Recovery of Locomotion After Ventral and Ventrolateral Spinal Lesions in the Cat. II. Effects of Noradrenergic and Serotonergic Drugs

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Brustein, Edna and Serge Rossignol. Recovery of locomotion after ventral and ventrolateral spinal lesions in the cat. II. Effects of noradrenergic and serotonergic drugs. J. Neurophysiol. 81: 1513–1530, 1999. The effects of serotonergic and noradrenergic drugs (applied intrathecally) on treadmill locomotion were evaluated in two adult cats subjected to a ventral and ventrolateral spinal lesion (T13). Despite the extensive spinal lesion, severely damaging important descending pathways such as the reticulo- and vestibulospinal tracts, both cats recovered quadrupedal voluntary locomotion. As detailed in a previous paper, the locomotor recovery occurred in three stages defined as early period, when the animal could not walk with its hindlimbs, recovery period, when progressive improvement occurred, and plateau period, when a more stable locomotor performance was observed. At this latter stage, the cats suffered from postural and locomotor deficits, such as poor lateral stability, irregular stepping of the hindlimbs, and inconsistent homolateral fore- and hindlimb coupling. The present study aimed at evaluating the potential of serotonergic and/or noradrenergic drugs to improve the locomotor abilities in the early and late stages. Both cats were implanted chronically with an intrathecal cannula and electromyographic (EMG) electrodes, which allowed determination, under similar recording conditions, of the locomotor performance pre- and postlesion and comparisons of the effects of different drugs. EMG and kinematic analyses showed that norepinephrine (NE) injected in early and plateau periods improved the regularity of the hindlimb stepping and stabilized the interlimb coupling, permitting to maintain constant locomotion for longer periods of time. Methoxamine, the α1-agonist (tested only at the plateau period), had similar effects. In contrast, the α2-agonist, clonidine, deteriorated walking. Serotonergic drugs, such as the neurotransmitter itself, serotonin (5HT), the precursor 5-hydroxytryptophan (5HTP), and the agonist quipazine improved the locomotion by increasing regularity of the hindlimb stepping and by increasing the step cycle duration. In contrast, the 5HT1A agonist 8-hydroxy-dipropylaminotetralin (DPAT) caused foot drag in one of the cats, resulting in frequent stumbling. Injection of combination of methoxamine and quipazine resulted in maintained, regular stepping with smooth movements and good lateral stability. Our results show that the effects of drugs can be integrated to the residual voluntary locomotion and improve some of its postural aspects. However, this work shows clearly that the effects of drugs (such as clonidine) may depend on whether or not the spinal lesion is complete. In a clinical context, this may suggest that different classes of drugs could be used in patients with different types of spinal cord injuries. Possible mechanisms underlying the effect of noradrenergic and serotonergic drugs on the locomotion after partial spinal lesions are discussed.

INTRODUCTION

Adult cats subjected to a massive bilateral ventral and ventrolateral spinal lesion at the lowest thoracic level, severely damaging ascending and descending pathways, among others, the reticulospinal and vestibulospinal pathways, can recuperate voluntary quadrupedal locomotion (Bem et al. 1995; Brustein and Rossignol 1998; Gorska et al. 1993a,b; Rossignol et al. 1996). However, as described in detail in a preceding paper (Brustein and Rossignol 1998), the cats suffered from transient deficits, such as long-lasting postural and locomotor deficits, the severity of which depended on the extent of the spinal lesion. Early after the lesion (early period), none of the cats could walk or support the weight of their hindquarters, and they moved around using only the forelimbs. During the recovery period, which lasted from 3 days up to 3 wk depending on the extent of the spinal lesion, the cats gradually regained the ability to support weight and walk with their hindlimbs. However, even when having reached a plateau period during which there was no further improvement, the quadrupedal stepping overground and on the treadmill remained wobbly with poor lateral stability. The coupling phase between the homolateral fore- and hindlimb was highly variable, and, in the most lesioned cats, the fore- and the hindlimbs could walk at different frequencies, which caused continual deviations in the coupling phase between the two girdles. These locomotor deficits often resulted in stumbling or falling on one side, which limited the cats to only a few consecutive steps at a time and to low treadmill speeds (0.1–0.35 m/s). These locomotor deficits were more pronounced during walking uphill or downhill on inclined treadmill (10°), and the amplitude modulation (AM) of the hindlimb electromyographic (EMG) activity, usually seen when walking uphill, was absent.

The most extensively lesioned cats did not improve their locomotor skills very much with time and expressed these deficits even 300 days postlesion. It was suggested that after functional and anatomic reorganization, the remaining pathways in the dorsolateral funiculi, such as the corticospinal, the remaining reticulospinal, and possibly propriospinal, were sufficient to provide the necessary drive for initiation of locomotion as well as some postural control. However, their contribution is limited because the cats cannot maintain constant fore- and hindlimb coupling or adapt to more demanding situations such as inclined walking.

It is clinically relevant to try and improve the locomotor capacities in this animal model of incomplete spinal lesion because the relative percentage of partial versus complete spinal lesions is increasing in the population of patients (Tator et al. 1993). Patients with partial spinal lesions exhibit residual locomotor capacities but suffer from locomotor and postural deficits such as difficulties in bearing weight, maintaining...
balance, and adapting their walking speed (Barbeau and Rossignol 1994). Therefore the purpose of this study was to try and improve the residual voluntary locomotor function by applying different noradrenergic (NE) and serotoninergic (SHT) drugs through a chronic intrathecal cannula. These two groups of drugs were chosen because of their known involvement in initiation and/or modulation of locomotion in complete spinal cats (Barbeau and Rossignol 1994; Barbeau et al. 1993; Chau et al. 1998a,b).

