Exp Brain Res (1998) 122:351–361

RESEARCH ARTICLE

B. Fedirchuk · J. Nielsen · N. Petersen · H. Hultborn

Pharmacologically evoked fictive motor patterns in the acutely spinalized marmoset monkey (*Callithrix jacchus*)

Received: 6 November 1997 / Accepted: 21 April 1998

Abstract The existence of a spinal network capable of generating rhythmic alternating activity resembling locomotion still has not been firmly established in primates, including man, although evidence for one is accumulating. The present study investigated whether it is possible to activate such a network by administration of a variety of pharmacological agents to acutely spinalized marmoset monkeys (*Callithrix jacchus*) in the absence of phasic afferent input to the spinal cord. Fourteen marmoset monkeys were decerebrated, spinalized, and paralyzed. The nerves supplying both hindlimbs were cut and recorded from. In 5 monkeys the effect of electrical stimulation of the brainstem was investigated before spinalization. In 3 of these monkeys, rhythmic activity alternating between extensors and flexor nerves was seen. In the 2 other monkeys only synchronized activity was elicited. In acutely spinalized monkeys, administration of L-3,4-dihydroxyphenylalanine (L-dopa; 3–4 h after treatment with nialamide) failed to evoke any rhythmic alternating activity. In contrast, administration of clonidine elicited alternating activity in all of 8 monkeys tested. In 4 of these monkeys, the activity was restricted to alternation between ipsilateral and contralateral flexor nerves, whereas alternating activity between ipsilateral flexors and extensors was also seen in the other 4 monkeys. Administration of excitatory amino acids (NMDA or NMA) also elicited rhythmic alternating activity in 7 of 10 spinalized monkeys. In 4, rhythmic alternating activity was seen between extensors and flexors on one limb as well as between ipsilateral and contralateral flexors. In 3 monkeys NMDA/NMA produced alternation between extensors and flexors of one limb without alternation between the ipsilateral and contralateral sides. Administration of noradrenaline failed to elicit any rhythmic activity, but rather completely depressed already existing activity. Administration of serotonin (5-HT) was ineffective in facilitating alternating activity in 6 of 8 monkeys and was facilitatory to rhythmic activity in the other 2. We suggest that these data provide further evidence of a network capable of eliciting rhythmic alternating activity resembling locomotion in the primate spinal cord. The network, however, seems to be more difficult to activate pharmacologically in those conditions than in other mammals. This may especially be the case in higher primates, including man.

Key words Locomotion · Central pattern generator · Mesencephalic locomotor region · Clonidine · Intrathecal application · Monkey

B. Fedirchuk (✉)
Department of Physiology, University of Manitoba,
730 William Ave, BMSB 404, Winnipeg, MB, Canada R3E 3J7
e-mail: brent@scrc.umanitoba.ca, Fax: +1-204-789-3930

J. Nielsen
Department of Physiology, Christian-Albrechts-Universität zu Kiel,
Olshausenstrasse 40–60, D-24098 Kiel, Germany

N. Petersen · H. Hultborn
Department of Medical Physiology, Panum Institute,
University of Copenhagen,
Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Introduction

The existence of a spinal network capable of generating alternating activity typical for locomotion in the absence of supraspinal input and peripheral feedback (i.e., a spinal central pattern generator for locomotion; CPG) has been established for a variety of species by administration of pharmacological agents in both in vitro and in vivo preparations (see Grillner and Wallén 1985).

In contrast to other vertebrates, the existence of a corresponding spinal network in primates, including man, remains to be firmly established, although the putative existence of such a network impacts the potential treatment of spinal cord-injured patients (see Illis 1995; Bussel et al. 1989, 1996; Vilensky and O'Connor 1997). Eidelberg et al. (1981) failed to elicit any locomotor-like activity in acutely spinalized macaque monkeys by administration of L-3,4-dihydroxyphenylalanine (L-dopa), but they did observe alternating extensor-flexor movement following stimulation of mesencephalic locomotor centers and locomotor activity after severe incomplete chronic spinal le-

sions and treadmill training (see also Vilensky et al. 1992). In patients presumed to have complete spinal cord lesions, there have been anecdotal reports of involuntary, "locomotor-like" electromyographic (EMG) activity, which was assumed to arise from a spinal locomotor network (Calancie et al. 1994; Bussel et al. 1996) and an almost normal EMG pattern may be observed in patients with incomplete spinal cord injury walking on a treadmill after a training period (Stewart et al. 1991; Wernig and Müller 1992; Wernig et al. 1995). Recent experiments have also demonstrated that rhythmic alternating activity may be elicited in spinal cord-injured patients by electrically stimulating the spinal cord epidurally (Dimitrijević et al. 1996; Gerasimenko et al. 1996; Shapkova et al. 1997).

Although these studies provide accumulating evidence suggesting the existence of a spinal CPG for locomotion in primates, the difficulty in assessing both the completeness of the spinal lesions in the human subjects and the possible contribution of peripheral-afferent mediated reflexes in producing the movements limits the value of the observations in confirming the existence of a *central* and *spinal* CPG in primates. One of the purposes of this study was therefore to provide experimental evidence for spinal locomotion in a completely spinalized primate (marmoset monkey) in the absence of peripheral feedback (i.e., spinal CPG). Another purpose was to elucidate some of the pharmacological properties of the putative primate spinal CPG. This information may prove to be useful for studies attempting to rehabilitate walking in patients with spinal cord injury. Such studies have already been initiated (Stewart et al. 1991; Wernig and Müller 1992; Wernig et al. 1995). Based on the experience on drug-induced spinal locomotion in the cat, neonatal rat, rabbit, lamprey, xenopus embryo, and chick embryo (see Discussion for references), we concentrated on two main pharmacological strategies: (1) administration of monoaminergic agents (mainly dopa and clonidine, sometimes also serotonin and noradrenaline); and (2) application of NMDA, sometimes in combination with the uptake blocker dihydrokainate (DHK). Naloxone and 4-AP were used to facilitate the locomotion activity. Part of this study has previously been published in abstract form (Hultborn et al. 1993).

Materials and methods

Experiments were conducted on 14 adult marmosets (*Callithrix jacchus*), which are primates in the suborder Platyrrhini (New World monkeys). The animals were of either sex, weighing from 330 to 475 g, and were treated in accordance with the National Institutes of Health *Guide for the care and use of laboratory animals* (NIH publication no. 86–23, revised 1985). Anesthesia was induced by intramuscular injection of Saffan (alphaxalone-alphadolone acetate 18 mg/kg) and maintained with supplemental doses (intravenous, 1.2–1.8 mg) every 15–30 min or whenever the animal's blood pressure increased to more than 120 mmHg.

The animal was intubated, and catheters were placed in one carotid artery for monitoring of blood pressure and in both femoral veins for administration of intravenous drugs. Atropine (intramuscular, 0.05 mg/kg) and dexamethasone (1–2 mg/kg) were given early in the surgery. A glucose and phosphate buffer solution (intravenous, 0.3–0.75 ml/h) was administered. The common femoral nerves of both hindlimbs were dissected and placed in implanted cuff electrodes. The common peroneal nerves (Per), the common tibial nerves (Tib), and occasionally the mixed hamstring nerves of both hindlimbs, were cut distally and freed from connective tissue for later mounting on bipolar silver hook electrodes for recording and stimulation. Laminectomy of either the T11–12 (n=10) or the C2 (n=4) vertebrae was performed for the spinalization and of the L2 vertebra to expose the dorsum of the spinal cord.

