Recovery of Locomotion After Ventral and Ventrolateral Spinal Lesions in the Cat. I. Deficits and Adaptive Mechanisms

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Brustein, Edna and Serge Rossignol. Recovery of locomotion Rossignol 1994 . After a complete spinal transection at the low thoracic level, adult cats can walk with the hindlimbs and adaptive mechanisms. J. Neurophysiol. 80: 1245 ± 1267, 1998. on a moving treadmill, with weight support of the hindquarters and proper plantar foot placement. This indicates that the spinal cord, together with peripheral afferents, in isolation from descending supraspinal inputs, can generate hindlimb locomotion (Barbeau and Rossignol 1987; Bélanger et al. 1996; Grillner 1981; Rossignol 1996; Rossignol et al. 1996). With the addition of an intensive training program and/or injection of noradrenergic agonists, these locomotor capacities can be expressed earlier and better (Barbeau et al. 1993; Barbeau and Rossignol 1987, 1991; Chau et al. 1998). However, the hindlimbs’ locomotion remain limited; it is not initiated voluntarily and it cannot obviously be expressed in coordination with the forelimbs. In addition, there are often deficits such as paw drag during the swing (Bélanger et al. 1996).

Because in humans, the percentage of partial versus complete spinal lesion is increasing in the population (Tator et al. 1993), there is a need for a better understanding of such conditions. The work of colleagues (Jiang and Drew 1996) has already shown that lesions limited to the dorsolateral pathways result in prolonged steps, paw drag, and an inability to step properly over obstacles, but, otherwise, there are only transient deficits in posture and interlimb coupling, suggesting that ventral and ventrolateral pathways are capable of controlling quadrapedal voluntary locomotion.

The present complementary work aims at describing the consequences of lesions to the ventral and ventrolateral parts of the spinal cord that carry pathways such as the vestibular and the reticulospinal, while leaving the dorsolateral ones intact. Former studies in chronic adult cats (Afeet 1974; Eidelberg 1981; Eidelberg et al. 1981; Windle et al. 1958) have suggested that pathways in the ventral and ventrolateral quadrants are essential for the recovery of voluntary quadrapedal locomotion (for more detailed review see Rossignol et al. 1996). These conclusions are, however, mainly based on spinal lesions, sparing only small patches of tissue in the ventral and the ventrolateral quadrants that were found to be sufficient to sustain locomotion. Similarly, in the acute decerebrate cat (Noga et al. 1991; Steeves and Jordan 1980) it was demonstrated that initiation of locomotion by stimulation of the mesencephalic locomotor region (MLR) depends on the integrity of ventrolateral tracts containing the reticulospinal pathways. However, there are now mounting evidence in the cat (Gorska et al. 1990, 1993a,b), in the monkey (Vilensky et al. 1992), as well as in human (Nathan 1994).

INTRODUCTION

It is of major clinical importance to identify and understand the adaptive locomotor capacities of the nervous system after spinal cord injuries if one is to design and implement rehabilitation strategies for locomotion (Barbeau and Rossignol 1994). After a complete spinal transection at the low thoracic level, adult cats can walk with the hindlimbs on a moving treadmill, with weight support of the hindquarters and proper plantar foot placement. This indicates that the spinal cord, together with peripheral afferents, in isolation from descending supraspinal inputs, can generate hindlimb locomotion (Barbeau and Rossignol 1987; Bélanger et al. 1996; Grillner 1981; Rossignol 1996; Rossignol et al. 1996). With the addition of an intensive training program and/or injection of noradrenergic agonists, these locomotor capacities can be expressed earlier and better (Barbeau et al. 1993; Barbeau and Rossignol 1987, 1991; Chau et al. 1998). However, the hindlimbs’ locomotion remain limited; it is not initiated voluntarily and it cannot obviously be expressed in coordination with the forelimbs. In addition, there are often deficits such as paw drag during the swing (Bélanger et al. 1996).

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that long-term recovery of voluntary locomotion is possible despite extensive lesions of the ventral and lateral quadrants, even if there are major locomotor deficits such as disrupted interlimb coupling (Gorska et al. 1993a).

In these studies, however, the period of gradual recovery of locomotion was only briefly mentioned, and none have analyzed and compared the deficits observed during the recovery period to those observed long term postlesion, after reaching a stable locomotor behavior (plateau period), or followed their evolution with time, nor have appropriate histological analysis been done to evaluate the extent of the spinal lesion. The locomotor deficits expressed in early days after the lesion probably reflect primarily the lack of the normal contribution of the lesioned pathways to locomotion, whereas the long-term deficits reflect the limit of the compensation that can be achieved by remaining pathways to the recovery of locomotion.

The evaluation of such a recovery process requires chronic recording methods. Therefore cats were implanted with chronic electromyographic (EMG) electrodes, which allows one to identify changes in EMG activity under constant recording conditions in the same cat before and after a lesion restricted to the ventral and ventrolateral pathways and document the locomotion characteristics both during the recovery period and long after achieving a stable locomotor behavior. The EMG activity was recorded daily and was synchronized to video images to allow detailed kinematic analysis. It also seemed important to evaluate more precisely the extent of the spinal lesion. Because remaining viable axons of descending pathways cannot be identified by inspection of histological sections only, wheat germ agglutinin–horseradish peroxidase (WGA-HRP) was injected caudal to the site of lesion at the end of the recovery period, and labeled cells were counted in the brain stem nuclei and in the motor cortex, the origin of descending corticospinal pathway.

It will be shown that, after small lesions, the locomotor deficits are minimal, but after large spinal lesions, cats can still initiate voluntarily quadrupedal locomotion. The step cycle structure of the individual limb is minimally affected, but there are severe locomotor deficits such as perturbed fore- and hindlimb coupling. There are also major changes in the adaptation of the hindlimb locomotion to more demanding situation such as walking on slopes. However, the cats can still perform such task through a greater use of the forelimbs.

**METHODS**

**General experimental protocol**

Experiments were carried out on eight adult cats (2.5–4.8 kg) that were trained to walk on a treadmill at different speeds (0.2–0.7 m/s) and with different inclines (10° uphill and downhill). When the cats could consistently maintain regular locomotion for periods of ~20 min, they were chronically implanted with electrodes to record EMG activity from fore- and hindlimb muscles. After recovery from surgery, 7–12 control experiments were made over a 1- to 6-wk period to determine the control (intact) EMG and kinematic values. Thereafter, the cats were submitted to the ventral-ventrolateral spinal lesion at thoracic level (T11 or T12), and their locomotor recovery was followed and documented daily. No special training program was applied, and the recordings on the treadmill started when the cat could walk voluntarily with all four limbs at a minimal treadmill speed of 0.1 m/s. When the cats did not show any further locomotor improvement (48–343 days postlesion, depending on the extent of the lesion), WGA-HRP was injected caudal to the site of the spinal lesion (L4). Then, 3 days later, the cats were perfused and the spinal cord, i.e., the site of HRP injection, and the site of lesion were taken for histological processing, as well as the brain stem and the motor cortices. All the surgical procedures and experimental protocols were reviewed and approved by the University Ethics Committee.

**Surgical procedures**

**EMG IMPLANTATION.** The bipolar EMG electrodes were chronically implanted under sterile conditions. First, the cats were premedicated with acepromazine maleate (Atarvet, 0.1 mg/kg sc), Atropine (0.05 mg/kg sc), and Penicillin G (40,000 IU/kg im) and then anesthetized with pentobarbital sodium (Somnotol, 35 mg/kg iv). Two 15-pin connectors (TRW Electronic Components Group, Elk Grove Village, IL) were fixed to the skull, and 14 pairs of Teflon-coated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA), soldered to the connector pins, were directed subcutaneously to different muscles acting around the fore- and hindlimb joints. The portion of the wire that was inserted into the muscle belly was exposed for 1–2 mm and then fixed in situ by a silk thread. The EMG electrodes were implanted in the following muscles, listed according to their main function: in the hindlimbs, the hip flexors, ilioptos (Ip) and sartorius (Srt); the knee flexor, semimembranosus (Sm); the ankle flexor, tibialis anterior (TA); the knee extensor, vastus lateralis (VL); and the ankle extensors, gastrocnemius medialis (GM) and lateralis (GL); in the forelimbs; the elbow flexor, cleidobrachialis (CIB), and the elbow extensor (lateral head), triceps brachii (TrL).