The specific purpose of this study was then to investigate the effects of noradrenergic and serotoninergic drugs on the treadmill locomotion of two cats subjected to extensive but partial spinal lesions of the ventral and ventrolateral quadrants. We wanted first to try and express locomotion in the early period after the lesion when there is practically no hindlimb locomotion and second to improve the locomotor capacities (walking maintenance and regularity, coupling between the fore- and the hindlimbs and speed adaptation) at a later period when voluntary quadrupedal walking was reestablished but is deficient.

Therefore, the different drugs were applied in two cats through an intrathecal cannula with its tip ending caudal to the spinal site of lesion. The locomotor activity was compared before and after the drug application using chronically implanted electrodes to identify changes in EMG activity under constant recording conditions in the same cat and to follow the progressive evolution of its locomotor capacities during the recovery period up to the point of achieving a stable locomotor behavior (plateau period).

Our results show that some drugs (noradrenaline, methoxamine, and quipazine) improved the walking capacities of the cats, resulting in a more stable and maintained locomotor performance. However, other drugs, such as clonidine, caused a deterioration of the locomotor performance. The mechanisms for the action of the drugs in partial spinal cats are discussed with reference to clinical situations.

METHODS

General experimental protocol

Experiments were carried out on two adult cats (EB7 and EB8) weighing 4.1 and 2.8 kg, respectively, which were among the most lesioned cats reported in our previous study (Brustein and Rossignol 1998). The cats first were trained to walk on a treadmill at different speeds (0.2–0.7 m/s) until they could maintain a regular locomotion for periods of ~20 min. Then the cats were anesthetized and implanted with EMG electrodes and an intrathecal cannula. Control experiments (n = 7–12) were made during a period of 1–6 wk to obtain EMG and kinematic values in the intact state. Thereafter the cats were submitted to the ventral and ventrolateral spinal lesion at the thoracic level (T13), and their locomotor recovery was followed and documented daily as well as long after no further recovery could be observed.

Different adrenergic and serotoninergic drugs were tested, and their effects on locomotion were followed for several hours and compared with the performance of the cat immediately before drug application.

At the end of the experimental series, wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected caudal (L3) to the site of spinal lesion (at days 340 and 280 postlesion for cats EB7 and EB8, respectively). Then 3 days later, the cats were perfused and the spinal site of lesion and the spinal site of WGA-HRP injection as well as the brain stem and the motor cortices were processed histologically. All the surgical procedures and experimental protocols were reviewed and approved by the University’s Ethics Committee.

Surgical procedures

During the first surgery, performed in sterile conditions and under general anesthesia, a cannula was inserted into the intrathecal space of the spinal cord and EMG electrodes were implanted in various fore- and hindlimbs muscles.

EMG IMPLANTATION. The surgical procedure for implantation of EMG electrodes and the function of the implanted muscles were detailed in the preceding paper (Brustein and Rossignol 1998). The implanted muscles included, in the hindlimbs: ilioptosae (Ip) and sartorius (Sr), semitendinosus (St), tibialis anterior (TA), vastus lateralis (VL), and gastrocnemius medialis (GM) and lateralis (GL); in the forelimbs: cleidobrachialis (CIB) and the lateral head of the triceps brachii (TrIL). Although all muscles were implanted both on the left (L) and on the right (R) limbs, only the left side of the animal facing the video camera was used to illustrate the kinematics.

INTRATHECAL CANNULA. A Teflon cannula (Teflon tube-thinwall, size 24 gauge) was inserted through an opening made in the atlanto-occipital ligament and gradually pushed through the intrathecal space, so that the tip was located at L3–L4 as measured before the insertion using external landmarks. The cephalic end of the cannula was inserted into a right-angle plastic port fixed to the skull by dental acrylic, next to the EMG connectors, and served for administration of drugs or sterile saline (0.9%). The inlet was capped to reduce contamination and was opened only at the time of drug or saline application. The dead space of the cannula was measured before the insertion and was determined to be ~100 μl. To ensure its patency, the cannula was flushed with a bolus of 100 μl sterile saline solution on nonexperimental days.

VENTRAL AND VENTROLATERAL SPINAL LESION. The ventral and ventrolateral spinal lesions were performed in a second surgical intervention, under general anesthesia, when the recordings of the control period were completed (see Brustein and Rossignol 1998).

Recordings and data analysis

EMG activity and the video images used for kinematic analyses were recorded simultaneously. They were synchronized using a SMPTE time code (time code generator Skotel TCG-80N and time code reader TCR-80N), recorded on both analog and video tapes (for details, see Brustein and Rossignol 1998).

In addition to quantitative kinematic analysis, the video tapes were reviewed frequently to obtain a better overall impression of the drug effects because the kinematic analyses in one plane gives only a partial assessment of the locomotor improvement. In some cases, the cats also were filmed walking from above to better evaluate the body orientation during walking and its improvement after drug injections (not illustrated).

Drug application

The tested drugs are listed in Table 1 together with their main action and the given doses. All the drugs were administered to both cats and were generally repeated in at least three separate experiments at different days after the partial spinal lesion (also indicated in the rightmost columns in Table 1).

