Locomotion of the Hindlimbs After Neurectomy of Ankle Flexors in Intact and Spinal Cats: Model for the Study of Locomotor Plasticity

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Carrier, Lynda, Edna Brustein, and Serge Rossignol. Locomotion of the hindlimbs after neurectomy of ankle flexors in intact and spinal cats: a model for the study of locomotor plasticity. *J. Neurophysiol.* 77: 1979–1993, 1997. To study the potential plasticity of locomotor networks in the spinal cord, an important issue for locomotor rehabilitation after spinal injuries, we have investigated the locomotor performance of cats before and after a unilateral denervation of the ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) both in cats with intact spinal cord and after spinalization. The effects of the inactivation of the ankle flexors were studied in three cats with intact spinal cord during periods of 4–7 wk. Cats adapted their locomotor performance very rapidly within a few days so that the locomotor behavior appeared to be unchanged practically. However, kinematic analyses of video records often revealed small but consistent increase in knee and/or hip flexion. These changes were accompanied by some increase in the amplitude of knee and hip flexor muscle activity. Cats maintained a regular and symmetrical walking pattern over the treadmill for several minutes. Two of these cats then were spinalized at T13 and studied for ~1 mo afterward. Whereas normally cats regain a regular and symmetrical locomotor pattern after spinalization, these cats had a disorganized and asymmetrical locomotor pattern with a predominance of knee flexion and absence of plantar foot contact of the denervated limb. Another cat first was spinalized and allowed to recuperate a regular symmetrical locomotor performance. Then it also was submitted to the same unilateral ankle flexor inactivation and studied for ~50 days. The cat maintained a well-organized symmetrical gait although there was almost no ankle flexion on the denervated side. There was no exaggerated knee hyperflexion and gait asymmetry as seen in the two previous cats spinalized only after they had adapted to the denervation of ankle flexors. It is concluded that, after muscle denervation, locomotor adaptation is achieved through changes occurring at different levels. Because cats spinalized after adaptation to the neurectomy had an asymmetrical locomotor pattern dominated by hyperflexion, it is suggested that the spinal circuitry has been modified during the adaptive process, presumably through the action of corrective supraspinal inputs. Indeed spinal cats do not normally display such abnormal flexions, and neither did the one cat denervated after spinalization. On the other hand, because the modified locomotor pattern in the spinal state is not functional and contains only some aspects of the compensatory response seen before spinalization, it is suggested that the complete functional adaptation observed in intact cats after peripheral nerve lesions may depend on changes occurring at the spinal and the supraspinal levels.

INTRODUCTION

One of the major hopes in the field of locomotor rehabilitation after spinal cord injury is that the combined approach of pharmacology, locomotor training, and functional electrical stimulation could lead to a gradual improvement of the residual locomotor capabilities (Barbeau and Fung 1994; Barbeau and Rossignol 1994; Dietz et al. 1994; Rossignol and Barbeau 1993, 1995; Wernig and Muller 1992; Wernig et al. 1995). The successful outcome of such rehabilitation strategies assumes that the locomotor control mechanisms have some degree of plasticity and that the improvement of the residual locomotor function can be retained. Various observations may argue for or against such locomotor plasticity.

It currently is believed, on the basis of a large body of experimental evidence from several animal species, that the basic locomotor pattern is generated largely at the spinal cord level and is regulated through afferent feedback and descending commands, as summarized in various reviews (Barbeau and Rossignol 1994; Delcomyn 1980; Grillner 1981; Pearson 1993; Rossignol 1996; Rossignol and Dubuc 1994). In that framework, one could ask how animals adapt their locomotion to new conditions such as damages to peripheral nerves, muscles, or joints and where do these adaptive changes take place? Although there is some evidence of anatomic plasticity (sprouting) in descending pathways and afferent pathways after spinal lesions or dorsal root sections (Goldberger and Murray 1982) that may underlie functional changes (Goldberger and Murray 1978), it is not clear, however, if the spinal locomotor generating circuits are modifiable functionally. Some of the evidence from cats (Forssberg and Svartengren 1983; Gordon et al. 1986) and rats (Sperry 1940–1942) indicates that, after transposing antagonist muscles of the ankle, the functions of the transposed muscles are retained, suggesting that there is little plasticity in the spinal locomotor mechanisms. For instance, the transposed ankle extensor muscle continues to discharge not in accordance with its new biomechanical function (ankle flexion) but according to its former function (ankle extension) and connectivity (hardwired networks).

