Early Locomotor Training With Clonidine in Spinal Cats

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Chau, Connie, Hugues Barbeau, and Serge Rossignol. Early locomotor training with clonidine in spinal cats. J. Neurophysiol. 79: 392–409, 1998. Clonidine, a noradrenergic alpha-2 agonist, can initiate locomotion early after spinalization in cats. Because this effect lasts 4–6 h, we have injected clonidine daily, intraperitoneally or intrathecally, and intensively trained five spinal cats to perform hindlimb walking on a treadmill starting at day 3 and continuing until 10 days posttransection. Each day, clonidine was injected to induce locomotor activity and cats were trained to walk with as much weight support as possible and at different speeds during multiple (1–5) locomotor training sessions, each lasting from 10 to 20 min, until the effects of clonidine wore off. Electromyographic (EMG) activity synchronized to video images of the hindlimbs were recorded before and after each clonidine injection. The results showed, first, a day-to-day change of the locomotor pattern induced by clonidine from the 3rd to the 11th day including an increase in the duration of the step cycle, an increase in the duration of extensor EMG activity, and an increase in total angular excursion of the hip, knee, and ankle joints. Second, after 6–11 days of this regimen, there was an emergence of a coordinated locomotor pattern with weight support of the hindquarters that was visible even before that day’s clonidine injection. The results suggested that daily injection of clonidine followed by early and daily interactive locomotor training can enhance the recovery of locomotion in spinal cats.

INTRODUCTION

It is well established that a few weeks after a complete spinal section at the thoracic level (Th13), adult cats can recover locomotion of the hindlimbs on a treadmill provided that there is adequate interactive training (reviewed in Rossignol 1996). Training long has been suggested to play an important role in the ability of cats to walk after spinal cord transection. Shurrager and Dykman (1951) reported an improvement in overground walking behavior of spinal kittens that received electrical stimulation of the hindlimb; the walking response of the hind legs became stronger, more precise and more functionally effective as training was continued. We have studied previously the recovery of locomotion in cats after spinal cord transection and showed that training played an important role in enhancing the recovery process (Barbeau and Rossignol 1987; Barbeau et al. 1993; Belanger et al. 1996; Rossignol et al. 1982, 1986). After 3–4 wk of training, the adult spinal cat (Th13) attained good locomotor function with large steps, bilateral plantar foot placement and weight support of the hindquarters for >3 min (Barbeau and Rossignol 1987). Furthermore, the spinal cat was able to adapt its locomotion for treadmill speeds ≤1.0 m/s. Belanger and colleagues (1996) found that spinal cats that received daily training could fully support the weight of the hindquarters at 14–24 days posttransection. Smith and colleagues (1982) found in 12-wk spinal cats (Th12) that the exercised group showed a performance superior to that of the nonexercised group. The untrained spinal cats performed poorly with only occasional plantar foot placement and were unable to support their weight during treadmill locomotion. The trained spinal cats, on the other hand, exhibited excellent weight support during locomotion and adapted to treadmill speeds ≤0.8 m/s (Rossignol et al. 1982; Smith et al. 1982). Cats spinalized as adults could bear the full weight of their hindquarters, generate reciprocal stepping on a treadmill, and showed EMG and kinematic patterns remarkably similar to those of normal cats (Belanger et al. 1996; Edgerton et al. 1991; Hodgson et al. 1994; Lovely et al. 1990).

Interactive locomotor training, during which the experimenter adjusts the weight supported by the animal according to its capacity on any particular day, is important during the recovery period (Belanger et al. 1996). However, in the first 7–10 days posttransection, the animals make only small hindlimb movements and cannot advance the hindlimb in front of the hip or to make foot contact with the plantar surface, and thus there is little or no weight support. Thus interactive locomotor training during that period is not optimal. In contrast, pharmacological stimulation during that period can induce locomotion. Specifically, noradrenergic alpha2 agonists have been shown to trigger locomotion with large steps in acutely spinalized cats (Forssberg and Grillner 1973) or in the early posttransection period (Barbeau et al. 1987; Rossignol et al. 1995) in chronic spinal cats. This effect lasts 4–6 h, during which time, the animal can be trained to walk. Consequently, we planned to evaluate the effect of daily training on the recovery process of locomotion during this early period using clonidine.

The implication of this approach is that there is some plasticity in the spinal mechanism responsible for generating locomotion. This central plasticity could be reflected by the evolution of the locomotor pattern with time. Barbeau and Rossignol (1991) recorded locomotion in chronic spinal cats at day (d) 2 posttransection and d7 posttransection after clonidine injection (150 μg/kg ip) and found that the locomotor pattern seen after clonidine given on d2 was different from that given on d7. There was an increase in the duration of the step cycle for the same treadmill speed from d2 to d7 accompanied by a gradual increase in the duration of stance. Concomitant temporal changes in EMG activity revealed that from d2 to d7, the EMG activity of extensor muscles was prolonged and that of the flexor muscles was shortened (Barbeau and Rossignol 1991). In a preliminary study, we showed in one cat a progressive improvement in the locomotor ability from d2 to d9 as reflected by the grad-
ual increase in cycle duration and weight support of the cat during this period.

Similarly, the ‘‘fictive’’ locomotor pattern evoked in early-spinal and late-spinal cat was found to differ, the latter having more characteristics of the normal adult pattern (Pearson and Rossignol 1991). Finally, Edgerton suggested that the spinal cats could be trained specifically to stand or to walk, and their motor abilities were specific to the type of training received (Edgerton et al. 1991; Hodgson et al. 1994).

Because clonidine can unmask the locomotor circuitry, which is capable of undergoing plastic changes after spinalization, it is possible that this spinal circuitry can be shaped and reinforced by early training after spinalization. The aim of the experiments therefore was to study in detail, after daily injection of clonidine, the effects of early training and clonidine on locomotor recovery after spinalization. This study can help determine whether early locomotor training in patients with spinal cord injury could be beneficial to enhance their functional recovery.

METHODS

Five normal adult cats were trained for periods ranging from 1 to 4 wk to walk at constant speeds on a motor driven treadmill belt enclosed by a transparent plexiglas box. All trained animals were capable of maintaining a steady and continuous locomotion at different speeds (0.2–0.7 m/s) for ≥20–25 min. After this training period, all animals were prepared to undergo surgical implantation of EMG recording electrodes and, in one cat (CC4), an intrathecal cannula at the time of EMG implantation. After these implantations, the locomotion of the cats was recorded to establish the baseline values of the control period (referred to as intact), before spinalization.

Surgical procedures

All operations were performed under general anesthesia (pentobarbital 35 mg/kg) and aseptic conditions. Surgeries performed for these experiments included the following: implantation of EMG electrodes for chronic recording, intrathecal catheterization, and spinalization.

IMPLANTATION OF CHRONIC EMG ELECTRODES. Briefly, except for HB6, which was implanted daily with pairs of enamel-insulated copper wire electrodes inserted percutaneously into the bellies of a few hindlimb muscles after spinalization, all other cats underwent chronic electrode placement. One or two multipin head connectors (TRW Electronic Components Group, Elk Grove Village, IL) were used. Fifteen Teflon-insulated stainless steel wires (Cooner Wire, Chatsworth, CA, AS633) were soldered to each connector a few days before surgery. With the animal secured in a stereotaxic frame, the connectors were placed on its skull using acrylic cement. The stainless steel wires then were led subcutaneously to various muscles. A pair of stainless steel wires then was inserted into each muscle. Unpaired wires, from the last pin of each connector, were placed under the skin of the neck to serve as a ground. Before muscle insertion, a small portion of the Teflon coating was removed from the stainless steel wires and then the wires were sewn into the bellies of selected flexor and extensor muscles of both hindlimbs. The implanted muscles were the following: iliotibialis (IP), hip flexor; gluteus medius (Gl), hip abductor and extensor; sartorius (Srt), hip flexor and knee extensor; semitendinosus (St), knee flexor and hip extensor; vastus lateralis (VL), knee extensor; gastrocnemius medialis (GM), ankle extensor and knee flexor; gastrocnemius lateralis (GL), ankle extensor and knee flexor; and tibialis anterior (TA), ankle flexor.

