Ankle extensor group I afferents excite extensors throughout the hindlimb during fictive locomotion in the cat

P. Guertin, M. J. Angel, M.-C. Perreault and D. A. McCrea*

Department of Physiology, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3

1. The effects of stimulating hindlimb extensor nerves (100–200 ms trains, 100 Hz, ⩽ 2 times threshold) during the flexor and extensor phases of the locomotor step cycle were analysed in the decerebrate, paralysed cat during fictive locomotion evoked by stimulation of the mesencephalic locomotor region.

2. Stimulation during extension of either the medial gastrocnemius (MG), lateral gastrocnemius–soleus (LGS) or plantaris (Pl) nerves was equally effective in increasing the duration and amplitude of electroneurogram (ENG) activity recorded in ipsilateral ankle, knee and hip extensor nerves. Enhancement of extensor ENG activity could be evoked with near threshold stimulation intensity and appeared within 10–40 ms of the onset of ankle extensor nerve stimulation. Stimulation of anterior biceps during extension occasionally evoked a modest increase in the duration of activity of hip, knee and ankle extensors. Stimulation of quadriceps during extension enhanced the activity of proximal extensors and soleus, but inhibited other ankle extensors.

3. Selective activation of ankle extensor Ia spindle afferents by muscle stretch also enhanced ipsilateral extension. It is argued that both muscle spindle and tendon organ afferents can contribute to the increase in extensor nerve activity evoked by group I stimulation intensity during fictive locomotion.

4. During flexion, stimulation of either the MG, Pl or LGS nerves at group I strength terminated on-going activity in ipsilateral flexors and initiated a burst of activity in ipsilateral hip, knee and ankle extensors, i.e. reset the step cycle to extension.

5. Low strength stimulation of the mixed muscle and cutaneous nerve innervating the plantar aspect of the foot produced extension enhancement and resetting similar to that evoked by group I muscle afferent stimulation. Stimulation of the cutaneous nerve supplying the dorsal aspect of the foot during extension enhanced extensor activity, and during flexion, enhanced the activity of flexors.

6. The effects reported here during fictive locomotion may also occur during overground locomotion with natural activation of group I muscle spindle and tendon organ afferents. Extensor spindle and tendon organ afferents may thus serve as an excitatory reflex system helping to shape the amplitude, duration and timing of ipsilateral extensor activity. Increased or unexpected activation of group I ankle extensor afferents or plantar foot afferents during locomotion could also compensate for increased loading of the limb.

It is well known that activation of hindlimb extensor group Ib Golgi tendon organ afferents at rest results in inhibition of homonymous and synergistic motoneurones (for review see Jami, 1992). Recent investigations, however, have revealed different actions of extensor group I afferents during fictive locomotion. Delivered during flexion, plantaris (Pl) nerve stimulation at group I strength terminates flexion and initiates extension (Conway, Hultborn & Kiehn, 1987). Delivered during extension, group I ankle extensor stimulation increases the locomotor activity of other extensor nerves including synergists (Conway *et al.* 1987; Pearson & Collins, 1993). This increase in extensor activity during fictive locomotion includes an increased amplitude and duration of the locomotor extensor phase. The

* To whom correspondence should be addressed.

entrainment of locomotion to the frequency of stimulation of ankle extensor muscle nerves at group I strength further illustrates the ability of extensor group I afferents to influence the spinal circuitry for locomotion (Conway *et al.* 1987; Pearson, Ramirez & Jiang, 1992). Stimulation of group I afferents in the quadriceps (Q) nerve can also influence the step cycle in both *in vivo* cat (Conway *et al.* 1987; Gossard, Brownstone, Barajon & Hultborn, 1994) and *in vitro* neonatal rat spinal cord preparations (Kiehn, Iizuka & Kudo, 1992). Evidence to date suggests that afferents from group Ib Golgi tendon organs are mainly responsible for effects evoked at group I stimulation strength during fictive locomotion (Conway *et al.* 1987; Pearson & Collins, 1993; Gossard *et al.* 1994).

Recently, it has been shown that activation of group I ankle extensor afferents during extension can result in disynaptic EPSPs in other ankle extensor motoneurones (Angel, Guertin, Jiménez & McCrea, 1994; McCrea, Shefchyk, Stephens & Pearson, 1995). These EPSPs replace (McCrea *et al.* 1995) the classical Ia and Ib non-reciprocal inhibition of synergists (see Jami, 1992). Disynaptic group I excitation of extensors is, therefore, one mechanism by which ankle extensor group I afferents can influence the activity of hindlimb extensors during mesencephalic locomotor region (MLR)-evoked fictive locomotion. This excitation is in addition to the longer latency excitation of extensor motoneurones evoked through spinal locomotor circuitry (Pearson & Collins, 1993; Gossard *et al.* 1994; Guertin, Angel, Jiménez & McCrea, 1994; McCrea *et al.* 1995) and monosynaptic group Ia excitation (Eccles, Eccles & Lundberg, 1957; Eccles & Lundberg, 1958; Edgley, Jankowska & McCrea, 1986). Locomotor disynaptic EPSPs appear only during MLR-evoked fictive locomotion and not in acute spinal animals (McCrea *et al.* 1995). Thus, the effects of activation of group I afferents during MLR-evoked fictive locomotion may differ from results of previous investigations in which, in many cases, locomotion was induced by drug administration in acute spinal cats.

It is unknown whether activation of group I afferents in each of the ankle extensors produces similar actions on the fictive locomotor step cycle. The present study compares the effects of stimulating the medial gastrocnemius (MG), lateral gastrocnemius–soleus (LGS), Pl and the separated lateral gastrocnemius (LG) and soleus (Sol) nerves on the amplitude, duration and timing of locomotor electroneurogram (ENG) bursts. While previous studies have usually illustrated the effects of activation of ankle extensor group I afferents on activity recorded in a single hindlimb extensor, the present study examines effects recorded simultaneously in ipsilateral hip, knee and ankle nerves as well as some contralateral nerves. Furthermore, the effects of activation of hip and knee extensor group I afferents are reported and compared with those produced by activation of ankle extensor group I afferents in the same preparations. Finally, the effects of activation of nerves innervating the skin of the foot and following selective activation of ankle extensor group Ia muscle spindle afferents are documented. In all experiments, fictive locomotion was evoked by stimulation of the MLR in decerebrate and paralysed cats. The accompanying paper (Perreault, Angel, Guertin & McCrea, 1995) presents results obtained with stimulation of hindlimb flexor nerves.

Preliminary results have been presented in abstract form (Guertin, Angel, Perreault, Carr & McCrea, 1993*a*, *b*).

METHODS

Preparation

Experiments were performed on eighteen male or female cats weighing 2–3 kg. Animals were anaesthetized with halothane (~1·5%) delivered in a mixture of oxygen and nitrous oxide (70%). Following induction of anaesthesia a tracheal catheter was inserted and cannulae were placed into jugular and hindlimb veins for administration of fluids and drugs. Blood pressure was monitored from one carotid artery and the other was dissected free and tied loosely for reversible ligation during the decerebration. Buffer solution (5% glucose and 0·85% $NaHCO_3$) was infused (5 ml h^{-1}) throughout the experiment. Atropine (0·05 mg kg^{-1}) was given s.c. and dexamethasone (2 mg kg^{-1}) I.V.

Combinations of the following hindlimb nerves were dissected and cut: Q, usually with the rectus femoris portion removed; posterior biceps and semitendinosus (PBSt); sartorius (Sart, medial and lateral branches combined); semimembranosus (Sm) and anterior biceps (AB), which were sometimes combined as SmAB; Pl; MG; LG and Sol, which were usually kept together (LGS) but were dissected separately in one experiment; flexor digitorum longus (FDL); flexor hallucis longus (FHL); the remaining part of the posterior tibial, which includes the muscular and cutaneous innervation of the plantar foot (Tib); tibialis anterior (TA); and the superficial peroneal (SP) nerve. The Q and Sart nerves were placed in cuff electrodes while the remaining nerves were mounted on conventional bipolar silver chloride hook electrodes. In the right (contralateral) hindlimb only three to five nerves were taken to monitor locomotion. Other branches of the sciatic femoral and obturator nerves as well as the tendons inserted around the iliac crest were cut bilaterally.

After laminectomy exposing the lumbar spinal cord segments, the animal was transferred to a rigid frame and the head positioned in a stereotaxic apparatus. Mineral oil pools were constructed from hindlimb and back skin flaps. Following a craniotomy over the occipital and parietal cortices, a precollicullar–postmammillary decerebration was performed by blunt transection. All brain tissue rostral to the transection was removed and anaesthesia was discontinued. The animal was paralysed with intravenous gallamine triethiodide (2–3 mg kg^{-1} h^{-1}) and artificially ventilated to maintain end-expired CO_2 between 3 and 5%. Blood pressure below 80 mmHg was counteracted by injection of Dextran and sometimes slow infusion of noradrenaline (1 mg kg^{-1} h^{-1} in 3 experiments). Animal temperature was maintained with radiant heat.