On the other hand, there is the view that there can be some functional plasticity within certain simple spinal reflexes that may even persist after spinalization. Wolpaw has shown, with operant conditioning, that monkeys can be trained to upregulate or downregulate the monosynaptic H-Reflex and that this “learned” asymmetry may persist after spinalization (Wolpaw 1994; Wolpaw and Lee 1989; Wolpaw and O’Keefe 1984; Wolpaw et al. 1983a,b). Work on the flexion reflex in the cat also suggests some degree of plasticity even in spinal animals (Durkovic 1975, 1983; Durkovic and Damianopoulos 1986; Misulis and Durkovic 1982, 1984), as previously suggested (Shurrager and Culler 1940; Dykman and Shurrager 1956).
Other types of evidence of spinal plasticity also have been obtained from the study of locomotion in spinal animals. After spinalization, kittens (Forsberg et al. 1980a,b) as well as adult cats (Barbeau and Rossignol 1987, 1994; Belanger et al. 1996), can walk with the hindlimbs on a treadmill, and the quality of such locomotion is a function of regular weight-bearing training (see also Edgerton et al. 1983; Smith et al. 1982). Early locomotor training using clonidine, an alpha-2 noradrenergic receptor agonist that can induce locomotion in the first hours after a spinalization (Forsberg and Grillner 1973) also appears to accelerate the recuperation of locomotion (Barbeau and Rossignol 1994; Barbeau et al. 1993; Rossignol and Barbeau 1993). Finally, it has been postulated that there is specificity in the training of spinal cats such that cats trained to stand or to walk will have a better standing or walking performance, respectively (Hodgson et al. 1994). This combined work then suggests some degree of plasticity within the spinal cord and more specifically in the locomotor control mechanisms. The muscle transposition paradigm described above may represent an extreme case of the demand on the CNS for functional locomotor plasticity, which requires the rewiring of neural circuits to adapt the function of transposed muscles. Other strategies may be more economical and efficient. As a matter of fact, work on tendon transposition or axotomy of specific hindlimb nerves (Gordon et al. 1980) often refer to "tricks" (Sperry 1942) developed by the animal to compensate for their neuromuscular deficit. For instance, to obviate the deficit in one joint due to muscle transposition of that joint, the animal can increase the excursion of other joints. Where are these tricks generated in the tripartite control scheme for locomotion mentioned above? Indeed, when an organism adapts its locomotor behavior to permanent changes in its limbs or joints, are the adaptive changes incorporated eventually within the spinal circuitry so that descending voluntary commands trigger spinal circuits, which already have been modified to take into account the state of the peripheral apparatus? Another alternative is that these adaptive changes are achieved by modifying descending the control commands themselves to the spinal circuits so that each step is modified appropriately to offset the deficit? In the first alternative, most changes would occur in the spinal cord, and in the second alternative, most changes would occur in the descending commands.

To study this question, we have investigated the locomotor behavior in cats before and after a unilateral denervation of two large ankle flexors of one limb, tibialis anterior (TA) and extensor digitorum longus (EDL). After a period of adaptation of treadmill walking with this new neuro-muscular state, two of the cats were spinalized at T13, and their locomotor performance studied for several days. In another cat, the same neuromectomy was performed after spinalization at a time when the cat already had regained its locomotor capabilities. The data suggest that, in normal cats, adaptation to such neuromuscular impairments involves changes both at spinal and supraspinal levels. The results of this study have been presented in preliminary form (Carrier et al. 1992).

**METHODS**

**General experimental protocol**

All the surgical procedures described below were reviewed and approved by the University Ethics Committee. Four adult cats, purchased through a local supplier approved by the university, were trained progressively during a period of 2–4 wk to walk on a treadmill, in a Plexiglas enclosure, at constant speeds ranging from 0.2 to 0.8 m/s so that they became accustomed to the laboratory environment and were able to maintain a nearly constant position on the treadmill. Electromyographic (EMG) electrodes then were implanted and several control recording sessions of EMGs and kinematics were taken for several days to establish reliable baseline control values for each cat (43 for cat 1, 27 for cat 2, 33 for cat 3, and 12 for cat 4). Thereafter, the ankle flexor muscles TA and EDL were denervated and cats that were studied again for several days (51 for cat 1, 27 for cat 2, and 40 for cat 3). Two of the cats (cat 1 and cat 3) were spinalized, and their locomotor performance studied for 24 and 32 days, respectively. In cat 4, the order was reversed; the cat was spinalized 12 days after implantation, the neuromectomy was performed 22 days after the spinalization, and the cat was killed 52 days later. Cat 2 was used only to investigate the effect of neuromectomy and was not spinalized. The number of recording sessions in between these events also varied from 3 to 18 depending on cats. Only certain recordings on specific days were used for complete analysis and display.

**Training procedures after spinalization**

From 3 to 4 days after spinalization, training of the spinal cat was performed one or twice daily (a recording session could replace a training session). Cats were trained to walk with their forelimbs standing on a platform placed a few centimeters above the belt and a Plexiglas separator placed so that the hindlimbs would not impede each other as can happen when cats have an exaggerated adduction after spinalization. In the early days after spinalization the weight of the hindquarters was supported partially by holding the tail, and locomotion was facilitated by manual perineal stimulation. In the two cats in which neuromectomy was performed before spinalization, clonidine 150–200 μg/kg was given intraperitoneally on four occasions to initiate walking movements (Barbeau et al. 1987) mainly in the first week postsurgical so that the expression of the locomotor pattern could be studied during that period without further plastic changes that could be brought about by the subsequent intensive training.