INTRATHECAL CATHERETERIZATION. An intrathecal cannula (Teflon 24LW tubing) was implanted in one cat, CC4, before spinalization. One end of the cannula was connected to a cannula connector, which was cemented on the skull together with the head connectors. The other end of the cannula was inserted into the intrathecal space through an opening in the atlanto-occipital ligament down to L4–L5.

SPINALIZATION. A laminectomy was performed at the Th13 vertebra. The dura was removed carefully and lidocaine hydrochloride (Xylocaine, 2%) was applied topically on the area of spinal cord to be transected. The spinal cord was severed completely with a pair of surgical scissors so that the ventral surface of the spinal canal could be visualized clearly. Absorbable hemostat (Surgicel) then was used to fill the space between the rostral and caudal ends of the spinal cord thus helping hemostasis. The wound then was sutured in layers.

Postoperative care

After all operations, animals were placed in an incubator until they regained consciousness before returning to their cages with ample food and water. Torbugesic (Butorphenol tartrate, 0.05 mg/kg sc) also was given in the first postoperative day (every 6 h) for analgesia. All spinal cats were placed in individual cages (104 × 76 × 94 cm). The cages were lined specially with a foam mattress in addition to the usual absorbent tissues to reduce the risk of developing skin ulcers. They were attended to at least twice daily for manual bladder expression, general inspection, and cleaning of the hindquarters. All procedures followed a protocol approved by the local ethics committee, and the well-being of the cats always was ensured.

Histology

When the animals were killed with an overdose of pentobarbital sodium, the spinal cord was removed for histological analysis (Kluver-Barrera method) to ensure the completeness of the spinal transection. Sagittal sections of 10-μm thickness were cut, including the area of the transection.

Recording and analysis procedures of locomotor performance

The locomotor performances of the cats were recorded (EMG synchronized to the video images) under the following different conditions: 1) intact, after chronic electrode implantation but before spinalization; 2) spinal predrug, after spinalization just before any drug injection; and 3) spinal postdrug, after spinalization and at different time intervals after drug injection. The pre- and post-drug trials, carried out on the same day, then were compared with the intact trials of the same cat (a within subject design where each animal has its own baseline for comparison).

During recording of the intact locomotion, cats were placed on the treadmill belt enclosed by the plexiglas, and free walking at different speeds was recorded. During recording of spinal locomotion, the forelimbs of the spinal cat were placed on a platform and the hindlimbs on the moving treadmill belt. Early after spinalization, the experimenter supported the weight of the hindquarters of the cat and provided equilibrium. With time, the cats could walk with complete weight support of the hindquarters and the experimenter only held the tail to provide lateral stability.

Reflective markers were placed on the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal joint (mtp) and the tip of the third toe. The video images of the
side view of the cat were captured using a digital camera (Panasonic 5100, shutter speed 1/1,000 s) and recorded on a video cassette recorder (Panasonic AG 7300). The side facing the camera was the ipsilateral side. Calibration markers (10 cm distance) were placed either on the treadmill frame or on the trunk of the cat to reduce parallax errors.

The EMG signals were amplified differentially (bandwidth of 100 Hz to 3 kHz). Twelve channels were recorded with a Vetter Digital (model 4000A PCM recording adapter) on a VHS video tape. The frequency response of the tape recorder was 1.2 kHz per channel.

The EMG recording was synchronized to the recorded video images by means of a digital Society for Motion Picture and Television Engineers time code. This time code (Skoteln time code generator model TCG-80N) was recorded simultaneously on the EMG tape and the audio channel of the VHS tape and also was inserted into the video images themselves.

The recorded EMG data during locomotion were played back on an electrostatic polygraph (Gould, Model ES 1000) and representative records of the animal’s performance before and after drug injections were selected for analysis. The EMG signals were digitized at 1 kHz and the onset and offset of bursts of activity were detected first automatically then verified manually and corrected where necessary. The duration and amplitude of the muscle bursts were measured using custom designed software. The mean amplitude was calculated as the integral of the rectified EMG divided by the burst duration. The rectified EMGs signals then were normalized and averaged using the knee flexor iSt as a trigger.

Kinematic analysis of the hindlimb began with the digitization of the selected video images using a two-dimensional PEAK Performance system (Peak Performance Technologies, Englewood, CA). Displacement data, encoded by the X and Y coordinates of different joint markers (iliac crest, femoral head, knee joint, lateral malleolus, mtp joint, and the 3rd toe) were measured at 60 fields/s, (temporal resolution of each images is therefore 16.7 ms). Angular displacement data and joint angles also were calculated automatically (e.g., hip joint angle was calculated based on the relative position of the iliac, hip, and knee markers). From both X-Y coordinates of the recorded markers, displacement data and the calculated joint angle data, displays of stick diagrams, trajectories, or normalized average joint angular displacement plots (filter = 3) were generated using custom-made software. Stick diagrams of one step cycle consisted of reconstruction of the actual hindlimb movements during the stance and swing phases (Fig. 1C). Trajectories, or the course of a single joint, also were reconstructed from the X-Y coordinates (Fig. 1D). Normalized average joint angular plots showed the changes in the angular excursion during one normalized step cycle.

A complete step cycle consists of a stance and a swing phase (Fig. 1A). The stance phase begins as soon as the foot contacts the supporting surface, in this case the treadmill belt, and terminates when the foot starts its forward movement. The swing phase begins at the onset of forward movement and terminates as the foot strikes the treadmill belt again. Foot drag is not normally seen but was seen after spinalization, resulting from an inadequate clearance of the foot during swing, and is defined as the initial period during which the dorsum of the paw touches the treadmill belt during the forward movement of the foot.

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** A: averaged joint angular displacement (mean ± SD) of hip, knee, ankle, and metatarso-phalangeal (mtp) joint for 6 normalized step cycles (the cycle is repeated twice) of a cat during the intact condition (before spinalization). See text for definitions of F. E1, E2, and E3, T, paw lift; ‡, paw contact. B: rectified, normalized and averaged electromyographic (EMG) recordings during the same 6-step sequence synchronized to foot contact of the hip, knee, and ankle extensor muscles, Glu, VL, and GL, respectively, occurred during the stance phase. Hip and knee flexor muscles, Srt and St, respectively, were activated during the swing phase. Onset of hip flexor iSt was later than the knee flexor iSt. There was also a double burst of activity seen in coSt. C: stick figures of the hindlimb illustrating the swing and stance phases of 1 step cycle. Each stick figure was formed by drawing lines between the different reflective markers. Stick diagrams shown were reconstructions of the actual hindlimb movements during the stance and the swing phases. Each frame was displaced from the previous frame by the distance traveled by the foot. Thus the stick figures are “spread out” horizontally to allow a better illustration of the limb movements. Note that the calibration of the x axis is twice that of the y axis. D: trajectory of each marker point, as indicated, during a complete step cycle.
Figure 1 shows the normalized average angular plots, with the swing and stance phases subdivided into different components (F, \( E_1 \), \( E_2 \), and \( E_3 \)) based on Philippion (1905). The swing phase begins with flexion (F) of all joints, and during late swing, while the hip continues to flex, the knee and ankle start to extend (\( E_1 \)). The stance phase begins when the paw contacts the treadmill (\( E_2 \)), at which point the knee and ankle flex passively (yield) as the hindlimb bear the weight of the body, then the knee and ankle extend again (\( E_3 \), 3rd extension) to propel the body forward.

The different parameters of locomotion we have investigated are defined below.

1) **Step cycle duration** was defined as the time (ms) elapsed between two successive contacts of the same foot. It was calculated by multiplying the number of video fields by 16.7 ms (interval between each field).

2) **Step length** was defined as the horizontal distance (mm) traveled between two successive contacts of the same foot. Thus, the step length comprised the stance length and swing length. It is important to note that the step length measurement only takes into account the horizontal distance (only \( \times \text{axis} \)) traversed by the foot from one foot contact to the subsequent foot contact without taking into account the vertical trajectory of the movement. The values at foot contact and foot lift were taken from the displacement data file generated by the Peak Performance calculation program. The stance length was the distance traveled when the foot was in contact with the belt. The swing length was calculated by measuring the distance traveled from foot lift to the most forward horizontal distance reached by the foot.