All muscles were implanted both on the left (L) and on the right (R) side of the animal. Usually the left side of the animal faced the video camera, except for cat EB5, which faced the camera from the right side. After the implantation, the cats were monitored closely. They were placed in an incubator to maintain body temperature, and an analgesic, buprenorphine hydrochloride (Temgesic, 0.005–0.01 mg/kg sc) was given every 6–8 h for 24–48 h. In addition, lactate Ringer dextrose 5% was administered intravenously. Twenty-four to 48 h later, the cat was returned to its individual cage and was given amoxicilline (Amoxil 22 mg/kg) orally twice a day for 10 days. In addition to implantation of EMG electrodes, two bipolar nerve cuffs were placed around the superficial peroneal nerve on each side and secured using a thin layer of impression material (light body viscosity Hydrophilic Vinyl Polysiloxane, Reprosil HF). These were used to stimulate the nerve at rest and during locomotion. In cats EB7 and EB8, a Teflon cannula (Teflon tube-thinwall, size 24 gauge) was inserted into the intrathecal spinal space at C1 and lowered so that the tip was located at L4–L5. The rostral end of the cannula was fixed into dental acrylic, next to the EMG connectors, and served to administer drugs intrathecally (these effects will be reported in a subsequent paper). The presence of the cannula is quite conspicuous in the cat EB7.

**VENTRAL AND VENTROLATERAL SPINAL LESIONS.** After the 7–12 control experiments (after the EMG implantation) the ventral and ventrolateral spinal lesion was performed. The procedure was done under general anesthesia as described above. A low thoracic vertebra was exposed, T7, for cats EB1–6 and T11 for cats EB7–8, and two small openings were drilled through the vertebral pedicles to approach the spinal cord laterally on both sides. After making a small incision in the dura, a microknife and/or a small forceps were inserted through the openings to lesion the ventral half of the
spinal cord while leaving the dorsal portion intact and protected by the overlying laminae. Afterward, the incision was closed in anatomic layers. The postoperative cares were the same as after the EMG implantation. It must be noted that all cats, in contrast to the complete spinal cats where bladder had to be expressed manually (Bélanger et al. 1996), controlled their micturition and did not need manual voiding.

Recordings and data analysis

EMG activity and the video images used for kinematics analyses were recorded simultaneously and were synchronized using a SMPTE time code (time-code generator Skotel TCG-80N and time-code reader TCR-80N).

EMG. The amplified and filtered (100 Hz to 3 kHz) signals were recorded on an analog VHS tape recorder (Vetter 4000A PCM Recording Adaptor with cutoff frequency of 1.25 kHz) and later played back and printed out using an electrostatic polygraph (Gould ES-2000). Walking sections, representing the locomotor capacities of the cat at that day, were chosen for detailed analysis and were digitized at 1 kHz using an AT/486 computer.

KINEMATICS. All the walking sections selected for EMG analysis were also used for limb movement analysis as well as for defining the interlimb coupling. Six reflective markers (3 M reflective tape) were placed over the skin of the following bony landmarks: the rostral tip of the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal (MTP) joint and the tip of the third toe.

The cats were videotaped walking on the treadmill using a Panasonic digital 5100 shutter camera (resolution of 16.7 ms/field) and a videotape recorder (Panasonic AG 7300). The camera was adjusted to get a clear image (shutter between 1/500 and 1/1,000). The position (x-y coordinates) of the reflective markers was digitized offline using a 2D PEAK Performance System. The coordinates of the position of each one of the markers were used to calculate the joint angular displacement and to reconstruct stick diagrams of single or several consecutive step cycles. In addition to digitizing the position of the reflective markers, the SMPTE time codes engraved on the video film were also used to determine the exact time of paw lift and paw contact of each one of the four limbs. The time of each of these kinematics events was then used to calculate the duration of the step cycle, the swing, and the stance and to construct footfall diagrams for each one of the consecutive steps in the walking sequence. The footfall diagrams were grouped according to interlimb coupling types and then normalized and averaged. From the averaged and normalized footfall diagram, the percentage of time the cat supported its weight with two, three, or four limbs was calculated and presented in a table form (see Table 3).

All the recordings were reviewed and analyzed to determine the evolution of the recovery. However, we illustrate mainly representative data taken during the recovery period and from the plateau period when the cats were already walking at their best. These data were compared with those obtained in the intact state.

Histological analysis

HISTOLOGICAL EVALUATION OF THE SPINAL SITE OF LESION. The damage of the spinal lesion was evaluated by inspecting, under light microscope, the physical damage and the extent of cellular and fibrous necrosis in consecutive cross sections of spinal cord stained with Kluver-Barrera (cats EB6–EB8, 8 μm thick, 1 in 50 saved) or with Cresyl violet methods (EB1–EB5, 40 μm thick, 1 in 5 saved). Whereas with the first method myelinated axons can more easily be identified, in the latter it is very well possible under the microscope to identify regions of intact axons that appear as regular densely packed profiles in areas of the white matter. Following these observations the total extent of the damage was reconstructed, and its severity in each region was indicated using different graphic patterns as illustrated in Fig. 1.

WGA-HRP LABELING AND CELL DISTRIBUTION ANALYSIS. The method of WGA-HRP injection and the related histological procedure have been described earlier (Jiang and Drew 1996). Briefly, after a recording period of 48–343 days, when the cat showed no more locomotor improvement, WGA-HRP (2%) was pressure injected, a few segments caudal to the site of the spinal lesion, usually at L2. The penetrations were done in two parallel frontal planes that permitted the whole cross-section of the spinal cord to be covered with the WGA-HRP solution (total of 20–30 μl). Three days later, the cat was perfused with 2 l of phosphate-buffered saline 0.9%, 2 l fixative (glutaraldehyde 1.25%, paraformaldehyde 1%, sucrose 1% in phosphate buffer at pH 7.4), and 1 l buffered sucrose solution 4%. The whole brain was removed, the brain stem and motor cortices were cut sagitally (40 μm), and every third section (1/5 saved) was taken for precipitating the HRP reaction products using the tetramethylbenzidine (TMB) method (Mesulam 1978). The reaction was carried as well on cross sections (1/5 saved) taken from the site of injection to verify that it was completely covered with HRP reaction products. Then the HRP-labeled cells in the red nucleus and the lateral vestibular nucleus were counted in all the sections. Moreover, in the motor cortex and the reticular formation the location of the labeled cells was digitized as well (adapted from Matsuyama and Drew 1997).

Briefly, the contours of histological sections were traced using a pantograph and then digitized using Autocad. The digitized sections were centered and oriented according to anatomic coordinates (Berman 1968) using a custom-made software. The digitization of the cells position, directly onto the computer image, extended in the brain stem from the midline to 2.5 mm on each side and was done on alternating sections (every 240 μm). Then the cells were divided into two major groups according to stereotaxic coordinates (adapted from Matsuyama and Drew 1997). The first group (A0 to P6) corresponds to the pontine reticular formation (PRF), which includes the nucleus reticularis pontis oralis and nucleus reticularis pontis caudalis, whereas the second group (P6 to P11), corresponds to the medullary reticular formation (MRF), which encompassed the nucleus reticularis gigantocellularis and the nucleus reticularis magnocellularis. For the motor cortex, digitization was done in all the sections (every 120 μm) up to 10 mm from the midline (Jiang and Drew 1996). The values were compared with data from three intact cats (1-tail Student’s t-test), after verification of the normality of the distribution of the intact values using Kolmogorov-Smirnov test for goodness of fit with Lilliefors correction (Stephens 1986; Zar 1996). Missing values, resulting from technical problems in processing the perfused tissue, were identified in Table 1 as “not available” (NA).

In this paper, only counts are presented, whereas the detailed cells distribution in the brain stem nuclei and in the motor cortex will be presented in a forthcoming paper.

RESULTS

Evaluation of the extent of spinal lesions

To facilitate the description of the results, we have defined two groups of cats: EB1–EB4 and EB5–EB8. This grouping was based both on the extent of the spinal lesion (evaluated histologically) and on the cat’s locomotor capacities during
the recovery period and the plateau period when they were already walking at their best.

The reconstructed lesion sites for the eight cats used in this study (Fig. 1) are based on inspection of consecutive spinal cord cross-sections stained with Kluver-Barrera (EB6–EB8) or Cresyl violet methods (EB1–EB5) for physical damage at the site of the lesion, as well as for the extent of cellular and fibrous necrosis. The related counts of WGA-HRP-labeled neurons are summarized in Table 1, and the total counts are illustrated next to each lesion site in Fig. 1.

The sections are presented in an order (EB1–EB8) that reflects the gradual augmentation of the extent of the damage applied and the area it encompassed in the spinal cord. The first group (EB1–EB4) had incomplete lesions, restricted to the ventral or lateral spinal cord, whereas the second group (EB5–EB8) showed bilateral damage to both ventral and ventrolateral funiculi. It is important to note that in some of the cats (EB1, EB6, EB7, and EB8) a cavity (syrinx) developed so that the damage was eventually larger than initially intended.

The counts of HRP-labeled cells in the brain stem nuclei and the motor cortex completed the histological evaluation of the spinal lesion. The number of labeled cells in the pontine nuclei was taken as an indicator of the extent of the lesion to the pontine reticulospinal pathways whose axons descend mainly ipsilaterally and are located around the ventral median fissure. The number of HRP-labeled cells in the medullary reticular formation nuclei was taken as an indicator of the extent of lesion to the medullary reticulospinal pathways. This pathway descends bilaterally in the spinal cord; however, the ipsilateral contribution dominates (Brodal 1969; Kuypers 1981; Kuypers and Maisky 1975, 1977). Most of the MRF axons descend in the ventrolateral funiculus; however, they are found as well in the dorsolateral funiculus (Kuypers and Maisky 1977; Petras 1967).