MLR-evoked fictive locomotion

Fictive locomotion was evoked using monopolar varnish-insulated electrodes (80 μm exposed diameter) to stimulate (80–200 μA, 1 ms duration rectangular pulses at 5–30 Hz) the MLR (posterior, 1–2 mm; lateral, 4 mm; 3–6 mm below the surface of the colliculi; Shik, Severin & Orlovskii, 1966). Unilateral (either left or right) MLR stimulation was usually used but sometimes bilateral stimulation was required to evoke locomotion. Stimulus parameters and location were optimized in each experiment. Rhythmic alternating activity in ipsilateral flexor and extensor muscle ENGs and co-ordination between the hindlimbs were used as the criteria for fictive locomotion. Data on unstimulated and stimulated step cycles were collected during 60–120 s bouts of MLR-evoked fictive locomotion.

Peripheral nerve stimulation

The strength of the nerve stimulation was expressed as multiples of the threshold (T) of the most excitable fibres as measured from the cord dorsum in L6 or L7. The afferent volley was examined and the threshold stimulus intensity determined throughout each experiment. Only results when the group I volley became maximal at $< 2{\cdot}5T$ are included. Cord dorsum records were not collected. Stimulus trains to peripheral nerves (usually 20 shocks, 0·1 ms duration, 100–200 Hz) were delivered during either the extensor or flexor phase of the fictive locomotor cycle. The onset of locomotor activity in a particular nerve was used to trigger the stimulus train following a predetermined delay. A computer was used to control stimulus delivery. Relays allowed switching between stimulation or recording of the peripheral nerves.

Activation of group I a muscle afferents

In one experiment, small amplitude stretches of the triceps surae and Pl muscles were used to activate ankle extensor group I a afferents. Using brief (2 ms), small amplitude ($\leqslant 40$ μm) stretches of triceps surae and Pl muscles (initial tension, 3·5 N), group I a muscle spindle afferents can be activated without activation of group I b Golgi tendon organ afferents (Fetz, Jankowska, Johannisson & Lipski, 1979; Jankowska, McCrea & Mackel, 1981*a*). The nerves to triceps surae and Pl muscles were left intact with their muscles but freed for mounting on bipolar hook electrodes. The calcaneus bone was cut and attached to an electromagnetic muscle puller. Trains of stretches similar in frequency and duration to those used for electrical stimulation were delivered during flexion or extension and effects on the fictive locomotor bursts analysed.

Analysis

Ipsi- and contralateral nerve activity (a total of 8–10 nerves) was recorded during MLR-evoked fictive locomotion in each experiment. ENGs were amplified and filtered (gain, 5000–50000; 3 dB high-pass, 30 Hz; low-pass, 3 kHz) before rectification and integration (envelope follower with a time constant of 100 ms). ENGs were recorded on-line (sampling rate, 500 Hz) along with stimulus markers indicating MLR and peripheral nerve stimulation (captured at 2 kHz) using a Concurrent 5450 computer. Later analysis consisted of selection of periods of stable fictive locomotion and calculation of the duration of locomotor bursts (time between onset and offset of each burst) and the duration of the fictive locomotor step cycles (time between the onset of two consecutive bursts in a particular nerve). ENG activity was also averaged and aligned according to the onset of the stimulus train. Only some of the ENG traces recorded are displayed in the figures.

Results are given as means ± S.D.

RESULTS

Stimulation of ankle extensor nerves during extension

Figure 1*A* illustrates the effects of Pl nerve stimulation at $1{\cdot}6T$ using a twenty shock train on the fictive step cycle. The filled rectangles indicate the duration of stimulation which was triggered from extensor ENG (MG) activity with a delay of 130 ms. The dashed lines at the onset of stimulation facilitate comparisons between ENG traces. The 200 ms stimulus train to the Pl nerve during extension increased the duration of AB, Q and MG activity. In this example, the amplitude of extensor ENG activity, particularly in AB and MG nerves, increased within 15–30 ms of the onset of Pl nerve stimulation. This figure illustrates one of the main findings of the present study: low intensity stimulation of ankle extensor nerves with short duration trains during extension enhanced the activity of hip, knee and ankle extensor nerves during fictive locomotion. This enhanced extensor activity was seen as an increase in the amplitude, duration or both amplitude and duration of the integrated–rectified ENG records. Stimulation of ankle extensor nerves at group I intensity clearly increased the total number of action potentials recorded in extensor nerves. The use of whole nerve ENG recordings precluded a direct assessment of the changes in firing rates of individual units. Although the minimum stimulation required to elicit extension enhancement varied between experiments, it was readily demonstrated in all but one experiment (17/18). The simultaneous enhancement of the activity of hip, knee and ankle extensors following stimulation of a single ankle extensor illustrated in Fig. 1 extends previous results obtained with stimulation of the Pl nerve showing an increase in MG (Pearson & Collins, 1993) and Q (Conway *et al.* 1987) activity during fictive locomotion. While no attempt was made to maximally activate the group I afferents and determine the minimum latency of enhanced extensor activity, increases in the amplitude of hip, knee and ankle extensor ENGs were most often seen within 10–40 ms of the first shock in the stimulus train.

The present results also indicate that stimulation of any of the ankle extensor nerves during extension can prolong and increase the activity of ipsilateral hip, knee and ankle extensors. Figure 1*B* shows that MG stimulation at $1{\cdot}6T$ produced larger bursts in hip and ankle extensor ENGs. Figure 2*A* is an example of the effects of LGS stimulation at low strength ($1{\cdot}28T$, 20 shocks) and shows the resulting increase in burst amplitude and duration in ipsilateral extensor nerves. The enhancement of extensor activity clearly outlasted the duration of the stimulus train. In the one experiment in which LG and Sol branches of the LGS nerve were separated, stimulation of either LG or Sol during extension enhanced extension (not illustrated). Thus, stimulation of any of the four ankle extensor nerves

at group I strength during extension can enhance ipsilateral extensor activity. There were no obvious differences between the four ankle extensors in their ability to evoke enhancement of ipsilateral extensor activity.

The distribution of enhanced extension in the ipsilateral limb was also examined following group I intensity stimulation of ankle extensor nerves during extension. Increased burst durations and amplitudes were observed in AB, Sm, Q, Pl, MG and LGS nerves. Increased duration of ipsilateral extensor bursts was the most common effect seen following the 100–200 ms duration stimulus trains used in the present study. An increase in the amplitude of extensor ENGs was more variable and, while occurring in most trials, was rarely observed simultaneously in all extensor nerves. Nevertheless (except for the LG and Sol nerves), an enhancement of the amplitude of each extensor nerve was observed in at least two experiments. LG and Sol nerves were separated in only one experiment; both displayed increased burst activity following stimulation of the Pl nerve (Fig. 5*C*).

One characteristic of MLR-evoked fictive locomotion is the absence of overlapping ENG activity in ipsilateral flexor and extensor nerves. This was maintained during stimulation of ankle extensor nerves with flexor nerves remaining silent until the termination of extensor activity. This is illustrated in Fig. 1*B* in which a prolongation of the inactive phase in Sart and TA nerves accompanied the prolongation of extensor activity. In the present preparation, the PBSt nerve was usually active when Sart and TA were active or for a brief period following the termination of extensor activity (Fig. 1*B*). This suggests that PBSt motoneurones innervating bifunctional muscles (Perret & Cabelguen, 1980) were most often active as flexors. When the PBSt nerve showed activity during extension, this activity was enhanced by stimulation of ankle extensor nerves at group I strength (not illustrated). Although examined in only two experiments, the activity of FDL and FHL occurred mainly during the transition from extension to flexion. Neither FDL (Fig. 1*B*) nor FHL (not illustrated) were activated by ankle extensor nerve stimulation. Thus, activation of group I ankle extensor afferents during extension delayed the onset of activity in PBSt, FHL and FDL nerves as well as the flexors TA and Sart.

