutes a significant mechanism for modulating the efficacy of regulatory reflexes.

*Identification of antidromic afferent activity in intact cats*

Several alternative explanations must be considered before ascribing a potential recorded from a mixed peripheral nerve to antidromic activity in a particular class of afferent fibers.

First is the possibility of cross talk from adjacent sources, particularly EMG from muscles. The tripolar design of the electrode is quite effective at excluding virtually all of the naturally occurring EMG activity (Stein et al. 1977); even large, synchronous activity such as the M-waves from the heteronymous muscle can barely be detected in the nerve cuff (Fig. 4*B*), although they are clearly discernible as short-latency cross talk in EMG recordings (Fig. 4*A*). Furthermore, the correlation analyses (e.g., Fig. 4*C*) established that the amplitude variation of the putative nerve volley was opposite to the EMG amplitude of the MSR and unrelated to the M-waves, which were generally constant. The only other muscles close enough to the cuff electrodes to be plausible sources of cross talk are the PBST group, the distal parts of which come close to the cuff electrodes when the knee is flexed. The sharp burst of PBST EMG activity that normally occurs just at the time of foot lift during walking was not discernible in the nerve-cuff records, and these flexor muscles did not exhibit any reflexive recruitment at short latency after stimulation of LG or MG nerves.

If the volleys recorded in the nerve cuffs were produced by nerve fibers, then the fiber class must be identified. The absence of similarly modulated EMG activity in the homonymous muscle eliminates the possibility of any significant contribution from $\alpha$-motoneurons. In fact, the number of motoneurons required to generate the large MSR at the beginning of the extension phase (Fig. 4*A*) is apparently too small to generate any detectable nerve volley at a latency earlier than that of the EMG reflex (Fig. 4*B*). This is not surprising, because individual axons generate very small currents, and this stimulus probably recruits only a small proportion of the ~140 $\alpha$-motoneurons that supply MG (Burke et al. 1977).

Activity in smaller diameter fibers, either afferent or efferent, can be ruled out on two grounds. First, most of the antidromic activity occurred with a very short latency that barely leaves time for conduction delays in the ascending and descending parts of this pathway when the maximal conduction velocities are assumed in both parts (see below). Second, the amplitude of the neurogram that can be produced by nerve fibers decreases rapidly with decreasing fiber diameter. In nerve cuffs of this length, Marks and Loeb (1976) calculated that extracellularly recorded, unitary action potentials should have an amplitude proportional to fiber diameter. For example, $\gamma$-motor axons have diameters ~25% of $\alpha$-motor axons (Burke 1983) and are less numerous than $\alpha$-motor axons (~60% according to Boyd and Davey 1968; 33% according to Burke et al. 1977; Weeks and English 1985). Even if all of the $\gamma$-motoneurons were recruited, the resultant volley would probably be no larger than that produced by the $\alpha$-motoneurons responsible for the MSR. Thus the nerve volleys in question seem necessarily to have been produced by a large number of large-diameter sensory axons, namely Group I afferents. Unfortunately, there is no way to distinguish between Ia spindle afferents, which are known to receive PAD, and Ib Golgi tendon organ afferents, which have been less well studied but appear to display similar changes in excitability during fictive locomotion (Eidelberg et al. 1985).

*Mechanisms for producing DRRs*

Antidromic activity could be produced by a large enough PAD in the terminal arborizations of afferent fibers. The antidromic volleys recorded in the peripheral nerves of intact cats occurred with a latency that ranged from 3.5 to 4.5 ms depending on the cats and distances between the spinal cord and the stimulating and recording cuff electrodes on the LG or MG nerves. We estimate the minimum time of conduction as 1.45–1.95 ms for the fastest Group Ia–afferent fibers of gastrocnemius-soleus (120 m/s) (Jimenez et al. 1984; Rindos et al. 1984) and a similar time of conduction back out along the same class of fibers. The remaining latency indicates a central delay of 0.6–1 ms for the generation of antidromic action potentials in Group I afferents of these muscles. This limits the possibilities to ephaptic or monosynaptic connection between primary afferents. However, no monosynaptic-presynaptic connections among homonymous Group I afferents have been described. With the use of stimulation of a cutaneous nerve, Rudomin, Solodkin, and Jimenez (personal communication) have observed an antidromic response in primary afferents of the MG-LG nerve that appeared to be ephaptic, because the spikes were unaccompanied by any PAD. The terminal arborizations of the spindle afferents from all of the ankle extensor muscles are richly elaborated and closely intertwined in the ventral horn, which would facilitate such interactions mediated by electrotonic fields or potassium fluxes. Alternatively, the antidromic activation of triceps surae motoneurons by stimulating the muscle nerve can also fire primary afferents if they have been depolarized to near-threshold, according to Decima and Goldberg (1970).