**Surgical procedures**

**EMG IMPLANTATION.** The general methods have been described earlier (Belanger et al. 1996). After fasting overnight, cats were premedicated with Acepromazine (Atravet, 0.1 mg/kg sc) and atropine (0.05 mg/kg sc) and then given a dose of pentobarbital (Somnotol 35 mg/kg iv). Penicilline G (40,000 U/kg im) also was given at the time of surgery. Up to 14 muscles were implanted chronically. Teflon-insulated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA) were soldered to two 15-pin connectors (TRW Electronic Components Groups, Elk Grove Village, IL) cemented to the skull of the animal and the other end of the wires was led subcutaneously to selected muscles. After removing the insulation for ~3 mm of the wires inserted in the muscle belly itself, each wire was attached individually by a silk thread at its entry in the muscle, and both wires were tied together at their exit from the muscle belly. The following muscles were implanted on both sides (i = ipsilateral to the camera, usually the left of the animal; co = contralateral): flexor muscles, IP, ilioïspoas; Srt, sartorius anterior; St. semimembranosus and extensor muscles; Glu, glutaeus; VL, vastus lateralis; GL, gastrocnemius lateralis; and GM, gastrocnemius medialis. Before denervation or spinalization, several recording sessions ensured that the EMG signals were stable and consistent so that the changes seen after such procedures were not due to changes in recording conditions. After surgery, cats were placed in their individual cage with an infrared light to maintain temperature overnight. Buprenorphine hydrochloride (Temgesic,
Recording and analyses

EMG. The EMG signals were amplified differentially using a band-pass filter of 100 Hz to 3 kHz and recorded on a 14-channel VHS tape recorder (Vetter model 4000A with a typical rise time at half-amplitude of 200 μs at the speed of recording). The EMGs were played back on an electrostatic polygraph (Gould 1000) and selected sequences were digitized at 1 kHz using a PDP 11/34 computer. An interactive custom made EMG analysis program was used to extract cycle and EMG burst values for statistical analyses and display. Furthermore, the EMG bursts could be averaged and normalized over many cycles using either one of the EMGs or a kinematic event such as paw contact as a trigger.

Kinematics. To analyze the kinematics of locomotion, six light-reflecting disks (made from 3 M reflecting tape) were stuck on the tip of the iliac crest, the great trochanter, the knee’s lateral condyle, the lateral malleolus, the fifth metatarsophalangeal joint, and the tip of the third toe. The cat walked freely on the treadmill, being connected to the equipment only through the flexible cables from the head plugs to the preamplifier input box. Locomotion was recorded using a Digital 5100 shutter camera and a Panasonic AG 7300 video tape recorder. With an exposure time varying between 0.5 and 2 ms, it was possible to have a sharp image of the reflective markers for every field, thus a time resolution of ±1 field (16.7 ms). Using a two-dimensional peak performance system, the six points were digitized for every field. From the x and y coordinates of each point, the kinematics of locomotion could be reconstructed and displayed as stick diagrams, trajectories of the points, or joint angular excursions of the hip, knee, ankle, metatarsophalangeal (MTP) joints. In all angular displays, flexion is downward except for the MTP joint where dorsiflexion of the toes corresponds to an upward deflection of the trace. Other events, such as paw contact and lift off, were identified and used to align normalized joint excursions or EMG signals. Finally, the EMG signals and the video images were synchronized using a SMPTE time code (time code generator Skotel TCG-80N and time code reader TCR-80N) recorded simultaneously on the analogue tape containing the EMG data as well as on one of the voice channels of the video tape and on the image itself.

RESULTS

Kinematics and EMG changes after neurectomy

All cats were tested on the treadmill 2–3 days after the neurectomy. We were struck by the fact that all cats showed very little locomotor deficits at slow walking speeds of 0.3–0.5 m/s. They had more difficulty walking at moderate speeds of 0.7–0.8 m/s and tired more rapidly. Careful visual observation suggested a decreased ankle flexion but a more conspicuous hip abduction during swing. This hip abduction disappeared after the first week in all but one cat, in which it persisted at a low level.

Figure 1 shows two step cycles at 0.4 m/s taken during the control period (Fig. 1A) and 25 days after the TA-EDL neurectomy (Fig. 1B). In Fig. 1A, the synchronized kinematics and EMGs illustrate a normal locomotor pattern. Just before lift-off, the knee flexor St discharges as a very brief burst, which, together with foot muscles (not illustrated), would clear the paw from the ground and start the swing phase. The hip, knee, and ankle flex while the toes dorsiflex during the first half of swing, a phase referred to as the flexion phase (F) in the step cycle defined by Philippson (1905). During this flexion period, the hip flexors, IP and Srt, start their discharge, which continues into the next phase, the first extension phase (E1), during which the knee and ankle extend to contact ground for the next stance phase. Extensor muscles, such as iGL, are activated just before foot contact, which starts the second extension phase (E2), and are active for most of the stance phase.

After the neurectomy of TA and EDL, the kinematics changes of the various joints (except for the ankle) were subtle (Fig. 1B) and hardly could be detected by eye only. As expected, there was a marked reduction, but not a complete abolition, of ankle excursion (from 30 to 13°, see Table 2) and a reduced dorsiflexion of the MTP joint (from 66 to 43°, see Table 2). The knee and hip showed an increase of peak-to-peak excursion of only a few degrees. These small kinematic changes, however, were accompanied by an increase in the amplitude of the St EMG, as well as a marked increase in the amplitude and duration of the hip flexor Srt. The delay seen normally between the onset of ST and the onset of IP or Srt was reduced after neurectomy (compare Fig. 1, A and B; see also Fig. 4), and the onset of the hip flexor discharge was more abrupt.