3) **Joint angular excursion** was defined as the difference in degrees between the maximum and minimum values reached by each joint during a complete step cycle. The angles were calculated by the Peak Performance calculation program, which generated the angular displacement data.

**Experimental protocol**

Before spinalization, EMG signals and kinematic patterns were recorded at various speeds on the treadmill. Different recordings were made on several days ranging from 5 to 14 days. The intact locomotion would later serve as the control reference for each cat.

After spinalization, experimentation began at 3 days posttranssection after the cat had recuperated from the surgery. For each following day (for 10–11 days), the locomotor performance of the cat was recorded (spinal predrug) to set the baseline recording for that day. Then clonidine was injected intraperitoneally (150–250 \( \mu \text{g/kg} \)) or intrathecally (100 \( \mu \text{l} \) of 4 \( \text{mM} \)) and the locomotor performance was evaluated again (spinal postdrug).

During the evaluation of the locomotor performance after spinalization, the hindlimbs of the cat were placed on the treadmill belt, whereas the forelimbs were placed on a platform. The experimenter lifted the hindquarters of the spinal cat to provide for weight support and equilibrium as required. Locomotion was evaluated at various speeds starting from 0.2 to 1.0 m/s. Perineal stimulation was given to enhance stepping. During predrug trials, especially during the early posttranssection days, moderate to strong perineal stimulation was given in an attempt to elicit locomotion as much as possible. During the postdrug trials, on the other hand, only light perineal stimulation was needed to enhance stepping. The effect of clonidine on locomotion lasted for 4–5 h, thus in this time period some locomotor training was possible.

We did not measure the level of clonidine in the blood. Pharmacokinetic study has shown that in humans that received an intravenous injection of clonidine (300 \( \mu \text{g} \)), the plasma clearance was 1.9–4.7 ml • min \(^{-1}\) • kg \(^{-1}\) of body weight and that renal elimination of the unchanged drug constitutes 60% of the drug clearance. The half-life averaged 8.5 h (Davies et al. 1976).

**Locomotor training**

Multiple (1–5) short training sessions were given daily after clonidine injection. These training sessions are additional to the recording sessions that were made at 30–45 min after clonidine injection. The length of each training session usually lasted ~10–20 min, depending on the locomotor capability of the cat on a particular day (see Table 1). During the training sessions, the hindlimbs were placed on the treadmill belt, and the cat exercised at different speeds as soon as the locomotor pattern appeared after the clonidine injection. During each training session, the experimenter lifted the hindquarters of the spinal cat to provide some weight support and equilibrium as required, with the goal being to let the animal support its own weight as much as possible during locomotion at all times. Stimulation also was given to the animal by lightly pinching the perineum. As the effects of clonidine wore off, the ability of the cat to walk consistently on the treadmill decreased and the training periods had to be shortened. Usually, by 4–5 h after clonidine injection, it was difficult to elicit proper locomotion and training was stopped. The cats then were returned to their cages and were not trained again until the following day.

**RESULTS**

Data obtained from five spinal cats were used. Table 1 indicates the profile of the experimental cats including the dosage of clonidine and the training received. The characteristics and progressive changes of the locomotor pattern observed in intact and spinal cat (both predrug and postdrug conditions) were examined.

**Intact locomotion**

The locomotion of cat CC2 during the intact condition (Fig. 1) will serve as a reference for locomotion of the same cat after spinalization as shown in subsequent figures. The characteristics of the locomotion are reflected in the normalized angular plots of the hip, knee, ankle, and mtp joints (the averaged cycle was repeated twice (Fig. 1A), and the stick diagrams representing one step cycle (Fig. 1C)). Figure 1D shows the trajectory of the different markers during a step cycle. The variability seen in the averaged angular plots was attributed to the cat’s difficulty in the intact condition to maintain a steady speed at such a low treadmill speed (0.2 m/s). However, it is important to show the locomotion at this speed to compare with the locomotor pattern after spinalization. The averaged EMG activity of the corresponding stepping sequence is shown in Fig. 1B. The EMGs signals were synchronized on foot contact. In intact locomotion, the timing of EMG activity is more complex than a simple alternation between flexor and extensor muscles. For example, the onset of knee flexor, iSt, was later than the onset of knee extensor, iVl. Double bursting can be seen in iSt as well.

**Overview of the recovery of locomotion**

Clonidine was effective in triggering locomotion in all adult chronic spinal cats a few minutes after the injection, and this effect gradually changed with time after spinalization. To describe this in more detail, the results of one representative spinal cat (CC2) are shown.

At 3d postspinalization, before clonidine injection, the cat
was only capable of showing some very occasional movements of the hindlimbs when placed on the moving treadmill belt (Fig. 2A) even when strong perineal stimulation was given. The movements were so small that they were almost not seen in the angular traces of the hip, knee, ankle, and mtp joints. The foot constantly was dragging on the dorsum without any plantar foot placement or weight support of the hindquarters. Within 30 min after clonidine injection, ip 200–250 μg/kg, and with perineal stimulation, despite some foot drag, the spinal cat was able to step consistently on the toes and partially support the weight of the hindquarters (Fig. 2F). All joints participated in these locomotor movements (7–8 step cycles) but the limb tended to remain behind the hip and therefore there was little efficient weight support in this position. The cat could walk at 0.1 and 0.2 m/s but could not walk at a speed of 0.3 m/s. The cat then was trained to walk at 0.2 m/s for two sessions of 10 min each.

The following day (d4), the effect of clonidine had completely worn off and there was again very little hindlimb movement before clonidine injection despite the training of the previous day (Fig. 2B). After clonidine injection, the steps were larger but not robust. There was an increase in angular excursion, step cycle duration and step length compared with the pattern on d3 (Fig. 2G). The hindlimbs were placed in a more forward position as compared with that of d3, and the cat could accept some weight during each step. The cat could walk at 0.3 m/s. At d5 and d6 (not shown in the figure), the cat could walk at 0.5 m/s. A toe drag during swing, indicated by a horizontal line under the foot, was observed frequently after clonidine injection (Fig. 2F and G). By d7 postspinalization during the preclonidine period (Fig. 2C), after 4 consecutive days of clonidine injection (d3–d6) and 15 training sessions, there was still no apparent improvement in the spontaneous locomotion during this predrug period. However, a well-coordinated locomotor pattern was elicited readily a few minutes after clonidine injection (Fig. 2H). There was an increase in all joint angular excursions, and an hyperflexion during swing, which might be related to the perineal stimulation. The cat could walk at 0.6–0.7 m/s. Therefore, although there was no apparent carry-over effect of the training from the previous day in the predrug period, the postclonidine period clearly indicated that changes were occurring within the spinal cord.

The next day, by d8 (Fig. 2D) before the injection of clonidine, it was indeed possible to obtain a good locomotor pattern with increased joint excursion although the peak values obtained were not as large as those obtained in the intact, prespinalization period (compare Fig. 1A). This was not due to the residual clonidine because the effects of clonidine dissipate 6 h postinjection as seen on previous days. After clonidine (Fig. 2F), there was a further increase in cycle duration, step length, and joint angular excursions approaching intact. The cat could walk at 1.0 m/s. However, there was a marked toe drag during the swing phase as indicated by the horizontal line underneath the stick figures (Fig. 2F).

On d9 (Fig. 2E), the predrug locomotor pattern was further improved and more robust as seen by the increase in all joint angular excursions. The locomotion better resembled that seen in the intact condition, indicative of a recovery of locomotion (see Fig. 1). The criteria for locomotor recovery as seen during spinal-predrug trials was as follows: consistent stepping, minimum 7–8 consecutive steps, stepping with plantar foot placement, and weight support of the hindquarters subjectively assessed by the examiner of the ability of the cat to accept weight during stance. Perineal stimulation was sometimes applied to enhance stepping. The amount of perineal stimulation given always was kept to the minimum; usually just a light pinch to the perineal area was sufficient. After clonidine injection (Fig. 2J), there was a further but small increase in the stance and swing duration as well as in angular excursion, and the cat could walk at speeds ≤1.0 m/s.