Effects of activation of ankle extensor group I afferents during extension on the period of the fictive step cycle are

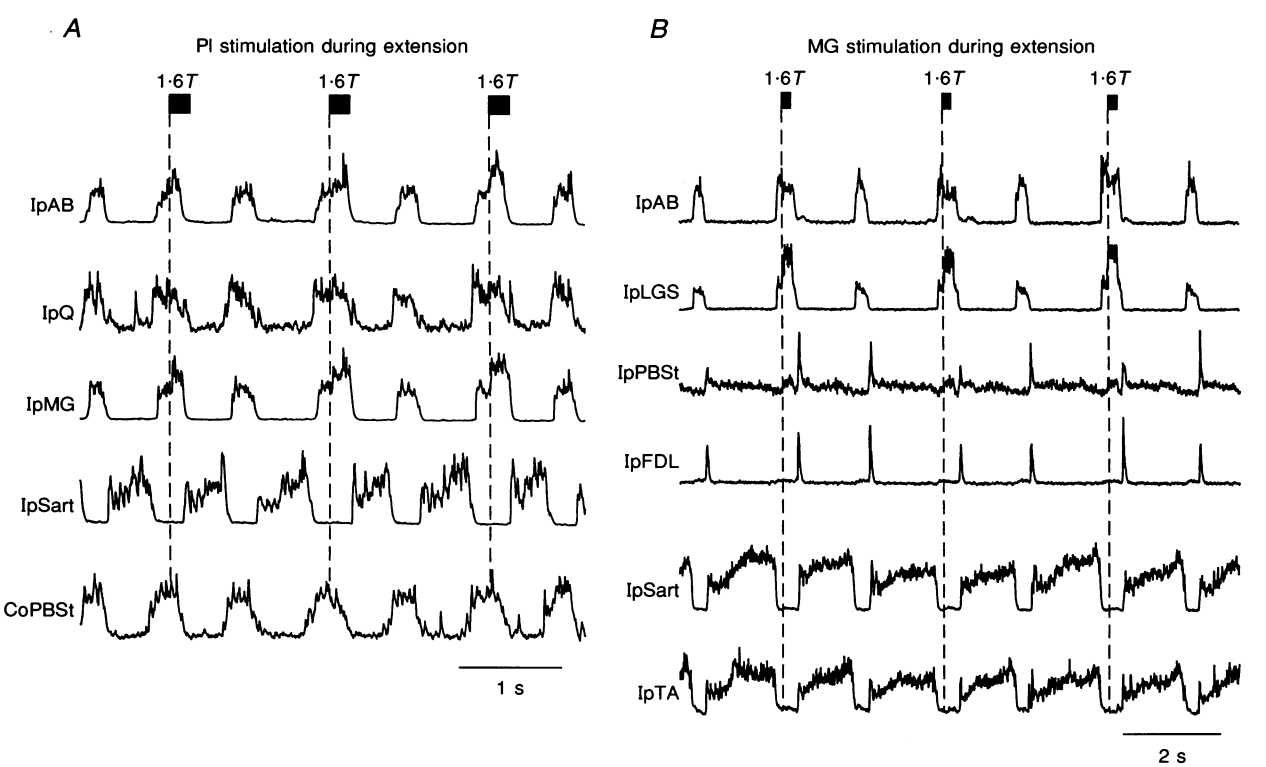

Figure 1. Enhanced ipsilateral extension following stimulation of ankle extensor group I afferents during MLR-evoked fictive locomotion

Traces are integrated and rectified ENGs of extensor (AB, Q, MG, LGS) and flexor (Sart, PBSt, FDL, TA, CoPBSt) nerves. Ip, ipsilateral; Co, contralateral. Filled rectangles indicate the duration of the stimulus train and the vertical dashed lines indicate stimulus onset. *A*, Pl stimulation (1·6*T*, 20 shocks) was triggered every second step approximately 130 ms after the onset of AB locomotor activity. Pl stimulation enhanced AB, Q and MG ENG locomotor bursts. *B*, in another experiment, stimulation of MG (1·6*T*, 20 shocks, 100 Hz) every second extensor burst also enhanced AB and LGS activity.

illustrated in Fig. 2*B*. The LGS nerve was stimulated following the onset of every second burst in AB. Cycle period, measured as the time between the onset of consecutive AB ENG bursts, and burst duration are plotted. The mean cycle period and burst duration of non-stimulated (control) steps are shown as continuous lines and their standard deviations as dotted lines. For all but four trials, the duration of AB activity increased following LGS stimulation. The periods of step cycles during which the LGS nerve was stimulated, however, were usually within one standard deviation of the mean control cycle period. Thus, increased duration of extensor nerve activity can occur without change in the onset of the next fictive step cycle. Results presented in Figs 4 and 5 and elsewhere (Conway *et al.* 1987; Pearson & Collins, 1993) show that stimulation of group I ankle extensor afferents can increase the cycle period or entrain locomotion (Conway *et al.* 1987; Pearson *et al.* 1992). Figure 2 illustrates that with the appropriate stimulation parameters, small perturbations of the step cycle can occur without altering its fundamental frequency. In this example, the increased duration of the extensor phase occurred at the expense of the flexor phase.

Contralateral ENG activity was also affected by activation of ipsilateral ankle extensor group I afferents during extension. Figure 1*A* illustrates that, compared with locomotor bursts without stimulation, bursts in CoPBSt (active during contralateral flexion) were prolonged by activation of group I afferents. Ipsilateral extensor nerve stimulation had little or no effect on the amplitude of contralateral flexor nerve activity in all experiments in which this was examined.

Figure 3 illustrates the effects of different LGS stimulus strengths on AB nerve activity during extension. The averaged traces ($n = 10$) were aligned at the onset of LGS nerve stimulation. Note the increase in amplitude beginning about 10 ms after the onset of stimulation for all stimuli above 1·16*T*. The amplitude of AB activity was increased by near threshold stimulation intensity (1·16*T*) and doubled with stimulation strengths as low as 1·28*T*. A prolongation of AB activity that outlasted the duration of the stimulus train was obtained with 1·4 and 1·75*T* stimulation. It is unlikely that monosynaptic excitation produces the enhancement of Q and AB bursts during fictive locomotion illustrated in Figs 1, 2 and 3 because such connections between ankle extensor group I afferents and hip and knee extensor motoneurones are weak (Edgley *et al.* 1986).

The effectiveness of very low stimulation strengths (Figs 2 and 3) is compatible with the possibility that group Ia muscle afferents in ankle extensor nerves may contribute to

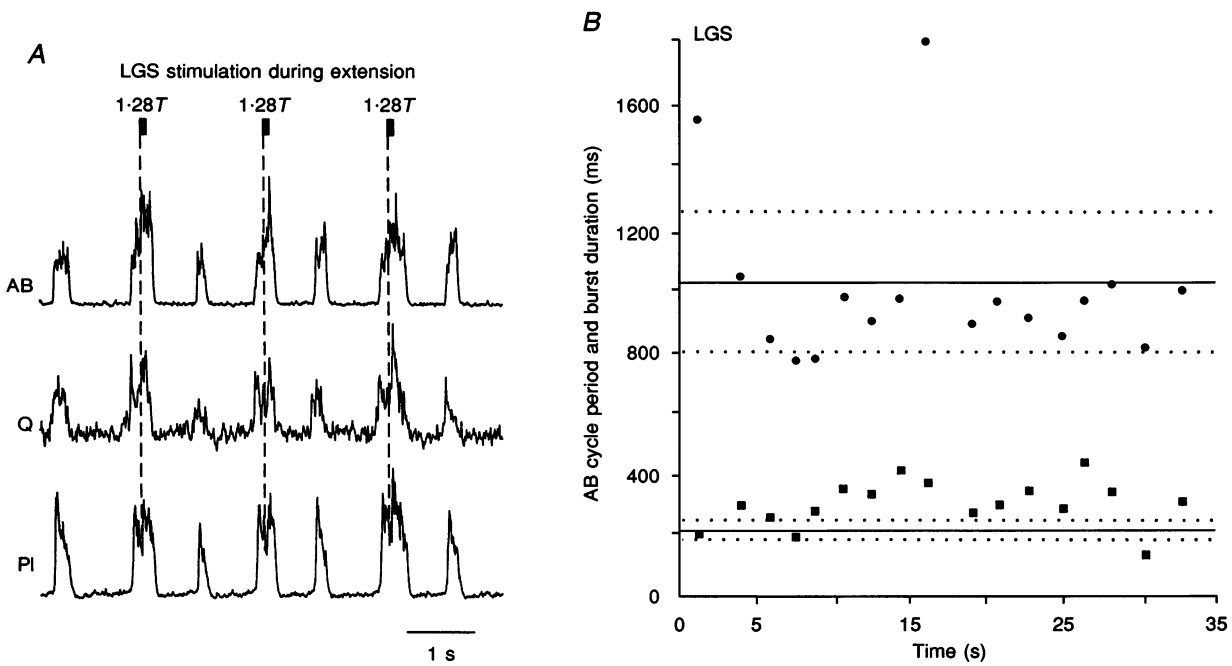

Figure 2. LGS stimulation during extension enhances the activity of other hindlimb extensors

A, stimulation of LGS (1·28*T*, 20 shocks at 200 Hz) every second extensor burst enhanced the amplitude and duration of ankle (Pl), knee (Q) and hip (AB) extensor locomotor bursts. *B*, the cycle period (●) and burst duration (■) of AB nerve activity during LGS nerve stimulation is plotted for a 35 s period of fictive locomotion. The continuous horizontal lines indicate the mean and the dotted lines the standard deviation for steps in which no stimulus was delivered. Stimulation of LGS substantially increased the AB burst duration in 13/17 trials. The majority of the cycle periods, however, did not change significantly following LGS stimulation.