The cycle duration and structure was similar for the same belt speed (0.4 m/s) before and after neurectomy when the animal had recovered fully as seen in Fig. 1. Table 1 shows that the cycle duration pre- and postneurectomy was similar for the same belt speed in cat 1. In cat 2, the overall cycle remained also the same although there was a small (but significant) increase in the swing phase duration. In cat 3, the cycle duration was decreased significantly from 996 to 851 ms due to a shorter stance. This decrease of cycle duration, although initially seen at 0.6 m/s, recovered after 4 wk so that pre- and postneurectomy step cycles were the same.

Figure 2, A and C, illustrates one step cycle before and after neurectomy in a stick figure format for the same cat. Again the changes are very subtle if any. There is a significant reduction in ankle and MTP excursion as well as a
somewhat greater flexion of the knee at the end of the swing phase after neurectomy. The overall shift of the hip toward more flexion is due to a more ventral placement of the light reflective disk at the iliac crest. In the angular traces (Fig. 2D), the total peak-to-peak hip excursion is the same as in Fig. 2B, but a study of the first derivative of such traces indicated a small increase in hip flexion velocity after neurectomy. In this example, the angular traces of the knee joint before and after neurectomy can be superposed, although the knee is somewhat more flexed at the onset of E1. Table 2 shows that for the hip and knee, the peak-to-peak excursions (dif) are very similar before and after neurectomy. Although the ankle and MTP joints excursions are reduced, the cat managed to clear the tip of the foot from the belt as seen in the trajectory of the foot point in Fig. 1C. Thus by very small step-by-step adjustments that tend to get blurred in the averaging process, the animal managed to clear the foot from the ground. In cat 2, the changes observed were somewhat more pronounced. For example, both before and after neurectomy, the hip reached a maximum extension of ~120° but reached a peak flexion of 85° after neurectomy compared with 95° before. Similarly, the overall peak-to-peak excursion of the knee was 33° compared with 27° before neurectomy. In cat 3, the total peak-to-peak excursion of the hip was increased by 4.5° and the knee by 5–6°. These changes were consistent and were augmented with increasing speed of the treadmill.

In Fig. 2, A and C, the trajectory of the foot marker below the stick figures also shows a somewhat greater vertical component after neurectomy. The vertical position of the toe point or the marker placed on the MTP joint was measured before and after neurectomy can be superposed, although during the swing phase for the three cats, and it was found that the foot was raised up to 5–7 mm more during swing than in the control period. In summary, then, it appears that through the combined effects of small increases in the flexion of hip and/or the knee, the animals could raise the paw a few millimeters higher during the swing to compensate for the reduced ankle flexion.

Figure 3 shows the averaged EMG of >110 cycles taken in different control recording sessions and a similar number of cycles postneurectomy at a speed of 0.7 m/s and mainly serves to illustrate the overall envelope of the EMG bursts. There was a marked increase in amplitude of all flexor bursts on the ipsilateral side whereas the changes in contralateral flexors were minimal (coIP, coSrt) or absent (coSt). It should be noted that St had a small second burst of activity.

**Fig. 1.** Kinematics and electromyographic (EMG) activity of locomotion at 0.4 m/s before and after neurectomy of tibialis anterior (TA) and extensor digitorum longus (EDL) in cat 1. In A, 2 consecutive cycles from control period are illustrated. For hip, knee, and ankle, downward deflection of traces indicate flexion whereas for metatarsophalangeal (MTP), upward deflection indicates plantar flexion. Note that for knee, apparent flexion occurring during stance is due to a gradual slippage of joint marker due to skin movements. i, paw contacts, †, paw lifts, ——, stance period. Vertical lines are aligned with downgoing arrows. In B, similar displays taken from 2 cycles 25 days postneurectomy. Gains of EMG amplifiers were same before and after neurectomy.
TABLE 1. Changes in overall cycle, swing, and stance duration

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cat 1</th>
<th>Cat 2</th>
<th>Cat 3</th>
<th>Cat 4</th>
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<tr>
<td>Control</td>
<td>23</td>
<td>18</td>
<td>72</td>
<td>9</td>
</tr>
<tr>
<td>Cycle</td>
<td>1,009 ± 83</td>
<td>1,004 ± 74</td>
<td>996 ± 107</td>
<td>1,010 ± 70</td>
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<tr>
<td>Swing</td>
<td>313 ± 32</td>
<td>257 ± 18</td>
<td>316 ± 42</td>
<td>302 ± 38</td>
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<tr>
<td>Stance</td>
<td>696 ± 63</td>
<td>747 ± 73</td>
<td>680 ± 90</td>
<td>708 ± 72</td>
</tr>
<tr>
<td>Postneurectomy</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>Cycle</td>
<td>953 ± 85</td>
<td>1,068 ± 79</td>
<td>851* ± 55</td>
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<tr>
<td>Swing</td>
<td>286 ± 44</td>
<td>305* ± 35</td>
<td>324 ± 40</td>
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<tr>
<td>Stance</td>
<td>667 ± 62</td>
<td>763 ± 61</td>
<td>527* ± 32</td>
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<td>n</td>
<td>18</td>
<td>NA</td>
<td>6</td>
<td>11</td>
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<tr>
<td>Cycle</td>
<td>621 ± 43</td>
<td>775 ± 172</td>
<td>726* ± 21</td>
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<tr>
<td>Swing</td>
<td>309 ± 43</td>
<td>226* ± 54</td>
<td>201* ± 18</td>
<td></td>
</tr>
<tr>
<td>Stance</td>
<td>312* ± 42</td>
<td>547 ± 151</td>
<td>526* ± 23</td>
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<td>Postspinal</td>
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<tr>
<td>n</td>
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<tr>
<td>Cycle</td>
<td>672* ± 16</td>
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<tr>
<td>Swing</td>
<td>243* ± 18</td>
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<tr>
<td>Stance</td>
<td>428* ± 18</td>
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Values of means ± SD are in ms. Changes are presented for all conditions in all cats walking at 0.4 m/s. The statistical significance (*P < 0.01) of changes in a given condition is established in relation to the immediately preceding condition.