Whether monosynaptic, ephaptic, or postsynaptic, the nonmotor nerve volleys that were observed might be made

to vary in strength, as observed, by modulating levels of PAD onto the afferents that produce the antidromic activity. Direct evidence for such PAD has been obtained in fictively locomoting cats, where PAD parallels a similar reduction of the MSR during the flexion phase as seen here (Duenas and Rudomin 1988; however, see Shefchyk and Jordan 1985).

A similar analysis applies to DRRs in decerebrate cats elicited by electrical stimulation to SAPH and PT nerve. The central delay for the early dorsal root reflex response (2.9-ms total latency) is consistent with the presence of one synapse or none. Eccles et al. (1961) also observed a DRR, elicited by stimulating muscle-nerve–Group I fibers, with a central latency of only 0.35 ms. They suggested an ephaptic connection or a spinofugal collateral (returning branch of the same fiber). Barron and Matthews (1935) and Chung et al. (1970) reported DRRs apparently lacking a central synapse that were nevertheless labile and intermittent; intermittent conduction in spinofugal collaterals was suggested. Our short-latency DRR was not constant; it was not phase dependent, but it almost disappeared during locomotion (Fig. 5*C, bottom trace*). Ephaptic transmission facilitated by a critical level of PAD could be responsible.

*DRR and PAD in reduced preparations*

The longer latency DRR reported here is similar to the DRRs in primary afferents reported by Brooks and Koizumi (1956) after stimulation of cutaneous and muscle afferents and by Eccles et al. (1961) stimulating muscle afferents only. They reported that the DRR had latencies in the 5- to 10-ms range, with central delay of 1.5–2.0 ms. Because Brooks and Koizumi (1956) found that the DRR increased in amplitude as the cord temperature decreased, it was important to be certain that the cord temperature was normal. Our animals were maintained at a temperature of 37°C by regulated radiant heat, which increased to 37.5–38°C during walking. The cats breathed at a normal rate that remained constant for 10–20 h.

Dubuc et al. (1985) observed spontaneous, rhythmic, antidromic discharges in primary afferents during fictive locomotion and in decerebrate locomotion on a treadmill. They found that the activity of different units had different phasing with respect to the step cycle, suggesting a role of the CPG in selective presynaptic control of afferent sensitivity. In the present experiments we could elicit DRRs in afferent fibers at a spinal level that projects to the triceps surae muscles (Fig. 4). This activity was maximal during flexion whereas DRRs in other rootlets could be minimal during flexion (Fig. 6). Duenas and Rudomin (1988) found that during fictive locomotion, the greatest PAD in Ia afferents of triceps surae also usually occurred during flexion. During fictive locomotion, the antidromic activity could be entirely attributed to the CPG. However, in walking cats it is also possible that such antidromic activity occurs from a PAD produced by other afferent fibers on the afferent fibers of the dorsal rootlet. During fictive locomotion in thalamic cats, phasic PAD on cutaneous afferent fibers can occur during both phases of the step cycle (Gossard et al. 1989). We note that in our decerebrate cats, some spontaneous antidromic activity also occurred during the extensor phase of the step cycle (see Fig. 5).

The level of PAD observed in any individual afferent probably represents the sum of several potential sources of PAD, each of which may itself be subject to PAD. A sustained and a phasic PAD on afferents fibers of SAPH and TP could contribute to the observed amplitude reduction and the phasic modulation in the cutaneous-evoked DRRs. There is evidence of a phasic PAD onto afferent fibers of SAPH nerve during fictive locomotion (Baev and Kostyuk 1982; Gossard et al. 1989). During fictive locomotion, an increase in excitability of SAPH and sural afferent fibers by intraspinal microstimulation has been noted (Duenas, unpublished observations).

It also seems likely that the net modulatory effects on different reflexes arise through different mechanisms and sources. For example, our decerebration procedure had little effect on the amplitude or modulation of the MSR, but it virtually abolished oligosynaptic FRA reflexes that were robust and deeply modulated during normal locomotion in the same cats (Duenas et al. 1984). At least several different descending pathways contribute variously to both PAD and interneuronal transmission of segmental reflexes (Carpenter et al. 1966; Holmqvist and Lundberg 1959; Kuno and Perl 1960; Rudomin et al. 1986).