To summarize, first, daily clonidine injection followed by locomotor training after spinalization resulted, by d8–d9, in the expression of a coordinated locomotor pattern without

### Table 1. Profile of the experimental cats used for early locomotor training experiments

<table>
<thead>
<tr>
<th>Cat</th>
<th>Gender</th>
<th>Condition</th>
<th>Time, Days</th>
<th>Daily Clonidine Doses*</th>
<th>Number of Training Sessions Each Day of Injection?</th>
<th>Postspinal Day With Weight Support During Locomotion Without Clonidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB6</td>
<td>Male</td>
<td>Spinalization</td>
<td>0</td>
<td>50–100 μg/kg ip (7)</td>
<td>1–2 (12–20)</td>
<td>9</td>
</tr>
<tr>
<td>CC1</td>
<td>Male</td>
<td>Control</td>
<td>−28</td>
<td>150–215 μg/kg ip (10)</td>
<td>1–2 (15)</td>
<td>11</td>
</tr>
<tr>
<td>CC2</td>
<td>Female</td>
<td>Spinalization</td>
<td>0</td>
<td>200–250 μg/kg ip (8)</td>
<td>3–5 (15–20)</td>
<td>9</td>
</tr>
<tr>
<td>CC3</td>
<td>Female</td>
<td>Control</td>
<td>−15</td>
<td>200 μg/kg ip (8)</td>
<td>3–5 (15–20)</td>
<td>9</td>
</tr>
<tr>
<td>CC4</td>
<td>Male</td>
<td>Spinalization</td>
<td>−5</td>
<td>100 μl of 0.4–4 mM it (8)</td>
<td>3–5 (15–20)</td>
<td>6</td>
</tr>
</tbody>
</table>

The control condition represents the time period before spinalization when the cat was being trained to walk on the treadmill and when recordings of intact locomotion were made for subsequent comparison. The time scale was set such that the day of spinalization was day 0. The cats were sacrificed at various days posttransection as indicated. Locomotor recovery ranges from 6 to 11d as indicated by the ability of the cat to walk on the treadmill with weight support of the hindquarters and foot placement even before clonidine injection. * Number of days when injection were given consecutively in parentheses. † Number of minutes per session in parentheses.
any further clonidine injection. Second, the recovery process during the first 9 days posttransection was a progressive one as seen from the gradual improvement in the locomotor pattern from d3 to d9 posttransection after clonidine injection. The steps were longer and more consistent with time, foot placement became more consistent, weight support increased, and there was a day-to-day increase in the ability to adapt to higher speeds. The maximum speed at which the cat could walk was 0.2 m/s at d3; 0.5 m/s at d5; 0.7 m/s at d7; and 1.0 m/s at d8–d9. Third, the gradual improvements in locomotion from d3 to d7 were only apparent after clonidine injection and not before, because there was simply no

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**FIG. 2.** A–E: progressive recovery of locomotion (cat illustrated in Fig. 1) after spinalization, before clonidine injection at 3, 4, 7, 8, and 9 days posttransection. F–J: corresponding locomotion of the spinal cat at 3, 4, 7, 8, and 9 days after clonidine injection. →, stance; ←, swing. Black bar under the stick diagram in F, G, and I represents the presence of foot drag during the onset of swing phase.
walking from d3 to d7 (Fig. 2, A–C). Thus it seems that clonidine can reveal an underlying recovery process in the spinal cord that would not otherwise be apparent. The result from this cat CC2 is representative of all experimental cats used in this study. The results of other cats will be summarized in following figures.

**Step cycle duration**

In Fig. 3, the step cycle duration in CC2 and CC4 during intact locomotion, spinal predrug, and postdrug conditions over the 9d posttransection period is shown. From d3 to d7 in CC2 and d3 to d4 in CC4, no value was given during the predrug trials (note the absence of the gray bar) because there was no locomotion during those periods. There were only some rudimentary rhythmic movements of the hindlimbs with strong perineal stimulation. The hindlimbs usually were extended with neither plantar foot placement nor any weight support of the hindquarters at all, so there were merely passive back and forth movements of the foot on the treadmill belt due to manipulations of the experimenter. At d8 and d9, the cycle duration of CC2 approached the value obtained during normal intact locomotion (shown as a dotted line) even before clonidine injection. At d6 of CC4, the cycle duration also approached the intact value. The effects of clonidine on step cycle duration are clear during the first 7 days (CC2) and 4 days (CC4) posttransection, when there was no locomotion during predrug trials. Once the locomotion was elicited (d8 for CC2 and d5 for CC4), the effect of clonidine became less dramatic; in other words, the relative increase in the cycle duration after clonidine compared with the predrug trial was less.

It is also interesting to note that, at d3, even after clonidine injection, the cycle duration in CC2 (as well as CC1 and CC3, not shown here) was below the intact value, except in CC4, in which the postclonidine cycle duration actually reached and somewhat exceeded the intact value (see also Table 2). For example, at d3, while the cycle duration postclonidine for CC1 and CC2 are, respectively, 29.4 and 33.3% below their respective intact values, CC4 is 7% above its normal value. It is important to note that while CC1, CC2, and CC3 received clonidine intraperitoneally, CC4 received clonidine intrathecally, suggesting that the intrathecal injection of clonidine may exert a more potent effect on the spinal cord during the early posttransectional period (d3). Other findings also support the suggestion that intrathecal clonidine injection produced a more potent effect than intraperitoneal injection of clonidine. At d3, after clonidine injection, cat CC4 could walk at 0.8 m/s as compared with 0.2 m/s observed in CC2.

A closer examination of the changes in the subcomponents of the step cycle duration is shown in Fig. 4, A and B, for two cats, CC2 and CC4, which received clonidine intraperitoneally and intrathecally, respectively. Similar results were obtained. During predrug trials, the swing duration remained relatively stable over time, whereas the stance duration significantly increased at d8–d9 approaching normal values. After clonidine injection, both stance and swing approached the normal values.

In CC2, after clonidine injection, the stance duration increased from d3 to d9 with an intermittent peak at d5, whereas the stance duration varied randomly before clonidine injection when the cat was not walking well. Before clonidine injection, from d3 to d7, the cat exhibited only rudimentary rhythmic movements of the hindlimbs. After clonidine injection, the stance duration was increased by 32% from d3 to d4 and by 14% from d4 to d5. The progressive increase in the stance duration is one indication of the progressive improvement of the locomotor performance. This progress was apparent only after clonidine injection as the cats were not walking before clonidine injection. The progressive improvement of locomotion was possibly due to the locomotor training made possible after clonidine injection.

**Step length**

After spinalization, the step length was very much reduced but clonidine restored it toward normal values. The relationship between the step length during predrug and postdrug trials in three spinal cats, CC2, CC3, and CC4, is shown in Fig. 5. In Fig. 5, A and B, the step length during the preclonidine trials from 3 to 7 days posttransection is small as indicated by the nearly horizontal slope, whereas the step length postclonidine was increased. From 7 to 9 days posttransection, there was a sharp increase in the step length in these two cats during the preclonidine trials to almost the intact values. Also, the data points formed a cluster along an oblique line, indicating that with time, there was increasing effect of clonidine.
Table 2. A summary of the numeric values of the cycle duration and step length on the day of recovery of spontaneous locomotion