Before drug application, a walking sequence on the treadmill was recorded to determine the predrug baseline performance, which varied with time after the lesion (see Brustein and Rossignol 1998). Then a bolus of 100 μl of the drug solution, prepared with sterile saline (0.9%), was administered through the intrathecal cannula using an adapted syringe to fit the inlet. The syringe was fastened to the rostral end of the cannula to avoid leakage and to ensure that the whole dose would be injected. Immediately afterward, a second bolus of 100 μl sterile saline was injected and served to flush the drug out of the dead space of the cannula. Usually one dose of one drug was given, unless
indicated otherwise, and consecutive drug experiments were separated by ≥48 h. The doses were kept as small as possible to minimize central effects or any other discomfort to the cat. The reaction time to the application of a drug, its maximal effect, and the fading time depended on the drugs. To cover the whole time course of the effects, recordings started as soon as 20–30 min after the drug application and were repeated each 30–45 min until the drug effects wore off (between 2 and 5 h). The results presented illustrate the maximal effects of the drug on any given day. The drugs were applied during the early, recovery, and plateau periods postlesion (see Table 1); however, the illustrated experiments were recorded in the early period when the cats exhibited consistent locomotor deficits. It should be emphasized that the duration of the different periods and the severity of the deficits depended on the extent of the spinal lesion (see Brustein and Rossignol 1998). For example, for the most severely lesioned cat (EB7), the early period lasted almost 4 wk, whereas it lasted only 1 wk for cat EB8.

Evaluation of the extent of the spinal lesion

Evaluation of the extent of the spinal lesion was done by combining conventional histological staining methods (cresyl violet, Klüver-Barrera) and by retrograde WGA-HRP labeling. The histological procedures were detailed in Jiang and Drew (1996), Matsuyama and Drew (1997), and also in the companion paper (Brustein and Rossignol 1998).

RESULTS

Extent of the spinal lesion and recovery of locomotion

The results of the histological evaluation of the spinal lesions of cats EB7 and EB8 were described in detail in the companion paper (Brustein and Rossignol 1998) (a summary is provided in Fig. 1). Briefly, examination of the site of spinal lesion of cat EB7 showed a major disruption of both ventral and ventrolateral funiculi. Intact tissue could be found only in the dorsal columns and in the left dorsolateral funiculus (DLF) (see Fig. 1A for the photomicrograph of the site of spinal lesion). The damage to the spinal quadrants was correlated with a pronounced decrease in the numbers of HRP-labeled neurons (expressed as percentage of the intact, Fig. 1A) found in brain stem nuclei corresponding to the origin of reticulo- and the vestibulospinal pathways descending in these quadrants (see Table 1 in Brustein and Rossignol 1998, for the number of HRP-labeled cells). The lesion of cat EB8 (Fig. 1B) encompassed the lateral vestibulospinal pathway, bilaterally, whereas

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**TABLE 1. Drugs injected**

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Role</th>
<th>Source</th>
<th>Dose (mM/100 μl)</th>
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<tbody>
<tr>
<td>Norepinephrine</td>
<td>Neurotransmitter</td>
<td>RBI</td>
<td>0.8–4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9, 14, 17, 34, 49, 104, 107, 147, 182)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>$\alpha_1$-noradrenergic agonist</td>
<td>Sigma</td>
<td>0.6–5.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(6, 33, 128, 169, 190, 205, 210)</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>$\alpha_2$-noradrenergic agonist</td>
<td>RBI</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(196, 198, 203, 239)</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>$\alpha_2$-noradrenergic antagonist</td>
<td>RBI</td>
<td>12.8–20.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(205, 210, 212)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Neurotransmitter</td>
<td>RBI</td>
<td>2.4–7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(42, 44, 120, 126, 175, 177)</td>
</tr>
<tr>
<td>Quipazine</td>
<td>5-HT agonist</td>
<td>RBI</td>
<td>0.6–2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(224, 227, 231, 234)</td>
</tr>
<tr>
<td>DPAT</td>
<td>5-HT$_{1A}$ agonist</td>
<td>RBI</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(266, 268)</td>
</tr>
<tr>
<td>SHTP</td>
<td>5-HT precursor</td>
<td>Sigma</td>
<td>1.1–2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(280, 282, 287, 289)</td>
</tr>
</tbody>
</table>

Drugs used in the different experiments are listed according to their role and commercial source. The range of doses tested in each cat is given together with the dose of the drug on any given day. The results presented illustrate the maximal effects of the drug on any given day. The drugs were applied during the early, recovery, and plateau periods postlesion (see Table 1); however, the illustrated experiments were recorded in the early period when the cats exhibited consistent locomotor deficits. It should be emphasized that the duration of the different periods and the severity of the deficits depended on the extent of the spinal lesion (see Brustein and Rossignol 1998). For example, for the most severely lesioned cat (EB7), the early period lasted almost 4 wk, whereas it lasted only 1 wk for cat EB8.

**FIG. 1.** Summary of the histological findings related to the extent of the spinal lesion. Photomicrographs (A and B, cats EB7 and EB8, respectively) of cross-sections taken from the spinal site of the lesion (stained with Klüver-Barrera) illustrate the extent of the maximal spinal lesion. Graph illustrates the number of horseradish peroxidase (HRP)-labeled cells found after the spinal lesion on the left and right pontine reticular formation (PRF), medullary reticular formation (MRF), lateral vestibular nucleus (LVN), red nucleus (RN), and motor cortices, expressed as percent of the mean values obtained from 3 intact cats. Shaded vertical bars crossing the 100 level illustrate the coefficient of variation of the average cell counts in the intact cats. Numbers of HRP-labeled cells can be found in the companion paper (Brustein and Rossignol 1998).
the reticulospinal pathways were damaged but to a lesser extent compared with cat EB7. In both cats, a major increase was observed in the number of HRP-labeled cells in the motor cortex contralateral to the less affected DLF (Fig. 1, A and B), as well as by changes in the distribution of the cells (Brustein et al. 1997).