*Role of PAD in presynaptic modulation of MSR transmission*

The present results suggest that PAD in the terminals of Group I proprioceptors is modulated during the step cycle and that it normally reaches a peak amplitude that is close to but does not exceed threshold for the initiation of antidromic action potentials. That is to say, it is as large as it can be without interfering with sensory information flow. The Ia-fibers presumably have typical resting potentials (−60 to −80 mV), thresholds (10- to 20-mV depolarization), and positive overshoots for their action potentials (+5 to +15 mV). Thus the maximal dynamic range of PAD can be no more than 15–20% of the maximal excursion of the action potential. If the release of transmitter at the Ia to motoneuron synapses depended linearly on this excursion, then this mechanism of presynaptic inhibition would be expected to have a modest effect at most. On the basis of single EPSP variability, it has been suggested that various branches of the terminal arborization might not always transmit action potentials because of failure to propagate into one or both daughter axons at branch points (Gu and Muller 1989; Luscher et al. 1983). Under these circumstances, depolarization of the arborizations should actually increase synaptic transmission by increasing the safety factor for such propagation.

Instead, it seems likely that the modulation of synaptic efficacy occurs locally at each bouton (Jack et al. 1981; Walmsley et al. 1988) and that it involves highly nonlinear, stochastic processes in which relatively small decreases in the amplitude of the action potential could effect large changes in probability of transmitter release (Clements et al. 1987). Such a local regulatory mechanism could account for frequency-dependent facilitations and depressions of EPSP size that appear to be individualized on the basis of the size of the postsynaptic motoneuron rather than generalized to a particular terminal arborization (Collins et al. 1984). The small motoneurons innervating type S

muscle fibers tend to receive larger EPSPs from Ia-fibers, but the amplitude of these EPSPs tends to be depressed during high-frequency activity in the Ia afferents; conversely, these same Ia-fibers provide small EPSPs onto large motoneurons, but these EPSPs tend to be potentiated at higher frequencies.

This raises the question of how these various nonlinearities would interact with PAD. This PAD may be applied selectively to individual boutons (Walmsley et al. 1987), but the depolarization seems likely to spread electrotonically to reach similar levels throughout a terminal arborization, based on the fact that it is readily recorded all the way out into the dorsal roots as the dorsal root potential. With the use of quantal analysis of single EPSPs, Clements et al. (1987) found a large range of presynaptic inhibitory effects in the triceps surae MSR after PBST stimulation. These effects were not correlated with starting EPSP amplitude and were not constant among several motoneurons receiving input from a single Ia afferent or among several afferents impinging on a single motoneuron. This would seem to favor highly localized processes such as direct calcium entry at the axo-axonic synapse, which would not necessarily be well correlated with gross PAD as measured directly or inferred from DRRs or from responses to intraspinal microstimulation.

The nature and magnitude of such possible complexities in synaptic transmission will need to be resolved to clarify the quantitative interpretation of classical paradigms such as the H-reflex and to consider their implications for more complex pathways involving interneurons. It remains possible, albeit unlikely, that the monosynaptic Ia system has been singled out for such Byzantine processes precisely because it offers no other opportunities for modulation of an otherwise large, fixed effect.

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## REFERENCES

ABRAHAM, L. D. AND LOEB, G. E. The distal hindlimb musculature of the cat. Patterns of normal use. *Exp. Brain Res.* 58: 580–593, 1985.

ABRAHAM, L. D., MARKS, W. B., AND LOEB, G. E. The distal hindlimb musculature of the cat. Cutaneous reflexes during locomotion. *Exp. Brain Res.* 58: 594–603, 1985.

AKAZAWA, K., ALDRIDGE, J. W., STEEVES, J. D., AND STEIN, R. B. Modulation of stretch reflex during locomotion in the mesencephalic cat. *J. Physiol. Lond.* 329: 553–567, 1982.

BAEV, K. V. Retuning of segmental responses to peripheral stimulation in cats during fictitious locomotion. *Neurophysiology* 13: 206–212, 1981.

BAEV, K. V. AND KOSTYUK, P. G. Polarization of primary afferent terminals of lumbosacral cord elicited by the activity of spinal locomotor generator. *Neuroscience* 7: 1401–1409, 1982.

BARRON, D. H. AND MATTHEWS, B. H. C. Intermittent conduction in the spinal cord. *J. Physiol. Lond.* 85: 73–103, 1935.

BOYD, I. A. AND DAVEY, M. R. *Composition of Peripheral Nerves.* Edinburgh: Livingstone, 1986.