<table>
<thead>
<tr>
<th>Cat</th>
<th>Condition</th>
<th>Days</th>
<th>n</th>
<th>Cycle Duration, ms</th>
<th>Percent</th>
<th>Step Length, mm</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB6</td>
<td>Intact</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>7d</td>
<td>4</td>
<td>1,029 ± 122</td>
<td>NA</td>
<td>194 ± 55</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>6</td>
<td></td>
<td>1,536 ± 157</td>
<td>NA</td>
<td>308 ± 37</td>
<td>NA</td>
</tr>
<tr>
<td>CC1</td>
<td>Intact</td>
<td>3</td>
<td></td>
<td>1,389 ± 173</td>
<td>NA</td>
<td>477 ± 138</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>11d</td>
<td>5</td>
<td>1,056 ± 229</td>
<td>77</td>
<td>236 ± 43</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>4</td>
<td></td>
<td>1,017 ± 78†</td>
<td>73</td>
<td>161 ± 12*</td>
<td>34</td>
</tr>
<tr>
<td>CC2</td>
<td>Intact</td>
<td>6</td>
<td></td>
<td>1,475 ± 184</td>
<td>NA</td>
<td>396 ± 54</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>8d</td>
<td>4</td>
<td>1,220 ± 101†</td>
<td>83</td>
<td>235 ± 31*</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>4</td>
<td></td>
<td>1,379 ± 37</td>
<td>94</td>
<td>314 ± 18</td>
<td>79</td>
</tr>
<tr>
<td>CC3</td>
<td>Intact</td>
<td>15</td>
<td></td>
<td>1,471 ± 218</td>
<td>NA</td>
<td>345 ± 43</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>9d</td>
<td>9</td>
<td>883 ± 112*</td>
<td>60</td>
<td>210 ± 26*</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>14</td>
<td></td>
<td>1,102 ± 68*</td>
<td>75</td>
<td>252 ± 22*</td>
<td>75</td>
</tr>
<tr>
<td>CC4</td>
<td>Intact</td>
<td>5</td>
<td></td>
<td>1,176 ± 78</td>
<td>NA</td>
<td>503 ± 30</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>6d</td>
<td>9</td>
<td>1,114 ± 168</td>
<td>95</td>
<td>373 ± 49*</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>8</td>
<td></td>
<td>1,091 ± 75</td>
<td>93</td>
<td>334 ± 44*</td>
<td>73</td>
</tr>
</tbody>
</table>

Values are means ± SD. Summary of values for all cats on the day of locomotor recovery when locomotion during predrug trials was observed, which ranges from 6 to 11 days posttransection (see column Days). The pre- and postdrug value is also expressed as a percentage of the intact value. Student’s t-tests were performed to compare if the pre- or the postdrug values were significantly different from the intact condition. * P ≤ 0.01. † P ≤ 0.05.

Treadmill speed 0.2 m/s.

In CC4, which received clonidine intrathecally (Fig. 5C), at 5 days posttransection, the step cycle length was very similar to that during the intact locomotion (86% of the intact). Also, as early as 6 days posttransection, the step length during preclonidine trials increased significantly and even slightly more than after clonidine injection. For all the data shown in Fig. 5C, the total dose of clonidine given each day was the same (100 μl of 4 mM it).

Table 2 shows the values of step cycle duration and step length for all of the cats when they were capable of walking (6–11 days). These are the data values used in the histogram (Fig. 3) and x-y plots (Figs. 4 and 5). In the table, the cycle durations during the pre- and postdrug trials also were expressed as percentages of the intact values. In all cats, ranging from 6 to 11 days posttransection, the cycle duration during predrug trials was similar to or lower than that seen during intact locomotion. After clonidine injection, cycle duration increased in most spinal cats. The cycle duration reached the intact value except in one case, CC3. The step length during pre- and postclonidine trials were all lower than the intact control, and the difference was statistically significant.

Angular excursion

The ranges of joint angles of one cat (CC2) during intact, pre- and postclonidine at 0.2 m/s are shown in Fig. 6. Before clonidine injection, on 3–7 days posttransection, there was very little movement in all joints as illustrated (Fig. 6, A–I). Beginning on d8, there was an increase in the movements at all joints. On d9 posttransection, the angular movements (shown both as ranges or maximum minus minimum angle) of the hip, knee, ankle and mtp (Fig. 6, B, D, F, and H), and the step cycle length (Fig. 6I) increased but remained below the intact values (horizontal dotted line). Also, there was a gradual increase in all joint excursions with a parallel
there was a significant increase in the angular movements in all joints compared with the predrug values. There was an increase in hip (Fig. 6K) and ankle (Fig. 6O) joint excursions that exceeded the normal joint movement after clonidine injection and tapered off by d8 and d9 but still remained above normal values at d9. Figure 6R showed that although there was a slight increase in the step length over time, it was still below normal values despite an above-normal increase in the hip and ankle joint excursion as described above.

This paradoxical decrease in step length despite the increase in angular excursion postclonidine can be related to the synchronous and exaggerated hip and knee flexion during swing followed by synchronous hip and knee extension before paw contact resulting in a decreased forward distance for each step. In normal cats, during the late swing phase (E1), the hip continues to flex while the knee begins to extend to promote forward placement of the paw at contact (see Fig. 1). In spinal cats postclonidine, the hip and knee flex and then extend at the same time before paw contact. A synchronous knee and hip extension results in a backward placement of the foot, reducing the step length measurement (see Fig. 2H). This observation was common among all experimental cats in this study. The exaggeration in the angular excursions can be caused partly by the perineal stimulation given that was required to initiate locomotion during the early days posttransection.

The values of all these joint angular movement ranges during intact, predrug and postdrug trials from five spinal cats on selected postspinal days showing locomotor recovery are shown in Table 3. These values also were expressed as percentages of the intact value for each cat. The values for the postspinal days shown here represents the first day that the cat could walk on the treadmill without need of clonidine injection. For example, in CC3 during the predrug trials, the hip angular excursion was 77% of the intact value, and on d9, the angular excursion increased to 114% of the intact value. For CC2, both d8 and d9 are shown, and an increase in hip, knee, ankle, and mtp joint excursion can be seen from one day to the next even during predrug trials. For example, in d8, the hip angular excursion was 77% of the intact value, and on d9, the angular excursion increased to 114% of the intact value. For CC4, which received intrathecal clonidine, the hip angular excursion increased during postdrug trials. The ankle angular excursion during the predrug trials was exaggerated (285%) and returned to a more normal value (160%) after clonidine injection.

**Speed adaptation**

All of the cats demonstrated, both pre- and postclonidine, a progressive ability to adapt their locomotor patterns to a range of treadmill speeds \( \geq 1.0 \text{ m/s} \). As seen in Fig. 7, in the intact condition (hatched area), step cycle duration decreased as the treadmill speed increased. During the intact condition, the locomotion was recorded between 0.2 and 0.6 m/s because this cat did not walk \( >0.6 \text{ m/s} \). The ability to adapt to treadmill speed also was found to be a progressive
process. On d4 (Fig. 7A), after clonidine injection, the cat was capable of walking ≤0.3 m/s, which was an improvement from the previous day (d3, not shown) when the cat could not walk >0.2 m/s. On the following 2 days (d5–d6), the cat could adapt to increasing treadmill speed ≤0.5 m/s with clonidine. By d7, the cat could walk at treadmill speeds ≥0.7 m/s after clonidine injection. Until this point (d4–d7), the cat still couldn’t walk at 0.3 m/s before cloni-
EMG

It is essential to examine the EMG changes accompanying the kinematic changes seen with clonidine injection and training after spinalization to better understand the possible underlying neurophysiological changes. Previously, in Fig. 2, we have shown the progressive kinematic changes in the locomotor pattern on different days posttransection; the corresponding EMG activity of the cat CC2 is shown in Fig. 8. All the EMG traces were synchronized to the iSt, and all the gains were kept constant to enable comparison of the EMG activity during intact (Fig. 1B), pre- and postclonidine conditions. The thin lines of Fig. 8J also shows the EMG signals in the intact state.

On d3 postspinalization, before clonidine injection, there was no organized EMG activity (Fig. 8A). There was some tonic activity in the flexors (iSrt and St) and no activity in the extensors (VL, GL, and Glu). After clonidine injection, clear alternating rhythmic bursting of the flexors and extensors can be seen during treadmill locomotion (Fig. 8F). However, activity of most proximal muscles such as the hip extensor (Glu) was much reduced compared with the intact condition. The burst duration of St also was prolonged greatly and the iSrt duration much shorter as compared with the intact pattern. The reduced activation of the proximal flexors such as iSrt may contribute to reduced flexion, and thus the extended position of the hindlimb throughout the step cycle.

