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Proprioceptive input resets central locomotor rhythm in the spinal cat

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Summary. The reflex regulation of stepping is an important factor in adapting the step cycle to changes in the environment. The present experiments have examined the influence of muscle proprioceptors on centrally generated rhythmic locomotor activity in decerebrate unanesthetized cats with a spinal transection at Th12. Fictive locomotion, recorded as alternating activity in hindlimb flexor and extensor nerves, was induced by administration of nialamide (a monoamine oxidase inhibitor) and L-DOPA. Brief electrical stimulation of group I afferents from knee and ankle extensors were effective in resetting fictive locomotion in a coordinated fashion. An extensor group I volley delivered during a flexor burst would abruptly terminate the flexor activity and initiate an extensor burst. The same stimulus given during an extensor burst prolonged the extensor activity while delaying the appearance of the following flexor burst. Intracellular recordings from motoneurones revealed that these actions were mediated at premotoneuronal levels resulting from a distribution of inhibition to centres generating flexor bursts and excitation of centres generating extensor bursts. These results indicate that extensor group I afferents have access to central rhythm generators and suggest that this may be of importance in the reflex regulation of stepping. Experiments utilizing natural stimulation of muscle receptors demonstrate that the group I input to the rhythm generators arises mainly from Golgi tendon organ Ib afferents. Thus an increased load of limb extensors during the stance phase would enhance and prolong extensor activity while simultaneously delaying the transition to the swing phase of the step cycle.

Key words: Spinal cord – Motoneurones – Fictive locomotion – Group I afferents – Resetting – Entrainment

Introduction

Spinal networks are capable of generating locomotor-like activity (Graham Brown 1911; see Grillner 1981). However, in order to compensate for unexpected postural disturbances, or changes in terrain, effective locomotor behaviour must utilize sensory feedback. Sensory regulation of stepping can occur via reflex pathways to motoneurones, thereby by-passing the locomotor rhythm generators. However, transmission in such pathways are generally modulated (or gated) by activity from the locomotor generator (Andersson et al. 1978a; Schomburg and Behrends 1978a, b; Forssberg et al. 1977; Forssberg 1979). Additionally sensory feedback may also act directly upon the rhythm generators themselves. Indeed, experiments on a variety of species have revealed that proprioceptive input can have access to rhythm generators (Pearson and Duysens 1976; Grillner and Rossignol 1978; Andersson et al. 1978b; Duysens and Pearson 1980; Andersson and Grillner 1981, 1983; Lennard 1985; Bässler 1986; Sillar et al. 1986). Further knowledge regarding interactions between proprioceptive input and rhythm generation is likely to reveal important aspects not only of the mechanisms underlying the reflex regulation of locomotion but also the intrinsic organisation of rhythm generators themselves.

In this study we have examined the action of extensor group I afferents on fictive locomotion in the spinal cat. Our results, from intracellular recordings from motoneurones and from gross elec-

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troneurogram (ENG) recordings, demonstrate that phasic activation of these afferents can reset rhythmic activity. The significance of this observation regarding the reflex regulation of stepping and the organisation of the locomotor rhythm generator in the cat will be discussed. Some of the results presented in this paper have appeared in abstract form (Conway et al. 1987a, b).

Methods

Preparation

Cats (n = 24; 2.5–4.0 kg) were anaesthetized with ether and N_2O. Once surgical anaesthesia was achieved the animals were anaemically decerebrated. Briefly, this consists of ligating the basilar artery in conjunction with both common carotid arteries. The ligature on the basilar artery was placed just cranial to the branch point of the posterior inferior cerebellar arteries (Pollock and Davis 1928). Blood pressure was monitored continuously via a cannula inserted into one of the carotids. In addition cannulae were inserted into the radial viens of both forelimbs.

Both hindlimbs were extensively denervated and selected nerves were dissected for stimulation and recording purposes. In the left hindlimb (ipsilateral limb) the following nerves were dissected free: posterior biceps, semitendinosus, anterior biceps-semimembranosus, sural, medial gastrocnemius, lateral gastrocnemius-soleus, plantaris, flexor digitorum longus, tibialis anterior, peroneus brevis and tertius, deep peroneal, sartorius, and quadriceps. In the right (contralateral) hindlimb only the sural nerve and the following muscle nerves were dissected: posterior biceps-semitendinosus, triceps surae and quadriceps. A laminectomy exposing lumbar spinal segments L4–S1 and a complete spinal section at T12 was performed. Following completion of surgery anaesthesia was discontinued and the cat transfered to a frame which rigidly secured the vertebral column and the hindlimbs. Large non-reactive pupils coupled with the development of strong extensor rigidity in the forelimbs and a lack of spontaneous movements were taken to indicate that the animals were decerebrate. The cats were then paralyzed (Pavulon, Organon 0.15–0.2 mg/kg supplemented every 40 min) and artificially ventilated. The end tidal PCO_2, body temperature and blood pressure were all maintained within normal physiological limits. Drops in blood pressure below 80 mmHg were counteracted by slow intravenous infusion of noradrenaline bitartrate (2 mg/ml). Skin flaps surrounding the exposed spinal cord and the cut hindlimb nerves were used to construct paraffin oil pools. The cut nerves were then mounted on bipolar silver electrodes for recording neural activity (ENG) or for electrical stimulation. Posterior biceps and semitendinosus were usually mounted together although in several experiments they were mounted on separate electrodes. The ventral nerves were mounted in cuff electrodes.

Generation of fictive locomotion

Rhythmic activity was induced by intravenous administration of the monoamine oxidase inhibitor nialamide (50 mg/kg; Sigma) followed by L-DOPA (45–100 mg/kg; Sigma) (Jankowska et al. 1967a, b; Viala and Buser 1971; Grillner and Zangger 1974). In all the experiments reported here the methyl ester of L-DOPA was used. This ester has a very high solubility and therefore dramatically reduces the volume of saline required as a vehicle for a specific dosage of L-DOPA. In the final 6 experiments carried out in this study carbidopa (1–8 mg/kg; carbidopa was a kind gift from Merck Sharp and Dohme, Copenhagen) was used in addition to L-DOPA. Carbidopa prevents peripheral decarboxylation and therefore prevents the appearance of cardiovascular changes which can result in unstable intracellular recording conditions. In both L-DOPA and L-DOPA plus carbidopa treated animals spontaneous rhythmic activity usually developed within 30 min of L-DOPA administration. In 7 experiments fictive locomotion did not develop or was poorly organised. Despite this, 'late reflexes' consistant with those described by Andén et al. (1966) following L-DOPA treatment could be observed.

Recording and stimulation

Intracellular recordings from antidromically identified hindlimb motoneurones were made with glass-capillary microelectrodes filled with 3 M K-acetate or 2 M KCl. KCl electrodes were only used in a few experiments in order to reverse IPSP's. After pulling the microelectrode tips were broken to give a diameter of approximately 1.5 μm and an impedence after filling in the range 3–7 MΩ. Intracellular potentials were recorded using conventional circuitry. Simultaneous ENG's were recorded from selected ipsilateral and contralateral muscle nerves. Afferent volleys were evoked by electrical stimulation (square wave pulses of 0.1 ms width; trains of stimuli 50–300 ips of varying duration were employed). The strength of stimulation for any given shock was expressed in multiples of threshold for the most excitable afferent fibres in the nerve stimulated (as judged from the incoming volley recorded from an electrode placed close to the dorsal root entry zone). Intracellular potentials, ENG's and afferent volleys were recorded on a 7 channel FM tape recorder (bandwidth from DC to 2.5 kHz) for later analysis. Segments of recorded data were then digitized (AD conversion frequency 12 kHz) and plotted using a laser printer.

Natural stimulation of extensor group I afferents

In 3 cats in this study the innervation of the lateral gastrocnemius and soleus muscles was left intact to allow for selective activation of muscle afferents. These muscles were dissected free from surrounding musculature and connective tissue with great care so as not to compromise there blood supply. Sutures were fixed to the periost and connective tissue at the distal tibia and to the Achilles' tendon. These sutures were placed in such a way that they were aligned when the ankle was held at 90°. Relative changes in muscle length were measured from this point. The calcaneus bone was cut and a hole was drilled in the bone, making it possible to attach the tendon via a thread and a stiff steelwire to a force transducer mounted on a muscle puller.