For all 8 cats, the histology of all the cats is displayed in 4 sets of columns. On the left, photomicrographs of the cross-sections taken from the spinal site of lesion, stained with cresyl violet or with Kluver-Barrera, to demonstrate the maximal extent of the lesion. To the right of the photomicrographs, a schematic reconstruction of the site of the lesion, evaluated using light microscope. For cats EB1–EB5, the total extent of the lesion is projected on a spinal cord section taken more rostrally, after inspecting several consecutive sections, whereas for cats EB6–EB8 the schemes illustrate the maximal site of lesion to emphasize the deformation of the spinal cord. The various textures identify the extent of damage. Intact: myelinated axons (or axonal profiles) appeared normal under the microscope; Severe damage: absent or highly fibrotic tissue; Moderate damage: some myelinated axons (or axonal profiles) of normal appearance within fibrotic and gliotic tissue; Syrinx: cyst. L = left; R = right. The next 2 adjacent columns illustrate sagittal brain stem sections (left and right) and summarizes the total values of horseradish peroxidase (HRP)–labeled cells (percent of control) found in the lateral vestibular nucleus (LVN), pontine reticular formation (PRF), and medullary reticular formation (MRF) for each cat when available (see Table 1).
MRF are the same on the right and on the left (64%), despite of the asymmetric lesion to the ventrolateral funiculi, suggesting also the existence of spared axons in the mass of necrotic tissue observed on the left ventrolateral funiculus. The second group includes the most lesioned cats (EB5–EB8) in which a significant decrease ($P < 0.01$) in MRF cells is observed on both sides, together with a significant reduction ($P < 0.05$) in PRF cell counts to levels below 30% of control values, at least on one side. In cat EB6, for example, intact tissue was observed in the dorsal columns, in the left dorsolateral funiculus (DLF), as well as some patches in the right DLF. However, the ventral half of the spinal cord is highly deformed and occupied by fibrotic tissue and a large syrinx. Despite the massive deformation and fibrotic tissue, the number of labeled cells in the right PRF was at the level of control value. On the left, however, the lesion was much more severe because only 14% HRP-labeled cells were found in the PRF. In cat EB7, with a very extensive spinal lesion sparing the dorsal columns and parts of the left DLF, the decrease in cell counts is striking (up to total of 18 and 23% of the intact values on the left and on the right brain stem, respectively), suggesting severe damage to most of the reticulospinal axons. It should be emphasized that the design of our lesion, aiming at damaging ventral and ventrolateral quadrants and keeping the dorsolateral quadrants intact, will preserve a certain population of reticulospinal axons descending in the dorsolateral funiculi of the spinal cord (Petras 1967). The HRP analysis is not available for cat EB5, which died unexpectedly. However, based on the physical damage at the site of the spinal lesion and its locomotor capacities during the recovery and the plateau periods, it was included in the second group.

In addition to damaging the reticulospinal tracts, a more pronounced damage was observed, in all cats, to the lateral vestibulospinal pathways. The axons of this pathway pass ipsilaterally in the ventromedial aspect of the ventral funiculus and originate in the lateral vestibular nucleus (LVN) (Brodal 1969; Kuypers 1981). The number of HRP-labeled cells in the LVN decreased remarkably to 0–33% of the intact values. In cat EB2 we have found only 13 and 10% of labeled cells in the right and left LVN, respectively. In cat EB7, however, 0.5% labeled cells were identified in the right LVN whereas only 0.1% were counted in the left LVN. The very low number of labeled cells in LVN corresponds to a real disruption of this pathway and was not related to a methodological artifact, because the spinal cord cross-section at the site of injection was completely filled with HRP reaction products, and there is a considerable number of heavily labeled cells in the PRF, whose axons descend in adjacent spinal cord areas.

As already mentioned, the aim of this study was to lesion the ventral and ventrolateral pathways while preserving important ascending and descending pathways in the DLF such as the rubrospinal and the corticospinal. Because both rubro- and corticospinal tracts are mainly crossed (Brodal 1969; Kuypers 1981), the HRP labeling in the red nucleus and motor cortex were taken as markers of the integrity of the contralateral DLF. In all cats, both the rubrospinal and the corticospinal pathways were somewhat affected by the lesion, as indicated in Table 1. For example, 1,486 HRP-labeled cells were counted in the right red nucleus of cat EB2, whereas on the left, 1,420 labeled cells were found. These values correspond to 63 and 60% of the intact values. In cat EB6, which had a very extensive lesion (including extensive damage to the right DLF) 2,013 labeled cells were found in the left cortex corresponding to 66% of the intact values. However, on the right cortex 4,155 cells were counted, i.e., to 136% of the control values. Such a phenomenon of pronounced increase in the number of labeled cells was also observed in the right motor cortex of cat EB7, which showed an even more outstanding increase to 192% of control values. The increase in the total number of labeled cells in the cortex of EB6 and EB7 was also accompanied by changes in the distribution of the cells. In the normal cat (Brustein et al. 1997; Jiang and Drew 1996) the maximal number of cells is found around the lip of the caudal bank of the cruciate sulcus and more laterally as well on the rostral bank. In cat EB7, however, in addition to the normal cell distribution, labeling appeared as well more laterally around the fundus of the cruciate sulcus and, in cat EB6, labeling appeared more medially on the rostral bank.

General remarks on the recovery of locomotion

Surprisingly, all the cats eventually recovered voluntary quadrupedal locomotion overground and on the treadmill. However, early after the lesion, the cats could not walk or even support their hindquarters and strolled around using their forelimbs only. For the group of cats, EB1–EB4, with relatively moderate lesions, this period was limited to 1–3 days. However, for the group of cats EB5–EB8, with most extensive lesions, it extended over >3 wk. Afterward, during the recovery period, the cats gradually regained some weight support of the hindquarters and the ability to stand and walk voluntarily with no external help. Yet, they all suffered from poor lateral stability and often fell on one side resulting in a wobbly, inconsistent walking for a few steps at a time. It is interesting to note, however, that the cats did not have problems negotiating obstacles placed in their way overground, or on a treadmill.

We have found that a good general indicator of the locomotor recovery with time after the lesion was the maximal treadmill speed the cat could attain and maintain at least for a few step cycles, as illustrated in Fig. 2. The group of cats EB1–EB4, with less extensive lesions, recovered quickly, as noted from the steep slope of the curves, and could achieve treadmill locomotion speeds between 0.5 and 0.7 m/s within 10 days of the lesion. On the other hand, cat EB7, as an extreme example of the most lesioned group (EB5–EB8), had a lesion damaging all areas except the dorsal columns and parts of the left dorsolateral funiculus (see Fig. 1 and Table 1), started walking quadrupedally (with no external support) only after 34 days and followed a slow recovery as indicated by the more gradual increase of the curve. Furthermore, it could never walk at speeds higher than 0.4 m/s and even later, its performance regressed with time, maybe due to the progression of a syrinx. However, it should be remembered that, even then, treadmill walking in cats with the largest lesions (group EB5–EB8) was irregular and wobbly, sometimes with a wide base of support of the hindlimbs, a diagonal orientation of the trunk, and occasional stumbling. These deficits were mostly transient for cats EB1–EB4;
however, lateral swaying of the hindquarters was still evident for a few weeks postlesion even when they were already walking at their best.

The irregularity of the hindlimb stepping is illustrated in Fig. 3 using stick figures of the right hindlimb (cat EB5) constructed for several consecutive steps at 0.4 m/s, before the lesion, during the recovery and the plateau periods. In the recovery period the steps are highly variable in duration, and the cat tends to stumble and struggle to maintain the imposed speed. The cat often lifts the feet higher than normal, as seen in the increased paw trajectories. During the plateau period, however, stability is much better as indicated by more regular steps and smoother trajectories.

The step cycle duration and its variability (mean ± SD), measured from successive onsets of St (EB2, EB3, and EB6) or Srt (cat EB7), pre- and postlesion, are illustrated in Fig. 4. For cats with a moderate lesion, represented by EB2 and EB3, the average cycle duration is not changed postlesion, but there is a tendency of increase in its variability as reflected by larger SD values relative to the control (see especially cat EB2 tested with variance ratio test). The more severely lesioned cats, such as cat EB6, show a sustained decrease in the cycle duration long-term postlesion (see as well Fig. 7, A and B, for cat EB5) and the most severely lesioned cat, EB7, showed as well a day-to-day variability in its locomotor performance reflected by very large SD. However, because of the small number of steps performed (6–13) in each trial, the postlesion values were not always found to be statistically different from the control ones. A comparison between the average cycle duration of the left and the right hindlimb showed no statistical difference even in cats with an asymmetric lesion such as cat EB7 (see Fig. 4, cat EB7). Despite the variability of the cycle duration, regression analysis showed that there are no major changes in the relationship between the duration of the step cycle subcomponents (swing and stance) and the overall cycle duration, obtained, however, in a more limited range of walking speeds, as illustrated in Fig. 5 for the most lesioned cats, EB5–EB7. Some changes were observed in the slopes and their correlation values (see Table 2 for slope values and coefficients), but they affected mainly the swing (see especially cat EB6), whereas those of the stance stay generally high as before the lesion. However, a comparison between the slopes of the intact situation and the recovery period or the plateau period was not found to be statistically different in the studied cats, neither for the stance nor for the swing.