Changes after spinalization

The changes in the locomotor pattern seen after spinalization were dramatic. Indeed, from previous experience (Barbeau and Rossignol 1987; Belanger et al. 1996), cats usually have a well-established regular and symmetrical locomotor pattern of the hindlimbs 3 wk after spinalization. In cat 1 (Fig. 5), 19 days after spinalization, on the lesioned side, the hip remained more control or less at 120 ± 130° while the knee performed large flexion movements (52 vs. 34° after neurectomy; see Table 2) powerful enough also to swing the ankle (46 compared with 13° postneurectomy; see Table 2). The MTP joint remained ventroflexed during the whole cycle, and the foot did not make any plantar contact. The activity in IP and St remained sustained throughout these prolonged swing phases whereas the activity in St was more discreet but much more prolonged than usual and certainly much longer than on the contralateral side. The ankle extensor GL started its discharge quite early before foot contact and was not maintained after foot contact, which was made on the foot dorsum. The locomotor pattern of the contralateral limb was not as well organized as normal presumably because of the very abnormal signals it received from the denervated limb. This resulted in a very asymmetrical locomotor pattern best seen in Fig. 5, which shows abnormally long swing phases (even longer than the stance phases) on the denervated side, and the reverse in the normal leg, which has to maintain weight for a greater proportion of time, the ipsilateral limb being incapable of supporting any weight. This asymmetry even increased as time progressed after spinalization (interlimb coupling going from $f_0.4$ to $f_0.8$). In cat 3, which also was submitted to the same sequence of neurectomy followed by spinalization, the results were very similar with a predominance of knee flexion (see Table 2). It is also worth noting that the disorganization of the movement is reflected in the very large SD, especially in cat 3.

This abnormal locomotor pattern and the asymmetry is well illustrated by the stick figures and averages of angular excursions shown in Fig. 5. During stance, the dorsum of the foot of the lesioned limb contacted the belt and the MTP joint plantarflexed. During swing, the more or less synchronous flexion at all joints results in an oval-shaped foot trajectory. On the intact side, the steps cycle were somewhat more normal as can be seen from the stick diagrams and foot trajectory (Fig. 5C). We will not attempt to describe these pathological locomotor patterns in much further details for each cat except to say that the walking pattern was, at times, even more abnormal with the lesioned limb performing two small incomplete steps for one step on the contralateral side.

The examples shown above were taken from the period where the cats had stable albeit abnormal locomotor performance. However, these anomalies could be seen earlier after spinalization. The first recording sessions were made 3–5 days after the spinalization. From previous experience, 5 days after spinalization cats have only small symmetrical rhythmic movements of the hindlimbs usually with the hip in extension (Belanger et al. 1996). In the cats presented here, the movements were disorganized and asymmetrical with frequent hyperflexions of the knee and hip on the lesioned side, and strong perineal stimulation was required to evoke movements. Only a few steps could be obtained at 0.2 m/s. To facilitate the induction of the locomotor pattern as early as possible after spinalization, clonidine (200 μg/kg ip) was injected (Barbeau et al. 1987); the results are...
FIG. 2. Kinematic of same control and neurectomy recording sessions as in Fig. 1. A and C: stick figures of 1 step cycle on treadmill together with trajectory of toe point for swing and stance. In this display, each figure of hindlimbs is displaced by an amount equal to linear displacement of paw between video fields so that horizontal calibration is larger than vertical one. B and D: averaged and standard deviation of angles calculated in 7 cycles before and 10 cycles after neurectomy are synchronized to left paw contact. Same step cycle is presented twice for more clarity. Philippson’s subdivisions of the step cycle (F-E₁-E₂-E₃) are presented on top.

TABLE 2. Angular maxima (Max), minima (Min) and differences (Dif) between Max and Min for the four joints in all cats for all conditions

<table>
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<tr>
<th>Cat</th>
<th>Number of Cycles</th>
<th>Hip MAX</th>
<th>MIN</th>
<th>DIF</th>
<th>Knee MAX</th>
<th>MIN</th>
<th>DIF</th>
<th>Ankle MAX</th>
<th>MIN</th>
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<td>167</td>
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Values are means ± SD in ms. All values were obtained from the reconstructed kinematics of several cycles recorded on video tape at 0.4 m/s. MTP, metatarsophalangeal.
Fig. 3. Averaged EMG recordings before and after neurectomy at 0.7 m/s. Averages are taken from 3 control sessions (means ± 1 SD of 37–113 cycles depending on EMGs) and 3 postneurectomy sessions (days 7, 26, and 46 postneurectomy). Averages are triggered on ipsilateral flexor semitendinosus muscle (iSt) and are normalized with respect to time and are repeated for 2 cycles.