Slow sinusoidal stretches and periods of vibration (10–200 μm, 100–200 Hz) of known amplitude and frequency were delivered by the puller during periods of fictive locomotion. Length and tension changes were continuously monitored. The amplitude of stretches and vibration was varied along with the resting tension in the muscles in order to gain an impression of the relative actions of group Ia and group Ib afferents on fictive locomotion. Passive stretches of low amplitude were used to provide natural stimulation of group Ia and group II spindle afferents. Increasing the amplitude of passive stretch lead to the development of high forces (in the order of 5 N) of sufficient magnitude to recruit Golgi tendon organ afferents to the stimulus (Houk and Simon 1967). Low amplitude, high frequency vibration was used as a means of selectively activating group Ia afferents (Brown et al. 1967; Fetz et al. 1979). Additionally, direct electrical

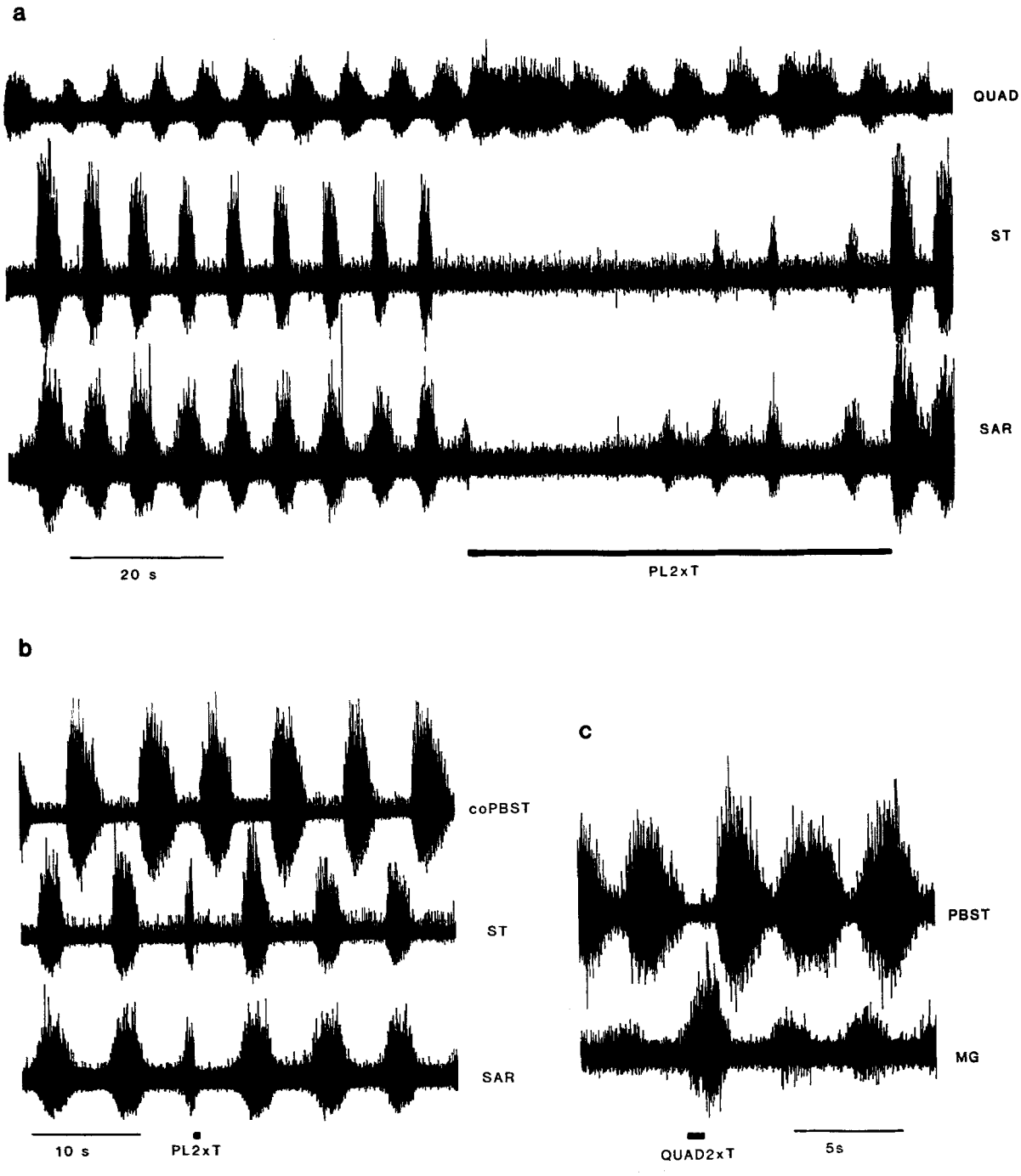

Fig. 1a–c. The effect on fictive locomotion of continuous and short-lasting electrical stimulation of extensor group I afferents in the spinal cat. The periods of stimulation are indicated by the solid bars in **a–c. a** ENG's recorded from the extensor nerve QUAD, (top trace) and flexor nerves, ST and SAR (middle and lower traces respectively) prior to and during continuous ipsilateral extensor group I afferent stimultion. The stimulus removes the tendency for the flexor nerves to display rhythmic activity for a considerable period, while simultaneously promoting activity in the extensor nerve. **b** ENG's recorded from ipsilateral flexor nerves (ST and SAR, middle and lower traces respectively) and contralateral flexor nerves (PBST, top trace) illustrating resetting of the locomotor rhythm by a short-lasting ipsilateral extensor group I afferent volley. The train of afferent volleys is presented during an ipsilateral flexor burst, terminating that burst and promoting an early onset of the burst in the contralateral flexor nerve. The locomotor rhythm following the stimulus is re-established from this reset position. **c** ENG's recorded from ipsilateral flexor (PBST, top trace) and extensor (MG, bottom trace) nerves. Here, the extensor group I afferent train is presented during an ipsilateral extensor burst and is seen to greatly enhance both the duration and amplitude of the extensor burst while delaying the appearance of the following flexor burst. Stimulation intensity was 2 × threshold for the most excitable fibres in the nerves stimulated in **a–c.** Rates of stimulation were 50 shocks per second in **a**, 200 shocks per second in **b** and 150 shocks per second in **c**. 50 mg/kg nialamide was given i.v. followed by 200 mg/kg and 100 mg/kg L-DOPA in **a–c** respectively

stimulation of the muscle via implanted intramuscular electrodes was used to evoke a twitch contraction and thereby activate Ib afferents in isolation from spindle afferents. Golgi tendon organs are particularly sensitive to active force generation in contracting muscle (Jansen and Rudjord 1964; Houk and Hennemann 1967) while spindle afferents will be silenced by muscle shortening.

Abbreviations used in text and figures: co – contralateral, QUAD – quadriceps, coQ – contralateral quadriceps, SAR – sartorius, ST – semitendinosus, PBST – posterior biceps with semitendinosus, MG – medial gastrocnemius, LG(-Sol) – lateral gastrocnemius (- soleus), Pl – plantaris, FDL – flexor digitorum longus, SUR – suralis, mn – motoneurone, xT – multiples of threshold, EPSP – excitatory postsynaptic potential, IPSP – inhibitory postsynaptic potential.

Results

Several advantages exist in studying afferent actions on fictive locomotion in the hindlimbs of the paralysed spinal (and decerebrate) cat. Firstly, all actions are by necessity limited to spinal networks. Furthermore, since no movement occurs there is no phasic modulation of sensory input and it is therefore possible to examine an afferent system independently of others. In the experiments reported here group I muscle afferents were activated mainly by graded electrical stimulation of the nerves, although, in several experiments stretch and vibration of the innervated triceps surae and plantaris muscles was employed.

Action of extensor group I afferents on fictive locomotion

In nialamide and DOPA treated animals burst generation reciprocates between flexors and extensors in the absence of phasic peripheral input to the spinal cord. However, this behaviour is disrupted during continuous extensor group I afferent stimulation (see Fig. 1a). Following the onset of stimulation (as indicated by the solid bar in Fig. 1a) the rhythmic activity is initially replaced by a more or less tonic firing in extensors (upper trace) and a suppression of flexor modulation (middle and lower traces). Given time, the rhythmic activity is re-established firstly in the extensors and later in the flexors. Thus it is apparent that extensor group I afferent stimulation can influence the generation of rhythmic activity.