BROOKS, M. C. AND KOIZUMI, K. Origin of the dorsal root reflex. *J. Neurophysiol.* 19: 61–74, 1956.

BURKE, D. Critical examination of the case for or against fusimotor involvement in disorders of muscle tone. In: *Motor Control Mechanism in Health and Disease,* edited by J. E. Desmedt. New York: Raven, 1983, p. 133–150.

BURKE, R. E. AND RUDOMIN, P. Spinal neurons and synapses. In: *The Handbook of Physiology. The Nervous System. Cellular Biology of Neurons.* Bethesda, MD: Am. Physiol. Soc., 1977, sect. 1, vol. 1, chapt. 24, p. 877–944.

BURKE, R. E., STRICK, P. L., KANDA, K., KIM, C. C., AND WALMSLEY, B. Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. *J. Neurophysiol.* 40: 667–680, 1977.

CARPENTER, D., ENGBERG, I., AND LUNDBERG, A. A primary afferent depolarization evoked from the brain stem and the cerebellum. *Arch. Ital. Biol.* 104: 73–85, 1966.

CHUNG, S. H., RAYMOND, S. A., AND LETTVIN, J. Y. Multiple meaning in single visual units. *Brain Behav. Evol.* 3: 72–101, 1970.

CLEMENTS, J. D., FORSYTHE, I. D., AND REDMAN, S. J. Presynaptic inhibition of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia axons. *J. Physiol. Lond.* 383: 153–169, 1987.

COLLINS, W. F., HONIG, M. G., AND MENDELL, L. M. Hetergeneity of group Ia homonymous $\alpha$-motoneurons as revealed by high-frequency stimulation of Ia afferent fibers. *J. Neurophysiol.* 52: 980–993, 1984.

DECIMA, E. E. AND GOLDBERG, L. J. Centrifugal dorsal root discharges induced by motoneurone activation. *J. Physiol. Lond.* 207: 103–118, 1970.

DUBUC, R., CABELGUEN, J. M., AND ROSSIGNOL, S. Rhythmic antidromic discharges of single primary afferents recorded in cut dorsal root filaments during locomotion in the cat. *Brain Res.* 359: 375–378, 1985.

DUENAS, S. H., LOEB, G. E., AND MARKS, W. B. A quantitative comparison of hindlimb muscle activity and flexor reflexes in normal and decerebrate cats during walking. *Soc. Neurosci. Abstr.* 10: 628, 1984.

DUENAS, S. H., LOEB, G. E., AND MARKS, W. B. Dorsal root reflex during locomotion in normal and decerebrate cats. *Soc. Neurosci. Abstr.* 11: 1028, 1985.

DUENAS, S. H. AND RUDOMIN, P. Excitability changes of ankle extensor group Ia and Ib fibers during spontaneous fictive locomotion. *Exp. Brain Res.* 70: 15–25, 1988.

ECCLES, J. C., KOSAK, W., AND MAGNI, F. Dorsal root reflex of muscle group I afferent fibers. *J. Physiol. Lond.* 159: 128–146, 1961.

ECCLES, J. C. AND LUNDBERG, A. Synaptic actions which may evoke the flexion reflex. *Arch. Ital. Biol.* 97: 199–221, 1959.

EIDELBERG, E., DUENAS, S. H., AND RUDOMIN, P. Excitability changes in ankle extensor group Ia and Ib afferents during spontaneous fictive locomotion. *Soc. Neurosci. Abstr.* 11: 1028, 1985.

GOSSARD, J. P., CABELGUEN, J. M., AND ROSSIGNOL, S. Intra-axonal recordings of cutaneous primary afferents during fictive locomotion in the cat. *J. Neurophysiol.* 62: 1177–1188, 1989.

GU, X. AND MULLER, K. J. Conduction block at CNS axon bifurcations: a central synaptic switch. *Soc. Neurosci. Abstr.* 15: 192, 1989.

HOLMQVIST, B. AND LUNDBERG, A. On the organization of the supraspinal inhibitory control of interneurones of various spinal reflex arcs. *Arch. Ital. Biol.* 97: 340–356, 1959.

HOUNSGAARD, J., HULTBORN, H., JESPERSEN, B., AND KIEHN, O. Bistability of $\alpha$-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J. Physiol. Lond.* 405: 345–367, 1988.

JACK, J. J., REDMAN, S. J., AND WONG, K. The components of synaptic potentials evoked in cat spinal motoneurones by impulses in single group Ia afferents. *J. Physiol. Lond.* 321: 65–96, 1981.