Despite the extensive lesion to the ventral and ventrolateral spinal quadrants damaging important reticulo- and vestibulospinal pathways, both cats recovered voluntary quadrupedal locomotion. Yet they suffered from long-lasting locomotor deficits, as illustrated in Fig. 2. From the consecutive stick figure diagrams and the angular excursion traces, it is noted that even at \( \sim 120 \) days postlesion (see Fig. 2, B and D), the hindlimb stepping was highly irregular compared with the intact situation (Fig. 2, A and C). The cats also suffered from poor lateral stability and sudden stumbling, which are reflected in abrupt amplitude increases of the EMGs bursts (see LSrt of cat EB7 and RSrt for cat EB8). In addition, the homolateral fore- and hindlimb coupling was affected as illustrated for the most lesioned cat, EB7, in Fig. 4A and in the phase plots of Fig. 8B. The coupling phase between the fore- and hindlimb shifted gradually, a result of different walking rhythms in the fore-and hindlimbs. For example, in Fig. 2B, six bursts were counted in RTril or in LTril compared with only five bursts in LVL. In cat EB8 the fore- and the hindlimbs stepped at the same frequency and the coupling shifts were within the range of one-step cycle duration. The ability to maintain a constant homolateral fore- and hindlimb coupling was permanently deficient for cat EB7 and transient for cat EB8. Cat EB8 not only maintained a more constant interlimb coupling but gen-

![Image](image_url)
erally did better long-term postlesion compared with cat EB7.
For example, cat EB8 could follow higher treadmill speeds of
0.5 m/s, whereas cat EB7 could hardly walk at 0.4 m/s, and
its daily performance was much more variable.

Drug applications

NE INJECTION IN THE EARLY PERIOD POSTLESION. The effects of
NE application, early after the spinal lesion, are illustrated
in Fig. 3 (cat EB7), and the mean changes in the EMG
activity (duration and amplitude) are summarized for both
cats in Table 2.

Early after the lesion (1 wk for cat EB8 and ≤34 days for cat
EB7), the cats could not support or walk with their hindlimbs,
and they moved around using the forelimbs only. Both cats had
poor lateral stability that resulted in difficulty in righting the
body. However, when light weight support such as holding the
tip of the tail was provided, a few highly disorganized quadru-
pedal steps could be performed at very low treadmill speeds
(0.1–0.2 m/s). Such a walking sequence is illustrated for cat
EB7 in Fig. 3B and should be compared with the intact state in
Fig. 3A. It is noticeable from the consecutive stick figures of
the left hindlimb that the cat had difficulty maintaining an
upright position even when the tail was held. The poor walking
is reflected in irregular angular excursion traces and in highly
disorganized EMG activity. Some episodes of exaggerated
EMG activity resulted from the cat’s attempts to recover from
a stumble. The disorganized stepping pattern of the hindlimbs
is further demonstrated by the foot fall diagram; while the left
hindlimb performed large steps, the right hindlimb performed
short ones. The coupling between the fore- and the hindlimbs
was also highly perturbed. The hindlimbs performed only three
consecutive steps, whereas the forelimbs performed five, indi-
cating that the hindlimbs and the forelimbs walked at different
frequencies of ~0.5 and 1 Hz, respectively.

Twenty minutes after injection of a third bolus of 1.6 mM/
100 μl NE (Fig. 3C) there was a considerable improvement in
the stepping pattern, which became more regular and sustained,
although it still was necessary to maintain part of the weight of
the cat by lightly holding the tail. The improvement in the
walking is evident from the stick figure diagram, which shows
five regular consecutive steps of the left hindlimb at a treadmill
speed of 0.4 m/s. The mean angular displacement measured in
all joints was much improved relative to the predrug condition,
and it was somewhat exaggerated compared with the intact
pattern (see Fig. 3A). For example, after NE application, the
mean angular displacement of the ankle was between 142 ± 4
TABLE 2. Amplitude and duration of EMGs after NE application (early after the spinal lesion)