Presented in Fig. 6. Whereas the nonlesioned side performed rather normal movements (not illustrated), the lesioned side illustrated here had very abnormal movements. As can be seen in the stick figures, the knee performed brisk hyperflexion movements more or less synchronous with hip flexions so that the limb was not brought forward and the paw landed on the dorsum of the toes and remained in that position for the rest of the stance, much as it was seen later without drugs as described above. The average EMG traces compares the activity in the control period (before neurectomy) and after neurectomy and spinalization. Very large almost synchronous bursts of activity are seen in all flexor muscles. Note that the activity in coSt starts at about 0.5 and peaks at 0.7, somewhat later than before spinalization, indicating that some symmetry was preserved at this early postspinal stage. This example thus illustrates that the abnormal movements described in previous figures, taken at day 19, already were present 6 days after spinalization, well before the cat...
FIG. 4. Two consecutive cycles taken 19 days after spinalization of a neurectomized cat. Presentation is the same as in Fig. 1 except for duty cycle shown here for 2 hindlimbs. Note marked asymmetry between 2 sides.

had any significant locomotor training on the treadmill. We have repeated this experiment three times in cat 3 and essentially obtained the same results. We thus could conclude that the abnormal pattern seen at a later stage was not due to an abnormal training because of the neurectomy but rather that the abnormal pattern was there very soon after the spinalization, suggesting that clonidine merely expressed an abnormal locomotor pattern already extant in the spinal cord.

The evolution of the EMG patterns as recorded with the same gains over the three conditions, i.e., control, postneurectomy, and spinal-postneurectomy at the same speed (0.4 m/s) is illustrated in Fig. 7. Activity of the ipsilateral flexor muscles was increased substantially after neurectomy whereas the contralateral St remains similar in both amplitude and timing. Activity of the extensor muscles also was increased, and there was more overlap between the extensor activity of both legs. After spinalization, there is not only a marked asymmetry (see displacement of coSt burst relative to iSt), but the flexor muscles showed a greater increase in amplitude and duration, and there was a substantial cocontraction between the hip flexor muscles and ankle extensor muscles. The combined timing and amplitude changes for the three conditions are illustrated more quantitatively in Fig. 8 for cat 1 at a speed of 0.4 m/s, a speed at which all conditions could be compared.

To evaluate the effect of the ankle flexor neurectomy in a spinal cat, the neurectomy was performed in cat 4 after it had been spinalized and had recovered hindlimb locomotion. Figure 9A shows one normal step in the control period with stick diagrams, trajectories, angular displacements, and EMGs. After spinalization, the swing was very smooth and the stance is shorter compared with normal, an common observation in spinal cats (Belanger et al. 1996). After neurectomy, the locomotor movements were symmetrical, regular, and smooth albeit with only very small flexion of the ankle and absence of plantar foot contact. The large knee hyperflexions seen in the spinal cats that had undergone neurectomy before spinalization were absent in this cat neurectomized after the spinalization. This suggested that the anomalies seen in the former two cats reflected spinal changes that had occurred during the period of adaptation before the spinalization.
DISCUSSION

Consequences of neurectomy

The results of these experiments show that, in normal conditions, cats adapted their locomotor pattern very well after the inactivation, by neurectomy, of two major ankle flexors TA and EDL. This adaptation involved small increases in knee and/or hip flexion just enough to clear the paw well above ground during the swing phase. The kinematic compensation was subtle and difficult to perceive by visual observation only. The EMG analyses showed that there was an increase in the knee flexor St and hip flexors IP and Srt. Therefore, the compensation is achieved mainly by changes occurring at more proximal joints, as described also by others. It indeed has been reported previously, although not analyzed in any detail, that after section of the whole common peroneal nerve, cats had a pronounced foot drop and compensated through a greater hip flexion (Gordon et al. 1980). In other circumstances, compensation also could take place in synergist muscles. For instance, in rabbits, progressive section of the tendons of ankle plantarflexors on one side led to a prolongation of the stance, hyperflexion during swing, and landing on the heel (Stewart 1937). When one of the muscles was biomechanically disconnected after tenotomy, other synergist muscles compensated so that several muscles have to be removed or tenotomized before any biomechanical changes could be seen in the locomotor pattern. Similar compensatory mechanisms have been described after the section of nerves to specific ankle extensors (Gordon et al. 1980; Wetzel et al. 1973; Whelan et al. 1995). Although TA and EDL are considered as the main flexors of the ankle, peroneus longus, which was not denervated in our experiments, is also an ankle flexor (Lawrence et al. 1993; Nichols 1994) with a significant moment arm in the plane of flexion when the ankle joint is flexed already such as during swing (Young et al. 1993). This might account for the fact that we still could measure ankle flexion during swing although some of this flexion also might be due to inertial forces, especially when the hip flexion movements were very brisk. This residual flexion was, however, insufficient to compensate for the inactivation of TA and EDL over the time span of the experiments, and therefore compensation from the more proximal flexors, as described above, was needed.