The animal was placed in a stereotaxic frame and after tying the remaining carotid artery a craniotomy was performed. The animal was then mechanically decerebrated at a pre-collicular, post-thalamic level. All brain tissue rostral to this level was removed and bleeding was controlled using suction and a monopolar cautery. After decerebration, anesthesia was discontinued. The animal was paralyzed with Pavulon (pancuronium bromide; intravenous, 0.15 mg/kg), supplemented every 40–45 min (0.05 mg), and artificially respirated.

Mineral oil pools were fashioned from the loose skin at the laminectomy and hindlimb wound margins. A monopolar recording electrode was placed on the dorsal surface of the lumbar spinal cord to record the afferent incoming volley associated with electrical stimulation of the peripheral nerves. The lowest intensity of stimulus (0.1-ms pulse) that produced a discernable incoming afferent volley was set as the "threshold stimulus" (T) for that nerve. All stimulus intensities are expressed in multiples of T.

A complete spinal transection was made under a dissecting microscope at either a thoracic or cervical level. A small amount of lidocaine noradrenaline solution (2%) was applied onto the segment to be lesioned prior to making the cut in an attempt to reduce spinal activity and local bleeding produced during the lesion.

Electrical brainstem stimulation

In five marmosets, prior to spinalization, the brainstem was electrically stimulated in an attempt to elicit fictive locomotion from the mesencephalic locomotor region (MLR). The stimulation was done with a steel monopolar cathode electrode, placed in the area of the cuneiform nucleus (Saavedra and Mazzuchelli 1969; AP0 to A 0.5; L 1.5 to 2.5; H +5 to +5.5), since this area has been demonstrated to be the anatomical correlate of the MLR in the cat and can elicit fictive locomotion in the macaque (Eidelberg et al. 1981). Rectangular 1 ms pulses were delivered at 20 Hz with intensities ranging from 50 to 150 μA.

Electroneurographic (ENG) recordings and the incoming volley recordings were amplified and continuously displayed during the experiment. They were also digitized (usually raw, occasionally rectified) using a Cygnus DAT tape drive and a Concurrent 5400 series computer (at sampling frequencies of 5 kHz or more).

Administration of pharmacological agents to produce locomotor activity

Drugs were administered to the paralysed and acutely spinalized marmosets either intravenously or intrathecally via a fine subdural catheter. This catheter was introduced at the thoracic level and pushed caudally in the ventral subdural space to the level of the lumbar spinal cord. Leakage of cerebrospinal fluid (CSF) associated with intrathecal perfusion was monitored either at the thoracic level, or in some cases, from a hole made in the dura matter at the lumbar level to allow fluid to escape. All intrathecally applied drugs were dissolved in an artificial monkey-CSF solution (NaCl 126.9 mmol/l, KCl 2.6 mmol/l, $CaCl_2$ 1.15 mmol/l, $NaHCO_3$ 24.97 mmol/l, Na_2HPO_4 1.13 mmol/l, MgCl 1.25 mmol/l), while intravenously applied drugs were dissolved in sterile saline.

Intravenously applied drugs were initially administered in doses that were less than those reported effective for other species and then increased to the equivalent or an even greater dose if the drug was deemed ineffective. The concentrations of intrathecally applied

Table 1 Summary of pharmacological agents administered and the resulting electroneurographic activity for each experiment (*F/E* alternation between flexor and extensor nerve activity on one limb, *F/F* alternation between left and right flexor nerve activity, *Synch* rhythmic synchronous activity, *dopa* L-3,4-dihydroxyphenylalanine (or methyl ester), *Nalox* Naloxone hydrochloride, *5-HT* serotonin, 5-hydroxytryptamine, *NA* Noradrenaline, *NMDA* *N*-methyl-D-aspartic acid, *NMA* *N*-methyl-aspartic acid (racemic), *4-AP* 4-aminopyridine, *Clon* clonidine, *DHK* dihydrokainic acid, 2-carboxy-4-isopropyl-3-pyrrolidineacetic acid)

Exp	Pharmacological agent administered																							
	1[a]	F/E	F/F	Synch	2[a]	F/E	F/F	Synch	3[a]	F/E	F/F	Synch	4[a]	F/E	F/F	Synch	5[a]	F/E	F/F	Synch	6[a]	F/E	F/F	Synch
1	dopa			•	Nalox			•	NMDA	•	•	•	5-HT			•	NA							
2	dopa				Nalox				NMDA		•		NMDA	•	•		NA							
3	dopa				Nalox			•	NMDA				NMDA				4-AP				dopa			
4	dopa				Nalox			•	NMDA	•	•	•	5-HT											
5	Clon				Nalox		•	•	Clon		•	•												
6	Clon		•	•	Nalox	•	•	•	Clon		•		Nalox		•		NMDA	•						
7	Clon & Nalox				Clon		•		Nalox		•		4-AP				dopa				DHK			
8	Clon		•	•	Nalox	•	•	•	4-AP	•	•		4-AP				Clon	•	•		Nalox	•	•	
9	Clon & Nalox		•	•	Nalox		•		4-AP		•		dopa				Nalox				5-HT			
10	Clon & Nalox	•	•	•	Clon & Nalox				NMA & DHK			•	5-HT				Clon							
11	Clon & Nalox	•	•	•	Saffan				Clon & Nalox			•	4-AP											
12	Clon & Nalox		•	•	4-AP			•																
13	NMDA				NMDA & DHK	•			Nalox	•			Nalox	•		•	5-HT				5-HT & NMDA			
14	NMDA				NMDA & DHK			•	NMDA			•	Nalox			•	NMDA & DHK				Clon & Nalox			•

Exp	Pharmacological agent administered (continued)																							
	7[a]	F/E	F/F	Synch	8[a]	F/E	F/F	Synch	9[a]	F/E	F/F	Synch	10[a]	F/E	F/F	Synch	11[a]	F/E	F/F	Synch	12[a]	F/E	F/F	Synch
1																								
2	NMDA	•	•																					
3	Nalox			•																				
4																								
5																								
6																								
7	NMDA				DHK	•		•	Nalox	•			NMDA & DHK	•			5-HT	•	•		NA			
8	5-HT	•	•		NMA	•	•		DHK & NMA	•			dopa	•			Clon				Nial			
9																								
10																								
11																								
12																								
13																								
14	5-HT			•	NMDA & DHK			•	5-HT			•	NMDA & DHK			•	4-AP							

[a] A mark in *F/E* or *F/F and Synch* indicates that although there was obvious alternation, synchronous bursts of activity also persisted

drugs were also usually increased after starting with lower concentrations and superfusing the spinal cord with a total volume of 0.75–1.0 ml. If the treatment was deemed ineffective, the concentration of the solution was increased and the spinal cord was superfused again. The catheter and subdural space were usually rinsed with 1.0 ml of artificial CSF before a new pharmacological agent was applied.