Intralimb coupling

The calculated joint angular displacement of the first step cycles illustrated in Fig. 3 for cat EB5 (representing the most lesioned group), from an intact and plateau period (88 days postlesion) trials, is presented in Fig. 6, A and B, respectively, with the averaged angle-angle plots (Fig. 6, C and D). The related raw EMGs are presented in Fig. 7. Notice that after the lesion, the cat’s step cycle is shorter (see as well Fig. 4), resulting in a greater number of steps for the same period of time. Some changes were observed in the magnitude of the angular excursion traces. For example, before the lesion, the hip excursion ranged, on the average, between 99 ± 1° and 130 ± 1.5° (mean ± SE), whereas 88 days postlesion, it was reduced (84 ± 4.1° to 105 ± 3.7°). Such changes were observed as well for the knee, ankle, and MTP joints and resulted in a general down displacement of the related angle-angle plots. Despite the excursion changes described above, the joint coupling is largely preserved. The only coupling change, which was specific to this cat, was observed for the knee relative to the ankle. A somewhat earlier extension of the knee relative to the ankle at the end of the swing results in an eight shape angle-angle plot.

Another important observation illustrated in Fig. 6 is the lack of paw drag at the onset of swing. Similarly, paw placement at the beginning of the stance was always on the plantar surface even during the recovery period (see Fig. 3).

Inspection of the related EMGs (Fig. 7) completes and supports our observations from the angular displacement analysis. The phase relations between the flexors RSt and RSrt are illustrated by heavy lines, aligned on the onset of RSt. The dashed lines in A and B are aligned on the onset of the bursts of the extensors RVL and RGM, to demonstrate their phase relationships. The burst relations of the extensors are not changed after the lesion. No changes were observed, as well, in the relations between the lift and the RSt burst onset, which always precedes the lift. However, the relations between the onset of the flexors RSt and RSrt is modified. In the control (intact) there is a constant delay in the onset
of RSrt burst relative to RSlt, whereas, after the lesion in cats EB5–EB7, they are activated almost at the same time. Yet this change in the bursting phase is not reflected as any detectable change in the coupling relations between the hip and knee, as already described above for their angle-angle plot. No such changes were observed for cats with smaller lesions (EB1, EB2, and EB4), which maintained the normal St-Slt burst onset relations after the lesion. This measure was not available for cats EB3 and EB8. The noticeable increase in the EMG amplitudes will be discussed separately (see EMG activity).

Interlimb coupling

It was demonstrated that the step cycle duration on the left and on the right hindlimbs is the same and varies together in the most extensively lesioned cats (see Fig. 4). Not only was the cycle duration the same, but, in addition, the lesion did not affect the alternating coupling pattern between the hindlimbs. This is demonstrated by the raw EMGs of the left and the right VL and the related footfalls illustrated for cat EB5 in Fig. 8, A, E, and I. In this example, the averaged phase difference between the right and left VL burst onset in the intact situation is maintained at 0.52, whereas for the recovery period and the plateau period it is 0.49 and 0.48, respectively. One should notice, however, that the individual phase onset values vary considerably after the lesion. This is easily seen from the consecutive phase values of the RVL burst onset (□) graphed in Fig. 8F.

One of the most pronounced deficits observed postlesion was a step-by-step inconsistent homolateral fore- and hindlimb coupling. The more extensive the lesion, the more perturbed were the coupling relations. Two examples are given to illustrate the most pronounced coupling changes. The first one is taken from cat EB5 (Fig. 8), which is also representative of the coupling deficits seen in cat EB6. Both of these cats improved their coupling deficits over time. The second example, taken from cat EB7 (Fig. 9), shows a perturbed interlimb coupling that did not improve even long term after reaching the plateau period. The coupling relations between the homolateral fore- and hindlimb are illustrated by comparing the phase of burst onset (relative to St) of the hindlimb extensor (VL) and the forelimb extensor (TriL) in the intact situation, during the recovery and the plateau periods, for up to 30 consecutive step cycles at 0.4 m/s. The intact cat maintains a phase difference of 0.2 on average (Fig. 8B). These constant phase relations are also indicated by the dashed lines connecting the related footfall (Fig. 8A). During the recovery period, cat EB5 (Fig. 8F) shows perturbed step-to-step interlimb coupling that is manifested in phase shifts ranging between 0 and 0.64.

To further describe the gradual and cumulative phase difference during the stepping sequence, a “cumulative difference” value was used (Fig. 8, D, H, and L). First, the phase
FIG. 4. Step cycles before and after lesions: the graphs of the average step cycle duration (mean ± SD), as a function of time after the spinal lesion are illustrated for cats EB2, EB3, EB6, and EB7. The shaded area represents the cycle duration ± SD of 3 control (Intact) experiments. The number of steps used for the calculations varied between 68 and 127 in the control experiments and between 5 and 40 following the lesion, depending on the cat’s walking deficits. L = left, R = right; *, results of t-test between the mean step cycle duration pre- and postlesion; *, results of variance ratio test between the variance pre- and postlesion (○ and *, $P < 0.05$; ○○ and **, $P < 0.01$).
of VL burst onset was calculated relative to TriL. The value obtained for the first step cycle was used as the reference, which was then subtracted from each of the values of the following step cycle. Thus a value of 0 is expected when the phase relations of VL versus TriL in one cycle is the same as for the reference. A value of 1 means that the hindlimbs deviated from the forelimbs by a whole step cycle. As predicted, the “cumulative phase difference” for the intact cat, with constant limb coupling, shows small fluctuations around 0 (Fig. 8D). However, postlesion, this value varies much more, but always remains in the range of one phase, i.e., within one cycle (Fig. 8H). Thus, for each step of the hindlimb, there is one step of the forelimb, and the 1:1 step cycle relationship is preserved. During the plateau period, when the cat is already walking at his best, it adopts a preferential homolateral in-phase coupling in which the fore- and the hindlimb of the same side are placed at the same time on the walking surface, as illustrated by the dashed lines in the footfalls of Fig. 8I. This is reflected as well in the cumulative phase difference values, which stabilize around 0 (Fig. 8L).

Cat EB7 (see Fig. 9) shows a more perturbed fore-hindlimb coupling, and it does not recuperate even long term postlesion (>300 days). There is a gradual phase deviation between fore- and hindlimb, which is illustrated by the cumulative difference (Fig. 9D) and the related EMGs and footfalls in Fig. 9E. For about every five hindlimb step cycles, the forelimb drifted from the hindlimbs for a whole step cycle. Thus, after 10 step cycles performed by the hindlimbs, the forelimbs have made 2 more steps as illustrated in Fig. 9E by the step cycle numbers on the footfalls. There is up to a 300-ms difference between fore- and hindlimb step cycle duration (see Fig. 9C), i.e., the hindlimbs and the forelimbs are walking at a different mean frequency (1 and 1.4 Hz, respectively). In contrast to cats EB5–EB7, cats EB1–EB4 as well as cat EB8 did not demonstrate such severe step-to-step fluctuations in fore- and hindlimb coupling. However, they all adopted some degree of homolateral in-phase coupling at least short term postlesion (see Table 3).