JANKOWSKA, E., MCCREA, D., RUDOMIN, P., AND SYKOVA, E. Observations of neuronal pathways subserving primary afferent depolarization. *J. Neurophysiol.* 46: 506–516, 1981.

JIMENEZ, I., RUDOMIN, P., SOLODKIN, M., AND VYKLICKY, L. Specific and nonspecific mechanisms involved in generation of PAD of group Ia afferents in cat spinal cord. *J. Neurophysiol.* 52: 921–940, 1984.

JORDAN, L. Factors determining motoneuron rhythmicity during fictive locomotion. *Symp. Soc. Exp. Biol.* 37: 423–444, 1983.

KANDA, K., BURKE, R. E., AND WALMSLEY, B. Differential control of fast and slow twitch motor units in the decerebrate cat. *Exp. Brain Res.* 29: 57–74, 1977.

KUNO, M. AND PERL, E. R. Alteration of spinal reflexes by interaction

with suprasegmental and dorsal root activity. *J. Physiol. Lond.* 151: 103–122, 1960.

LOEB, G. E. The control and responses of mammalian muscle spindles during normally executed motor tasks. *Exercise Sport Sci. Rev.* 12: 157–204, 1984.

LOEB, G. E. AND GANS, C. *Electromyography for Experimentalists.* Chicago, IL: Univ. of Chicago Press, 1986.

LUNDBERG, A. Inhibitory control from the brain stem of transmission from primary afferents to motoneurons, primary afferent terminals and ascending pathways. In: *Brain Stem Control of Spinal Mechanisms,* edited by B. H. Sjolund and A. Bjorklund. New York: Elsevier, 1982, p. 179–223.

LUSCHER, H., RUENZEL, P., AND HENNEMAN, E. Composite EPSPs in motoneurons of different sizes before and during PTP: implications for transmission failure and its relief in Ia projections. *J. Neurophysiol.* 49: 269–289, 1983.

MARKS, W. B. AND LOEB, G. E. Action currents, internodal potentials, and extracellular records of myelinated mammalian nerve fibers derived from node potentials. *Biophys. J.* 16: 655–668, 1976.

PERRET, C. Centrally generated patterns of motoneuron activity during locomotion in the cat. *Symp. Soc. Exp. Biol.* 37: 405–422, 1983.

PERRET, C. AND CABELGUEN, J. M. Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. *Brain Res.* 187: 333–352, 1980.

RINDOS, A. J., LOEB, G. E., AND LEVITAN, H. Conduction velocity changes along lumber primary afferent fibers in cats. *Exp. Neurol.* 86: 208–226, 1984.

RUDOMIN, P., SOLODKIN, M., AND JIMENEZ, I. PAD and PAH response patterns of group Ia- and Ib-fibers to cutaneous and descending inputs in the cat spinal cord. *J. Neurophysiol.* 56: 987–1006, 1986.

SCHOMBURG, E. D., ROESLER, J., AND MEINCK, H. M. Phase dependent transmission in the excitatory propriospinal reflex pathway from forelimb afferents to lumbar motoneurons during fictive locomotion. *Neurosc. Lett.* 4: 249–252, 1977.

SHEFCHYK, S. J. AND JORDAN, L. M. Synaptic transmission from muscle afferents during fictive locomotion in the mesencephalic cat. *J. Neurophysiol.* 54: 1101–118, 1985.

STEIN, R. B. AND CAPADAY, C. The modulation of human reflexes during functional motor tasks. *Trends Neurosci.* 11: 328–332, 1988.

STEIN, R. B., NICHOLS, T. R., JHAMANDAS, J., DAVIS, L., AND CHARLES, D. Stable long-term recordings from cat peripheral nerves. *Brain Res.* 128: 21–38, 1977.

WALMSLEY, B., EDWARDS, F. R., AND TRACEY, D. J. Nonuniform release probabilities underlie quantal synaptic transmission at a mammalian excitatory central synapse. *J. Neurophysiol.* 60: 889–908, 1988.

WALMSLEY, B., WIENIAWA-NARKIEWICZ, E., AND NICOL, M. J. Ultrastructural evidence related to presynaptic inhibition of primary muscle afferents in Clarke's column of the cat. *J. Neurosci.* 7: 236–243, 1987.

WEEKS, O. I. AND ENGLISH, A. W. Compartmentalization of the cat lateral gastrocnemius motor nucleus. *J. Comp. Neurol.* 235: 255–257, 1985.