Table 1 shows the experimental treatments for each of the 14 marmosets. Owing to the limited number of animals included in the study and our expectations of the effectiveness of some of the pharmacological agents tested, we decided not to apply the drugs in either a blind or random manner. Rather, we attempted to evaluate one of our three main pharmacological combinations (dopa and naloxone; clonidine and naloxone; NMDA and DHK) as the "first treatment" within any one experiment. Once the first treatment was administered we waited until effects, if any, were stable (usually 30–60 min) and then continued with either a repeated application (perhaps at a new dosage) or administration of a different pharmacological agent. For pharmacological agents administered later in the experiments we cannot exclude the possibility that some effects could be due to a prolonged response (or a synergistic effect) from a drug applied earlier in the experiment. For all experiments where dopa was to be administered, the monoamine oxidase inhibitor nialamide (intravenous, 0.5 mg/kg) was slowly administered early in the experiment.

Results

Brainstem-evoked motor patterns

Electrical stimulation of the brainstem could elicit fictive motor output in the paralyzed preparation. In two experiments, electrical stimulation of the brainstem produced rhythmic activity that was alternating between flexor and extensor nerves, with alternation between the left and right sides (Fig. 1). This pattern of motor activity resembles the "fictive locomotion" commonly elicited in the MLR-stimulated decerebrate cat and demonstrates the ability to elicit MLR-evoked fictive locomotion in the decerebrate marmoset. This activity was somewhat intermittent in that it did not persist throughout the duration of the brainstem stimulation. This might indicate that the brainstem stimulating electrode was in a suboptimal location for eliciting locomotion. Nonetheless, this demonstration of fictive locomotion in the marmoset is especially pertinent since it allows us to compare pharmacologically evoked fictive motor patterns in the spinal preparations to a fictive motor pattern that we are confident is locomotor related.

In three experiments, electrical brainstem stimulation produced ENG activity that was alternating between left and right flexor nerves, between flexor and extensor nerves on one side only, or synchronous discharges with no rhythmic alternating activity.

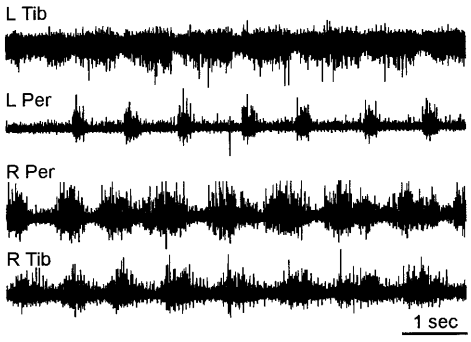

Fig. 1 Locomotor-like raw electroneurographic activity elicited by electrical stimulation of the brainstem (100 μA, 20 Hz) prior to spinalization (*Tib* common tibial nerve, *Per* common peroneal nerve)

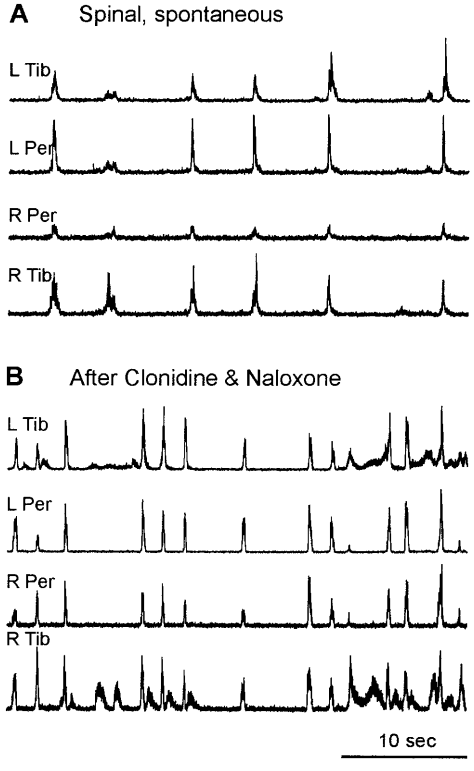

Fig. 2A, B Synchronous bursts of electroneurographic activity (shown rectified and smoothed) occurring spontaneously after spinalization (**A**) and after administration of clonidine and naloxone (**B**) in the same experiment. Clonidine and naloxone increased the amplitude (note *R Per*) and frequency of these synchronous discharges (corresponding traces in **A** and **B** are at the same gains and time scale)

Spontaneous activity following spinalization

In five experiments, there was spontaneous rhythmic activity evident within 30 min following the spinalization that was persistent. In all cases, this consisted of synchronous bursts of activity in multiple ENGs at a relatively low frequency (approximately 0.1–0.3 Hz; see Fig. 2A). To assess whether these rhythmic bursts were attributable to the level of spinalization, we spinalized the animal at C2 rather than at a thoracic level in four experiments. One of these cervically spinalized preparations displayed the same type of spontaneous synchronous activity, so the level of spinalization was deemed not to determine this pattern of activity.

We also considered that this pattern of synchronous rhythmic discharge could be solely attributable to the gross activity during spinalization and subsequent irrita-

tion at the site of spinalization, but such a conclusion seems unlikely for two reasons: (1) we did observe these types of synchronous rhythmic discharges in every experiment. In those cases where they did not occur spontaneously after spinalization, they could be elicited later in the experiment either by application of pharmacological agents (see Table 1) or by electrical or mechanical stimulation of the spinal cord (not illustrated); (2) in one experiment we reanesthetized the animal with Saffan (after decerebration) prior to spinalization in order to minimize spinal activity elicited by the spinalization. We monitored the depressed spinal activity during spinalization but found that rhythmic synchronous discharges were evident once the anesthetic wore off. Local anesthesia of the lesion site after spinalization or general anesthesia (Saffan) during the spinalization did not prevent the development of synchronous activity.

Whether this type of fictive motor pattern was present immediately after spinalization or became evident later in the experiment, it could coexist with the other motor patterns that were elicited by pharmacological means (see, e.g., Fig. 5). However, in some instances where the synchronous bursting was robust, it was evident that other motor patterns were being "interrupted" or interfered with. In two experiments, we gave diazepam (0.05 mg/kg) in an attempt to reduce the spontaneous activity and specifically to reduce the rhythmic synchronous discharges so that we could assess the effects of subsequent pharmacological treatments. Actually, administration of pharmacological agents could elicit synchronous bursts in preparations where they had been lacking (see Table 1), and monoaminergic agents could also increase both the frequency and the amplitude of ongoing synchronous rhythmic activity (see Fig. 2B). This effect seemed to be due to a nonspecific increase in spinal excitability, since both electrical stimulation and gentle mechanical prodding of the spinal cord could also elicit or enhance synchronous rhythmic bursts (not illustrated).

Effects of pharmacological agents

Table 1 summarizes the experimental regimen for each experiment and the motor pattern observed after each experimental treatment. The black dots in Table 1 denote whether rhythmic activity displayed alternation between peroneal and tibial nerves within one side (F/E), left and right peroneal nerves (F/F), or a pattern of rhythmic activity where there was alternation both between flexors and extensors on one side and between the two sides (both F/E and F/F marked). Also, the presence of rhythmic discharges in ENGs that were synchronous either between flexors and extensors on one side or between left and right sides is denoted by a black dot in the "Synch" column.