To further understand the gait modifications observed after the spinal lesion, footfall diagrams were constructed for each one of the cats for several consecutive step cycles, using kinematic events. After normalization and averaging, these footfall diagrams were compared pre- and postlesion for the number of limbs supporting the cat at each moment as well as to identify the predominant limb coupling pattern. Such a footfall diagram is shown in Fig. 10 (cat EB6), and the results are summarized for all cats in Table 3. In the control state, cat EB6 is supported mainly by two and three limbs (96% of the time) and by four limbs, in only 4% of the step cycle time. The associated coupling pattern between the homolateral fore- and the hindlimb, as already described using EMGs (see Figs.
TABLE 2. Results of the regression analysis between the cycle duration and the swing or the stance (see Fig. 4)

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Recovery Period</th>
<th>Plateau Period</th>
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</thead>
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<td>EB5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td>36</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Speed range, m/s</td>
<td>0.3–0.5</td>
<td>0.2–0.5</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Slope of swing</td>
<td>0.16 (0.80)</td>
<td>0.11 (0.27)</td>
<td>0.08 (0.28)</td>
</tr>
<tr>
<td>Slope of stance</td>
<td>0.85 (0.98)</td>
<td>0.87 (0.89)</td>
<td>0.91 (0.95)</td>
</tr>
<tr>
<td>EB6</td>
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<td></td>
<td></td>
</tr>
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<td>77</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>Speed range, m/s</td>
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<td>0.2–0.4</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>n</td>
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<td>16</td>
</tr>
<tr>
<td>Slope of swing</td>
<td>0.14 (0.63)</td>
<td>−0.012 (0.02)</td>
<td>0.33 (0.68)</td>
</tr>
<tr>
<td>Slope of stance</td>
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<td>1.01 (0.92)</td>
<td>0.66 (0.87)</td>
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<td>EB7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
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<td>245</td>
<td></td>
</tr>
<tr>
<td>Speed range, m/s</td>
<td>0.2–0.4</td>
<td>0.1–0.3</td>
<td>0.2–0.35</td>
</tr>
<tr>
<td>n</td>
<td>27</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Slope of swing</td>
<td>0.05 (0.28)</td>
<td>0.1 (0.4)</td>
<td>0.19 (0.7)</td>
</tr>
<tr>
<td>Slope of stance</td>
<td>0.94 (0.98)</td>
<td>0.89 (0.96)</td>
<td>0.80 (0.97)</td>
</tr>
</tbody>
</table>

Values in parentheses are correlations (r). Results of the regression analysis between the cycle duration and the swing or the stance, during the recovery period or the plateau period relative to the intact state (cats EB5–EB7). None of the values were found to be significantly different from control.

7 and 8), is on the average 0.2 in phase difference. After the lesion, there is a major increase in the time the cat is supported by four limbs: 17% (recovery period, day 77) and 33% (plateau period, day 141). At first, this is associated with a diagonal-like coupling type, in which the forelimb is placed almost at the same time as the hindlimb of the opposite side and later, during the plateau period, with homolateral in-phase coupling. Cat EB5 shows the same tendency of increased time supported by four limbs at a time. However, during the recovery period, it spent just 50% of the time in a diagonal coupling, whereas in the rest of the steps in the sequence, the coupling pattern was as in the control. The coupling pattern of cat EB7 was more variable. This cat spent one-third of the steps in the sequence in diagonal couplings, another third in homolateral in-phase couplings, and the rest as in the control. However, during the plateau period, only diagonal and in-phase type of coupling were observed. Cats EB1–EB3 as well as cat EB8 did not use the diagonal coupling pattern during the recovery period but adopted homolateral in-phase coupling, which, again, was associated with an increase in the time spent on four limbs. However, during the plateau period, only cat EB1 kept walking in homolateral in-phase pattern while cats EB2, EB3, and EB8 returned to control coupling and supporting limb pattern. This analysis was not applicable for cat EB4.

EMG activity

Comparison of the raw EMG activity, before and after the lesion (see Figs. 7–9), shows that generally the alternating activity between extensors and flexors of the same limb (see RSt and RGM of cat EB5 in Fig. 7 and LSt and LVL of cat EB7 in Fig. 9E) is maintained as well as the alternating activity between muscles of contralateral limbs such as RVL and LVL or RTrIL and LTrIL (see Fig. 8, A, E, and J). However, various changes were observed in the amplitude and burst duration of the hindlimb muscles after the lesion. These differed from one cat to another as well as with time postlesion. Two examples are given in Table 4 to demonstrate the variability of the pattern. The first example is taken from cat EB2, which represents the moderately lesioned cat group (EB1–EB4) in which the changes were mostly restricted to the recovery period. The second example is taken from cat EB6 and mostly demonstrates the changes after a more extensive lesion (EB5–EB8), which are sustained and observed also during the plateau period.

During the recovery period there was a significant decrease in the normalized amplitude of EB2 flexors to 38–71% of control values. A reduction was observed as well in the amplitude of the extensor LGM (48%), while VL amplitude on both sides increased. However, during the plateau period, only the increase in VLs amplitude and the decrease in LGM amplitude are maintained. The burst durations, however, are less affected, and these changes are noted only during the recovery period (see, LGM, RVL). In contrast, in cat EB6, all the recorded extensors and flexors of the hindlimbs show a significant increase in amplitude 77 days postlesion (122–433%) as well as 141 days postlesion, 110–141% (except LSt), and their burst duration is shorter. The variable changes observed for the different cats in the burst amplitude and duration are associated with an increased coefficient of variation (CV) or SD values that reflect the wobbly walking and possibly the step-to-step corrections to avoid falling.

Contrary of the variable changes in amplitude and duration of the bursts of activity seen in the hindlimb muscles, marked and consistent changes were observed, across all the cats, in the normalized amplitude of the forelimb extensor TriL. Table 5 presents these values for all the cats expressed as percent of the control. In general, a significant increase (P < 0.01) is observed during the recovery period (ranging from 124% and up to 337%) and is maintained long term postlesion, during the plateau period (117–330%). One exception is LTrIL of cat EB7, which is significantly decreased both during the recovery and the plateau periods. Other than that, EB8’s LTrIL is reduced, but only long term postlesion and the TrIL of EB5 shows no change during the recovery period (although on the long term it is significantly increased).

An important long-term deficit was observed in the AM of the hindlimb muscles activity when walking over a tilted treadmill (10°). Cats in the intact state had no difficulty adapting their walking to slopes (up or down) of the treadmill, even at speeds of up to 0.7 m/s, and their stepping was as regular and sustained as during level walking. During uphill or downhill walking, the activity in the fore- and the hindlimb muscles was modulated up and down, respectively, as illustrated in Fig. 11 for cat EB5 (see intact experiments). The normalized amplitude of RVL, RSt as well as of right cleidobrachialis (RCIB), during uphill walking increases by ~5–10%, compared with their amplitude measured during level walking, whereas downhill, a decrease of the same magnitude is observed. For this cat, however, the uphill modulation of RTrIL amplitude is more pronounced, up to 20% from its activity at level. Notice that for RCIB, only the EMG amplitudes recorded at the uphill and level situation are presented because of a major change in its activity pattern during downhill walking.
FIG. 6. Representative kinematic data recorded from the right hindlimb of cat EB5 in the intact state and 88 days post spinal lesion. The angular excursions (A and B) are related to the 1st step cycles illustrated in the stick diagram of Fig. 3. Notice that before the lesion (intact), the cat makes 3 steps, whereas after the lesion it makes 4 shorter steps for the same time interval. In the duty cycle traces the heavy line represents the stance whereas the down and up arrows represent foot contact and lift, respectively. The angle-angle plots in C (intact) and D (88 day postlesion) are the means of the whole step sequence, $n = 9$ (intact), $n = 11$ (postlesion). Arrows around the plots indicate the direction of the movements in various subdivisions of the step cycle as defined by Phillipson (1905): flexion (F) and 1st extension phase ($E_1$) in swing and the 2nd ($E_2$) and the 3rd ($E_3$) in the stance.

After the lesion, all the cats had major difficulties walking on a tilted treadmill even at very low speeds. All the deficits, such as poor lateral stability, inconsistent stepping, occasional stumbling, etc., described for their walking at level were more pronounced walking on a tilted treadmill and were observed even when their level walking was much improved. After the lesion, a major deviation from the normal modulation pattern was found, both in the fore- and the hindlimb muscles. As seen in Fig. 11, early on in the recovery period, RVL and especially RSrt amplitude at level is higher relative to the uphill one, a result of a wobbly and irregular walking during which the cats are producing large EMG bursts. Later, after day 40, there is no modulation in RVL amplitude, neither during uphill nor during downhilm walking, whereas for RSrt, no modulation is observed for uphill walking, but there is a pronounced reduction (50%) during downhill walking. At the same time, the forelimb muscles, in addition to a general increase of their amplitude, express a more pronounced AM. RTriL as well as RCIB, double their amplitude during uphill walking, whereas during downhill walking, RTriL amplitude decreases to control values. The changes in the modulation of the hindlimb and forelimb muscle amplitude seems to depend on the extent of the spinal lesion, as cats with smaller lesions ($EB1–EB4$) recover the ability to modulate their hindlimb muscle activity when walking on an inclined treadmill, and with it the modulation of TriL activity is set back to control levels as illustrated in Fig. 12 for cat $EB3$.