Using short stimulus trains the nature of this interference with rhythm generation becomes more clear. ENG and intracellular motoneuronal recordings during fictive stepping reveal that brief extensor group I afferent stimulation does not simply interrupt the rhythmic activity but resets it. An extensor group I afferent volley presented during a flexor burst will terminate that burst (Fig. 1b middle and lower traces, Fig. 2ai and b lower trace) and promote the generation of ipsilateral extensor (see Fig. 2b top trace) and contralateral flexor (Fig. 1b top trace, Fig. 2b middle trace) bursts with an earlier onset than would be anticipated without the stimulus. When delivered during an extensor burst, an extensor group I train will enhance the extensor activity and delay the appearance of the next flexor burst (see Fig. 1c). Group I afferents from knee and ankle extensors were capable of producing resetting while comparable stimulation of flexor nerves was never seen to cause resetting behaviour. Stimulation of flexor group I afferents was seen only to interfere with an ongoing burst and did not produce permanent phase shifts of the locomotor rhythm (not illustrated).

The abrupt termination of ipsilateral flexor bursts, seen during resetting behaviour, is apparent as a rapid hyperpolarization of flexor motoneurones following from which the cyclic membrane oscillations, that accompany rhythmic activity, start afresh (see Fig. 2ai). Interestingly, when a similar stimulus is presented when the flexor is inactive no potential shift can be detected in flexor motoneurones (Fig. 2aii), indicating that the hyperpolarization observed during flexor bursts is not due to postsynaptic inhibition of motoneurones but results from the removal of the excitation to the flexors (disfacilitation) (see also Figs. 3b and 4).

'Late reflexes' in flexors and extensors

In some preparations (7 cats) the administration of L-DOPA did not produce rhythmic activity but

Fig. 2a, b. Resetting of locomotor rhythm by extensor group I afferent stimulation. **a** Intracellular recording from a flexor motoneurone (ST, top trace in **ai** and **aii**) and its corresponding ENG (lower trace in **ai** and **aii**) during fictive locomotion. In **ai** a short-lasting train of extensor group I afferent volleys is presented during a flexor burst. The stimulus evokes a rapid termination of the flexor burst which is associated with a hyperpolarization of the flexor motoneuronal membrane. A similar stimulus is presented in **aii** during an interval between flexor bursts and induces no change in membrane potential. **b** Intracellular recording from an extensor motoneurone (LG, top trace) and simultaneous ENG recordings from ipsilateral and contralateral flexor nerves (bottom and middle traces respectively). The figure demonstrates the early excitation of extensor motoneurones associated with the resetting behaviour and the early initiation of a contralateral flexor burst provoked by extensor group I afferent stimulation. In **a** and **b** the period of stimulation is indicated by the solid bars. Stimulation intensity was 1.4 × T and the frequency of stimulation was 150 shocks per second. Full spike height is not shown in **b**. Voltage calibration in **a** refers to the intracellular records in **ai** and **aii**. Drugs: 50 mg/kg nialamide, 4 mg/kg carbidopa followed by 40 mg/kg L-DOPA. The recordings in **a** and **b** were obtained three and five hours after administration of L-DOPA

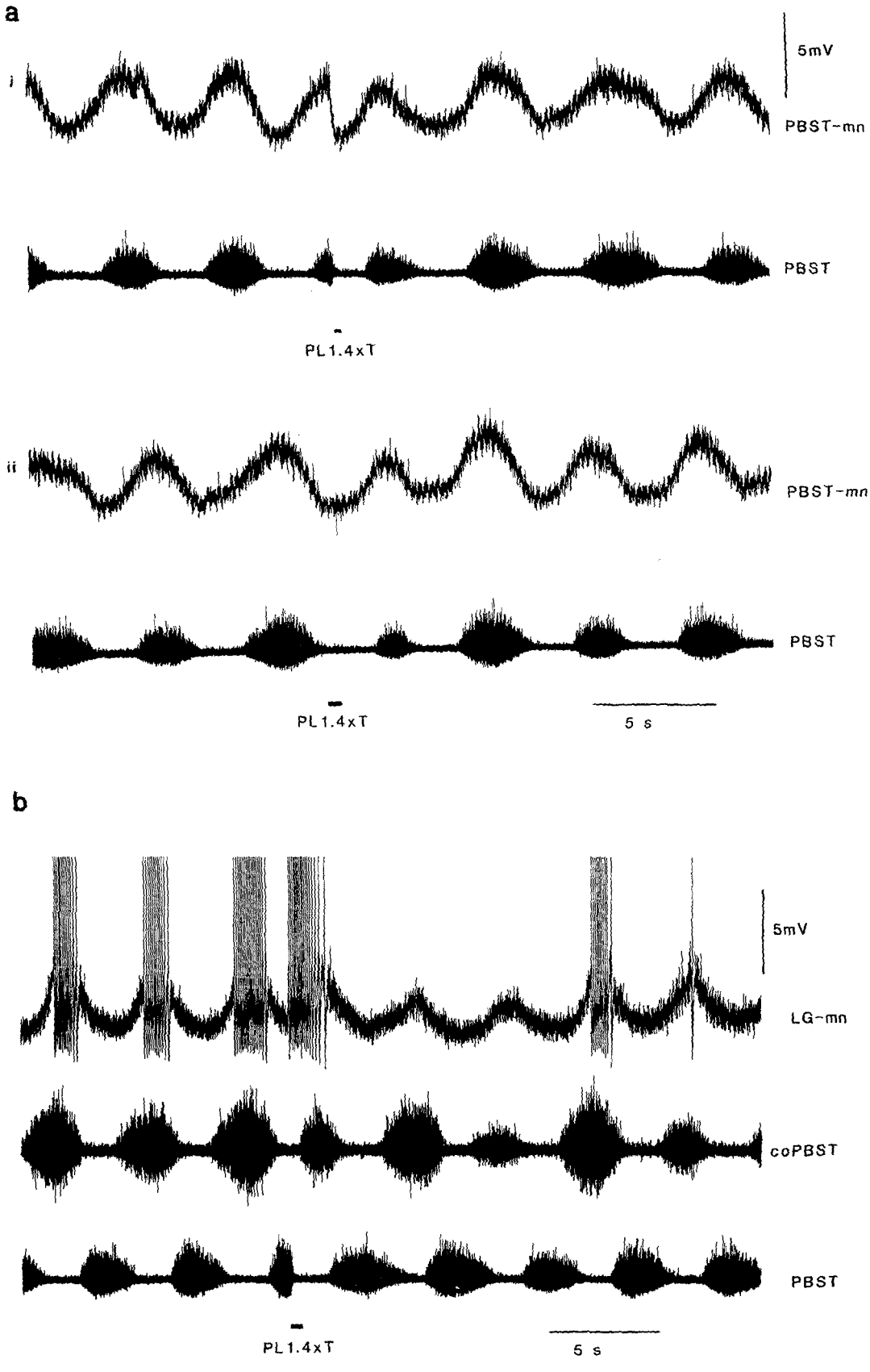
a
i
5mV
PBST-mn
PBST
PL 1.4xT
ii
PBST-mn
PBST
PL 1.4xT
5 s
b
5mV
LG-mn
coPBST
PBST
PL 1.4xT
5 s