Dopa and naloxone

Intravenously applied L-dopa or methyl-L-dopa (up to 100 mg/kg) failed to evoke any type of fictive activity in the acutely spinalized marmoset (n=4 as the first treatment). Using electrical stimulation of peripheral nerves (stimulus intensities ranging from 10 to 100 T) we could observe a buildup of long-latency reflexes following administration of dopa (see Fig. 3A). These long-latency reflexes evoked from high-threshold afferents (flexor reflex afferents, FRA; Eccles and Lundberg 1959) were enhanced by the administration of naloxone (0.5 mg/kg: see Fig. 3A). As illustrated in Fig. 3B, increasing the duration of the stimulus train did delay the onset of the late reflex discharge when measured from the onset of the train. This is characteristic for the late FRA-evoked reflex

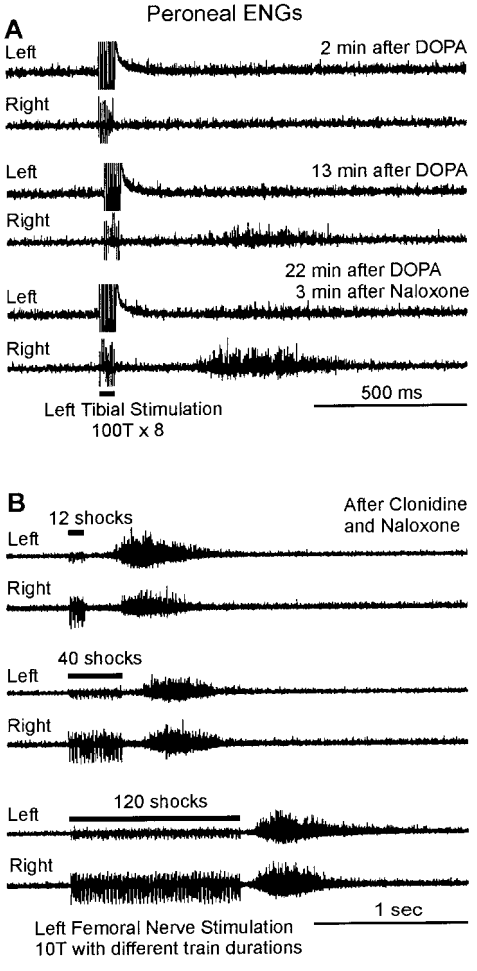

Fig. 3A, B Stimulation of high-threshold stimulation afferents in peripheral nerves following administration of dopa and naloxone (**A**) or clonidine and naloxone (**B**) could evoke long-latency discharges in flexor nerves (different experiments). In **A**, the discharges that were evoked by electrical stimulation of the left tibial nerve (100 T, 8 shocks), could be seen to build up following dopa administration. **B** Increasing the duration of the stimulus train (left femoral nerve; 10 T) delayed the onset of the long-latency discharges. Note: in **A** the discharges in the right peroneal nerve are elicited by a contralateral stimulus and in **B** the stimulus evokes bilateral discharges

discharges following dopa as described in the cat (Jankowska et al. 1967). Usually in the cat, the late FRA discharges released by administration of dopa are observed in ipsilateral flexor and contralateral extensor nerves and stimulation of nerves on both sides results in a mutual reciprocal inhibition between the ipsilateral and contralateral discharges (Jankowska et al. 1967). This pattern of reciprocal inhibition between the pathways activated from the ipsilateral and contralateral nerves is thought to be a key element in the generation of rhythmic alternating activity between the two sides following dopa application in the cat (Jankowska et al. 1967). However, this pattern of inhibition appears to be weak or lacking in the marmoset. In Fig. 3A the late FRA-evoked reflex discharge seen in the right peroneal nerve is evoked by a contralateral stimulus, while in Fig. 3B the stimulus elicits bilateral late reflex discharges in flexor nerves.

Despite the success of dopa (with naloxone) in eliciting late FRA-evoked reflex discharges, the pattern of slow alternating ENG activity shown in acutely spinalized cats was never observed in the marmosets. Also, continuous electrical stimulation of peripheral nerves or the spinal cord (a variety of strengths, 20 Hz) after dopa administration, which is effective in facilitating for enhancing the generation of rhythmic alternating discharges in the cat (Grillner and Zangger 1979), was ineffective in the marmoset.

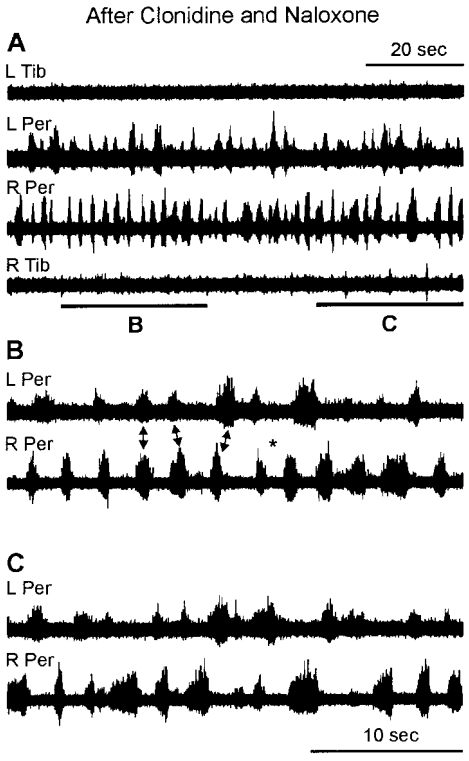

Fig. 4A–C Rhythmic activity in the left and right peroneal nerves following intravenous administration of clonidine and naloxone. **A** An extended period and, for the periods denoted by the *bars*, the left and right peroneal nerves are shown in **B** and **C** on an expanded time scale. In **B**, a section of activity where the bursts can be seen to change phase with respect to each other is marked by the *arrows*. From the time marked by the *asterisk* to the end of the recording period (shown in **C**), the bursts retained an alternating pattern

Clonidine and naloxone

In eight experiments, clonidine (n=5 intravenous, 0.5–1.0 mg/kg; n=3 intrathecal; 3.75 mM) followed by naloxone (intravenous, 0.5 mg/kg) were administered as the first treatment. In each case rhythmic ENG activity was elicited. Rhythmic bursts of activity in the left and right peroneal ENGs that were largely alternating was the characteristic pattern of ENG activity elicited by these agents (n=5; see Figs. 4, 5). This alternating flexor activity of the left and right limbs had a relatively low frequency (approximately 0.25–0.5 Hz) and could persist during the synchronous discharges that often occurred following spinalization (see Fig. 5, Table 1). The synchronous bursts could in some instances seem to disrupt the alternating activity, but this pattern would reappear if the synchronous burst became less vigorous (either spontaneously or by adding diazepam).