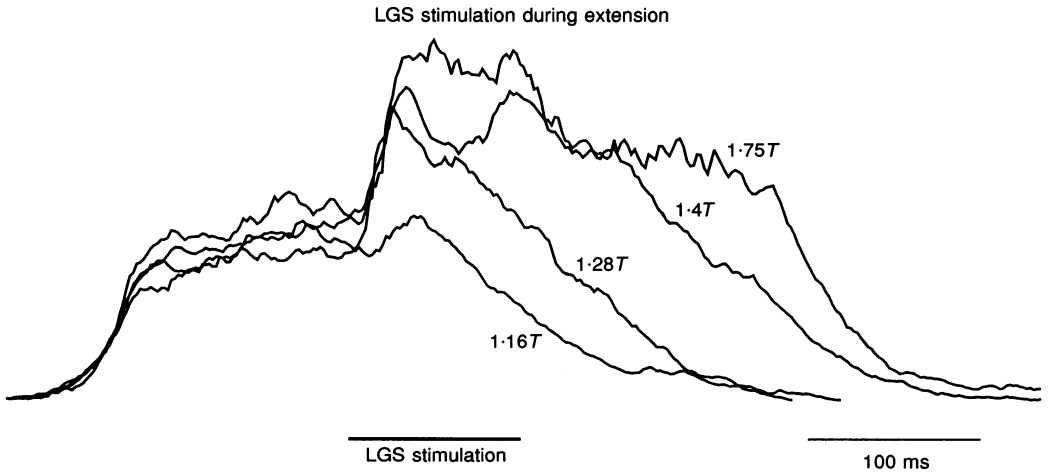

Figure 3. Enhancement of AB locomotor activity by low strength LGS nerve stimulation
Each trace is the average of 10 AB bursts in which the LGS nerve was stimulated at the strengths indicated (trains of 20 shocks, 200 Hz). The traces were aligned with the onset of the stimulus train which is indicated by the horizontal bar below the traces. Even with low stimulus strengths there was an increase in burst amplitude. Note that the duration of AB activity can outlast the stimulus train.

extension enhancement. To investigate this, Ia afferents were activated (see Methods) by short trains (20 stretches, 100 Hz) of small amplitude ($< 35\ \mu$m) stretches of the Achilles tendon. Such tendon vibration activates primary muscle spindle afferents in the triceps surae and Pl nerves without recruiting Ib tendon organ afferents. Figure 4 shows that such vibration delivered during extension increased burst duration in AB, Pl and MG and amplitude in Pl and MG nerves. Because the ankle extensor nerves were left intact with the muscle in this experiment, ankle

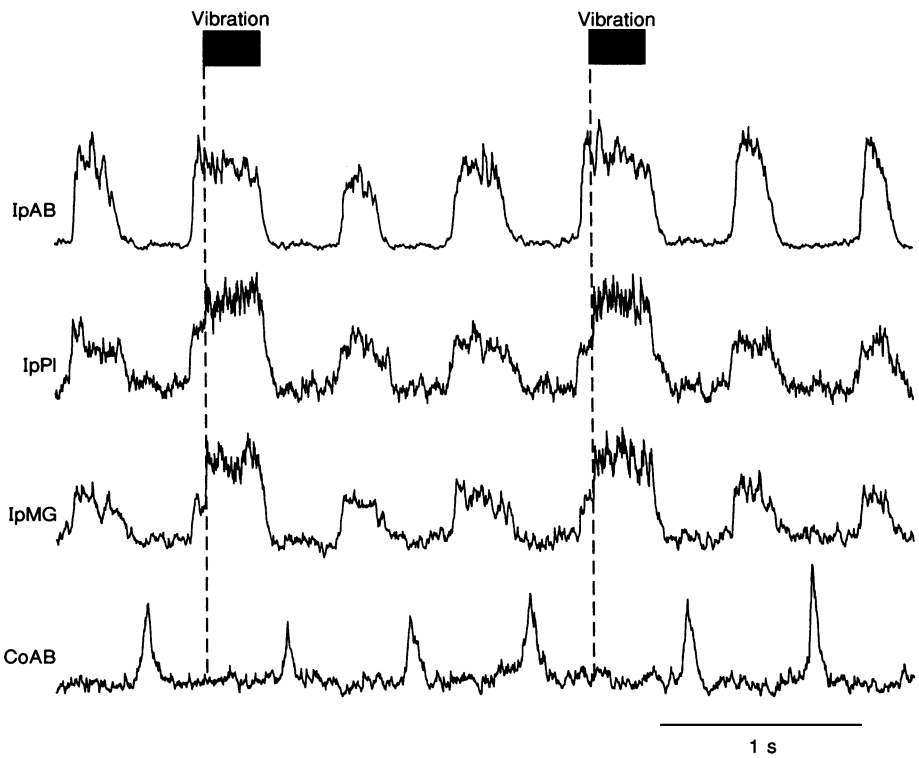

Figure 4. Selective activation of triceps surae and Pl Ia afferents enhances extension
Trains of 35 μm stretches of the Achilles tendon (20 stretches, 100 Hz, initial tension 3·5 N; indicated by the filled rectangles) were triggered from AB activity every third step with a delay of about 60 ms. Tendon vibration increased the duration of AB bursts and increased the amplitude and duration of Pl and MG bursts.

extensor ENG recordings will register both efferent discharges and the afferent activity produced by tendon vibration. It is difficult, therefore, to interpret the source of the increased amplitude of ankle extensor ENGs. Therefore, the important feature of Fig. 4 is the increased activity in the hip extensor (AB) ENG. Further evidence that the activation of Ia afferents can influence spinal locomotor circuitry is that the cycle period was increased, i.e. reset, by muscle vibration (Student's t test, $P > 0{\cdot}01$). The period of consecutive AB bursts for step cycles without muscle stretch was 628 ± 53 ms ($n = 56$) and 726 ± 51 ms ($n = 29$) for step cycles with stretch. Figure 4 also shows that vibration during extension prolonged the contralateral cycle period.

The step cycle period (interval between subsequent AB bursts) when vibration was delivered during flexion (triggered from the onset of TA activity) was 443 ± 49 ms ($n = 21$) and not significantly different ($P > 0{\cdot}1$) from the 457 ± 44 ms period of step cycles ($n = 20$) without vibration. Thus, vibration delivered during flexion did not reset the step cycle.

Stimulation of hip, knee and toe extensor nerves during extension

In four experiments the Q nerve was stimulated at $2T$ during extension. In all cases, Q stimulation increased the amplitude of hip extensor ENG activity. Figure 5*A*, *B* and *C* illustrate the increase in hip extensor activity evoked by stimulation of the Q nerve during extension in three experiments. Unlike the increase in the amplitude of hip, knee and ankle extensor ENG activity produced by activation of ankle extensor afferents, Q stimulation inhibited the activity of ankle extensors. In Fig. 5*A*, $2T$ Q stimulation silenced the activity of MG. This inhibition of MG indicates that Q group I afferents can activate pathways producing a net inhibition of the activity of MG during fictive locomotion. This point is further illustrated in Fig. 5*B* in which Q $1{\cdot}3T$ stimulation produced clear inhibition of Pl and LGS activity while increasing AB