**DISCUSSION**

Recovery of locomotion as a function of the extent of the spinal lesion

Recovery of locomotion in adult chronic cats was reported to depend on the integrity of the ventral and ventrolateral
spinal quadrants (Afield 1974; Eidelberg 1981; Windle et al. 1958). However, our experiments as well as those of others (Bem et al. 1995; Gorska et al. 1993a,b) show that cats with extensive lesions severing the ventral and ventrolateral quadrants, which eliminate the vestibulospinal and severely damage the reticulospinal pathways (see cats EB6 and EB7), but generally preserve pathways in the dorsolateral funiculus such as the corticospinal pathway, can recover quadrapedal voluntary locomotion. Our approach thus permitted to establish that voluntary quadrapedal locomotion is still possible after severe damage to the ventral and ventrolateral pathways, provided important ascending and descending tracts in the dorsolateral quadrants are present. This approach and conclusion are in contrast to that of others (Afield 1974; Eidelberg et al. 1981; Windle et al. 1958) who concluded that even minimal patches of intact tissue in the ventral quadrants in absence of all the other pathways was essential for hindlimb locomotion. Some of the lesions described in their studies have some similarities to ours. For example, Eidelberg et al. (1981) describe two cats in which only the dorsal columns were left intact and the HRP labeling was confined mainly to the nucleus gracilis; both these cats behaved as complete spinal cats. Afield (1974) describes a cat in which a patch of tissue remained in the medial DLF. This cat also behaved as a complete spinal cat. However, the extent of the lesion was evaluated only under light microscope and was not assessed by the HRP method. Thus it is possible that the number of functional axons in the patch was much smaller than could be evaluated, which could explain the spinal behavior of the cat.

Gorska et al. (1993a) and Bem et al. (1995) reported recovery of locomotion in cats with intact tissue only in the dorsal columns. This is in contrast to the above-mentioned study of Eidelberg. We believe, in light of our HRP experiments, as well as on Eidelberg’s comments (Eidelberg et al. 1981), that examining the site of lesion with light microscope only may not be sufficient to estimate the surviving axons, especially in a highly necrotic and deformed tissue as we have found for cat EB6 on the right PRF (see Fig. 1 and Table 1). Therefore it is probable that more axons have survived elsewhere in the preparation of Gorska et al. (1993a) and Bem et al. (1995), which could explain their recovery.

In our cats, there was a good correlation between the extent of damage to vestibulospinal, pontine reticulospinal and to the medullary reticulospinal pathways as evaluated by WGA-HRP labeling (see Table 2), the time for recovery of quadrapedal locomotion (Fig. 2), and the severity of the locomotor deficits. Such correlation justifies grouping the cats into two major groups, moderately lesioned (EB1–EB4) and extensively lesioned (EB5–EB8) despite the variability in the extent of spinal cord lesions in each of the cats. Severely lesioned cats (EB5–EB8) such as cat EB7 with the most extensive lesion including the majority of the lateral and ventral funiculi eliminating the vestibulospinal and severely damaging the reticulospinal pathways, walked quadrapedally after 34 days. However, its walking was limited to low treadmill speed, and was highly variable with severe and permanent interlimb deficits. Cats in which more reticulospinal cells have been spared (EB1–EB4) recovered more quickly and their locomotor deficits were transient, even though there was a major loss of vestibulospinal cells.

In summary, although others have shown that a small amount of intact tissue in the ventral and ventrolateral quadrants may be sufficient to maintain quadrapedal locomotion, we show that, even after very extensive lesions of those pathways, quadrapedal locomotion is possible, albeit with deficits that correlated with the extent of the lesion, as documented by HRP labeling of cells with descending spinal axons.

Recovery of weight support and lateral stability

In line with the observations of others (Bem et al. 1995; Gorska et al. 1993a), we report a correlation between time of recovery of quadrapedal voluntary locomotion and the extent of the spinal lesion. Cats with extensive lesions started walking after 10 days and up to 1 mo postlesion (see Fig. 2). Before that, as we have reported, the cats dragged their hindquarters and suffered from poor weight support and lateral stability. However, Gorska et al. (1993a) and Bem et al. (1995) observed poor lateral stability mainly during the period of recovery. The only cats that showed these long-term deficits (5–6 mo) were the ones described to have only the most dorsal parts of the DLF and the dorsal columns intact. Our cats, however, even the ones classified as less extensively lesioned (EB1–EB4), exhibited swaying of the hindquarters also long term postlesion when they were walking at their best. This difference cannot be attributed to a difference overground versus treadmill locomotion, because our cats showed the same deficits daily overground, as documented in preliminary observations of ground reaction forces (Brustein et al. 1995). It is interesting to note that the pro-
gressive recovery of weight support and walking was also observed after lesions sparing only the ventral quadrants (Afelt 1974; Eidelberg et al. 1981) and after lesions restricted to the DLF (Jiang and Drew 1996). However, these were transient, and independent walking over the treadmill at speeds of 0.35 m/s was reported in the latter paper, as soon as 3 days postlesion.

The observations on the correlation between recovery of weight support and walking is in line with Mori’s concept about the importance of postural control to the full expression of locomotion. The reduction of weight support probably resulted from damage to reticulospinal pathways that descend in the ventral and ventrolateral funiculi and were suggested to regulate muscle tone (Mori 1989; Mori et al. 1992). In addition, the decrease in weight support may also result from destruction of the vestibulospinal tract, which normally provides strong excitatory drive to extensors (Orlovsky 1972b). The quick recovery of weight support (2–3 days) in cats EB1–EB4, despite the elimination of most of the vestibulospinal pathway, could be due to the spared reticulospinal axons that may be sufficient to maintain the control of muscle tone after the lesion. However, in more extensively lesioned cats, such as EB7, in which a much smaller number of reticulospinal axons have survived, the time needed for recovery (up to 3 wk) may suggest that this low number of neurons (~20%) is not sufficient to maintain

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**FIG. 8.** Interlimb coupling in cat EB5: comparison of interlimb coupling between the right hindlimb and the right forelimb of cat EB5; Intact, 36 and 88 days postlesion. A, E, and I: representative raw EMGs and footfalls taken from the walking sequences used to calculate the phase of bursts onsets in B, F, and J, the cycle duration in C, G, and K, and the cumulative phase difference in D, H, and L, for each one of the individual consecutive step cycles. Tri, triceps brachii.
normal function (Sabel 1997) even after allowing for a long-term reorganization.

One explanation for the recovery of weight support in these cats could be an increased dependence on segmental reflexes to set the muscle tone as suggested for the recovery of weight support in complete spinal cats (Grillner 1972; Guertin et al. 1995; Pratt et al. 1994). However, this by itself may not be enough. Prentice and Drew (1996) suggest the existence of two alternative pathways for postural control during locomotion. One is active during unobstructed walking and the other function during gait modifications such as stepping over an obstacle. It is interesting to note that our cats showed no problems in executing a visuomotor task such as stepping over obstacles (unpublished observations), indicating that these skills were not affected. Implication of the motor cortex during execution of skilled locomotor tasks and gait modifications was shown by many (Armstrong 1986; Beloozerova and Sirota 1993; Drew 1991a; Widajewicz et al. 1994). It is possible that, when the regulation of posture (during level walking) through the reticular formation is insufficient, alternative pathways such as the one active during skilled gait modifications may take over and together with enhancement of segmental reflexes can reestablish some of the postural functions during locomotion, although this is not sufficient for the recovery of lateral stability (Brustein et al. 1995).

Initiation of locomotion

The involvement of pathways descending in the ventral and ventrolateral quadrants in the initiation of locomotion

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**FIG. 9.** Interlimb coupling in cat EB7: comparison of interlimb coupling between the left fore- and hindlimb of cat EB7 in the intact state and 51 days postlesion. A and B: individual values of left VL and TriL cycle duration in consecutive step cycles (as in Fig. 8, C, G, and K). C and D: cumulative phase difference for the same sequence as in A and B. E: raw EMGs and footfalls (51 days postlesion), from which the cumulative values in D were calculated, illustrate the different number of steps (indicated on the footfalls) done by the fore- and the hindlimbs.
TABLE 3. The average percent of time spent on 2, 3, or 4 supporting limbs in 7 of the cats, in the intact state, during recovery and plateau-periods

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>Day</th>
<th>n</th>
<th>Coupling Type</th>
<th>Day</th>
<th>n</th>
<th>Coupling Type</th>
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</table>

The values were calculated from the averaged and normalized footfall diagram, after grouping all the steps according to the various types of limb coupling. H, difference of zero phase between homolateral fore- and hindlimbs (in-phase coupling); D, difference of zero phase between diagonal fore- and hindlimbs (trot); I, difference of 0.25 phase between homolateral fore- and hindlimbs (walking); n, number of steps; day, day postspinal lesion.

by MLR and PLR stimulation was studied in acute decerebrate cats that were subjected to different spinal cord lesions at C2–C3 or the brain stem (Noga et al. 1991; Steeves and Jordan 1986). It was found that lesions to the ventrolateral funiculi abolish MLR initiation of locomotion, whereas any lesion in the dorsal half of the spinal cord had no effect. These findings suggested that MLR initiation of locomotion is conveyed through ventrolateral pathways (for reviews of brain stem centers involved in initiation of locomotor, see Armstrong 1986; Jordan 1991; Rossignol 1996). The inability of MLR stimulation to initiate locomotion after ventrolateral lesions observed in the acute experiments could actually