Fig. 2

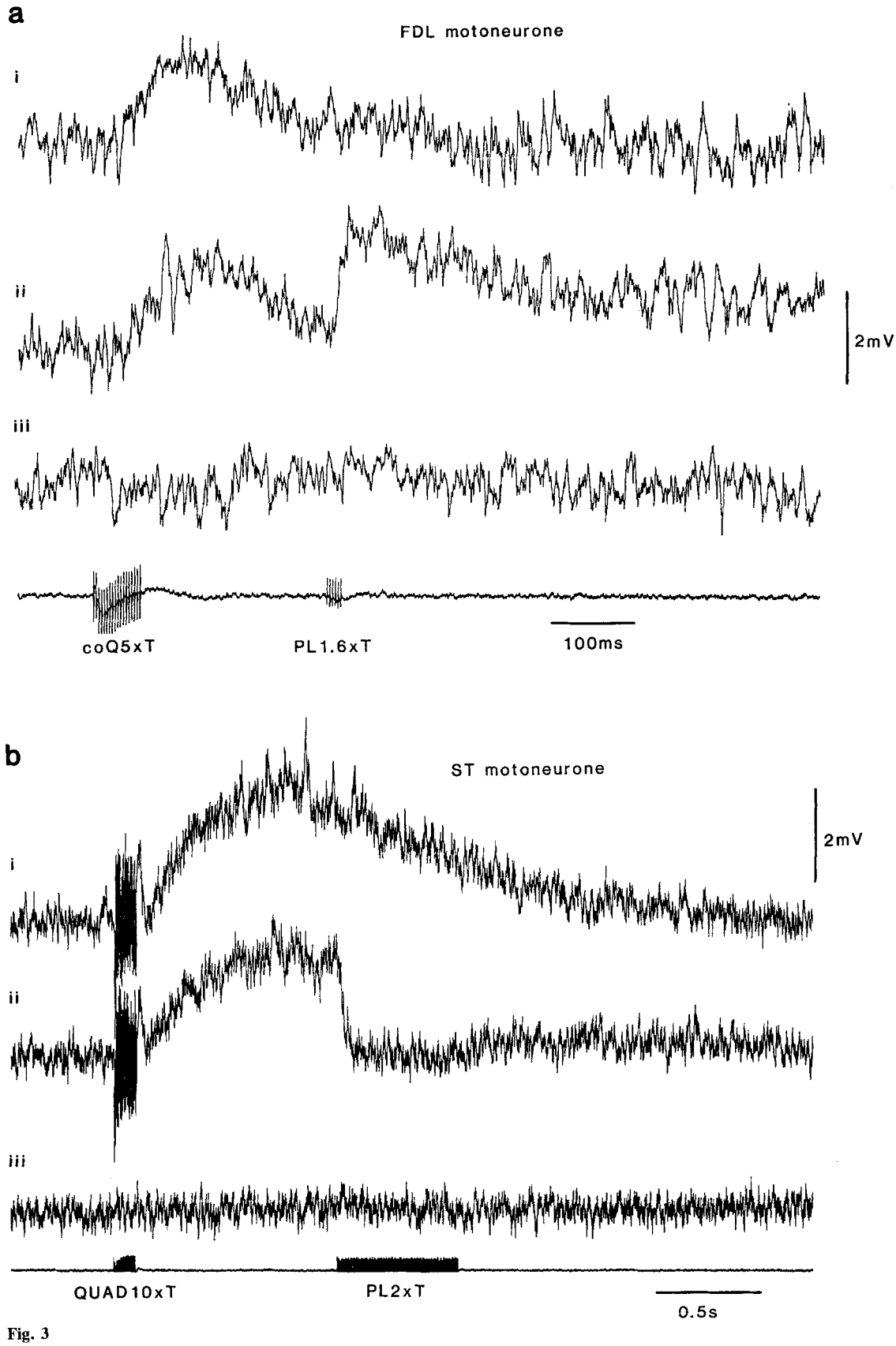
a
FDL motoneurone
i
ii
iii
2mV
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Fig. 3

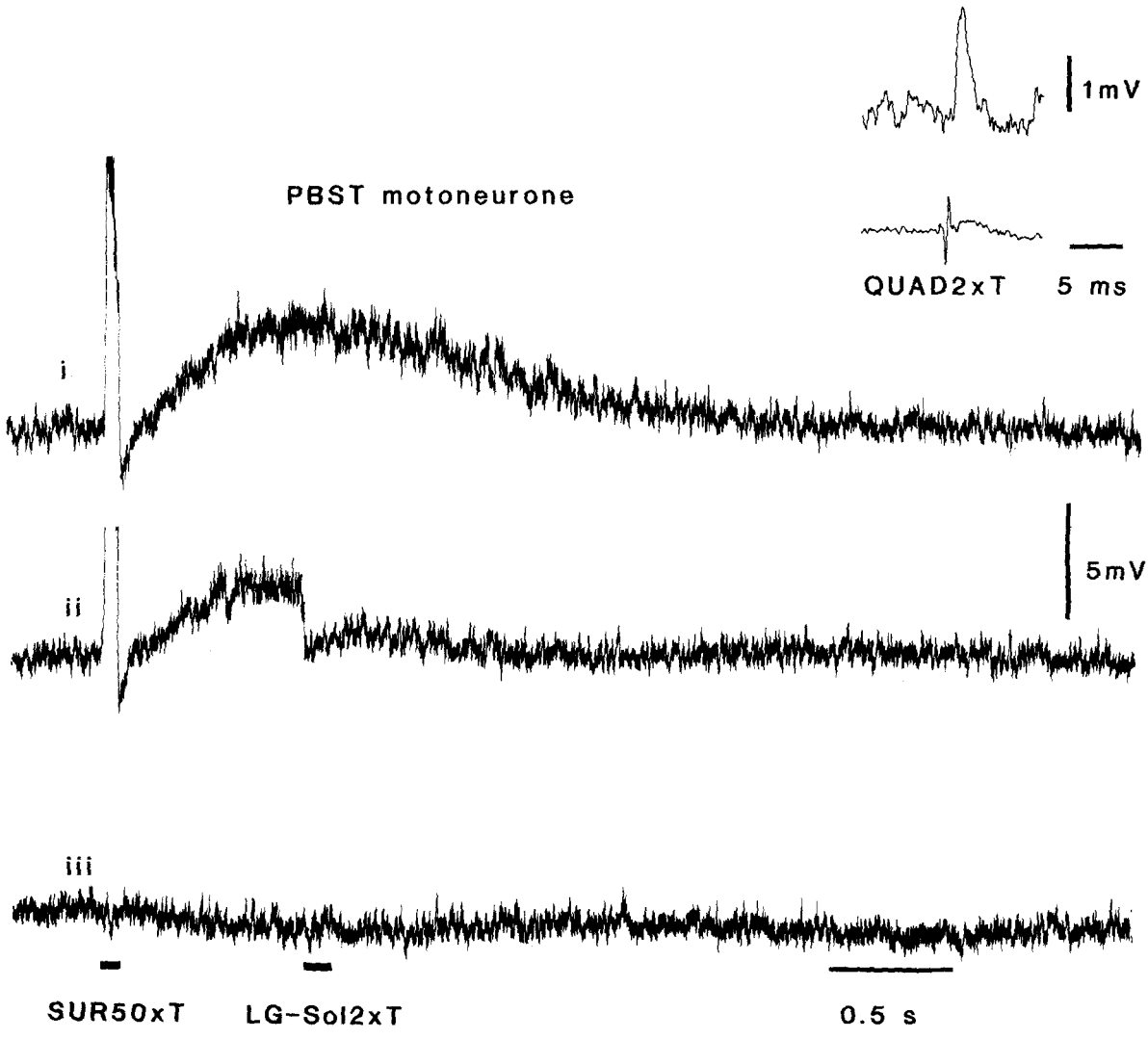

Fig. 4. Effect of intracellular chloride injection on group I afferent stimulation in a flexor motoneurone during FRA conditioning. The experimental paradigm is the same as illustrated in Fig. 3. Cl^- ejection from a KCl filled microelectrode was achieved by passing a hyperpolarizing current of 10–15 nA for 3–4 min. Recordings were made with a bias current of –5 nA. Trace *i* illustrates an intracellular recording from a flexor motoneurone (PBST) showing the 'late reflex' following an ipsilateral FRA stimulus train, while trace *ii* illustrates the conditioning ipsilateral FRA volley plus the extensor group I train. Trace *iii* depicts the action of extensor group I train alone. The insert shows the reversed disynaptic IPSP evoked in PBST from quadriceps (QUAD). It should be noted that despite the reversal in the IPSP a hyperpolarization still results following group I afferent stimultion in *ii*. This hyperpolarization is therefore not due to post-synaptic inhibition. The timing and duration of the stimulus volleys are indicated by the bars below the lowermost trace. The first stimulus train applies only to trace *i*and *ii*, the second stimulus train applies only to traces *ii* and *iii*. For insert note different time and voltage calibrations. Stimulation intensity for group I was 2 × T (LG) with frequency of stimulation 200 shocks per seconds. Drugs: 50 mg/kg nialamide was given i.v. followed by 80 mg/kg L-DOPA. The recordings were taken 3 h after injection of L-DOPA