The alternation between peroneal ENGs was quite stable (could persist for hours), but occasionally we could observe periods of activity were this alternation could "break down." During this time, it appeared as if the two peroneal ENGs were bursting at independent frequencies for a short time. In these cases, the bursts became "out of phase" and it did appear that the bursts were neither organized as being truly synchronous nor alternating (see Fig. 4B). This pattern was easily recognized and usually reverted to a stable alternating pattern after a short period (seconds; see Fig. 4A,C).

Electrical stimulation of peripheral nerves (2–20 T; 20–50 Hz) could occasionally (n=3 experiments) augment

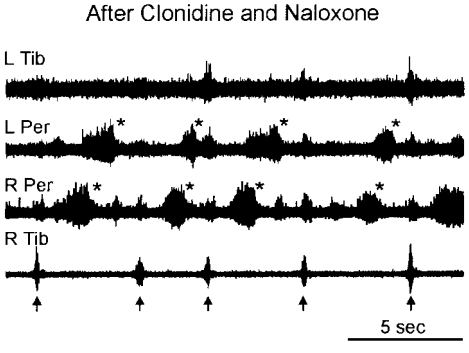

Fig. 5 The alternating pattern of activity (marked by *asterisks*) between the left and right peroneal nerves elicited by clonidine and naloxone could coexist with the synchronous bursts of activity as depicted in Fig. 2 (marked by *arrows*)

the rhythmic activity elicited by clonidine and naloxone, usually by evoking rhythmic activity in a previously inactive tibial ENG. For each of these cases the rhythmic activity in the tibial nerves had a degree of alternation with activity in the ipsilateral peroneal nerve. However, the bursts of tibial ENG activity were always transient, occurring at the onset of the peripheral nerve stimulation and failing after a few bursts (not illustrated).

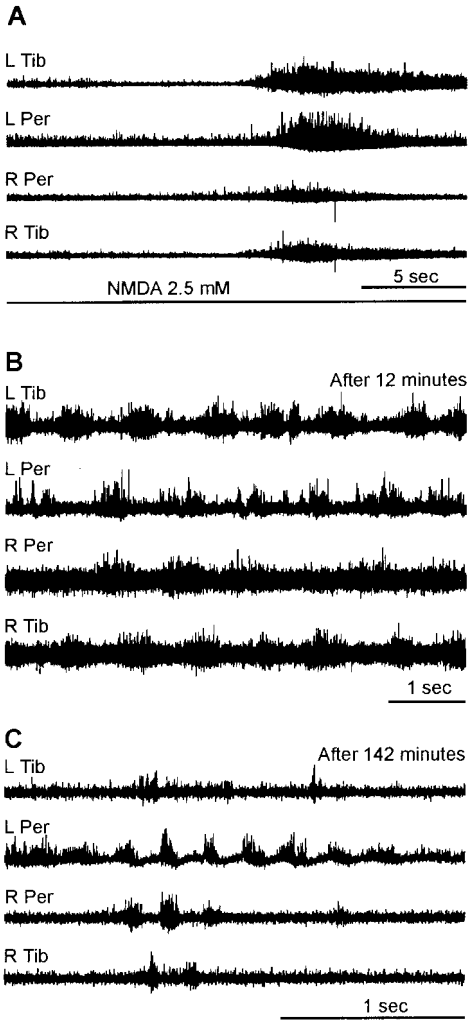

Fig. 6A–C Intrathecal application of 2.5 mM NMDA induced a slow increase in ENG activity (**A**). **B** Rhythmic activity with alternation between bursts in the tibial and peroneal nerves on both sides (12 min after **A**). The synchronicity of activity in the two peroneal nerves is more obvious in **C** (142 min after **A**). Note that the rhythmic bursts in **B** occur at approximately 1 Hz, while those in **C** are at approximately 5 Hz

There were no differences in the patterns of ENG activity elicited by clonidine, depending on the route of administration. Intrathecal application (3.75 mM) was, however, effective in avoiding drug-related blood pressure decreases seen with intravenous administration. Intrathecal administration was therefore the preferred method of application for the last experiments in which clonidine was tested as the first treatment. In cases where clonidine was applied intrathecally, the subsequent administration of naloxone was still delivered intravenously (0.5 mg/kg).

Mechanical stimulation of the perineal or scrotal skin, which has been shown to be effective in facilitating stepping in spinalized cats treated with clonidine and naloxone (Pearson and Rossignol 1991), was ineffective in enhancing the alternating fictive motor patterns elicited by these drugs in this study.

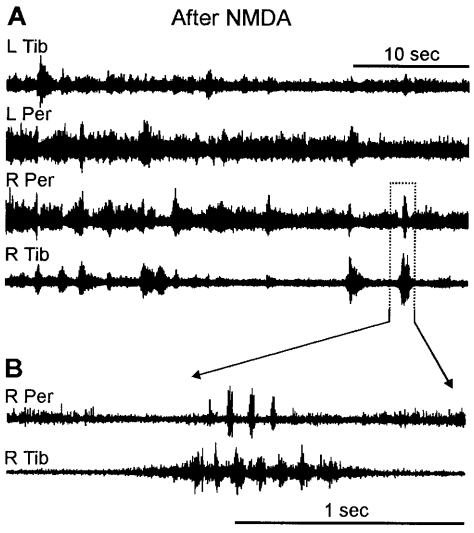

Fig. 7A, B The alternation in activity between peroneal and tibial nerves elicited by intrathecal NMDA could occur at a relatively slow frequency (**A**). The last burst in **A** is seemingly synchronous for both the peroneal and tibial nerves on the right side and is shown with an expanded time scale in **B**. The expansion reveals that the burst is comprised of high-frequency (about 10 Hz) alternation of activity in flexors and extensors

NMDA and DHK

Intrathecal administration of NMDA (0.2–5.0 mM) or NMA (2.5–5.0 mM), either mixed with or followed by intrathecal administration of DHK (2.5 mM), was able to elicit rhythmic activity in the spinalized marmoset in one of two experiments where it was the first agent administered. In addition, NMDA and DHK were able to elicit rhythmic activity in three experiments after dopa and naloxone had failed to produce any activity. Soon after the administration of NMDA, there was an appreciable increase in tonic activity (see Fig. 6A). Following this buildup in activity, spontaneous bursts of activity became apparent, with the prominent pattern being alternating activity in peroneal and tibial ENGs of an individual limb (Figs. 6, 7). In four experiments, NMDA or NMA produced not only alternation between flexor and extensor ENGs on one limb, but a flexor and extensor alternation of both limbs (see Fig. 6B,C). These bouts of activity were short-lived (seconds in duration) and occurred spontaneously. Their initiation was not promoted by electrical stimulation of peripheral nerves.

The frequency of alternating activity produced by administration of NMDA or NMA and DHK encompassed a large range. In Fig. 7A, the alternating activity between peroneal and tibial ENGs was at a slow overall rate (approximately 0.4 Hz), while the activity shown in Fig. 6B is rhythmic at approximately 1 Hz. In general, the frequency of rhythmicity increased as the NMDA concentration was increased (see Fig. 6C). As illustrated in Fig. 7, an even faster alternating rhythm could coexist with a slower pattern of rhythmic activity. In fact, some of the bursts of activity in the peroneal and tibial ENGs that ap-

pear to be synchronous when seen on a slow time scale are actually bouts of fast alternation (at approximately 10 Hz) between peroneal and tibial ENGs. This type of ENG activity following intrathecal administration of NMDA and DHK has been previously described in the acutely spinalized cat (Douglas et al. 1993) and has been postulated to be a fictive representation of "scratching" or "pawshake" (Pearson and Rossignol 1991). In Fig. 7B, only the left limb ENGs were active, but other examples of high-frequency alternating motor patterns were obtained where both left and right flexor and extensor ENGs were participating.