The section of a motor nerve also has sensory consequences. Denervation of TA and EDL eliminates potentially important sensory information from these muscles. Recent data show that stimulation of afferents from TA, possibly from group I and group II afferents, during the flexion phase of fictive locomotion, can reset the cycle to extension (Perreault et al. 1995). It was postulated that these effects could be due to group II spindle secondaries. It then should be
expected that, normally, stimulation of these afferents during stance where flexor muscles are stretched would provide some positive feedback to extensors and prolong the stance phase (indeed described for Srt stimulation but not for TA and PBSt). In absence of these afferents, this mechanism could participate in the decrease of the step cycle mainly due to a shortening of the stance phase seen in one of the cats at least in the first few weeks postneurectomy.

Functional plasticity

The above results show that cats adapted their locomotor pattern very well after the denervation of ankle flexors through compensatory movements of other joints. How are these compensatory changes achieved? There are several lines of evidence suggesting that changes in the state of the neuro-muscular apparatus will trigger anatomic and physiological changes in various structures. For instance, transposition of nerves from muscle containing different populations of muscle unit types can lead to changes of the distribution of unit type in the muscles such that slow motor units can be converted to fast units (for review, see Buller and Pope 1977). After nerve transposition, new monosynaptic connections are made between the axons originating from a gastrocnemius or plantaris nerve with peroneus longus motoneurons (Eccles et al. 1960, 1962). The distribution of afferents and efferents to transposed muscles in the various lumbo-sacral roots is also changed (Gordon et al. 1986).

Despite these changes in the reflex circuitry, a number of experiments suggest that there is little functional plasticity in locomotor mechanisms in very challenging situations such as after nerve or muscle transpositions. After transposing the gastrocnemius (G) muscle, an ankle extensor, to the distal tendon of the ankle flexor tibialis anterior (TA), it was found that the gastrocnemius, which normally discharges during stance, continued to discharge in stance despite its being now an ankle flexor (Forssberg and Svarten-gren 1983; Gordon et al. 1986). This was taken as evidence that the basic spinal locomotor circuitry had no significant plasticity, in support of the notion of an innate hardwired locomotor network. This is in agreement with the classical work of Sperry on tendon transposition in rats. After transposition of ankle antagonists muscles (Sperry 1941) or nerves (Sperry 1940) in the hindlimbs or muscles and nerves in the forelimbs (Sperry 1942), there was no adaptation even several months after the transposition and despite various attempts at training to force the proper use of the transposed muscles. Even during basic motor behaviors such as locomotion, rats contracted the gastrocnemius during stance, causing the ankle to flex. Similarly during swing, TA contracted and extended the ankle. Although the transposed muscle apparently did not adapt, other muscles at the thigh and hip.

**FIG. 6.** Kinematic and EMG recordings pre- and postneurectomy in cat 1, 6 days after spinalization and with 200 µg/kg ip of clonidine. A: stick figure of swing indicates large flexion of knee and hip. B: trajectories of marker points. C: kinematic analysis of 8 cycles is triggered on forward movement of paw although paw has not lifted yet because of foot drag. D: EMGs of control (prespinalization) and postspinalization postneurectomy are superimposed and triggered on onset of iSt, which is close to but not synonymous to onset of swing as seen in Fig. 1A.
modified their contraction pattern to prevent scraping the foot on the floor during swing. Sperry concluded that rats showed no plasticity in the function of transposed muscles. On the other hand, monkeys eventually were able to learn, several months after a transposition of elbow antagonist muscles to use the transposed muscles properly in certain particular tasks (Sperry 1947), suggesting that primates, including man, have more potential for plasticity than rats. Similar findings were made after muscle transposition in the cat forelimb in which some adaptive discharges were found (Yumiya et al. 1979). For instance, the palmaris longus muscle, which normally discharges during stance, now is active mainly during swing and only little during stance.

There is thus conflicting evidence on whether or not the CNS is capable of sufficient plasticity to reroute its command signals so that transposed muscles can be recruited in accordance with their new and opposite biomechanical functions. This is a very demanding situation, which may be at the very limit of adaptive mechanisms. On the other hand, it is clear, as shown in the present results, that it may be a more economical strategy to develop compensatory movements of other joints to maintain the locomotor function when one joint cannot function properly, as is the case here, with the drastic reduction of ankle flexion due to the denervation. The question then is where are these compensatory changes organized and controlled?