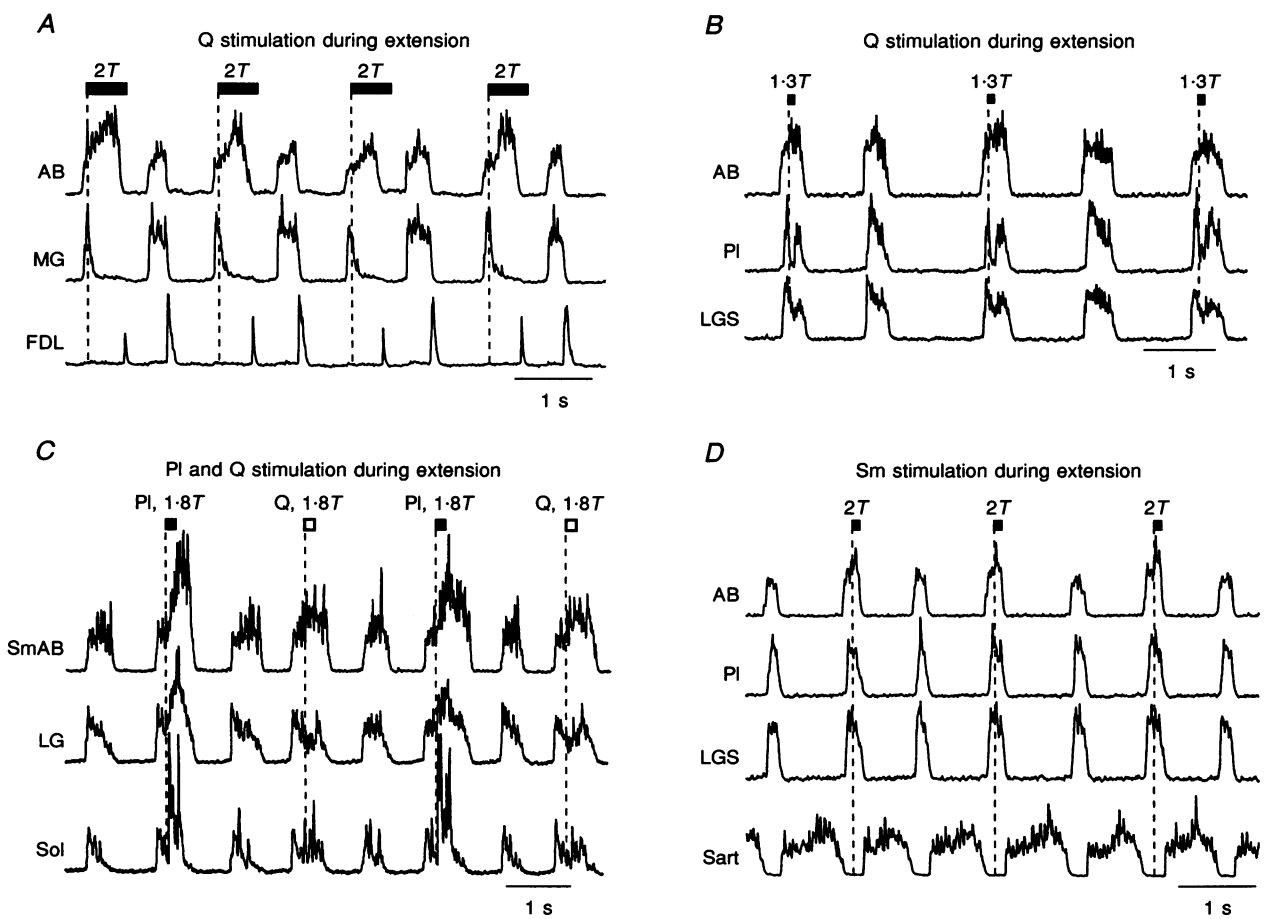

Figure 5. Stimulation of knee and hip extensor group I afferents is less effective than ankle extensors in enhancing extension

A, stimulation of Q ($2T$, 50 shocks, 100 Hz) increased AB activity, but terminated MG activity. *B*, stimulation of Q ($1{\cdot}3T$, 20 shocks, 200 Hz; triggered from AB activity) produced an enhancement of AB activity but a reduction in the amplitude of Pl and LGS bursts. *C*, alternating Pl (filled rectangles) and Q stimulation (open rectangles) shows the greater effect of ankle nerve stimulation (20 shocks to both nerves). *D*, stimulation of Sm ($2T$) slightly increased extensor activity.

activity. Since stimulation of Q at 1·3T would activate a substantial number of I a but relatively few I b afferents (see Jankowska *et al.* 1981*a*), it is likely that the inhibition of MG is evoked largely by activation of Q I a afferents. Figure 5*C* shows that Q stimulation inhibited LG but not Sol activity. This observation may be explained by the monosynaptic connections between Q I a afferents and some Sol but few LG motoneurones (Eccles *et al.* 1957). A comparison of the effects of Pl and Q nerve stimulation at the same intensity is also illustrated in Fig. 5*C*. As shown, Q stimulation (open rectangles) produced some enhancement of the duration and amplitude of SmAB activity but this was less pronounced than that produced by Pl stimulation (filled rectangles). One explanation for the weaker effects of Q stimulation on the amplitude of SmAB nerve activity may be the reciprocal inhibition of some SmAB motoneurones evoked from Q nerve stimulation (Eccles & Lundberg, 1958).

In confirmation of other studies (Conway *et al.* 1987; Kiehn *et al.* 1992; Gossard *et al.* 1994) the present results show that activation of Q group I afferents can perturb the activity of the locomotor central pattern generator. Note that the duration of the Q stimulation in Fig. 5*A* extended into the flexor portion of the step cycle. For the entire period of data collection, of which a portion is illustrated in Fig. 5*A*, the mean cycle period between subsequent AB

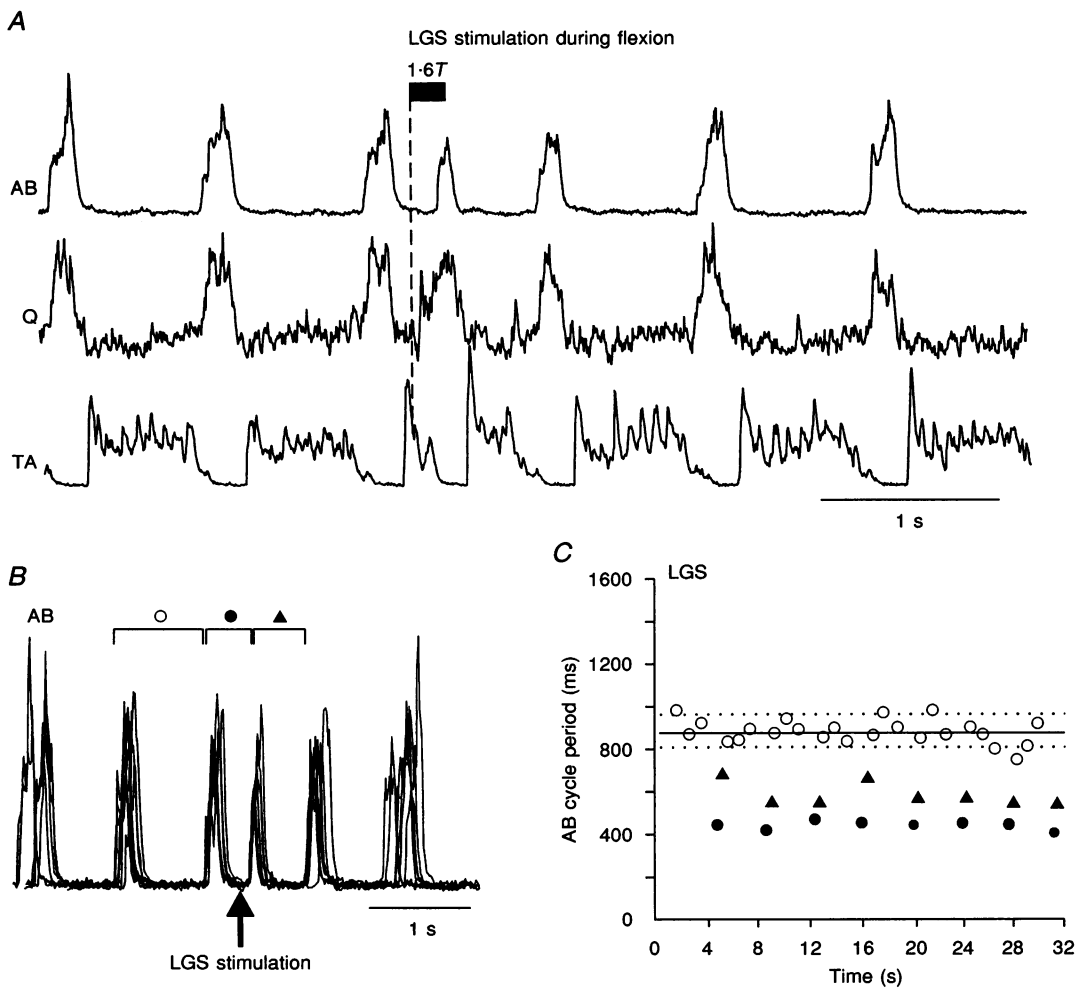

Figure 6. Stimulation of LGS group I afferents during flexion resets the step cycle to extension during MLR-evoked fictive locomotion