<table>
<thead>
<tr>
<th>Cat</th>
<th>Muscle</th>
<th>Normalized Amplitude</th>
<th>Duration</th>
<th>Normalized Amplitude</th>
<th>Duration</th>
<th>Normalized Amplitude</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact (0.4 m/s)</td>
<td>Predrug (0.2 m/s)*</td>
<td>Postdrug (0.4 m/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EB7</td>
<td>LSrt</td>
<td>100 ± 11 (17)</td>
<td>193 ± 19</td>
<td>211 ± 18† (8)</td>
<td>226 ± 111</td>
<td>548 ± 22† (12)</td>
<td>154 ± 46†</td>
</tr>
<tr>
<td></td>
<td>RSrt</td>
<td>100 ± 16 (16)</td>
<td>215 ± 29</td>
<td>227 ± 36† (7)</td>
<td>441 ± 228</td>
<td>302 ± 31† (11)</td>
<td>184 ± 52</td>
</tr>
<tr>
<td>EB8</td>
<td>LSrt</td>
<td>100 ± 10 (17)</td>
<td>303 ± 40</td>
<td>137 ± 41† (7)</td>
<td>466 ± 106</td>
<td>209 ± 24† (11)</td>
<td>380 ± 58</td>
</tr>
<tr>
<td></td>
<td>LGM</td>
<td>100 ± 9 (16)</td>
<td>678 ± 64</td>
<td>ND</td>
<td>ND</td>
<td>239 ± 35† (11)</td>
<td>521 ± 83†</td>
</tr>
<tr>
<td></td>
<td>RGM</td>
<td>100 ± 14 (17)</td>
<td>581 ± 81</td>
<td>245 ± 47† (7)</td>
<td>565 ± 228</td>
<td>113 ± 25 (7)</td>
<td>401 ± 85†</td>
</tr>
<tr>
<td></td>
<td>LTriL</td>
<td>100 ± 9 (16)</td>
<td>801 ± 47</td>
<td>47 ± 21† (6)</td>
<td>851 ± 142</td>
<td>41 ± 20† (12)</td>
<td>569 ± 110†</td>
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<tr>
<td></td>
<td>RTriL</td>
<td>100 ± 6 (17)</td>
<td>752 ± 77</td>
<td>111 ± 14† (8)</td>
<td>873 ± 175</td>
<td>132 ± 11† (12)</td>
<td>570 ± 110†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>step cycle</td>
<td></td>
<td>1,099 ± 57 (15)</td>
<td></td>
<td>2,084 ± 816† (4)</td>
<td>934 ± 125†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forelimb</td>
<td></td>
<td>1,098 ± 61 (15)</td>
<td></td>
<td>1,003 ± 189 (7)</td>
<td>770 ± 65†</td>
</tr>
<tr>
<td>EB8</td>
<td>LSrt</td>
<td>100 ± 10 (12)</td>
<td>178 ± 48</td>
<td>89 ± 17 (2)</td>
<td>76 ± 11‡</td>
<td>76 ± 31† (7)</td>
<td>103 ± 15†</td>
</tr>
<tr>
<td></td>
<td>RSrt</td>
<td>100 ± 9 (10)</td>
<td>144 ± 34</td>
<td>83 ± 25 (4)</td>
<td>154 ± 51</td>
<td>73 ± 18† (7)</td>
<td>124 ± 16</td>
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<tr>
<td></td>
<td>LSt</td>
<td>100 ± 12 (12)</td>
<td>383 ± 50</td>
<td>129 ± 15‡ (4)</td>
<td>644 ± 267†</td>
<td>133 ± 10† (7)</td>
<td>369 ± 22</td>
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<tr>
<td></td>
<td>LGM</td>
<td>100 ± 14 (12)</td>
<td>646 ± 81</td>
<td>ND</td>
<td>ND</td>
<td>57 ± 4† (7)</td>
<td>575 ± 77</td>
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<tr>
<td></td>
<td>RGM</td>
<td>100 ± 9 (12)</td>
<td>510 ± 83</td>
<td>75 ± 14‡ (4)</td>
<td>1,355 ± 171‡</td>
<td>82 ± 25† (7)</td>
<td>417 ± 79‡</td>
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<td></td>
<td>LTriL</td>
<td>100 ± 10 (12)</td>
<td>741 ± 77</td>
<td>70 ± 3‡ (3)</td>
<td>1,056 ± 307‡</td>
<td>90 ± 13‡ (7)</td>
<td>616 ± 55‡</td>
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<tr>
<td></td>
<td>RTriL</td>
<td>100 ± 7 (12)</td>
<td>612 ± 62</td>
<td>108 ± 12 (4)</td>
<td>1,228 ± 216†</td>
<td>103 ± 7 (7)</td>
<td>507 ± 54‡</td>
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<td>step cycle</td>
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<td>1,081 ± 170 (12)</td>
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<td>1,554 ± 635‡ (3)</td>
<td>852 ± 63†</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Forelimb</td>
<td></td>
<td>1,081 ± 193 (12)</td>
<td></td>
<td>1,239 ± 223 (4)</td>
<td>854 ± 69‡</td>
</tr>
</tbody>
</table>

The postdrug time period for cat EB7 was 20 min after three doses of NE (1.6 mM/100 μl) and for cat EB8 was 160 min after one dose of NE (1.6 mM/100 μl). Postlesion time was 9 and 6 days for cats EB7 and EB8, respectively. Number of average step cycles is enclosed in parentheses. The normalized and averaged amplitude of EMG bursts pre- and postnorepinephrine (NE) injected in the early period, are given for each one of the cats, as percent of the intact values ± the coefficient of variation (cv) and are listed for selected muscles in the fore- and the hindlimbs, together with their burst duration in real time (ms ± SD). ND = not detectable during the experiment although the signal was present in other circumstances. EMG, electromyogram; LSt and RSt, left and right semitendinosus; LSt, left sartorius; LGM and RGM, left and right gastrocnemius medialis; LTriL and RTriL, left and right lateral head of the triceps brachii. *This speed was 0.4 m/s for cat EB8. The statistical significance of all the mean values was tested against the intact values, using Student’s t-test († P < 0.01, ‡ P < 0.05).

and 83 ± 5° (means ± SE), compared with 134 ± 4 to 98 ± 3° in the intact situation. This pronounced increase in angular displacement was accompanied by a marked increase in the normalized EMG amplitude of the hindlimb muscles (see Table 2), especially of the flexors, which reached beyond values obtained at the same walking speed in the intact state. The fore- and hindlimb coupling pattern also was stabilized as reflected by more organized foot fall patterns for all four limbs. It should be noted that there was still a mismatch, although smaller, between the number of steps performed by the hindlimbs and the forelimbs (the walking frequencies in the fore- and the hindlimb were 1.3 and 1.1 Hz, respectively).