Other pharmacological agents

As can be seen from Table 1, other pharmacological agents that have been shown to affect motor output were also administered. These agents were always given later in the experiment, after administration of monoaminergic drugs or NMDA (or both) so it is possible that the effects that they occasionally elicited might involve some interaction with effects from the preceding pharmacological agents. Since noradrenaline, 5-HT, and 4-AP were not the primary pharmacological agents to be tested in this study, we limit our comments to reporting the incidence of discernable facilitation of alternating motor patterns and acknowledge that their effects might have been more favorable if they had been administered earlier in the experiments.

Intrathecal noradrenaline (10 mM or 20 mM) was not effective in eliciting any rhythmic activity (n=3 experiments). It did, however, cause a marked decrease in tonic activity of peripheral ENGs, indicating a reduction of spontaneous spinal activity. 5-HT applied intrathecally (0.5–2.0 mM) was effective in facilitating rhythmic activity in two of eight experiments where it was applied. Lastly, intravenous administration of 4-AP (0.5–1.0 mg/kg) was effective in eliciting or facilitating rhythmic alternating activity in two of seven experiments.

Discussion

In this discussion we will focus on the different types of fictive motor patterns that were observed in the present experiments, and on the actions by the various pharmacological agents. In both cases we will make particular reference to the differences observed in relation to the cat. Finally, our results are discussed in relation to the present research aiming to develop new strategies for rehabilitation of locomotor capability in spinal cord-injured humans.

Comments on marmoset fictive motor patterns

Firstly, we were able to demonstrate that electrical stimulation of the brainstem of the decerebrate marmoset can produce fictive activity having a pattern of alternation that resembles MLR-evoked fictive locomotion in the cat (Fig. 1). This observation is not surprising, since stimulation at or near the cuneiform nucleus has been previously shown to produce locomotor-like motor patterns in the primate (Eidelberg et al. 1981). Nevertheless this is an important observation in that it allows comparison to fictive motor patterns after spinalization.

Secondly, it was apparent that the amplitude of ENG activity was small for all types of evoked fictive motor activity, including that produced by brainstem stimulation prior to spinalization. The signal-to-noise ratio in this preparation was much smaller than that of a comparable cat preparation. It is often seen in the spinalized L-dopa-treated cat that the fictive motor output is more regular and stable when the amplitudes of ENG activity are large (unpublished observations from Hans Hultborn's laboratory). It therefore seems likely that the small ENG amplitudes and the feeble and unstable nature of the motor patterns evoked after spinalization in the present study reflect a very low level of activity at both the motoneuronal and the premotoneuronal levels of the spinal circuitry.

Thirdly, the bursts of synchronous activity in both ipsilateral and contralateral ENGs were striking in the spinalized marmoset. They were often spontaneously occurring, but could in other cases be enhanced by pharmacological treatment (see Table 1). We did consider that these synchronous bursts might be elicited by spinal stimulation or irritation at the lesion site, but we could not prevent the appearance of these synchronous bursts with our attempts to minimize the activity elicited by the spinalization (see Materials and methods). Similar bursts of synchronous fictive activity can occasionally be seen in L-dopa-treated spinalized cats, but their incidence is less frequent than what we have observed here in the marmoset and they develop late in the experiment when they do occur (unpublished observations from Hans Hultborn's laboratory). As described in the Results, the bursts of synchronous activity could coexist with other fictive motor patterns such as alternation between flexor and extensor activity of one limb, left-right alternating activity between flexor ENGs (see Fig. 5), and a putative paw-shake type of rhythmicity. Although the synchronous activity could partly interfere with the timing of the other motor patterns, their relative independence suggests that at least partly independent rhythm generators may be responsible for their production.

Finally, the coupling of activity between the hindlimbs was often unstable. For the alternating activity in flexor nerves of the hindlimbs evoked by clonidine, regular alternation could be replaced by periods during which the maintained rhythmic flexor bursts in each limb appeared virtually "uncoupled" from each other (see Fig. 4). After a while the alternation could be spontaneously reinstated. It therefore appears as if the "reciprocal inhibition" between the two sides of the spinal cord is very weak. There are two additional observations that support the notion of weak inhibition in the marmoset spinal cord – at least under the present experimental conditions. Firstly, following

dopa administration, late FRA-evoked reflexes could be evoked by stimulation of high-threshold afferents. To a large extent these reflexes had the same characteristics as FRA-evoked reflexes in the cat (long latency, delayed until after the end of the stimulus train; Jankowska et al. 1967). However, in the marmoset the stimulation evoked reflex discharges in both flexor and extensor nerves bilaterally without evidence of the reciprocal inhibition of the reflex responses on the two sides, which is seen in the cat (Jankowska et al 1967). Secondly, and even more surprisingly, we failed to demonstrate the disynaptic reciprocal inhibition between the peroneal and tibial nerves (Brent Fedirchuk, Jens Nielsen, and Hans Hultborn; unpublished observations). We therefore hypothesize that inhibition in general was much depressed under the present experimental conditions. Alternatively, if these observations are not attributable to reduced inhibition, they indicate major differences in the organization of the marmoset spinal network as compared to the cat. This is of particular interest as it has been proposed that the reciprocal inhibition may represent a crucial element of flexor and extensor alternation (see Jankowska et al. 1967).

Effects of pharmacological agents

Monoaminergic agents

Administration of the noradrenergic precursor L-dopa, when preceded by the monoaminoxidase inhibitor Nialamide, induces locomotor activity in spinalized cats (Budakova 1973; Grillner and Zangger 1979; Pearson and Rossignol 1991; see also Jankowska et al. 1967; Lundberg 1979). Pharmacological analysis suggests that in the cat, L-dopa primarily acts indirectly by increasing the release of noradrenaline from the terminals of descending noradrenergic pathways and to a lesser degree by direct activation of adrenergic α-receptors (Andén et al. 1966).

In contrast to these findings in the cat, L-dopa was never effective in producing fictive locomotor-like activity in the marmoset. Despite the previously reported inability of L-dopa to elicit locomotor activity in the macaque monkey (Eidelberg et al. 1981), the failure of L-dopa in the present study is somewhat surprising, since L-dopa (and clonidine) release the "late dopa-reflexes" (Fig. 3), which are postulated to show activation of the spinal locomotor circuitry (Jankowska et al. 1967; Lundberg 1979; Baldissera et al. 1981). One possibility is that the lack of reciprocal inhibition between the left and right sides might be related to the lack of locomotor activity.