Stimulation of LGS nerve (1·6T, 20 shocks, 100 Hz) was triggered approximately 40 ms from the onset of activity in TA, every fourth step. *A*, TA activity sharply declined soon after the stimulus onset and was followed by bursts in AB and Q nerves. *B*, 6 superimposed 4·5 s epochs of AB activity are superimposed from a 27 s bout of fictive locomotion and aligned at the onset of LGS stimulation (arrow). *C*, the interval between consecutive AB bursts (cycle period) of control (○), stimulated (●) and post-stimulated (▲) cycles are plotted as raw values from the traces in *B*. The continuous horizontal line indicates the mean and the dotted lines the standard deviation of control cycle periods. Following LGS stimulation (●), there was a reduction in the cycle period (i.e. resetting) that persisted through the next step (▲) before reaching the control value.

bursts with $2T$ Q stimulation (779 ± 75 ms) was significantly ($P > 0{\cdot}01$) shorter than for cycles without Q stimulation (926 ± 71 ms). We suggest that the continuation of Q stimulation into the flexion phase reset the step cycle to extension thus shortening the step cycle period (Conway *et al.* 1987; see below). The inhibition of ankle extensor activity indicates that, in addition to the excitatory actions on the central pattern locomotor circuitry, Q stimulation also activates other inhibitory pathways. To summarize, activation of Q group I afferents during extension produces a more restricted pattern of ipsilateral extension enhancement than that produced by activation of ankle extensor group I afferents.

Stimulation of hip extensor nerves during extension was performed in four experiments. In two experiments, SmAB, AB or Sm stimulation during extension failed to increase the duration of extensor activity. In the other two experiments, $2T$ stimulation of the Sm nerve increased the duration of both AB and ankle extensor activity. An example is shown in Fig. 5*D* in which $2T$ stimulation of the Sm nerve during extension slightly increased the duration of bursts in AB and the ankle extensors. Clear increases in the amplitude of ankle extensor ENG activity were never seen. FDL nerve stimulation had no effect on extensor ENG amplitude or duration in the one experiment in which this was attempted (not shown). Compared with the ankle extensors, stimulation of other extensor nerves during extension consistently failed to evoke the full pattern of enhancement of ipsilateral hip, knee and ankle extensor ENG duration and amplitude during fictive locomotion.

Stimulation of extensor nerves during flexion

Ankle extensor nerves were also stimulated during the flexor phase of the fictive locomotor cycle using parameters similar to those used during extension. Figure 6 shows the effects of LGS $1{\cdot}6T$ stimulation delivered during flexion at every fourth step and triggered with a 40 ms delay after

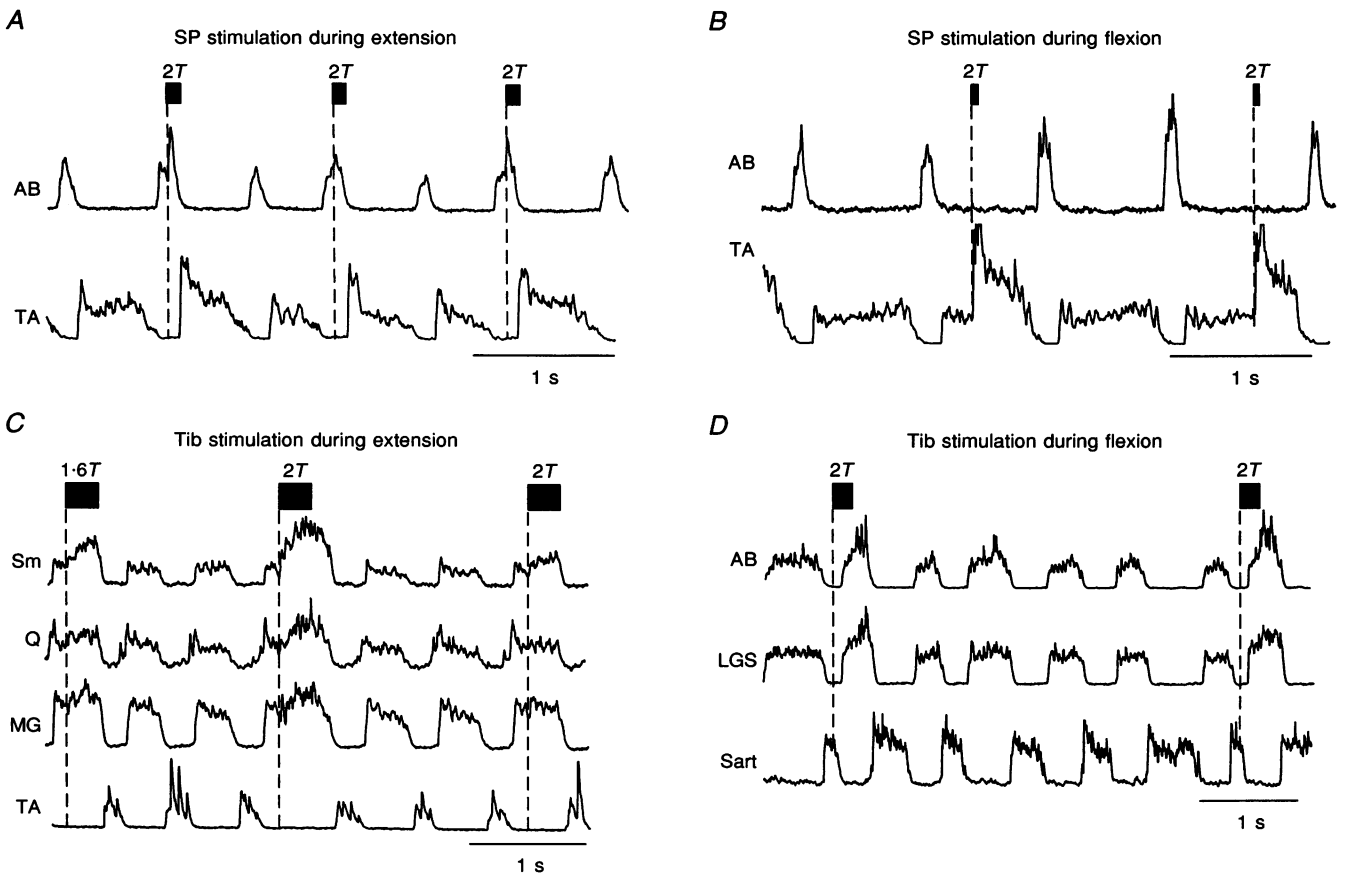

Figure 7. Effects of low strength stimulation of cutaneous nerves on fictive locomotion

A, stimulation ($2T$, 20 shocks, 200 Hz) of the SP nerve during extension every second step (triggered from AB activity) evoked an increase in AB burst amplitude, as well as an increase in the amplitude of the following flexor burst. *B*, stimulation of SP ($2T$, 10 shocks, 200 Hz) during flexion (triggered from TA activity) evoked an increase in TA burst amplitude and did not reset the locomotor step cycle. *C*, stimulation ($1{\cdot}6$ and $2T$, 20 shocks, 100 Hz) of the distal portion of the tibial nerve during extension (triggered from Sm activity) enhanced ankle, knee and hip extensor bursts. *D*, stimulation of Tib at $2T$ (20 shocks, 100 Hz) during flexion activity terminated flexion and initiated an early extension similar to that produced by activation of ankle extensor group I afferents.

the onset of TA activity. LGS stimulation terminated on-going TA activity within 150 ms of the onset of stimulation (Fig. 6*A*). This was followed by a burst of activity in extensor nerves (AB and Q). The consistency of this resetting of the fictive step cycle is illustrated in Fig. 6*B*. In *B*, a 27 s period of fictive locomotion was divided into six epochs of 4·5 s (including the trial in Fig. 6*A*). These epochs were superimposed and aligned at the onset of the LGS stimulation train (arrow). In each case, LGS stimulation evoked a burst in the AB nerve with a consistent delay (about 150 ms) following the onset of stimulation. The symbols in Fig. 6*B* are also used in the graph in Fig. 6*C* which illustrates the effects of LGS nerve stimulation on the cycle period. For all trials, the cycle period decreased during LGS stimulation. The subsequent poststimulation cycle period was also shorter than the non-stimulated cycle period. Thus, LGS stimulation at 1·6*T* during flexion consistently reset the step cycle and hastened the onset of the subsequent step. Similar resetting effects were observed with activation of MG and Pl afferents during flexion (not illustrated). There were no obvious differences in the abilities of the different ankle extensor nerves to reset the step cycle during flexion.