From d5 to d7, there was still no organized EMG activity before clonidine injection. After clonidine injection, there was an improvement in the EMG pattern as compared with d3 (Fig. 8G). The emergence of burst activity was seen in both the hip and the ankle extensors, iGlu and iGL, respectively. This increase in extensor activity may have contributed to the improved weight support of the cat at d5. A definite burst activity of a knee flexor (coSt) also was observed although the burst duration was very long.

On d7, after clonidine injection, there was a change of the EMG pattern as compared with d3 and d5 (Fig. 8H). The burst duration of the hip flexor (iSrt) increased significantly; this may explain the increased forward placement of the paw in front of the hip or aligned with the hip as opposed to behind the hip as seen in d3. Also, an increase in hip, knee, and ankle extensor (iGlu, iVL, and iGL) activity was seen. This increase in the hindlimb extensor muscle activity presumably contributed to the significant increase in the weight support of the animal at this stage. The burst duration of the contralateral knee flexor (coSt) was reduced compared with that seen on d5. Thus despite the lack of improvement in the EMG pattern before clonidine injection, a clear progression of the EMG pattern can be seen after clonidine injection at d7 as compared with d3 posttransection.

By d8, some rhythmic bursting in the flexors and extensors emerged before clonidine injection. For example, there was a reduction in tonic activity in knee flexors (St and coSt) as compared with d3 or d7 (Fig. 8D). Extensor (VL and GL) burst activity increased and appropriate St bursts were also seen. However, iGlu activity was still absent. After clonidine injection (Fig., 8I), there was an increase in the hip and knee extensor activity (Glu and VL) and a decrease in the GL activity approaching that seen in the intact cat.

At d9, there was a further increase in the activity of all muscles as compared with the pattern seen on d8. The iSt, iSrt, and iVL increased in amplitude and showed an appro-

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**TABLE 3.** Numeric values of the range of joint angular excursion on the day of recovery of spontaneous locomotion

<table>
<thead>
<tr>
<th>Cat</th>
<th>Condition</th>
<th>Day</th>
<th>Range</th>
<th>Percent</th>
<th>Range</th>
<th>Percent</th>
<th>Range</th>
<th>Percent</th>
<th>Range</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB6</td>
<td>Intact</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>7d</td>
<td>35 ± 14</td>
<td>NA</td>
<td>25 ± 12</td>
<td>NA</td>
<td>64 ± 18</td>
<td>NA</td>
<td>56 ± 27</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td></td>
<td>NA</td>
<td>35 ± 8</td>
<td>NA</td>
<td>35 ± 7</td>
<td>NA</td>
<td>35 ± 8</td>
<td>NA</td>
<td>50 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9d</td>
<td>30 ± 4</td>
<td>NA</td>
<td>29 ± 4</td>
<td>NA</td>
<td>33 ± 9</td>
<td>NA</td>
<td>45 ± 14</td>
<td>NA</td>
</tr>
<tr>
<td>CC1</td>
<td>Intact</td>
<td>11d</td>
<td>27 ± 10</td>
<td>135</td>
<td>20 ± 8</td>
<td>70</td>
<td>58 ± 23</td>
<td>175</td>
<td>27 ± 9</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>1d</td>
<td>35 ± 13</td>
<td>175</td>
<td>40 ± 12</td>
<td>138</td>
<td>91 ± 24*</td>
<td>276</td>
<td>29 ± 8</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Postdrug</td>
<td>4</td>
<td>22 ± 5</td>
<td>NA</td>
<td>39 ± 7</td>
<td>NA</td>
<td>26 ± 9</td>
<td>NA</td>
<td>55 ± 15</td>
<td>NA</td>
</tr>
<tr>
<td>CC2</td>
<td>Intact</td>
<td>8d</td>
<td>17 ± 6</td>
<td>77</td>
<td>16 ± 9*</td>
<td>41</td>
<td>27 ± 9</td>
<td>104</td>
<td>21 ± 10*</td>
<td>38</td>
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<tr>
<td></td>
<td>Predrug</td>
<td>7d</td>
<td>27 ± 6</td>
<td>123</td>
<td>22 ± 5*</td>
<td>56</td>
<td>48 ± 12*</td>
<td>185</td>
<td>69 ± 20</td>
<td>125</td>
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<tr>
<td></td>
<td>Postdrug</td>
<td>9d</td>
<td>25 ± 5</td>
<td>114</td>
<td>24 ± 7*</td>
<td>62</td>
<td>37 ± 10</td>
<td>142</td>
<td>46 ± 42</td>
<td>84</td>
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<td></td>
<td></td>
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<td>204</td>
<td>43 ± 13</td>
<td>78</td>
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<tr>
<td>CC3</td>
<td>Intact</td>
<td>9d</td>
<td>19 ± 5*</td>
<td>73</td>
<td>16 ± 5*</td>
<td>67</td>
<td>31 ± 9†</td>
<td>148</td>
<td>42 ± 43</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Predrug</td>
<td>9d</td>
<td>31 ± 4†</td>
<td>119</td>
<td>39 ± 5*</td>
<td>163</td>
<td>46 ± 8*</td>
<td>219</td>
<td>47 ± 30</td>
<td>100</td>
</tr>
<tr>
<td>CC4</td>
<td>Intact</td>
<td>5</td>
<td>28 ± 10</td>
<td>114</td>
<td>34 ± 7</td>
<td>97</td>
<td>57 ± 15*</td>
<td>285</td>
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<td>6d</td>
<td>32 ± 6</td>
<td>114</td>
<td>34 ± 7</td>
<td>97</td>
<td>57 ± 15*</td>
<td>285</td>
<td>62 ± 18</td>
<td>126</td>
</tr>
</tbody>
</table>

The averaged angular excursion (in degrees, mean ± SD) was obtained during intact, predrug trials and postdrug trials. The angular excursions during pre- and postdrug trials are also expressed as a percentage of the intact values. Student’s t-tests were performed to compare whether the pre- or the postdrug angular excursions were significantly different from the intact condition. MTP, metatarso-phalangeal. *P < 0.01. †P < 0.05.
priate bursting pattern (Fig. 8E). After clonidine injection, all EMG activity increased and very clear bursting of each muscle can be seen (Fig. 8J). This clonidine-induced locomotion at d9 also was compared with the normal locomotion (overlay on Fig. 8J, with EMG traces during intact condition shown as thinner lines). The EMG pattern resembled that seen in the intact condition, but some differences still were present. These differences included increased EMG amplitude in all muscles, prolonged ST burst duration, synchronous activation of iSrt and iSt as opposed to a later activation of iSrt to iSt in the intact. The difference in timing of muscle bursts also can be seen in Glu, a hip abductor and extensor. In the intact locomotion, the Glu had a much longer burst than it showed after spinalization; this may contribute to a longer step length than that seen in the spinal locomotion.

To summarize, after a daily clonidine injection followed by training, there was an organized EMG pattern seen as early as d9 posttransection without clonidine injection. There was a progressive improvement of the EMG pattern from d3 to d9 after clonidine injection. This progressive improvement included the emergence of an alternating bursting activity of flexor and extensor activity and increased EMG amplitudes.

There was a maturation of the EMG pattern over time. This is shown in Fig. 9 with results from a different cat, CC3, as an example. The cat was walking at 0.3 m/s. At 7 days posttransection, after clonidine injection, although there was an alternating pattern between the flexors and the extensors, they were almost of the same duration. The burst duration of hip flexors (iSrt, coSrt) and knee flexors (iSt and coSt) were much longer than was seen during the intact condition. For example, during the intact condition (Fig. 9A), the characteristic of St was a very short double burst, activation of iSrt and iSt as opposed to a later activation of iSrt to iSt in the intact. The difference in timing of muscle bursts also can be seen in Glu, a hip abductor and extensor. In the intact locomotion, the Glu had a much longer burst than it showed after spinalization; this may contribute to a longer step length than that seen in the spinal locomotion.