**Sites of functional plasticity**

Whereas the cats had adapted very well to the neurectomy when otherwise intact, the same cats showed a disorganized locomotor pattern soon after spinalization. The gait was characterized by irregular cycles with marked hyperflexions especially of the knee. The synchronous hip and knee flexion did not bring the limb forward but the limb rather was stepping in place. There was no plantar foot placement, and therefore the weight was supported mainly by the other hindlimb, leading to a quite asymmetrical gait. On the other hand, when the neurectomy was performed in a spinal cat that already had recuperated a stable locomotion, the locomotor pattern remained rather symmetrical and regular although there was no significant ankle flexion nor proper plantar foot contact. In other words, neurectomy after spinalization did not have the remarkable disorganizing effect that spinalization had on cats having recuperated from the same neurectomy when their spinal cord was intact. We interpret these data to mean that, when an animal modifies its locomotor pattern to compensate for a lesion of its neuro-muscular peripheral apparatus, long-term changes occur both at the spinal and the supraspinal levels. We postulate that, initially, step-by-step and asymmetrical descending signals from supraspinal structures are provided to the spinal cord to compensate for the peripheral deficits. These may, after some time, induce some long-term changes in the spinal cord that, for instance, could subserve the hip/knee hyperflexion seen as one of the main compensating mechanisms. Where could these changes occur? Part of the compensation, such as the increased flexion of the knee and/or the hip joints, as well as the subsequent placement of the paw may result from compensatory supraspinal inputs, such as the motor cortex, which has a cyclical discharge and is especially active during voluntary gait compensation, such as stepping over obstacles (Armstrong and Drew 1984, 1985; Drew 1993; Widajewicz et al. 1994; Yumiya et al. 1979). Given the extensive

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**Fig. 7.** Comparison of EMGs in control, neurectomized, and neurectomized-spinal states of cat 1. These values were taken from several step cycles (see Fig. 8 for number of cycles values). All gains are the same for each muscle in the 3 conditions.
FIG. 8. Bar diagram of EMG recordings in control, neurectomized, and spinal states at 0.4 m/s. EMG bursts are displayed as horizontal rectangles whose width represents average onset and offset of bursts + 1 SD in 1 normalized step cycle and whose height represents normalized amplitude of burst (normalized amplitude = integrated burst divided by its duration). n values beside muscle names indicate numbers of burst used to calculate these averages.

convergence of supraspinal and peripheral afferent inputs on spinal interneurons (Baldissera et al. 1981), it is interesting to postulate that long-term facilitation mechanisms in the cortico-spinal pathway (Iriki et al. 1990) could lead to long-term changes in the excitability of interneurons closely related to the spinal pattern generator. Increased descending inputs on the side of the lesion could induce changes in excitability that could persist after spinalization, leading to the abnormal hyperflexions seen at the hip and knee.

When these correcting supraspinal signals are removed by spinalization, the spinal pattern is expressed on its own without descending compensation. This pattern clearly expresses hyperflexion of the more proximal joints on the ipsilateral side and is asymmetrical. On its own, this modified
FIG. 9. Kinematics and EMGs recorded from cat 4 during control, post spinal (day 21), and spinal + neurectomy (day 29) treadmill locomotion at 0.4 m/s. A, D, and G: stick figures of swing and stance of a representative step cycle. Related trajectories of different joint markers are illustrated below each stick figure. B, E, and H: average of angular displacement of hip, knee, ankle, and MTP joints in 6–10 consecutive cycles. Normalized step cycle starts at left foot contact and is displayed twice. C, F, and I: related EMG activity presented after averaging ($n = 5–10$) and normalization. After spinalization, cat recuperated an organized, symmetrical locomotion. Note that sartorius anterior (Srt) muscle also displayed some activity in extension. After neurectomy cat continues to walk regularly but lacks ankle flexion. Note that Srt pattern has come back to a pattern closer to control period.
spinal pattern is, however, neither adequate nor sufficient to compensate for the peripheral lesion. The conclusion that the spinal cord has been modified on a long-term basis was reached because when the neururectomy is performed after spinalization, at a time when the cat has recuperated a stable locomotor pattern, the spinal pattern expressed does not include the significant ipsilateral hyperflexions of the proximal joints nor the marked asymmetry seen in the other cats that were spinalized only after the neururectomy. Their locomotor pattern is basically the one seen in normal (i.e., without neururectomy) cats after spinalization except that there is only little ankle flexion and therefore an improper foot placement.

Do the changes observed in cats spinalized after neururectomy represent some form of spinal “learning”? The idea of spinal learning has been around for many decades (see Sperry 1945; Wolpaw 1994 for review of early and more recent literature). There are indications that the monosynaptic reflex can change with operant conditioning and that these changes persist after spinalization (Wolpaw 1994). Similarly, there are indications that the flexion reflex can be conditioned classically in spinal cats (Durkovic 1975, 1996). Finally, the spinal cord appears capable of “learning” more complex and specific tasks (Hodgson et al. 1994). The evidence presented here does not suggest that the spinal cord has “learned” a complete locomotor strategy for the denervation of the pretibial muscles because the movements performed in the spinal state are inadequate and do not compare with the fine compensation seen before spinalization. However, these experiments do suggest that some changes have occurred in the spinal cord of cats, which previously had adapted to the lesion, because the locomotor pattern expressed after the spinalization is quite different from the pattern normally expressed by spinal cats and also quite different from the locomotor pattern seen when the denervation is performed after the spinalization.

In summary, this work suggests that there is some plasticity in the spinal locomotor network and that this plasticity could perhaps be used within the context of locomotor rehabilitation in patients with spinal injuries as suggested in the INTRODUCTION. Whereas the specific spinal circuitry involved in the control of specific muscles and their antagonists may have to be hardwired as suggested above, other control mechanisms can be modified to preserve the overall function of locomotion. Whether functional locomotor plasticity can be achieved after complete spinalization remains to be elucidated. The present work, however, suggests that such spinal plasticity could be meaningful after incomplete spinal cord lesions, which leave some of the descending command tracts intact and potentially capable of producing long-term changes in the spinal cord.

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