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**FIG. 10.** Footfall diagrams: representative averaged and normalized footfall diagrams taken from cat EB6 at intact, 77 and 141 days postlesion. Filled horizontal rectangles represent the stance of each one of the limbs; left (L) and right (R) hindlimb (H) and forelimb (F), respectively; LH, RH, LF, and RF. Vertical lines divide the step cycle according to the number of supporting limbs, illustrated as well, in the bottom figures in which the circles represent whether the limb is in contact with the treadmill (filled) or not (open). The total percent of the time the cat is supported by (2), (3), or (4) limbs is given by the stack bar diagram to the right.
TABLE 4.  Amplitude and duration of the hindlimb muscles (0.4 m/s)

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Recovery Period</th>
<th>Plateau Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Normalized amplitude</td>
<td>Duration</td>
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<tr>
<td>EB2</td>
<td>LS</td>
<td>100 ± 26 (124)</td>
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<td></td>
<td>LSt</td>
<td>100 ± 39 (120)</td>
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<tr>
<td></td>
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<td>EB3</td>
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</tbody>
</table>

The normalized and averaged EMGs amplitude, presented as a percent of the intact (% ± CV) and their average duration (means ± SD, in ms) taken from cats EB2 and EB6 (at 0.4 m/s) from intact, recovery, and plateau period trials. L, left; St, semitendinosus; Srt, sartorius; R, right; VL, vastus lateralis; GM, gastrocnemius medialis. * P < 0.05. † P < 0.01. Number of cycles are in parentheses.

correspond to the situation seen here in the early days postlesion in which no hindlimb locomotion was observed even up to several weeks after the largest lesions, as illustrated in Fig. 2. However, the chronic lesioned cats recover the ability to initiate voluntary locomotion. In the less lesioned cats that recover quickly, the spared reticulospinal axons may be sufficient to regain this function, as suggested before (see Recovery of weight support and lateral stability). However, for the most lesioned cats that started walking after >3 wk post spinal lesion, the participation of alternative pathways in the DLF cannot be excluded. After an extended dorsal hemisection, it was found that stimulation of the pontine locomotor region (PLR) could still evoke locomotion, although a high strength of stimulation was needed, suggesting that normally the PLR exerts its effects at least in part through the dorsolateral pathways (Noga et al. 1991). Such an indirect polysynaptic dorsolateral pathway was proposed by Shik to be involved in initiation of locomotion (Kazennikov et al. 1985; Shik 1983). The locomotion evoked by stimulation along this pathway cannot be differentiated from the one evoked through other locomotor regions, and it is thought to result from the convergence of inputs from the cortex and the reticular formation (Beresovskii 1990).

**Step cycle structure and intralimb coupling**

The voluntary and quadrupedal walking was, however, inconsistent as described by the variability of the hindlimbs step cycle duration (Fig. 4), as well from the increased CV and SD values of EMGs amplitude and duration summarized in Table 4. This variability may reflect both the poor lateral stability of the cats as well as the step-to-step adaptations to avoid falling and maintain stepping.

Despite the sustained decrease in the duration of the step cycle for the most lesioned cats (125–250 ms) and its variability, the lesions did not seem to affect the step cycle structure (Fig. 5), i.e., the swing and the stance varies with the step cycle duration as before the lesion. This is in line with others’ (Gorska et al. 1993a) results for three of their cats. However, in one cat with bilateral lesions to the DLF, and in cats after lesions to the lateral funiculi (Gorska et al.

TABLE 5.  TriL amplitude at 0.4 m/s

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Recovery Period</th>
<th>Plateau Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TriL</td>
<td>LTriL</td>
<td>RTriL</td>
</tr>
<tr>
<td>EB1</td>
<td>100 ± 10 (20)</td>
<td>100 ± 25 (31)</td>
<td>222 ± 13* (10)</td>
</tr>
<tr>
<td>EB2</td>
<td>100 ± 19 (26)</td>
<td>100 ± 19 (26)</td>
<td>150 ± 19* (9)</td>
</tr>
<tr>
<td>EB3</td>
<td>100 ± 17 (40)</td>
<td>100 ± 19 (44)</td>
<td>227 ± 17* (15)</td>
</tr>
<tr>
<td>EB4</td>
<td>100 ± 7 (20)</td>
<td>100 ± 7 (22)</td>
<td>337 ± 15* (16)</td>
</tr>
<tr>
<td>EB5</td>
<td>100 ± 13 (24)</td>
<td>100 ± 72 (25)</td>
<td>92 ± 17 (25)</td>
</tr>
<tr>
<td>EB6</td>
<td>100 ± 10 (28)</td>
<td>100 ± 7 (29)</td>
<td>124 ± 11* (19)</td>
</tr>
<tr>
<td>EB7</td>
<td>100 ± 9 (16)</td>
<td>100 ± 8 (17)</td>
<td>38 ± 25* (19)</td>
</tr>
<tr>
<td>EB8</td>
<td>100 ± 6 (14)</td>
<td>NA</td>
<td>93 ± 10 (7)</td>
</tr>
</tbody>
</table>

The normalized and averaged TriL amplitude presented as a percent of the intact values (% ± CV), for each one of the 8 cats from intact, recovery and plateau period trials. TriL, lateral head of triceps brachii; L, left; R, right; NA, not available. * P < 0.01. † P < 0.05.
the step cycle duration of the hindlimbs increased, and the stance varied less with the walking speed, much like the swing in intact cats. This discrepancy may result from a difference in the extent and location of the lesions or from a difference between overground and treadmill locomotion. However, this does not resolve the above differences, as even the complete spinal cat maintains normal relations between the step cycle duration and the swing or the stance (Barbeau and Rossignol 1987; Bélanger et al. 1996).

In addition to the preserved step cycle structure, even the most lesioned cats in our study generally maintained intralimb coupling as illustrated in Figs. 3, 6, and 7 using kinematic and EMG data. Even in cat EB5, which showed somewhat earlier extension of the knee during the stance relative to the ankle, the change may be interpreted as a compensating one to maintain the step length. Another change noted in cats EB5–EB7 was a decrease in the delay between the burst onset of Srt relative to St. However, the St maintained its relation to the paw lift. A change in the St-Srt bursting onset relations was observed in the complete spinal cat (Bélanger et al. 1996) as well as in cats subjected to lesions restricted to the DLF (Jiang and Drew 1996). However, in these cats the St activity is delayed relative to the lift, and in the complete spinal cat it is accompanied by an earlier onset of TA and a paw drag. Although TA recordings were not available in our cats, the observed St-Srt relations was not accompanied by a paw drag at any time. In this case a tighter Srt-St bursting relation may be beneficial for the cat, ensuring a synchronous strong flexion of both the hip and knee at the beginning of the swing, that the hindlimb is cleared of the walking surface to avoid stumbling.

Interlimb coupling

One of the most important deficits observed postlesion was a step-by-step inconsistent coupling between the homolateral fore- and hindlimb, calculated from the onset of EMG activity in the related extensors (Fig. 8) (see as well English 1979 for the intact cat). The larger the lesion, the more severe were the deviations of the fore-hindlimb coupling from the stable intact pattern. The deviations could be classified into three major classes in line with the observations of others (Bem et al. 1995), during overground locomotion. In the first class, the phase differences between the fore- and the hindlimbs vary in the limit of one step cycle, and the step cycle duration in the homolateral fore- and hindlimbs is maintained the same all along the stepping sequence. In the second class, the fore- and the hindlimb of the same side...
are walking at a slightly different frequency as illustrated by an up to 300-ms difference in their step cycle duration. This uncoupling results in a continuous phase drift between the two girdles. The third type is an in-phase coupling of the homolateral fore- and hindlimb, a walking pattern that is rarely seen for a trained cat walking at a moderate or high speed (English 1979; Hildebrand 1976). The first type of perturbation was observed in cats EB5 and EB6, whereas the second was observed for the most lesioned cat, EB7. All cats adopt the third, in-phase walking pattern except the most lesioned cat, EB7. In cats EB1–EB4 and EB8, this occurs during the recovery period, whereas for cats EB5 and EB6, it appears during the plateau period. Adopting homolateral in-phase walking allows the possibility to increase the period during which all limbs are on the ground to support the weight of the animal, which may contribute to the improvement in walking stability after the lesion and is therefore a compensatory mechanism (Blaszczyk and Loeb 1993; Hildebrand 1976).

Bem et al. (1995) observed such coupling changes during overground locomotion in cats with different ventral and lateral spinal lesions, using kinematics events for the analyses. However, the interpretation of the results is not the same as ours, and the homolateral in-phase coupling is related by these authors to be a source of destabilizing oscillations in the walking rhythm. However, if this was the case, our cats could not maintain up to 30 consecutive steps, most of them in in-phase coupling, during the plateau period. The values of cumulative phase difference (see Fig. 8, D, H, and I) support our interpretation. One can see that when the cat uses homolateral in-phase coupling (Fig. 8I) these values stabilize around 0, and only 3 step cycles out of the 30 show extreme deviation, suggesting the return of a more stable coupling pattern.