On 9 days posttransection, a more detailed and complex EMG pattern can be seen (Fig. 9C). For example, the duration of the flexor bursts (iSt, iSrt, coSt) was much shorter than at 7 days posttransection. A double burst also can be seen in St. Therefore, the characteristic short flexor and long extensor burst activity patterns were restored.
FIG. 8. Rectified, averaged, and normalized EMG activity of the cat CC2 (same cat as in Fig. 2) synchronized to iSt at 3, 5, 7, 8, and 9 days postspinalization, during preclonidine and postclonidine trials. Note that the EMG signals in J (9 days postspinalization) are superimposed with the intact EMG signals (as seen in Fig. 1B) indicated by the thin lines.
initially with time (d3–d5), the flexor burst duration did not. The progressive increase in the extensor duration therefore would contribute to the increase in the stance duration over time.

Table 4 shows the numeric values of the EMG burst duration and percentage of EMG amplitude of different flexor and extensor muscles of the five experimental cats on selected days when the cats were walking before clonidine injection. In general, the EMG burst duration of the hindlimb muscles during the predrug trials approached the intact values (CC1, CC2, CC3, and CC4), indicating locomotor recovery. For example, in predrug trials of CC2 on d9, the burst duration of iSt, iSrt, iVL, and iGL were 88, 79, 97, and 80% of the intact values, respectively. After clonidine injection, the EMG burst duration and the relative EMG amplitude of flexors (iSt or iSrt) also were increased as compared with the predrug trials. For example, in CC1, the EMG burst duration of iSt increased from 70 to 113%, and the EMG amplitude increased from 145 to 345%. Changes in EMG burst duration and amplitudes also were seen, although more variable, in the extensor muscles, iVL or iGL.

DISCUSSION

Overview

In the present study, we examined the recovery of locomotion of the hindlimbs after spinalization using early locomotor training made possible by the injection of clonidine. We found that the locomotor pattern elicited by clonidine was rudimentary soon after spinalization and became more complex with time, that a gradual improvement of locomotion during the first week after spinalization was revealed by daily injection of clonidine, and that early locomotor training under the influence of clonidine resulted in an early recovery (6d–11d) of a locomotor pattern that was similar in many respects to the intact pattern.

Evolution of locomotor recovery

In five spinal cats, which received daily injections of clonidine and early locomotor training, recovery of locomotion could be attained as early as 6–11 days posttraection with weight support and proper foot contact (see Table 1). This recovery period is shorter than that reported in the literature. The relative EMG amplitude and duration was calculated as a percentage of the corresponding intact value. In the predrug trials, no data were available before d8 as the cat was not walking without clonidine injection in that period. When the cat began to walk on d8 before clonidine injection, the data from d8 and d9 are shown by stippled lines. There was an increase of the EMG amplitude of flexor and extensor muscles from d8 to d9. On d9, the relative EMG amplitude of these muscles during predrug trials were close to the intact values. After clonidine injection, the iSt EMG amplitude was initially above normal and stabilized at 150% of the intact value, whereas the iVL amplitude continuously increased from d5 to d9 posttranssection.

In Fig. 10B, the EMG burst duration of different muscles after clonidine injection is shown. It shows that, although the extensor EMG burst duration (iGL and iVL) increased initially with time (d3–d5), the flexor burst duration did not. The progressive increase in the extensor duration therefore would contribute to the increase in the stance duration over time.
induced locomotion may contribute to an earlier recovery of locomotion.

Progressive changes in the locomotor pattern were observed over time. The gradual improvement of locomotion from d3 to d7 (see Figs. 5–7) was apparent only with clonidine injection because the animal could not walk without clonidine. There was a gradual increase in the step duration, in the ability to support weight, and in the adaptation to higher speed (see Figs. 5–7). This is in agreement with the results of Barbeau and Rossignol (1987) and Barbeau et al. (1993), who reported that in chronic spinal cats that received clonidine an improvement in the locomotor pattern (increase cycle duration and weight support) was seen from d2 to d7, and from d7 to d9 postspinalization.

The time-dependent improvement also was seen in the central locomotor pattern recorded after paralysis (Pearson and Rossignol 1991). The fictive locomotor pattern was recorded from hindlimb nerves in two groups of adult chronic spinal cats. One group was trained to step on the treadmill (late-spinal animals) and the other was not trained and was examined as early as 4 days after partial cord lesion in rats (Li and Raisman 1994). It is possible that early locomotor training may affect the evolution of the spinal cord undergoing plastic changes after spinalization. Thus locomotor training may change, enhance, or guide the underlying plastic changes that will optimize the locomotor recovery. These plastic changes may occur at different levels such as anatomic, physiological, or neurochemical.

Anatomic changes

Anatomic changes such as collateral sprouting can contribute to the recovery of function after injury. Collateral sprouting was found during the recovery period in different preparations including partially hemisected animals, complete unilateral hindlimb deafferented animal, and after partial unilateral rhizotomy or the spared root preparation (Goldberger and Murray 1974, 1982; Liu and Chambers 1958; Robinson and Goldberger 1986; Murray and Goldberger 1974, 1986; Zhang et al. 1995). In the partially hemisected cat, a lesion was made between T6 and L1 of the cat spinal cord sparing the dorsal column. It was found that the use of the limbs for standing and locomotion and the responses to segmental reflex stimulation (but not crossed reflex elicitation) progressively improved beginning at 2 wk posthemisection. Using radioautographic methods (injection of [H]-proline) they found evidence of collateral sprouting from dorsal roots at 20 days after hemisection, (Murray and Goldberger 1974). In the spared root preparation, where all dorsal roots caudal to L6 were cut except L6, they found that the L6 roots projected as far as T3 on both sides. That is, the increase in the amount of projection was confined to normal limits (Goldberger and Murray 1982). Also, using electron microscopy, they found morphological changes (complex terminals, originate exclusively form dorsal roots) in the dorsal horn. The number of complex terminals decreased acutely (3 days postop), representing a loss of terminals from the cut roots. The number returned to normal levels during the chronic stage (3–10 wk) (Zhang et al. 1995). Therefore, collateral sprouting in the adult lesioned cat can contribute to the recovery of function. In our study, a complete spinal transection was performed in all cats, preventing sprouting of the descending system below the lesion. However, we cannot rule out the contribution of sprouting of neurons such as primary afferents below the lesion to recovery at a later stage. Collateral sprouting usually is considered as a long process (3–10 wk), and it is unlikely that the early locomotor recovery (within 1st 10 days posttransection) observed can be attributed primarily to the anatomic plasticity. However, because it was found that sprouting can begin as early as 4 days after partial cord lesion in rats (Li and Raisman 1994), it is possible that early training may guide or enhance the ongoing sprouting process and promote the recovery of locomotion.
TABLE 4. Numeric values of the EMG burst duration and amplitude of flexor and extensor muscles

<table>
<thead>
<tr>
<th>Cat</th>
<th>Day</th>
<th>iSt</th>
<th>n</th>
<th>Intact</th>
<th>n</th>
<th>Burst Duration, ms</th>
<th>Predrug</th>
<th>n</th>
<th>Postdrug</th>
<th>Burst Amplitude, %</th>
<th>Predrug</th>
<th>Postdrug</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB6</td>
<td>9</td>
<td>NA</td>
<td>7</td>
<td>300 ± 87</td>
<td>7</td>
<td>624 ± 148</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>iSt</td>
<td>8</td>
<td>178 ± 49 (70)</td>
<td>13</td>
<td>286 ± 60 (113)</td>
<td>148</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC1</td>
<td>9</td>
<td>iSrt</td>
<td>6</td>
<td>298 ± 99</td>
<td>14</td>
<td>262 ± 95 (88)</td>
<td>245</td>
<td>87</td>
<td>82</td>
<td>174</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>CC2</td>
<td>6</td>
<td>iSt</td>
<td>10</td>
<td>159 ± 49 (69)</td>
<td>16</td>
<td>182 ± 50 (80)</td>
<td>202</td>
<td>33</td>
<td>57</td>
<td>147</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC3</td>
<td>15</td>
<td>iSt</td>
<td>11</td>
<td>122 ± 20 (82)</td>
<td>9</td>
<td>188 ± 43 (154)</td>
<td>669</td>
<td>85</td>
<td>72</td>
<td>287</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>CC4</td>
<td>6</td>
<td>iSrt</td>
<td>33</td>
<td>154 ± 35 (54)</td>
<td>191</td>
<td>33 ± 145</td>
<td>338</td>
<td>192</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iVL</td>
<td>100</td>
<td>839 ± 169 (103)</td>
<td>671</td>
<td>103 ± 82 (103)</td>
<td>247</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iGL</td>
<td>118</td>
<td>883 ± 195 (130)</td>
<td>736</td>
<td>117 ± 109 (109)</td>
<td>131</td>
<td>138</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values taken from five experimental cats when the cat could walk during predrug trials (6–11d). The burst durations during pre- and postdrug trials are expressed in milliseconds as means ± SD and as a percent of the intact values in parentheses. Burst amplitudes were expressed as a percent of the intact values. i, ipsilateral; St, semitendinosus; Srt, sartorius; VL, vastus lateralis; GL, gastrocnemius lateralis.