It is also surprising that intrathecal noradrenaline did not induce or facilitate locomotor activity as has been shown in the cat (Kiehn et al. 1992), but instead abolished all activity. Indeed, of the monoaminergic agents tested, only the α-adrenergic receptor agonist clonidine initiated alternating activity as it does also in the cat (Forssberg and Grillner 1973; Barbeau et al. 1987; Pearson and Rossignol 1991).

These differences between the cat and marmoset may be explained by differences in the distribution of different monoaminergic systems and their receptor systems. If, for example, the dopaminergic system is more prominent in the marmoset than the cat, L-dopa administered to a spinal marmoset would result in more transmitter release from spinal dopaminergic terminals than from noradrenergic terminals. The fact that the noradrenergic agonist clonidine could evoke rhythmic activity in the present study indicates the presence of α_2-adrenergic receptors capable of generating rhythmic activity. The failure of intrathecally applied noradrenaline to evoke rhythmic activity in this study might be attributed to its nonselective activation of both α_1-and α_2-adrenergic receptors or, alternatively, simply because it was administered relatively late in the experiment after other pharmacological agents.

In chronic spinal cats, 5-HT modulates ongoing locomotor activity rather than initiating it (Barbeau and Rossignol 1990, 1991). In the present study, intrathecal 5-HT application did reinstate or enhance locomotor activity in two instances (see Table 1), so it is possible that 5-HT has a similar modulatory influence in the marmoset. Although 5-HTP has been demonstrated by Viala and Buser (1971) to initiate locomotion in the spinal rabbit and 5-HT induces fictive locomotion in the neonatal rat spinal cord (Smith et al. 1988; Cowley and Schmidt 1995), we did not try 5-HT as the first pharmacological treatment and therefore cannot make firm conclusions regarding its ability to initiate locomotion in the marmoset.

It should, finally, be noted that the facilitation of rhythmic activity that we observed following administration of 4-aminopyridine (4-AP) and naloxone is in accordance with their action in the cat spinal cord (Zangger 1981; Dubuc et al. 1989; Pearson et al. 1992). Both agents are thought to facilitate neural transmission in a rather nonspecific way; 4-AP through a general facilitation of synaptic transmission and naloxone through an increase in the excitability of spinal circuits. It is therefore not surprising that the two agents have similar actions in the cat and marmoset spinal cord.

Excitatory amino acid agents

The excitatory amino acid (EAA) transmitter agonist NMDA has been demonstrated to activate the spinal locomotor circuitry in a variety of preparations including the lamprey (Grillner et al. 1981), the *Xenopus* embryo (Dale and Roberts 1984), the neonatal rat (Kudo and Yamada 1987; Smith et al. 1988), the embryonic chick (Barry and O'Donovan 1987), the rabbit (Fenaux et al. 1991), and the cat (Douglas et al. 1993). The EAA uptake blocker DHK has been shown to potentiate NMDA induced locomotor activity in lamprey (Brodin and Grillner 1985), rat (Smith et al. 1988), and cat (Douglas et al. 1993).

In the present study, intrathecally applied NMDA or NMA were also effective in initiating alternating activity, primarily between flexors and extensors of individual limbs. The coordinated alternation between the two limbs

was often weak, however (see Fig. 6B,C). The frequency of the rhythm was probably dose-dependent (as in the lamprey and rat) and was generally higher than for both the brainstem-evoked and the monoamine-evoked rhythmicity. These observations agree with the NMDA effects in other preparations. Also, we observed faster bouts of rhythmic activity within a slower alternating pattern (Fig. 7) that are very similar to those produced by NMDA in the cat (Douglas et al. 1993) and have been postulated to represent fictive paw-shake or scratching.

Relevance to rehabilitation of spinal cord-injured patients

If locomotion is defined as alternating activity in flexor and extensor nerves that is coordinated bilaterally, then we have failed to demonstrate the existence of a spinal locomotor network in the marmoset. We have, however, found component fictive patterns evoked by different pharmacological agents (clonidine, NMDA, 5-HT), which if combined would resemble a true locomotor pattern. This suggests that a spinal locomotor network exists, but that we have not completely activated it with the pharmacological interventions tested in this study. There may be many reasons, some of which are given above, why this may be the case. Perhaps the preparation of the animal has led to pathological transmission in the spinal circuits (i.e., lack of inhibition, occurrence of synchronized bursts), thereby obstructing the demonstration of the full locomotion pattern. Another alternative, as suggested by Eidelberg et al. (1981), is that the primate spinal network is simply more difficult to activate in the absence of supraspinal control than in other species. It could also be that we have not tried the right combination of pharmacological agents. The distribution and relative importance of the different descending monoaminergic systems (and their receptors) may have a different profile in different species. It therefore might be useful to compare the capability of a pharmacological agent in evoking locomotor activity with the profile of that endogenous pharmacological system for that species. A correlation would suggest that determining these profiles also for the human spinal cord would aid in selecting both the most appropriate animal model and the pharmacological treatments to be developed for spinal cord-injured patients.

Rossignol and coworkers have also shown the importance of training in pharmacologically treated spinalized cats to promote coordinated treadmill locomotion (Barbeau and Rossignol 1987; Barbeau et al. 1991). This demonstration underscores the role of phasic afferent input in reinforcing and modifying motor output. The importance of appropriate afferent input has also been discussed in relation to the locomotor output seen by Eidelberg et al. (1981) in chronically spinalized macaques (Vilensky et al. 1992). Perhaps appropriate proprioceptive afferent inputs would assist the pharmacologically evoked motor patterns, which this study has shown can be generated *centrally* within the primate spinal cord to settle into a more stable locomotor-like pattern. We therefore: (1) do find it likely that there is a network capable of generating locomotion in the primate spinal cord and, (2) propose that this network may be fully activated with the right combination of pharmacological agents, training and, possibly electrical stimulation of either the spinal cord or peripheral nerves.

Such strategies will also eventually be necessary in the rehabilitation of walking in patients with spinal cord injury and have already been initiated by several groups. Dimitrijević et al. (1996), Gerasimenko et al. (1996), and Shapkova et al. (1997) have demonstrated with some success that epidural stimulation of the spinal cord may elicit locomotor-like activity in spinal cord-injured patients, and, of direct interest to the present study, Stewart et al. (1991) found that clonidine could greatly improve the walking ability of some patients with partial spinal cord lesions. As discussed above, there may be a considerable species differences in the pharmacology of the spinal locomotor network, also between the human and marmoset primates, so therefore extrapolation from these experiments to the human spinal cord should be done with caution. Nevertheless, based on the present study it seems reasonable to hypothesize that noradrenergic and possibly even EAA agonists are more promising pharmacological agents for activating the human locomotor circuitry than those that act by promoting transmitter release from endogenous synapses. More important probably, the present observations can be used to stress the importance of a combination of alternative strategies (pharmacological, physiotherapeutical, electrical stimulation) in future attempts to rehabilitate walking in the human.

Acknowledgements The authors wish to thank Dr. R. Brownstone for his participation in some experiments and Lillian Grøndahl, Ingrid Kjær, and Maria Setterbom for their excellent technical assistance. B. Fedirchuk received a fellowship from the Medical Research Council of Canada. This work was supported by the Danish Research Council and the Danish Society of Multiple Sclerosis.