NE improved the regularity, maintenance, and walking speed of cat EB8 as well. For example, predrug, the step cycle duration of the fore- and the hindlimbs did not differ significantly; nevertheless, they were highly variable (for mean values ± SD, see Table 2). After NE, the cycle duration in the fore- and the hindlimbs showed a better match and a much reduced variability (variance ratio test, P < 0.01). Quantitative analyses of the EMG activity and the kinematics showed that NE effects in cat EB8 (see Table 2) were less pronounced compared with that observed in cat EB7. For example, in cat EB8, the mean range of angular excursion increased in all the joints but to a lesser extent compared with cat EB7 (9, 8, and 3° in the hip, knee, ankle, and metatarsophalangeal (MTP) joints, respectively), and the EMG amplitude of the hindlimb muscles was changed only slightly (see Table 2). These quantitative differences are probably a result of the different predrug locomotor capacities of each cat, which correlated with the extent of the spinal lesion (see Fig. 1).

The effects of NE were similar in cats EB7 and EB8 in all the experiments done during the early period (2 and 3 experiments, respectively) in accordance with the different time duration of this period in the two cats (for more details see Table 1).

Application of NE during the plateau period

The injection of NE in cat EB7 was repeated further during the plateau period (this experiment was not done in cat EB8). A representative example for such an experiment is given in Fig. 4 and summarized in Table 3.

Before the drug (Fig. 4A), the cat could follow a treadmill speed of 0.3 m/s. But its walking pattern (compared with the intact state, see Figs. 3A and 2A) was irregular as reflected in the abrupt changes in the joint angular traces and in the variability of the EMG activity. The coupling phase between the fore- and the hindlimbs was also variable as illustrated by the dashed lines connecting the foot falls of the homolateral limbs. As with application of NE in the early period (Fig. 3), NE improved locomotion also when given during the plateau period. In the two situations, the stepping of the hindlimbs became much more regular (see foot fall diagrams and smoother angular excursion traces in Fig. 4B) and there was a general increase in the EMG amplitude (see Table 3). However, there were some differences between NE effects in the early and the plateau periods. For example, during the plateau period, the increase in the joint angular excursion was limited to the knee joint only (from 12 to 34°) and more importantly, NE stabilized both the coupling pattern and the phase drifts...
between the homolateral fore- and the hindlimbs (see foot falls diagrams in Fig. 4B) to a pattern which resembles the intact one (Fig. 3A).

Clonidine

In light of the improvement in the walking after injection of NE, we further tested the \( \alpha_2 \)-noradrenergic agonist, clonidine because it is implicated in the initiation and the modulation of locomotion in the completely transected spinal cats (for review see Rossignol 1996). The effects of intrathecal injection of clonidine in the early period were quite different from those observed in the complete spinal cat, and they were also different from the effects of NE in the partial spinal cat at that period (see Fig. 3). Not only clonidine did not improve the locomotor capability of the cats, but it appeared to impair it. However, because the predrug pattern was highly deficient (see Fig. 3B) it was difficult to assess the changes quantitatively.

In the plateau period, the adverse effects of clonidine were very obvious in both cats. At that stage, even one bolus of clonidine (0.8 – 1.9 mM/100 \( \mu l \)) was sufficient to cause a major reduction in the weight support of the hindlimbs, to increase swaying of the hindquarters, stumbling, and falling, which limited the cats to a few steps at a time at very low treadmill speeds (0.2 m/s). The effects of clonidine on the locomotion of cat EB7 are illustrated in Fig. 5B.

Forty-five minutes after injection of clonidine (1.9 mM/100 \( \mu l \)) the cat could barely support its hindquarters and could hardly step. As illustrated in Fig. 5B, the stick figure diagram, the joint angular excursion traces and the EMG activity, are extremely disorganized (see Fig. 6A for the average changes in EMGs amplitude). These detrimental effects were long-lasting and the return to predrug walking capacities could not be observed for the following five recording hours.

The effect of clonidine could be reversed mostly by an injection of yohimbine (\( \alpha_2 \)-noradrenergic antagonist). As illustrated in Fig. 5C, 60 min postinjection of yohimbine (20.5 mM/100 \( \mu l \)), given 45 min postinjection of clonidine, thus well within the period of maximal action of clonidine (Fig. 5B), the weight support of the hindquarters improved and so did the regularity of the stepping, and the cat’s ability to walk at higher

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**Fig. 4.** NE injection during the plateau period (cat EB7). Representative walking sections taken at the same treadmill speed (0.3 m/s) to compare the locomotion of cat EB7 in A, predrug, and in B, 20 min after intrathecal injection of 3.2 mM/100 \( \mu l \) NE (for details see Fig. 3). Related averaged bursts amplitude and duration are given in Table 3.
speeds (±0.3 m/s). Yohimbine also improved the regularity of the EMG pattern, which returned to predrug level (for quantitative effects on the EMG activity in both cats, see Fig. 6).

**Methoxamine**

Because there was a major locomotor improvement after injection of NE, but none with the $\alpha_2$-noradrenergic agonist, clonidine, we further tested the effects of an $\alpha_1$-noradrenergic agonist, methoxamine on the locomotion (applied only during the plateau period). The results of such experiments, are illustrated in Figs. 7 and 8, and the EMG values are summarized in Fig. 9. These results with methoxamine were reproduced in both cats in all the experiments (for details, see Table 1).