Q nerve stimulation delivered during flexion produced effects in four out of five experiments. In two cats a clear resetting to extension was seen and in the other two cats Q stimulation produced an excitation of hindlimb flexors and no resetting to extension. Hip extensors were not stimulated during flexion.

Stimulation of nerves innervating dorsal and plantar surfaces of the foot

Nerves innervating the dorsal (SP) and plantar surface (Tib) of the foot were stimulated during fictive locomotion. SP stimulation at 2*T* during extension increased the amplitude of on-going bursts in the AB nerve and also enhanced the beginning of the subsequent burst in the TA nerve (Fig. 7*A*). SP stimulation during flexion (Fig. 7*B*) increased the amplitude of the activity in the flexor, TA. Effects of SP stimulation during flexion were thus unlike the resetting observed with ankle extensor stimulation during flexion (Fig. 6). Stimulation of the Tib nerve (plantar foot cutaneous and muscular innervation) during extension in three experiments produced an increase in the duration and amplitude of ipsilateral extensors. Figure 7*C* shows the increase in ipsilateral hip, knee and ankle extensor activity evoked by Tib stimulation at 1·6 and 2*T* during extension. In one experiment, direct stimulation of the central foot pad with percutaneous electrodes produced extension enhancement similar to that illustrated in Fig. 7*C*. This suggests that cutaneous afferents in the Tib nerve can evoke effects similar to those evoked by activation of ankle extensor group I afferents. During flexion, Tib stimulation reset the step cycle by terminating flexion and initiating extension (Fig. 7*D*). Conway, Scott, Riddell & Hadian (1994) reported that stimulation of the lateral portion of the Tib (plantar) nerve in DOPA-treated spinal cats terminated on-going flexor and promoted extensor activity. Stimulation of the medial plantar nerve produced a facilitation of flexor activity (Conway *et al.* 1994). The contribution of muscle afferents to effects evoked by Tib nerve stimulation remains to be determined.

DISCUSSION

The principle findings of this study are that there are several afferent systems that, when activated during extension, can enhance ENG activity recorded in hindlimb nerves. The most consistent effects were evoked by stimulation of ankle extensor nerves with the MG, LG, Sol and Pl nerves being equally effective. Group I strength stimulation of any of the ankle extensors produced increased amplitude and/or duration of extensor nerves innervating hip, knee and ankle extensors as well as PBSt motoneurones (when active during extension). Stimulation of hip and knee extensor nerves can also result in extension enhancement but these effects were more variable and less widely distributed than those resulting from ankle extensor stimulation. These observations complement those of Conway *et al.* (1987), Pearson *et al.* (1992) and Pearson & Collins (1993) showing that the step cycle can be entrained or reset with stimulation of extensor nerves, particularly the Pl nerve. Another new observation is that selective activation of ankle extensor muscle spindle afferents can produce larger and longer duration locomotor discharges in extensors. Stimulation of cutaneous afferents innervating the plantar foot also evoked extension enhancement. While the present emphasis is on the enhancement of on-going extensor activity during locomotion, stimulation during flexion of any of the ankle extensors or the Tib nerve terminated flexion and reset the step cycle to extension.

Afferent systems involved in extension enhancement and resetting

Previous studies have presented direct evidence that activation of Golgi tendon organ (group Ib) afferents in ankle extensor nerves is responsible for resetting and extension enhancement (Conway *et al.* 1987; Pearson *et al.* 1992; Pearson & Collins, 1993). The present results are in accord with those observations since even the low intensity stimulation illustrated in Figs 2 and 3 will recruit a portion of the Ib afferents of ankle extensor nerves (Jack, 1978). While previous studies failed to demonstrate a contribution of group Ia afferents to extension enhancement (Conway *et al.* 1987; Pearson *et al.* 1992; Pearson & Collins 1993; Gossard *et al.* 1994), present results show that activation of ankle extensor primary muscle spindle afferents alone can increase the amplitude and/or duration of hip and ankle extensor activity and reset the step cycle.

We offer two explanations for the present demonstration of Ia afferent-evoked extension enhancement. The first is the simultaneous activation of Ia afferents from the four ankle

extensors and not, as in previous studies (Conway *et al.* 1987; Pearson *et al.* 1992; Pearson & Collins 1993; Gossard *et al.* 1994), from a single ankle extensor nerve. The second reason is that the present experiments employed a locomotor preparation in which group Ia afferents can activate a disynaptic excitatory pathway during extension (see introduction). This disynaptic excitation would increase the effectiveness of ankle extensor Ia afferents in evoking locomotor effects and would be inoperative (see McCrea *et al.* 1995) in many of the previous experiments assessing group Ia actions during fictive locomotion in spinal preparations. It has been postulated that Ib actions during locomotion are mediated by actions on the spinal locomotor pattern generator circuitry (Conway *et al.* 1987; Pearson & Collins, 1993; Gossard *et al.* 1994). To this we now add a contribution from ankle extensor group Ia afferents. The suggestion that the effects of Ia afferent activation may be weaker than that evoked from Ib afferents is based both on the failure of previous studies to demonstrate these actions and the present failure of Ia afferent activation to terminate flexion and reset the cycle to extension. This contrasts with the resetting of the step cycle seen following electrical stimulation of single extensor nerves recruiting both Ia and Ib afferents. Despite the weaker actions of Ia afferents in resetting, we suggest that the term 'group I' is preferable to the term 'Ib' when describing the effects of electrical stimulation of extensor nerves during locomotion. The question also remains as to whether the similar actions of Ia and Ib effects during fictive locomotion are mediated by common or parallel sets of spinal interneurones.

Because some group II afferents are activated by stimulation strengths as low as $1{\cdot}5T$ (Jack, 1978), it was important to demonstrate extension enhancement by lower strength and near threshold stimulation strength (Figs 2 and 3). While extension enhancement clearly can occur without recruitment of group II afferents, the possibility that they could contribute to these effects was not explored. This is because the powerful effects of activation of group I afferents make it difficult to assess additional contributions from extensor group II afferents. During DOPA-induced fictive locomotion in acute spinal cats, extensor group II stimulation did not enhance group I EPSPs and may have produced other reflexes (Gossard *et al.* 1994). This suggests that extensor group I and II afferents do not act on the same neuronal systems to enhance extension during fictive locomotion. The accompanying paper reports resetting evoked by activation of group II afferents in flexor nerves (Perreault *et al.* 1995).

While a possible contribution to resetting of systems activated antidromically by electrical stimulation of peripheral nerves cannot be completely ruled out (Hammond, Miller & Scott, 1981; also see Discussion in Perreault *et al.* 1995), muscle stretch avoided antidromic activation of motoneurone axon collaterals as did stimulation of cutaneous afferents in the Tib nerve. The resulting extension enhancement shows that the motoneurone axon collateral Renshaw cell system is not required for extension enhancement.

Neuronal pathways producing extension enhancement

Activation of group I afferents during fictive locomotion can excite hindlimb extensor motoneurones through a number of pathways including the monosynaptic excitatory connections between homonymous and synergistic motoneurones (Eccles *et al.* 1957). In accord with the pattern of monosynaptic excitation, ENG amplitude increases were seen most consistently in ankle extensor nerves some of which are known to have strong heteronymous Ia monosynaptic connections (Eccles *et al.* 1957). The recent observations that activation of ankle extensor group I afferents evokes disynaptic EPSPs in hip extensor motoneurones (Angel *et al.* 1994; Guertin *et al.* 1994) as well as other ankle extensors (Angel *et al.* 1994; McCrea *et al.* 1995) suggest that, while the pattern of group I-evoked excitation during fictive locomotion may conform to that of monosynaptic excitation, disynaptic pathways may also be responsible for some of the excitation during extension enhancement. The monosynaptic EPSPs evoked in some knee and hip extensor motoneurones from ankle extensor Ia muscle spindle afferents are small (Edgley *et al.* 1986). In non-locomoting preparations, electrical stimulation of ankle extensor group I afferents produces non-reciprocal inhibition of about 70% and excitation of about 25% of SmAB motoneurones (Jankowska *et al.* 1981*a*; Jankowska, McCrea & Mackel, 1981*b*). The large increases in hip extensor activity following activation of ankle extensor group I afferents during fictive locomotion in Figs 1, 2 and 3 are, therefore, largely the result of pathways activated during locomotion. These would include the longer latency excitation of extensor motoneurones evoked through spinal locomotor circuitry (Gossard *et al.* 1994; Guertin *et al.* 1994; McCrea *et al.* 1995) as well as disynaptic group I excitation. An assessment of the relative contributions of monosynaptic, disynaptic and other pathways to extension enhancement must await the conclusion of experiments involving intracellular recording from extensor motoneurones (Guertin *et al.* 1994). Finally, the reduction in ankle extensor ENG activity following Q stimulation (Fig. 5) in the present MLR fictive locomotion preparation differs from the increase in ankle extensor ENG reported in the acute spinal DOPA preparation (Conway *et al.* 1987). At present we have no explanation for this difference in Q group I actions between the preparations.