Neurochemical changes

There is also evidence of modification of spinal receptor activity after complete spinal cord transection. For example, specific receptor supersensitivity after spinal cord transection was reported (Barbeau and Bedard 1981). Denervation supersensitivity can be attributed to the gradual disappearance of the noradrenergic terminals below the transection (Haggendal and Dahlstrom 1973). Recently, Giroux et al. (1995) reported, in the spinal cord of chronic spinal cats (Th13), an upregulation of serotonin 1A receptors, α1-noradrenergic receptors, and α2-noradrenergic receptors labeling below the lesion 15–30 days after spinalization (Giroux et al. 1995). In rats, a significant increase in α1- and α2-adrenoceptors densities also was found after a complete transection of the spinal cord at vertebral level T5–T9 (Roudet et al. 1993, 1994).

It is possible that the effects of clonidine in mediating the locomotor recovery may be related partly to these receptor changes after spinal cord transection. Indeed, recent findings in our laboratory show that intrathecal injection of clonidine in intact cats produced much less pronounced effects than those observed in spinalized animals. Thus it is suggested that the effect of clonidine in spinalized animals is related to the changes in the receptor sensitivity. To what extent the daily injection of clonidine could change the fate of the receptors is still unknown.

Physiological changes

PLASTICITY OF NEURONAL CIRCUITRY. Although it has been suggested that the spinal cord circuitry generating locomotor function is hard-wired and has a limited capacity to reorganize itself after injury (Forssberg and Svartengren 1983; Sperry 1940, 1941), there is some evidence that the spinal circuitry can undergo some physiological changes.

Early studies using spinal kittens and one spinal puppy demonstrated that chronic spinal mammals were capable of acquiring motor conditioned responses through training (Shurrager 1955). Experiments on classical conditioning of the flexion reflex in spinal cats (Durkovic 1983) and operant conditioning of the H-reflex experiment in monkey (Wolpaw and Chong 1989; Wolpaw et al. 1989) also showed the capacity for functional changes at the spinal cord level. In these studies, the simple monosynaptic spinal reflex was suggested to have undergone adaptive changes at the segmental level in the presence of supraspinal influences.

In our laboratory, we also have demonstrated the functional plasticity of the spinal cord after a unilateral lesion of the ankle flexor nerves (Carrier et al. 1992, 1997). In neurontomized cats that already have compensated successfully for the loss of ankle function by an increase of hip or knee flexion, a superimposed spinalization revealed an asymmetrical spinal locomotor pattern, with large hyperflexion of the knee on the lesioned side. It was suggested that readjusted descending input after neuromectomy in the otherwise normal cat may have caused plastic changes in the spinal circuitry to maintain locomotion, and these adaptive changes became evident when all descending inputs were removed as in the case of spinalization. These findings are in accordance with the suggestion by Wolpaw and Carp that exposure of the spinal circuitry to supraspinal influences can induce intrinsic and long-term changes in the spinal cord (Wolpaw and Carp 1993). These studies support the notion that the spinal cord is capable of adaptive plasticity when there are changes in the supraspinal and/or peripheral inputs.

TRAINING EFFECT. As mentioned in the introduction, training plays an essential role in the recovery of locomotion in adult spinal cats. It is possible that locomotor training can induce and/or may enhance plastic changes within the spinal cord (deprived of all descending inputs) responsible for the gradual recovery in locomotor function with time.

Edgerton and colleagues reported that training could in-
duce functional changes in the spinal circuitry in generating a motor task (Edgerton et al. 1991; Hodgson et al. 1994). They showed that cats trained to stand (standing-trained) had great difficulty stepping, and the stepping-trained cats had great difficulty in maintaining a standing posture. Because the musculature between cats that were trained to stand and cats that were trained to walk were similar, they suggested that the training effect on locomotor recovery was of neural origin rather than of muscular origin.

Viala and colleagues (1986) also showed that spinal rabbits exhibit different locomotor stepping pattern depending on the types of training they received. Infant spinal rabbits that were trained on a motor-driven “bicycle,” which moves the hindlimbs either synchronously or alternatively exhibited synchronous stepping or alternating stepping locomotor pattern, respectively, after training.

Thus these findings not only suggest that the spinal cord is capable of learning (through training), but they also demonstrate a high level of specificity in learning the motor task in the spinal cord. These studies also suggest that peripheral afferent inputs could strongly affect the plasticity of central locomotor networks during early development.

In the present study, the amount of peripheral afferent input also may help determine the outcome of training. For example, the cat that received clonidine intrathecally (CC4) fared better than cats that received clonidine intraperitoneally (HB6, CC1, CC2, and CC3). CC4 was capable of generating a well-organized locomotor pattern at 6 days posttranssection as opposed to 9–11 days in the other cats. It is possible that 100 μg clonidine intrathecally was a high enough dose to activate the locomotor pattern more strongly soon after spinalization as compared with the other cats. The relatively more forceful locomotor pattern elicited by clonidine then may provide a more adequate afferent inflow to the spinal network generating locomotion early on rather than a rudimentary locomotor pattern, and this may have contributed to the earlier recovery in CC4.

Clinical significance

Studies involving the recovery of locomotor functions of incomplete paraplegic patients showed that treadmill training with a body weight support system improves significantly the locomotor pattern in these subjects (Barbeau et al. 1992; Dietz et al. 1994, 1995; Fung et al. 1990; Visintin and Barbeau 1992; Wernig and Muller 1992). After 1–7 mo of training, marked improvements were seen in weight support capability, the walking speed, and the timing and coordination of the EMG pattern (Barbeau et al. 1992; Dietz et al. 1994, 1995; Wernig and Muller 1992). Recent studies using another form of locomotor training, functional electrical stimulation-assisted walking, have shown improvements of walking speed after 1 yr of training in incomplete spinal cord injured subjects (Wieler et al. 1995). Barbeau and colleagues reported improvements in locomotion in two subjects with chronic incomplete spinal cord injuries after a treatment regimen that incorporated the combined effects of clonidine and cyproheptadine (a serotonergic antagonist) together with a treadmill training program while the subject was supported by a body weight support harness system. The weight-bearing ability of the subjects improved, their posture became upright, the flexor spasms decreased, the walking speed and the stride length also increased (Barbeau et al. 1992; Fung et al. 1990; Visintin and Barbeau 1989). Taken together, locomotor training alone or in combination with pharmacological intervention was found to be beneficial in these subjects.

As indicated in the present animal study, it is possible that earlier training started as soon as possible after spinal injury also might be beneficial and need further investigation in spinal cord injured subjects.

In summary, the present results support the idea that the spinal cord undergoes plastic changes after spinal injury and that locomotor training given during the early postspinal period may be beneficial. The locomotor recovery we observed might be a result of an interaction between the central plasticity and peripheral input. Although clonidine may uncover or “release” the rudimentary locomotor rhythm, the spinal locomotor network might be further consolidated or molded through the peripheral afferent inflow during locomotor training. Early locomotor training may activate peripheral afferents, including cutaneous and proprioceptive, which interact with the normal plastic changes (physiological, anatomical, or neurochemical) and probably reinforce the spinal locomotor network.

The understanding of adaptive capacity of the spinal cord may lead to new and better approaches to the abnormal segmental function caused by spinal cord injury, stroke, or other central lesions. This study provides a rationale for a treatment strategy that incorporates drug therapy and training and might offer hope to improve the recovery process in spinal cord injured patients.

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