Changes in the interlimb coupling were observed as well after lesions sparing the ventral and ventrolateral quadrants during overground (Afelt 1974) and treadmill locomotion (Eidelberg 1981) and also after lesions restricted to the DLF (Jiang and Drew 1996), after damage to long propriospinal neurons (English 1989; see, however, Kato et al. 1984) and to the ventral spino cerebellar pathway. No changes were observed after lesions to the dorsal spino cerebellar tract (English 1985), or to the dorsal columns (Gorska et al. 1993b; Jiang and Drew 1996; see, however, English 1980). Thus the fore- and hindlimb coupling pattern is suggested to result from the contribution of many converging inputs, peripheral, segmental, propriospinal and supraspinal on to the stepping generators (English and Lennard 1982; Miller and Van der Meche 1976; Rossignol 1996; Rossignol et al. 1993). This is in line, therefore, with the suggestion of others (Jiang and Drew 1996), that the deficits in interlimb coupling are related to the extent of the spinal lesion and not necessarily to a specific damage to one pathway. However, a common aspect to all the extensive lesions in our studies as well as those reported here is the damage in the lateral funiculus. Kato et al. (1984) suggested that supraspinal descending tracts are involved in conveying information adjusting the timing of activity in the fore- and hindlimb. One of the most prominent candidates is the reticulospinal tract. Reticulospinal neurons not only are active and modulated during locomotion (Orlovsky 1970), but single-unit recordings in the intact cat (Drew et al. 1986; Drew and Rossignol 1990a) and in the fictive locomotion preparation (Perreault et al. 1993) have shown that reticulospinal neurons discharge in correlation with the activity of flexors or extensors of more than one limb. Furthermore, microstimulation of these neurons at rest evoked compound movements including one limb or more as well as the neck (Drew and Rossignol 1990a,b). The responses were organized reciprocally between limbs of the same girdle and were simultaneous, as well in the forelimb. During locomotion, the evoked responses, excitatory or inhibitory, were organized with respect to the step cycle and in the thalamic cat (Drew and Rossignol 1984) and in the fictive preparation (Perreault et al. 1994) or the intact cat (Drew 1991b) caused changes in the amplitude as well in the duration of the muscle or in the
related nerve activity up to resetting the existent locomotor rhythm. It was therefore suggested that the signal coming from a single reticulospinal neuron could be carried by one axon to more than one muscle in more than one limb to reinforce, on a step-by-step basis, the ongoing locomotor rhythm or even reset it. Taken together, these observations suggest implications of the reticulospinal pathways in the control of the coordination between the limbs (Drew 1991b; Rossignol et al. 1993).

Thus, if the contribution of the reticulospinal pathways to the control of fore- and hindlimb coordination is of major importance, severe damage to its axons will produce coupling instability between the two girdles. In that context, it is most interesting that up to a certain extent of the lesions (see cats EB5 and EB6), a stable in-phase coupling can still be expressed, indicating preserved functional interactions between the girdles. However, after more extensive lesions, such as in cat EB7, the step-to-step inconsistencies in the fore- and hindlimb coupling is sustained even long term postlesion. In this cat, the different reticulospinal pathways are severely damaged (see Table 1), and the remaining DLF pathways such as the corticospinal or the propriospinal and the ascending pathways in the dorsal columns are not sufficient to keep the stability of the interlimb pattern. Gorska et al. (1995) have tested the effect of damage to the caudal pole of the nucleus pontis oralis and to the rostral pole of the nucleus reticularis pontis caudalis on the fore- and hindlimb coupling. They reported a transient effect on the interlimb coordination. The transitory effect is probably due to the limited extent of the lesion, because we have noted that permanent perturbations in the interlimb coupling is associated only with damage to most of the reticulospinal neurons in the pontine and medullary reticular formation nuclei.

**EMG activity**

Various changes were observed in the amplitude and duration of the EMG activity as demonstrated in Table 4. The EMG activity of the most lesioned cats were more affected and showed, in general, a pronounced and sustained increase in amplitude and as well a reduction in their duration, which together permitted the cat to produce quick steps at the same length, to maintain the imposed treadmill speed. Another possibility is that the increase in the EMG’s amplitude results from permanent changes in the motor units population such as that observed after complete spinal lesion (Cope et al. 1986; Edgerton et al. 1983). However, these changes are associated with muscle atrophy, which was not apparent here.

An important change is related to the deficits in the AM of EMG activity when walking uphill or downhill on a treadmill. The increase in the EMG amplitude during uphill walking (see, as well, Pierotti et al. 1989 for the intact cat) is absent for the most lesioned cats, whereas for the less extensively lesioned animals, the loss of modulation is transient and the cats recover it with time. The lack of AM of the hindlimb extensors can be attributed to the absence or reduction of inputs from vestibulo- and reticulospinal pathways, as well as to damage of ascending pathways such as the ventral spinocerebellar tract, which could lead to a reduction or alteration in descending inputs. The vestibulospinal pathway exerts strong excitatory influence on extensor motoneurons during locomotion (Orlovsky 1972b), whereas the reticulospinal pathways may also exert powerful excitatory effects on the extensor muscles when evoked in the appropriate phase of the step cycle (Drew and Rossignol 1984; Perreault et al. 1994), although its effects have often been associated with flexors (Orlovsky 1970, 1972a). Elimination of these inputs may result in a more restricted firing rate and modulation pattern of the individual motoneuron, which will limit the muscle force production.

The direct implication of vestibulo- and reticulospinal pathways in the modulation of the EMG activity during locomotion on inclined surface has been suggested by Matsuyama and Drew (1996). They recorded vestibulo- and reticulospinal neurons in chronic cats during walking over a tilted treadmill. The vestibulospinal neurons showed increased depth of discharge modulation during inclined locomotion as well as changes in peak discharge. The latter effects, together with an increase in discharge, were observed as well for reticulospinal neurons. Thus, in the case of the most extensively lesioned cats in which both the reticulo- and vestibulospinal pathways are severely damaged, such a modulation is absent, and there is no recovery, whereas, in the less lesioned cats long term postlesion, the larger number of surviving reticulospinal axons are probably sufficient to maintain that function. When the EMG modulation is absent for the hindlimb muscles, we observed a more pronounced modulation in Tri amplitude, which is sustained for the extensively lesioned cats and transient for the less lesioned cats. Together this implies that the observed increase in TriL modulation can be interpreted as an adaptive mechanism that is applied as needed. Thus the cat, by exerting control over the forelimb muscles, can compensate for the deficits in the hindlimb muscle activation. Preliminary data obtained using force platform suggest that the forelimbs contribute to propulsion in these cats (Brustein et al. 1995).

**Conclusions**

Sparing the ventral and ventrolateral funiculi was shown by Windle et al. (1958), Afelt (1974), and Eidelberg (1981) to be sufficient for recovery of locomotion. Our results, as well as those of others (Bem et al. 1995; Gorska 1993a,b), show that quadrupedal voluntary locomotion is possible also after extensive lesions to the ventral and ventrolateral pathways, yet sparing pathways in the DLF. However, the cats suffer from major locomotor and postural deficits. Immediately after the lesion and for a few weeks, the most lesioned cats could not support or walk with their hindlimbs, indicating the importance of the ventral and ventrolateral pathways to the normal locomotion, which probably depends heavily on their inputs for initiation of locomotion and for maintaining posture. The time for recovery of weight support and walking in the most extensive lesioned cats also implies some reorganization in the spared pathways, suggesting that they may not be the main contributor or be directly involved in the
normal control of these aspects of the locomotion in the intact cat. The deficits observed during the plateau period indicate, however, that DLF pathways, including the residual population of reticulospinal neurons, can be sufficient to provide the necessary drive to initiate locomotion as well as for some locomotor-related postural adjustments. Although the activity in these pathways is sufficient to maintain the intralimb coupling, they are not sufficient to maintain the interlimb coordination or modulate the EMG amplitude during locomotion on an inclined treadmill, nor to maintain lateral stability. These functions are probably normally executed mainly by the brain stem pathways such as the reticulo- and vestibulospinal. The ‘functional taking over’ after partial lesions was suggested by Altermark et al. (1987) for the recovery of food reaching in cats, by Vilensky et al. (1992) in the monkey as well as in human (Nathan 1994). The basis for such reorganization is suggested to be due to plastic changes such as sprouting (Aoki et al. 1988; Goldberger and Murray 1978; Jiang and Drew 1996; Kimura et al. 1994; Kozlowski et al. 1996). We have observed such changes in the motor cortex of cats EB6 ± EB8, which show up to a twofold increase in the number of labeled cells, as well as appearance of labeled cells in regions other than those related to the hindlimbs (Brustein et al. 1996). The suggested innervation of the lumbar spinal cord by axons of cells originating in more rostral levels tracts. The BELOOZEROVA, I. N. AND SIROTA, M. G. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. J. Physiol. (Lond.) 461: 1–25, 1993.


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