Methoxamine considerably improved the locomotion of cat EB7. An hour and 30 min after application of 4 mM/100 μl methoxamine, the steps were more robust and regular, with forceful foot placement and were performed with a better lateral stability. The regularity of the steps is noted clearly when comparing the consecutive stick figures diagram before (Fig. 7A) and after methoxamine (Fig. 7B). The related angular excursion traces show that the movements of the joints are smoother and reproducible from cycle to cycle because all the abrupt changes characteristic of the predrug walking disappeared. The average range of movement at the hip, ankle, and MTP joints decreased after the application of methoxamine (from 20 down to 14°, 33 to 22°, and from 57 to 44°, respectively) whereas a slighter decrease was observed in the range of the knee movement (from 27 to 23°). The activity of the hindlimb muscles markedly changed. There was a general increase in the normalized amplitude compared with the predrug situation (see especially RGM). In addition, the duration of LSt increased significantly (see average EMG values in histograms of Fig. 9).

### Table 3. Amplitude and duration of EMGs after NE application (plateau period)

<table>
<thead>
<tr>
<th>Cat</th>
<th>Muscle</th>
<th>Predrug</th>
<th>Postdrug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normalized Amplitude</td>
<td>Duration</td>
</tr>
<tr>
<td>EB7</td>
<td>LSt</td>
<td>100 ± 30 (18)</td>
<td>175 ± 78</td>
</tr>
<tr>
<td></td>
<td>LSrt</td>
<td>100 ± 13 (18)</td>
<td>327 ± 95</td>
</tr>
<tr>
<td></td>
<td>RSrt</td>
<td>100 ± 15 (16)</td>
<td>250 ± 53</td>
</tr>
<tr>
<td></td>
<td>LGM</td>
<td>100 ± 6 (17)</td>
<td>685 ± 135</td>
</tr>
<tr>
<td></td>
<td>RGM</td>
<td>100 ± 43 (17)</td>
<td>538 ± 87</td>
</tr>
<tr>
<td></td>
<td>LTEIL</td>
<td>100 ± 9 (18)</td>
<td>659 ± 119</td>
</tr>
<tr>
<td></td>
<td>RTrIL</td>
<td>ND</td>
<td>1,065 ± 152 (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>931 ± 108 (18)</td>
</tr>
</tbody>
</table>

The normalized and averaged EMG amplitude pre- and post-NE injection with their mean duration, during the plateau period (same format as Table 2, except that the postdrug values are tested statistically against the predrug values). Postdrug period was 20 min after NE injection (3.2 mM/100 μl) 104 days post lesion (0.3 m/s). *P < 0.01. †P < 0.05.

FIG. 5. Injection of clonidine followed by yohimbine (cat EB7). Treadmill stepping sequences taken 210 days postlesion. A: predrug; B and C: postdrugs. Consecutive stick figures of the left hindlimb, the angular displacement and the raw EMGs in B illustrate that, 45 min after intrathecal injection of 1.9 mM/100 μl clonidine, the cat sagged and could not maintain the weight of the hindquarters nor walk. However, as illustrated in C, 60 min after intrathecal injection of 20.5 mM/100 μl yohimbine, given 45 min after clonidine, the walking of the cat improved considerably as noted from regular stick figure diagrams, joint angular displacement traces and raw EMG taken at treadmill speed of 0.3 m/s.
After the administration of methoxamine, the ability of the cat to follow higher treadmill speeds did not improve. Nevertheless there was a major improvement in the ability of the cat to maintain locomotion at the same speed range for longer periods of time. Predrug, the cat could make about 6 consecutive steps, whereas after application of methoxamine, it easily did 20 steps.

The coupling phase between the homolateral fore- and hindlimb also changed after the drug application, as illustrated in Fig. 8. The graphs of the phase of burst onset of LTriL and LVL show that, predrug (Fig. 8B), the homolateral coupling phase varied between 0.4 – 0.8, whereas post methoxamine (Fig. 8C), it was within the intact range (Fig. 8A) and fluctuated around a value of 0.25. Further, the coupling pattern was changed from a diagonal type predrug (Fig. 8B) to almost an intact pattern with the difference that the forelimbs were leading. The general improvement in the locomotion after methoxamine was long-lasting and could be observed for 4 – 5 h.

Methoxamine improved the walking of cat EB8 as well (for the average EMG values see Fig. 9). As with cat EB7, the regularity of the hindlimbs stepping improved. This is indicated by a decrease in the variability of the hindlimb step cycle duration (variance ratio test $P < 0.01$, see mean values in Fig. 9D). There was also a stabilization of the coupling between the homolateral fore- and hindlimbs, around a phase value of 0.2, which resembles the intact situation. Methoxamine also caused a decrease in the average range of angular displacement in the hip, knee, and ankle joints (from 39 down to 33°, 38 to 26°, 30 to 19°, respectively), much like what was observed in cat EB7. These changes, in contrast to the observed in cat EB7, were accompanied by a decrease in EMG amplitude (see Fig. 9).

**Serotonergic drugs**

Several serotonergic drugs were tested: the neurotransmitter itself, 5HT; the 5HT1,2,3 agonist, quipazine; the 5HT precursor, 5-hydroxytryptophan (5HTP); and a 5HT1A agonist, 8-hydroxy-dipropylaminotetralin (DPAT). All these drugs, except the agonist DPAT, improved locomotion in a similar manner. They differed mainly in their time of action; 5HT and 5HTP did not last long (<2 h), whereas the effects of quipazine could be observed for $\geq 5$ h after the application. DPAT was detrimental to locomotion of EB7 by inducing, as soon as 20 min postapplication, a severe foot drag of both hindlimbs causing the cat to stumble very often. This effect faded away after 1 h.