Functional significance

During real locomotion the activity of extensor Ib afferents innervating Golgi tendon organs is greatest during contraction of the homonymous muscle (for references see

Jami, 1992), i.e. during the extensor portion of the step cycle. On the other hand, the activity of Ia afferents during locomotion is complex, governed both by muscle length and activity of the γ-motor system. Many ankle extensor Ia afferents display significant activity both at maximal muscle length (i.e. just before foot contact) and during contraction of the ankle extensors (i.e. during extension) (Prochazka, Trend, Hulliger & Vincent, 1989). Taking Ia and Ib afferent activity together, there will be considerable activity in extensor group I afferents during extension. Because both Ib and Ia afferents can enhance extension, their activity during real locomotion would suggest that they can function as a feedback system producing excitation of on-going extensor activity. This view is supported by recent observations showing that activation of ankle extensor nerves in decerebrate cats can prolong the activity of ipsilateral extensors and reset the step cycle during treadmill locomotion (Whelan, Hiebert & Pearson, 1995).

If the group I afferent system is used to continually regulate the duration and force of extensor activity during real locomotion, afferents located in the ankle extensor muscles and from the plantar foot would be ideally suited to sense foot contact and loading of the limb during locomotion of the cat. This might explain the relatively greater effectiveness of ankle extensor than hip and knee extensor afferents in influencing extensor activity during MLR-evoked fictive locomotion observed in the present experiments. If group I extensor afferents fulfil a similar function in regulating locomotion during plantigrade locomotion in man, the relative effectiveness of hip, knee and ankle extensor afferents may well be different from that in the cat. The present results suggest that the simultaneous activity of group I afferents from several muscles during real locomotion would result in a considerable strengthening of the contraction of extensors and influence the duration of the extensor phase of locomotion. Using short duration stimulus trains we have shown that extension prolongation can occur without much change in the duration of the locomotor cycle (Fig. 2). Under other conditions with prolonged activity of group I afferents, group I activity could increase the duration of the extension phase to the extent that the frequency of the step cycle is altered. Such actions are suggested by experiments showing that the locomotor frequency can be entrained by electrical stimulation at group I strength (Conway *et al.* 1987; Pearson *et al.* 1992). While the functional significance of the termination of flexion and resetting of the step cycle to extension is unclear, Fig. 7 shows that group I activation during flexion perturbed not only the immediate but also the next step cycle. This further supports the notion that group I afferents can access central locomotor circuitries affecting both the amplitude and timing of motoneurone activity. We suggest that, during real locomotion, extensor group I afferents act during extension to continually shape and assist the timing of the activity of ipsilateral hindlimb extensors.

Angel, M. J., Guertin, P., Jiménez, I. & McCrea, D. (1994). Disynaptic autogenic and heterogenic group I excitation of hindlimb extensors during fictive locomotion. *Society for Neuroscience Abstracts* **20**, 648.7.

Conway, B. A., Hultborn, H. & Kiehn, O. (1987). Proprioceptive input resets central locomotor rhythm in the spinal cord. *Experimental Brain Research* **68**, 643–656.

Conway, B. A., Scott, D. T., Riddell, J. S. & Hadian, M. R. (1994). Effects of plantar nerve stimulation on the transmission of late flexion reflexes in the decerebrate spinal cat. *Journal of Physiology* **479P**, 145–146*P*.

Eccles, J. C., Eccles, R. M. & Lundberg, A. (1957). The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *Journal of Physiology* **137**, 22–50.

Eccles, R. M. & Lundberg, A. (1958). Integrative pattern of Ia synaptic actions on motoneurones of hip and knee muscles. *Journal of Physiology* **144**, 271–298.

Edgley, S., Jankowska, E. & McCrea, D. (1986). The heteronymous monosynaptic actions of triceps surae group Ia afferents on hip and knee extensor motoneurones in the cat. *Experimental Brain Research* **61**, 443–446.

Fetz, E. E., Jankowska, E., Johannisson, T. & Lipski, J. (1979). Autogenetic inhibition of motoneurones by impulses in group Ia muscle spindle afferents. *Journal of Physiology* **293**, 173–195.

Gossard, J.-P., Brownstone, R. M., Barajon, I. & Hultborn, H. (1994). Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. *Experimental Brain Research* **98**, 213–228.

Guertin, P., Angel, M. J., Jiménez, I. & McCrea, D. (1994). Both disynaptic and longer latency interneuronal pathways mediate extension enhancement evoked by group I muscle afferent stimulation during fictive locomotion. *Society for Neuroscience Abstracts* **20**, 648.8.

Guertin, P., Angel, M. J., Perreault, M.-C., Carr, P. & McCrea, D. A. (1993*a*). Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion. *Society for Neuroscience Abstracts* **19**, 61.15.

Guertin, P., Angel, M. J., Perreault, M.-C., Carr, P. & McCrea, D. A. (1993*b*). Ankle extensor group I afferents excite extensors throughout the hindlimb during MLR-evoked fictive locomotion. *The Physiologist* **36**, 14.18.

Hammond, G. R., Miller, S. & Scott, P. D. (1981). The effect of antidromic stimulation of hindlimb nerves during fictive locomotion in low spinal cats to test a model of a spinal locomotor generator. In *Advances in Physiological Science. Regulatory Functions of the CNS. Motion and Organization Principles*, vol. 1, ed. Szentagothai, J., Palkovits, M. & Hamori, J., pp. 213–217. Pergamon Press, Budapest.

Jack, J. J. B. (1978). Some methods for selective activation of muscle afferent fibres. In *Studies in Neurophysiology*, ed. Porter, R., pp. 155–176. Cambridge University Press, UK.

JAMI, L. (1992). Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. *Physiological Reviews* **72**, 623–666.

JANKOWSKA, E., MCCREA, D. & MACKEL, R. (1981*a*). Pattern of 'non-reciprocal' inhibition of motoneurones by impulses in group Ia muscle spindle afferents in the cat. *Journal of Physiology* **316**, 393–409.

JANKOWSKA, E., MCCREA, D. & MACKEL, R. (1981*b*). Oligosynaptic excitation of motoneurones by impulses in group Ia muscle spindle afferents in the cat. *Journal of Physiology* **316**, 411–425.

KIEHN, O., IIZUKA, M. & KUDO, N. (1992). Resetting from low threshold afferents of *N*-methyl-D-aspartate-induced locomotor rhythm in the isolated spinal cord–hindlimb preparation from newborn rats. *Neuroscience Letters* **148**, 43–46.

MCCREA, D. A., SHEFCHYK, S., STEPHENS, M. J. & PEARSON, K. G. (1995). Disynaptic group I excitation of synergist ankle extensor motoneurones during fictive locomotion. *Journal of Physiology* (in the Press).

PEARSON, K. G. & COLLINS, D. F. (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *Journal of Neurophysiology* **70**, 1009–1017.

PEARSON, K. G., RAMIREZ, J. M. & JIANG, W. (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Experimental Brain Research* **90**, 557–566.

PERREAULT, M.-C., ANGEL, M. J., GUERTIN, P. & MCCREA, D. A. (1995). Effects of stimulation of hindlimb flexor group II afferents during fictive locomotion in the cat. *Journal of Physiology* **487**, 211–220.

PERRET, C. & CABELGUEN, J.-M. (1980). Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. *Brain Research* **187**, 333–352.

PROCHAZKA, A., TREND, P., HULLIGER, M. & VINCENT, S. (1989). Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. *Progress in Brain Research* **80**, 61–74.

SHIK, M. L., SEVERIN, F. V. & ORLOVSKII, G. N. (1966). Control of walking and running by means of electrical stimulation of the mid-brain. *Biofizika* **11**, 659–666.

WHELAN, P., HIEBERT, G. W. & PEARSON, K. G. (1995). Stimulation of group I extensor afferents prolongs the stance phase in walking cats. *Experimental Brain Research* **103**, 20–30.

Acknowledgements

This study was supported by the Medical Research Council of Canada. M.-C.P. and M.J.A. were supported by the Rick Hansen Man in Motion Legacy Fund. The skilful assistance of Sharon McCartney throughout these experiments is gratefully appreciated. We also thank Drs Patrick Carr and Ismael Jiménez for their help in collecting some of the present results.

Received 11 July 1994; accepted 6 February 1995.