Fig. 3a, b. Conditioning of extensor group I afferent stimulation by 'late reflexes' evoked in extensors and flexors by contralateral and ipsilateral FRA stimulation respectively. **a** Intracellular recording from an extensor motoneurone (FDL) demonstrating the facilitatory action of an extensor group I train following contralateral FRA stimulation. Trace **ai** shows the action of the contralateral FRA stimulus alone, while trace **aii** illustrates the action of the conditioning contralateral FRA volley plus the extensor group I train. Trace **aiii** illustrates the action of the group I train alone, without prior conditioning by the contralateral FRA. Without prior conditioning the group I train is completely ineffective in producing an EPSP in the extensor motoneurone. **b** Intracellular recording from a flexor motoneurone (PBST) following a similar experimental paradigm as illustrated in **a**. Trace **bi** depicts a 'late reflex' recorded following ipsilateral FRA stimulus train, while trace **bii** illustrates the action of an extensor group I train following ipsilateral FRA conditioning. Trace **biii** depicts the action of the extensor group I stimulation alone without prior conditioning. The timing and duration of stimulus volleys are represented by the incoming volleys illustrated by the lowermost traces in **a** and **b**. The first stimulus train (FRA volleys) apply only to traces *iii* and *iii*. Drugs: in both **a** and **b** 50 mg/kg nialamide was given i.v. followed by 100 mg/kg and 45 mg/kg L-DOPA in **a** and **b** respectively. In **b** 8 mg/kg carbidopa was injected before administration of L-DOPA. The recordings were taken 1 and 4 h after administration of L-DOPA in **a** and **b**

released the transmission of 'late reflexes' on high threshold afferent (FRA) stimulation (Andén et al. 1966). Transmission of these 'late reflexes' is believed to involve the same networks that generate rhythmic activity (Jankowska et al. 1967a, b; Lundberg 1979). Therefore, by using the 'late reflexes' in the absence of spontaneous locomotion it is possible to test the action of a group I afferent volley under the following conditions: (1) when the interneurones generating extensor bursting have been activated by contralateral FRA stimulation, (2) when interneurones generating flexor bursting have been activated by ipsilateral FRA stimulation and, (3) when the interneurones generating locomotor activity are inactive.

By utilizing the above experimental paradigm, it is possible to demonstrate that a very short extensor group I train, although incapable of provoking any response in an extensor motoneurone, will evoke a large EPSP if it is conditioned by a 'late reflex' in that motoneurone (Fig. 3a). This observation suggests that a common interneuronal pathway mediates the 'late reflex' to extensor motoneurones and the excitation evoked by extensor group I afferents. The disfacilitation of flexor motoneurones seen during resetting can be similarly demonstrated by conditioning an extensor group I train with a 'late reflex' to flexor motoneurones (Fig. 3b). Here again it is possible to demonstrate that the group I evoked hyperpolarization only occurs when the motoneurone is depolarized by the 'late reflex', demonstrating that the group I train acts to inhibit the interneurones transmitting 'late reflexes' to flexor motoneurones.

That the hyperpolarization of flexor motoneurones by extensor group I afferent stimulation represents a disfacilitation and not postsynaptic inhibition is further emphasized by Fig. 4. In this experiment Cl^- was injected into a flexor motoneurone by passing a hyperpolarizing current (-10 nA) through a KCl filled microelectrode until the reversal potential for IPSP's was passed (Coombs et al. 1955). The resulting reversal of the disynaptic IPSP from quadriceps to posterior biceps-semitendinosus under these conditions is illustrated by the insert in Fig. 4. The persistance of the extensor group I evoked hyperpolarization after FRA conditioning following Cl^- injection (Fig. 4ii) confirms that the observed hyperpolarisation is due to a disfacilitation of flexor motoneurones.

In the original papers on the effects of DOPA on spinal reflex transmission (Andén et al. 1966; Jankowska et al. 1967a, b) it is seen that the early flexor reflex response to high threshold afferent stimulation is completely suppressed and a 'late reflex' develops within a considerable latency after termination of the train. The finding that the 'late' response does not develop during a long stimulus train was explained by short-lasting inhibition of the interneurones by the afferent impulses (Jankowska et al. 1967a, b). In the present study responses to FRA stimulation sometimes seemed more variable than described by Andén et al. (1966) with incomplete suppression of the early response (e.g. Fig. 3b and Fig. 5; the Ia inhibition from quadriceps to semitendinosus is expected to remain after DOPA, but the accompaning excitation should have been suppressed) and/or a rather short latency onset of the 'late' response (e.g. Fig. 3b). This variability may have several causes. Firstly, the suppression of the early component is dependent both on the dose of DOPA and the time interval between administration and recording. In the present series results were obtained also in periods when the DOPA effect was declining. Secondly, in order to study spatial facilitation we often used relatively low stimulus intensities to evoke submaximal 'late reflexes' (cf. Fig. 3a). The use of less intense FRA stimuli may indeed contribute to a weaker inhibition (during the stimulus train) of the interneurones mediating the 'late discharge' and thus results in responses of shorter latency.

Selectivity in group I actions

By using the same strategy employed in Figs. 3 and 4 it is possible to demonstrate, by grading the strength of the test stimulus, that the resetting behaviour seen with low threshold electrical stimulation is due to activation of group I and not group II afferents.

Figure 5 illustrates the effect upon the 'late reflex' in a flexor motoneurone of varying the strength of extensor afferent stimulation. The figure illustrates that even at stimulus strengths as low as 1.3 × T (Fig. 5ii) there is a suggestion of some disfacilitation of the 'late reflex'. This disfacilitation is clearly apparent at 1.4 × T (Fig. 5iii) and becomes maximal with 1.6 × T stimulation (Fig. 5iv). Electrical stimulation at 1.6 × T (and above) will certainly recruit some low threshold group II afferents. However, it would seem unlikely that significant numbers of group II afferents could contribute to the 'late reflex' disfacilitation observed with 1.4 × T stimulation (Fig. 5iii). Furthermore, if a stimulus of sufficient strength to recruit large numbers of group II afferents (but not group III or IV afferents) is presented following 'late reflex' conditioning (Fig. 5v), rather than suppressing the reflex, the stimulus initiates a new flexor reflex. This, and the observation of disfacilitation with low strengths of stimulation suggests that the disfacilitation of the 'late reflex' (and the resetting behaviour illustrated in Figs. 1 and 2) results from the activation of group I afferents.

Entrainment of the rhythmic activity

Given that low threshold electrical stimulation of extensor afferents can reset fictive locomotion we