References

Andén NE, Jukes MG, Lundberg A (1966) The effect of dopa on the spinal cord. 2. A pharmacological analysis. Acta Physiol Scand 67:387–397

Baldissera F, Hultborn H, Illert M (1981) Integration in spinal neuronal systems. In: Brooks VB (ed) Motor control. (Handbook of physiology, sect 1, The nervous system, vol II). American Physiological Society, Bethesda, pp 509–595

Barbeau H, Rossignol S (1987) Recovery of locomotion after chronic spinalization in the adult cat. Brain Res 412:84–95

Barbeau H, Rossignol S (1990) The effects of serotonergic drugs on the locomotor pattern and on cutaneous reflexes of the adult chronic spinal cat. Brain Res 514:55–67

Barbeau H, Rossignol S (1991) Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. Brain Res 546:250–260

Barbeau H, Julien C, Rossignol S (1987) The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. Brain Res 437:83–96

Barbeau H, Chau C, Rossignol S (1991) Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats. Brain Res Bull 30:387–393

Barry MAJ, O'Donovan MJ (1987) The effects of excitatory amino acids and their antagonists on the generation of motor activity in the isolated chick spinal cord. Brain Res Dev Brain Res 36:271–276

Brodin L, Grillner S (1985) The role of putative excitatory amino acid neurotransmitters in the initiation of locomotion in the lamprey spinal cord. II. The effects of amino acid uptake inhibitors. Brain Res 360:149–158

Budakova NN (1973) Stepping movements in the spinal cat after dopa injection (in Russian). Fiziol Zh SSSR 59:1190–1198

Bussel B, Roby-Brami A, Yakovleff A, Bennis N (1989) Late flexion reflex in paraplegic patients. Evidence for a spinal stepping generator. Brain Res Bull 22:53–56

Bussel B, Roby-Brami A, Rémy Néris O, Yakovleff A (1996) Evidence for a spinal stepping generator in man. Paraplegia 34:91–92

Calancie B, Needham–Shropshire B, Jacobs P, Willer K, Zych G, Green BA (1994) Involuntary stepping after chronic spinal cord injury. Evidence for a central rhythm generator for locomotion in man. Brain 117:1143–1159

Cowley KC, Schmidt BJ (1995) Effect of inhibitory amino acid antagonists on reciprocal inhibitory interactions during rhythmic motor activity in the in vitro neonatal rat spinal cord. J Neurophysiol 74:1109–1117

Dale N, Roberts A (1984) Excitatory amino acid receptors in *Xenopus* embryo spinal cord and their role in the activation of swimming. J Physiol (Lond) 348:527–543

Dimitrijević MR, Halter JA, Gerasimenko Y, Sherwood AM, McKay WB (1996) Central pattern generator in human: motor responses evoked by and spinal cord evoked potentials recorded from epidural electrodes at different locations. Soc Neurosci Abstr 22:543.8

Douglas JR, Noga BN, Dai X, Jordan LM (1993) The effects of intrathecal administration of excitatory amino acid agonists and antagonists on the initiation of locomotion in the adult cat. J Neurosci 13:990–1000

Dubuc R, Rossignol S, Lamarre Y (1989) The effects of 4-aminopyridine on the spinal cord: rhythmic discharges recorded from peripheral nerves. Brain Res 369:243–259

Eccles RM, Lundberg A (1959) Synaptic actions in motoneurones by afferents which may evoke a flexion reflex. Arch Ital Biol 97:199–221

Eidelberg E, Walden JG, Nguyen LH (1981) Locomotor control in macaque monkeys. Brain 104:647–663

Fenaux F, Corio M, Palisses R, Viala D (1991) Effects of an NMDA-receptor antagonist, MK-801, on central locomotor programming in the rabbit. Exp Brain Res 86:393–401

Forssberg H, Grillner S (1973) The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res 50:184–186

Gerasimenko Y, McKay WB, Pollo FE, Dimitrijević MR (1996) Stepping movements in paraplegic patients induced by epidural spinal cord stimulation. Soc Neurosci Abstr 22:543.5

Grillner S, Wallén P (1985) Central pattern generators for locomotion, with special reference to vertebrates. Annu Rev Neurosci 8:233–261

Grillner S, Zangger P (1979) On the central generation of locomotion in the low spinal cat. Exp Brain Res 34:241–261

Grillner S, McClellan A, Sigvardt K, Wallén P, Wilén M (1981) Activation of NMDA-receptors elicits "Fictive locomotion" in lamprey spinal cord in vitro. Acta Physiol Scand 13:549–551

Hultborn H, Petersen N, Brownstone R, Nielsen J (1993) Evidence of fictive spinal locomotion in the marmoset (*Callithrix jacchus*). Soc Neurosci Abstr 19:225.1

Illis LS (1995) Is there a central pattern generator in man? Paraplegia 33:239–240

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

Kiehn O, Hultborn H, Conway BA (1992) Spinal locomotor activity in acutely spinalized cats induced by intrathecal application of noradrenaline. Neurosci Lett 143:243–246

Kudo N, Yamada T (1987) *N*-Methyl-D,L-aspartate-induced locomotor activity in a spinal cord-hindlimb muscles preparation of the newborn rat studied in vitro. Neurosci Lett 75:43–48

Lundberg A (1979) Multisensory control of spinal reflex pathways. Prog Brain Res 50:11–28

Pearson KG, Rossignol S (1991) Fictive motor patterns in chronic spinal cats. J Neurophysiol 66:1874–1887

Pearson KG, Jiang W, Ramirez JM (1992) The use of naloxone to facilitate the generation of the locomotor rhythm in spinal cats. J Neurosci Methods 42:75–81

Saavedra JP, Mazzuchelli AL (1969) A stereotaxic atlas of the brain of the marmoset (*Hapale jacchus*). J Hirnforsch 11:105–122

Shapkova HY, Shapkov YT, Mushkin AY (1997) Spinal cord stimulation-evoked locomotion in paraplegic children (abstract). Acta Physiol Scand 159:33A

Smith JC, Feldman JL, Schmidt BJ (1988) Neural mechanisms generating locomotion studied in mammalian brain stem-spinal cord in vitro. FASEB J 2:2283–2288

Stewart JE, Barbeau H, Gauthier S (1991) Modulation of locomotor patterns and spasticity with clonidine in spinal cord injured patients. Can J Neurol Sci 18:321–332

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

Vilensky JA, O'Connor BL (1997) Stepping in humans with complete spinal cord transection: a phylogenetic evaluation. Mot Control 1:284–292

Vilensky JA, Eidelberg E, Moore AM, Walden JG (1992) Recovery of locomotion in monkeys with spinal cord lesions. J Mot Behav 24:288–296

Wernig A, Müller S (1992) Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. Paraplegia 30:229–238

Wernig A, Müller S, Nanassy A, Cagol E (1995). Laufband therapy based on "rules of spinal locomotion" is effective in spinal cord injured persons. Eur J Neurosci 7:823–829

Zangger P (1981) The effect of 4-aminopyridine on the spinal locomotor rhythm induced by L-dopa. Brain Res 215:211–223