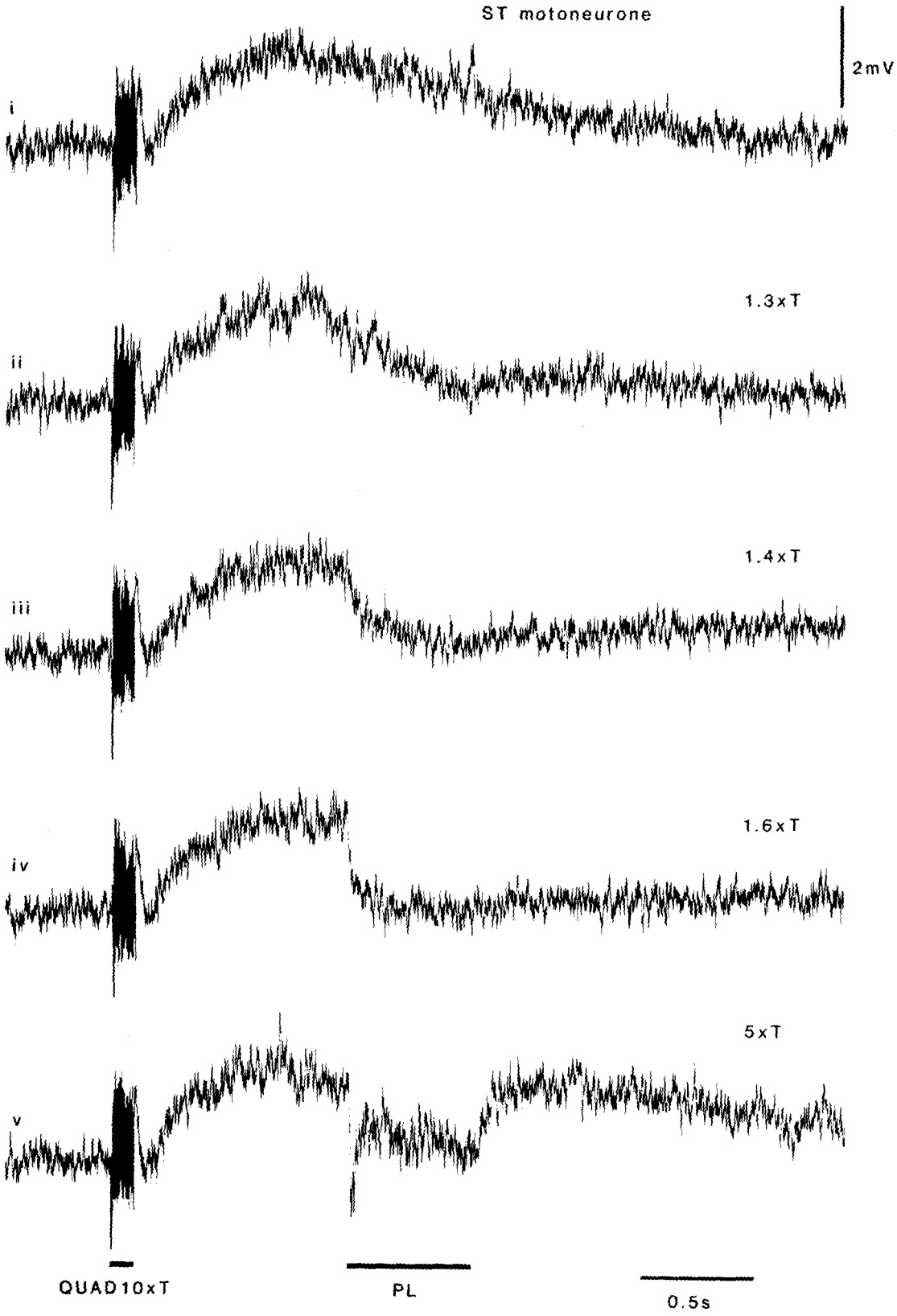

Fig. 5. Effect on 'late reflexes' in a flexor motoneurone of grading afferent stimulation. Same experimental arrangement as in Figs. 3 and 4. *i* intracellular recording from a flexor motoneurone (PBST) showing a 'late reflex' following ipsilateral FRA stimulation (QUAD 10 × T). Traces *ii–v* illustrate the actions of grading the strength of an extensor train (PL) following FRA conditioning. A disfacilitation of the 'late reflex' is apparent at 1.3 × T (*ii*) and becomes maximal with stimulation of 1.6 × T (*iv*). Stimulation in the group II range (*v*) initiates a new flexor reflex rather than a disfacilitation of the 'late reflex'. The timing and duration of stimulus volleys are indicated by solid bars below the lowermost trace. The extensor train does not apply for trace *i*. Same cell as in Fig. 3b

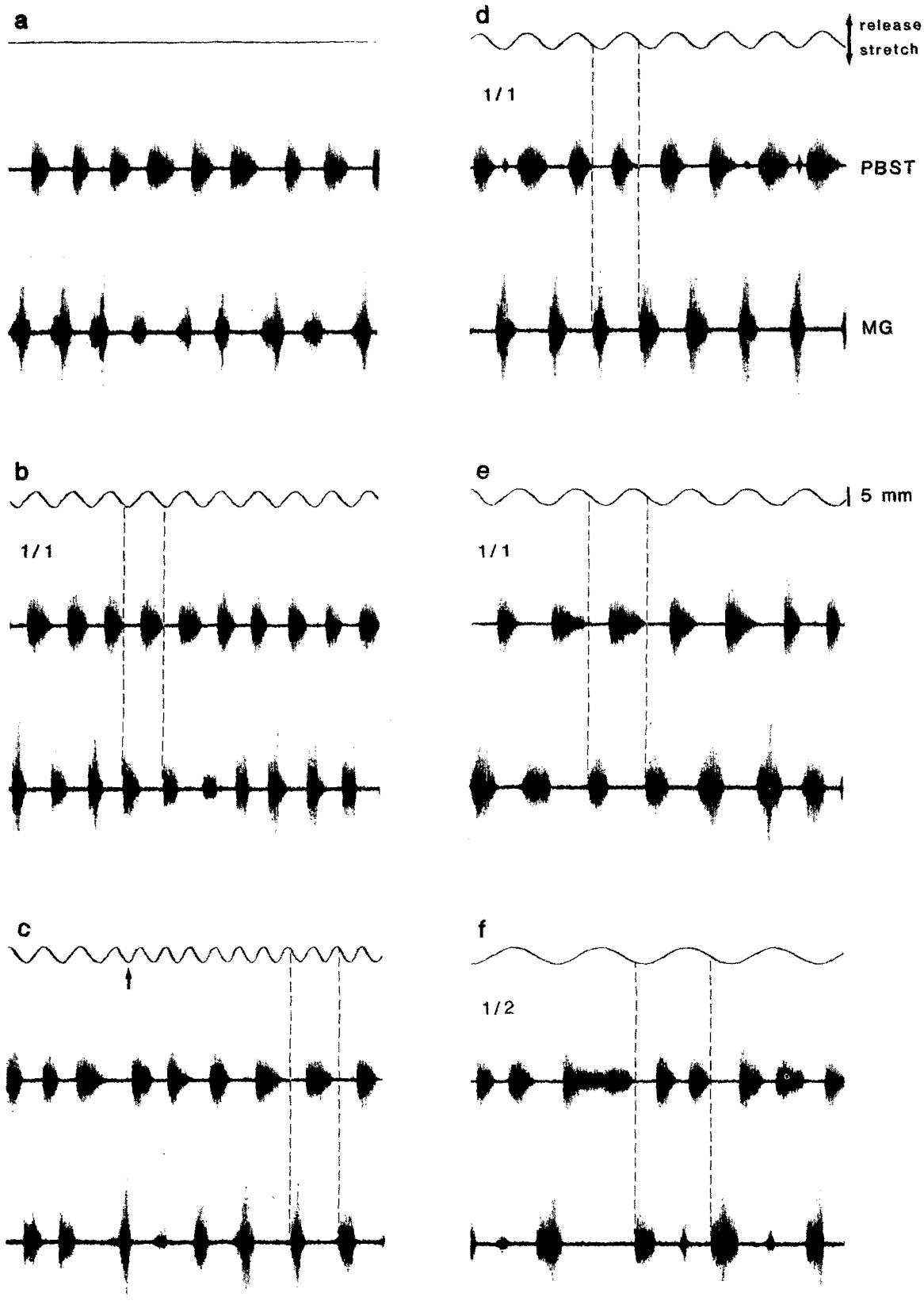

Fig. 6

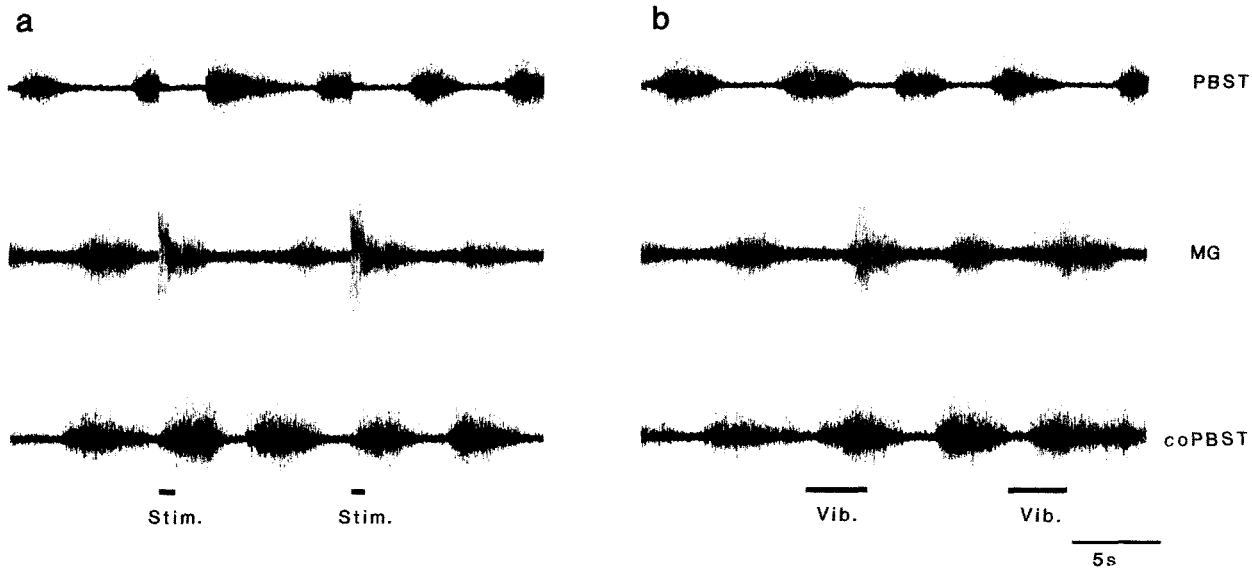

Fig. 7a, b. Effects on fictive locomotion of **a** direct muscle stimulation and of **b** low amplitude vibration of the lateral gastrocnemius-soleus muscle group. Both **a** and **b** show ENG's recorded from ipsilateral flexor and extensor nerves (PBST and MG, upper and middle traces respectively) as well as that from the contralateral nerve (coPBST, lower trace). The periods of stimulation ("stim.") and vibration ("vib.") are indicated by solid bars in **a** and **b** respectively. **a** Illustrates that a short-lasting contraction of the triceps surae muscle evoked by electrical stimulation (via implanted electrodes) can produce effective resetting of the locomotor rhythm (compare to Fig. 2b). Conversely, **b** illustrates that low amplitude, high frequency vibration does not reset fictive locomotion. The resting tension in **a** and **b** was 5 N and rates of stimulation were 200 shocks per second in **a** and 150 shocks per second in **b**. Amplitude of vibration was 50 μm. Drugs: 50 mg/kg nialamide and 80 mg/kg L-DOPA

examined if phase modulation of the sensory input could entrain on-going rhythmic activity. In the experiments described below sinusoidal stretches of the innervated lateral gastrocnemius-soleus muscles were used to induce modulated afferent input to the spinal cord. The ability of naturally evoked afferent input to entrain locomotor activity was studied over a range of frequencies and amplitudes of stretch. Note that the preparation still was paralyzed and that efferent activity thus did not cause contraction and movement related sensory feedback.

Figure 6 illustrates the effect of sinusoidal stretch on fictive locomotion recorded from representative flexor (PBST) and extensor (MG) nerves. The spontaneous rhythm observed with the muscle held with a resting tension of 1.7 N is shown in Fig. 6a. Imposing sinusoidal stretches to lateral gastrocnemius soleus at frequencies above (Fig. 6b) and below (Fig. 6d, e) the spotaneous cycle frequency could produce entrainment only if peak tensions approaching 5 N were achieved. At these tensions a considerable contribution of Ib afferent discharge to the overal afferent signal may be expected (Houk and Simon 1967). Perfect 1 : 1 entrainment (Fig. 6b, d, e) could be achieved over a narrow bandwidth (0.17–0.26 Hz) centred around the spontaneous locomotor fre-

Fig. 6a–f. Entrainment of the rhythmic activity by sinusoidal stretch of the lateral gastrocnemius-soleus muslce. Traces **a–f** show fictive locomotion represented by ENG recordings from flexor (PBST) and extensor (MG) nerves. Trace **a** illustrates spontaneous rhythmic activity recorded with a low resting tension in the triceps surae (1.7 N). While traces **b** to **f** depict the effect of imposing sinusoidal stretches to the triceps tendon. Length records are shown above each segment of fictive locomotion; an upward deflection represents release while stretch is shown as a downward deflection. Addition of sinusoidal stretching results in an entrainment of the intrinsic burst activity of both flexors and extensors. In **b** the intrinsic bursting frequency increases from the spontaneous frequency of 0.22 Hz to 0.26 Hz, the frequency of the imposed stretches. The burst activity in **b** is phase-locked to the stretch cycle. Extensor bursts always occur on the stretch phase of the length change. Increasing the frequency of stretch above that in **b**, as is shown following the arrow in **c**, results in a breakdown of entrainment. Decreasing the frequency of stretch below the spontaneous burst frequency also produces entrainment. In **d** and **e** the burst frequency is decreased to 0.19 Hz and 0.17 Hz respectively corresponding to the imposed frequency of stretching. When the external perturbation frequency is further decreased as in **f** the flexor and extensor burst fails to follow in a strictly entrained pattern. However, a clear relationship exists between the large extensor bursts and the stretch cycle. Bursts with small amplitude corresponding to the extensor phase in PBST are sometimes seen in **d** (cf. Engberg and Lundberg 1969). The resting tension in **a** is 1.7 N while peak tension during stretch is 5 N. Drugs: 50 mg/kg of nialamide was given i.v. followed by 80 mg/kg of L-DOPA

quency (0.22 Hz). Outwith this frequency range the locomotor rhythm no longer showed a strict dependence on the peripheral input and perfect entrainment failed. Increasing the frequency of stretching beyond the range where 1 : 1 entrainment occured resulted in a reversion to the spontaneous locomotor rhythm (Fig. 6c). Despite this breakdown of entrainment following transition to higher frequencies of stretch, extensor bursting would always be phaselocked to the stretch phase of a sinusoidal movement (see latter part of Fig. 6c). Decreasing the frequency of stretching would also allow the intrinsic locomotor rhythm to break through (Fig. 6f). However, in these instances the slow stretches would result in the appearance of large extensor bursts which coincided with the development of peak tension in the muscle. It would therefore appear that outwith the frequency range where perfect entrainment could be observed the peripheral input, although inadequate to clamp the rhythm generating mechanism, is still capable of influencing the relative timing and amplitude of alternating bursts.

Sinusoidal stretches of low amplitude or amplitudes that were ineffective in generating large passive tensions within the muscle never produced an entrained locomotor rhythm. The inference of this observation is that load and not muscle length is the critical factor in producing entrainment (cf. Duysens and Pearson 1980). In agreement with this, it was observed that resetting could be seen following a contraction of the innervated lateral gastrocnemius-soleus muscles (Fig. 7a; contraction, evoked by direct electrical stimulation via implanted electrodes) while no such behaviour could be observed following high frequency low amplitude vibration of the muscle (Fig. 7b). It would therefore appear that resetting and entrainment result mainly from the activation of extensor group Ib afferents.

Discussion

During actual locomotion it has earlier been demonstrated that both the hip extension (hip angle) and the load of the hindlimb extensor muscle have important effects on the locomotor rhythm. Grillner and Rossignol (1978) first described in the chronic spinal cat during treadmill locomotion that the normal transition from extension to flexion did not occur if hip extension was manually prevented. On subsequent slow manual hip extension the limb flexion was initiated as the hip angle exceeded a certain limit. These experiments were confirmed and extended by Andersson and Grillner (1978b, 1981) during fictive locomotion (a preparation where complications arising from movement related sensory feedback are eliminated). It was demonstrated how imposed sinusoidal hip movements could entrain the central generated locomotor rhythm (Andersson and Grillner 1983). Pearson and Duysens (1976); Duysens and Pearson (1980) working with high decerebrated cats have studied the effect of altering the load acting on extensor muscle during treadmill locomotion. In these experiments one hindlimb was denervated, except for the nerves to the ankle flexors and extensors, and held fixed. They described that increased stretch of triceps surae (largely increasing activity in Golgi tendon Ib afferents during active extensor contraction) abolished the locomotor rhythm and induced tonic extensor activity of the ipsilateral limb, while the rhythm of the contralateral limb remained unchanged. In our present experiments on fictive locomotion it was demonstrated that stimulation of extensor group I afferents promotes extensor burst activity ipsilaterally and flexor burst activity contralaterally. This differences between our result and those of Pearson and Duysens probably reflects differences in the experimental situations. It is likely that the virtual lack of effect on the contralateral limb during treadmill locomotion can be ascribed to the overriding effect of movement related sensory feedback from the walking limb, while the crossed effects are more readily seen during fictive locomotion, where the sensory input is experimentally controlled.

Our present results demonstrate that stimulation of extensor group I afferents not only interrupts the locomotor rhythm, but actually reset the ongoing activity and entrain the central generated rhythm. In resetting or entraining the locomotor rhythm it is apparent that stimulation of extensor group I afferents must act upon the interneurones that generate the rhythmic activity. Our results demonstrate that extensor group I afferents distribute excitation (ipsilaterally) to cells generating extensor bursts and inhibit those generating flexor bursts. These observations appear to support the hypothesis that rhythmic locomotor activity is a consequence of phasic activity in reciprocally organised interneuronal pools consistent with the original 'half-centre' theory of Graham Brown (1911, 1924). As described above the interneuronal pools controlling the two hindlimbs are coordinated. During alternating activity (hindlimb fictive locomotion) extensor group I afferents stimulation (ipsilaterally) results in coordinated reciprocal actions onto the contralateral side, i.e. excitation of cells generating flexor bursts and inhibition of those generating extensor bursts. The present series of experiments cannot answer whether this bilateral coordination relies on crossed pathways from exten-

sor group I afferents to contralateral rhythm generators, or if the effect is secondary to a primary action on the ipsilateral locomotor center.

In this study the capability to reset fictive locomotion, appears to be a feature displayed only by extensor group I afferents and in particular extensor group Ib afferents. Low threshold flexor muscle afferents were never observed to reset rhythmic activity. Stimulation of extensor nerves at strengths sufficient to excite a significant population of group II afferents could not enhance the resetting behaviour seen with lower strengths of stimulation. Similarly, an important contribution from spindle afferents (group I and II) in producing resetting or entrainment seems unlikely due to the inability of low amplitude sinusoidal stretch (group Ia and II) or vibration (group Ia) to entrain or reset fictive locomotion. In contrast, the ability to entrain fictive locomotion with stretches of sufficient amplitude to excite both Golgi tendon organs and spindle afferents suggests that extensor Ib afferents provide the effective stimulus in this respect. Furthermore, the involvement of tendon organs in this behaviour is strengthened by the observation that resetting can be seen following a twitch contraction of triceps surae (a procedure which should excite tendon organs but silence spindle afferents). Thus it would appear that loading of extensor muscles provides the adequate stimulus (via Golgi tendon organs) in resetting the locomotor rhythm. It should be realized that resetting involves an obvious threshold phenomenon. It may therefore be difficult to appreciate small contributions from an afferent system and we cannot exclude that muscle spindle afferents have an accessory action to the effect produced by Golgi tendon organs. It has recently been shown that there is indeed an extensive convergence between Ia and Ib afferents on interneuronal pathways (Jankowska and McCrea 1983).

The demonstration that load receptors can influence the phase relations of the step cycle has important functional implications. For example, an unfavourable load distribution at the end of the stance phase would prolong the extensor activity and inhibit the initiation of the swing (flexion) phase (see Pearson and Duysens 1976 and 1980). Conversely, if a limb fails to meet a surface during the extensor phase of the step, or if the surface suddenly gives away, the lack of activation of these 'load' receptors will provoke an early flexor phase ipsilaterally and a prolonged stance phase contralaterally. The extra load acting on the contralateral limb will also help to promote the ipsilateral flexion in such a situation. Thus the afferent feedback from the extensor load receptors will provide an important element in the timing and shaping of the centrally generated activity pattern of extensors and flexors during locomotion.

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References

Andén N-E, Jukes MGM, Lundberg A, Vyklicky L (1966) The effect of DOPA on the spinal cord 1. Influence on transmission from primary afferents. Acta Physiol Scand 67: 373–386

Andersson O, Forssberg H, Grillner S, Lindquist M (1978a) Phasic gain control of the transmission in cutaneous reflex pathways to motoneurones during 'fictive locomotion'. Brain Res 149: 503–507

Andersson O, Grillner S (1981) Peripheral control of the cat's step cycle. I. Phase dependent effects of ramp-movements of the hip during "fictive locomotion". Acta Physiol Scand 113: 89–101

Andersson O, Grillner S (1983) Peripheral control of the cat's step cycle. II. Entrainment of the central pattern generators for locomotion by sinusoidal hip movements during "fictive locomotion". Acta Physiol Scand 118: 229–239

Andersson O, Grillner S, Lindquist M, Zomlefer M (1978b) Peripheral control of the spinal pattern generators for locomotion in cat. Brain Res 150: 625–630

Bässler U (1986) Afferent control of walking movements in the stick insect Cuniculina impigra. II. Reflex reversal and the release of the swing phase in the restrained foreleg. J Comp Physiol A 158: 351–362

Brown MC, Engberg I, Matthews PBC (1967) The relative sensitivity to vibration of muscle receptors of the cat. J Physiol 192: 773–800

Brown TG (1911) The intrinsic factors in the act of progression in the mammal. Proc R Soc Lond B 84: 308–319

Brown TG (1924) Studies in the physiology of the nerveous system. XXVIII: absence of algebraic equality between the magnitudes of central excitation and effective central inhibition given in the reflex centre of a single limb by the same reflex stimulus. Q J Exp Physiol 14: 1–23

Conway BA, Hultborn H, Kiehn O (1987a) Effects of extensor group I afferent stimulation on rhythmic activity in the spinal cat during locomotion. Neurosci Lett Suppl 29: S64

Conway BA, Hultborn H, Kiehn O (1987b) Resetting rhythmic activity by extensor group I afferent stimulation in the spinal cat during fictive locomotion. Acta Physiol Scand 129 (3): C11

Coombs JS, Eccles JC, Fatt P (1955) The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. J Physiol 130: 326–373

Duysens J, Pearson KG (1980) Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. Brain Res 187: 321–332

Engberg I, Lundberg A (1969) An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. Acta Physiol Scand (75): 614–630

Fetz EE, Jankowska E, Johannisson T, Lipski J (1979) Autogenic inhibition of motoneurones by impulses in group Ia muscle spindle afferents. J Physiol 293: 173–195

Forssberg H (1979) Stumbling corrective reaction: A phase-dependent compensatory reaction during locomotion. J Neurophysiol 42: 936–953

Forssberg H, Grillner S, Rossignol S (1977) Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion. Brain Res 132: 121–139

Grillner S (1981) Control of locomotion in bipeds, tetrapods, and fish. In: Brooks VD (ed) Handbook of physiology, Sect 1. The nervous system. II. Motor control. American Physiological Society, Maryland, Waverly Press, pp 1179–1236

Grillner S, Rossignol S (1978) On the initiation of the swing phase of locomotion in the chronic spinal cats. Brain Res 146: 269–277

Grillner S, Zangger P (1974) Locomotor movements generated by the deafferented spinal cord. Acta Physiol Scand 91: 38A–39A

Houk J, Henneman E (1967) Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. J Neurophysiol 30: 466–481

Houk J, Simon W (1967) Responses of Golgi tendon organs to forces applied to muscle tendon. J Neurophysiol 30: 1466–1481

Jankowska E, Jukes MGM, Lund S, Lundberg A (1967a) The effect of DOPA on the spinal cord 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexor and extensors. Acta Physiol Scand 70: 369–388

Jankowska E, Jukes MGM, Lund S, Lundberg A (1967b) The effect of DOPA on the spinal cord 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. Acta Physiol Scand 70: 389–402

Jankowska E, McCrea DA (1983) Shared reflex pathways from Ib tendon organ afferents and Ia muscle spindle afferents in the cat. J Physiol 338: 99–111

Jansen JKS, Rudjord T (1964) On the silent period and Golgi tendon organs of the soleus muscle of the cat. Acta Physiol Scand 62: 364–379

Lennard PR (1985) Afferent perturbations during "monopodal" swimming movements in the turtle: phase-dependent cutaneous modulation and proprioceptive resetting of the locomotor rhythm. J Neurosci 5: 1434–1445

Lundberg A (1979) Multisensory control of spinal reflex pathways. In: Granit R, Pompeiano O (eds) Reflex control of posture and movement. Prog Brain Res, Vol 50. Elsevier Biomedical Press, Amsterdam, pp 11–28

Pearson KG, Duysens J (1976) Function of segmental reflexes in the control of stepping in cockroaches and cats. In: Herman RM, Grillner S, Stein PSG, Stuart DG (eds) Neural control of locomotion. Plenum Press, New York, pp 519–537

Pollock LJ, Davis L (1923) Studies in decerebration I. A method of decerebration. Arch Neurol Psychiat Chicago 10: 391–398

Schomburg ED, Behrends HB (1978a) Phasic control of the transmission in the excitatory and inhibitory reflex pathways from cutaneous afferents to alpha-motoneurones during fictive locomotion in cats. Neurosci Lett 8: 277–282

Schomburg ED, Behrends HB (1978b) The possibility of phasedependent monosynaptic and polysynaptic Ia excitation to homonymous motoneurones during fictive locomotion. Brain Res 143: 533–537

Sillar KT, Skorupski P, Elson RC, Bush BMH (1986) Two identified afferent neurones entrain a central rhythm generator. Nature 323: 440–443

Viala D, Buser P (1971) Modalités d'otention de rythmes locomoteurs chez le lapin spinal par traitements pharmacologiques (DOPA, 5-HTP, d'amphétamine). Brain Res 35: 151–165

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