A representative example for the effects of quipazine on the locomotion of cat EB7 is given in Figs. 10 and 11, and the quantitative values of the EMG activity are summarized in the histograms of Fig. 12. Predrug, the cat walked irregularly, as illustrated in the stick figure diagram (Fig. 10A) showing five consecutive steps, each of a different duration. In addition, the
angular movement of the joints was variable and included abrupt, sudden changes. The raw EMGs also reflected this disorganized walking. The EMG activity of the different muscles not only varied in amplitude and duration but also in their time course (see especially right and left GM).

After injection of quipazine (1.2 mM/100 μl), the cat’s walking improved considerably. It had better lateral stability and it could take long regular steps at a constant speed of 0.3 m/s (see Fig. 12), which were, still, shorter than the mean intact step cycle duration at 0.3 m/s (1,372 ± 62 ms). After the drug, the angular excursion traces were more regular and stable. In addition, there was a major increase in the average range of angular excursion of the hip, knee, and ankle: from 17 to 27°, 21 to 29°, and from 19 to 28°, respectively. The values of angular excursion, after quipazine, resembled more the intact values (26° in the hip, 32° in the knee, and 36° in the ankle).

No change was observed in the average range of angular excursion of the MTP joint, which stayed lower relative to the intact. The EMG activity also reflected the increase in regularity and prolongation of the steps. There was a general tendency for prolongation of the burst duration relative to the predrug situation (see Fig. 12B), especially in the extensors. The latter was even significantly longer than the intact burst duration. Quipazine also stabilized the interlimb coupling, as illustrated in the average foot fall diagrams in Fig. 11. Predrug the coupling pattern was variable, as expressed by a continual shift between three main types of coupling patterns, homolateral in-phase coupling, diagonal coupling (Fig. 11A), and almost a normal coupling pattern (see average foot fall diagram of the intact in Fig. 8A). After quipazine, the cat was walking consistently with an homolateral, in-phase, coupling pattern, which is illustrated in the foot fall diagram in Fig. 11B, averaging 13 consecutive steps. This more robust coupling pattern probably resulted from an improved hindlimb stepping regularity (variance ratio test P < 0.01) and not from a better coupling between the fore- and the hindlimbs because even postdrug, the drift in the coupling phase persisted (see the related phase plot).

Combination of methoxamine and quipazine

As detailed in the preceding sections, both methoxamine and quipazine improved locomotion but in a different way. The first drug increased the amplitude of the EMGs, decreased the cycle duration and the joint angular excursion while the later prolonged the step cycle duration. Therefore both drugs were given at the same time in one bolus. The effects on stability of the walking is illustrated in Fig. 13, whereas the quantitative values are given in Fig. 14.

The cat walking (regularity, maintenance, and adaptation to speeds) is illustrated in Fig. 13 by plotting the step cycle duration of a whole consecutive stepping sequence performed
during one recording session. Before the drug (Fig. 13B), the cat had major difficulty maintaining a treadmill speed of 0.35 m/s for >10 step cycles, and after several steps in which it touched the back of the treadmill, the speed of the treadmill was decreased to 0.3 m/s. Even then the cat walked irregularly as reflected by a step to step fluctuation in the cycle duration.

Sixty-five minutes postapplication of one bolus of the combined solution, which contained only half the doses (2 mM methoxamine + 1.2 mM quipazine/100 μl) used when each one of the drugs was tested separately, an elegant and sustained walking was observed, with much improved lateral stability. This is indicated by a much reduced variability of the step cycle duration, which resembled generally the intact situation (Fig. 13A). Notice, however, that in the intact situation, walking at 0.3 m/s was somewhat too slow for this cat, and this was expressed in higher step cycle variability. In addition to improve regularity there was also an improvement in the speed range the cat could follow and maintain (≤0.4 m/s). Generally, the stepping was characterized by somewhat shorter steps (see Fig. 14B) and by a corresponding slight reduction in the average range of the angular displacement (not illustrated). The EMG duration and amplitude showed various changes as summarized in Fig. 14, A and B.

The application of the combined solution also improved cat EB8’s walking. In this cat we have observed an increase in the step cycle duration (see Fig. 14D). Changes also were found in the average range of angular excursion of the hip, ankle, and MTP, which decreased from 29 to 23°, 29 to 21°, and 48 to 43°, respectively. Significant increases were found in the amplitudes of all the flexors (see Fig. 14, C and D). The extensor amplitudes did not change, but their duration increased significantly compared with the predrug situation.

**DISCUSSION**

In this work, we have demonstrated that different noradrenergic and serotoninergic drugs can improve or deteriorate walking of cats with partial but large ventral and ventrolateral lesions of the low thoracic spinal cord. First the extent of the spinal lesion with special emphasis on the damage to descending noradrenergic and serotoninergic tracts will be discussed. Then different possible mechanisms by which the drugs may exert their effects will be suggested as well as possible clinical applications.

**Extent of the spinal lesions in relation to descending noradrenergic and serotoninergic pathways**

The extent of the damage to noradrenergic and serotoninergic pathways after the ventral and ventrolateral spinal lesions only could be evaluated indirectly by comparing the results of the evaluation of the extent of the spinal lesion (for details, see
the preceding paper, Brustein and Rossignol 1998) to what is known from the literature about the noradrenergic and serotonergic pathways in the spinal cord.

Previous studies using histofluorescence and retrograde HRP labeling showed that noradrenergic pathways originating in nucleus locus coeruleus, the main source of spinal noradrenergic fibers, project primarily through the ventrolateral funiculus. In addition, nucleus subcoeruleus, nucleus Kolliker-Fuse, and cell bodies in A5 send fibers through both the ventrolateral and the dorsolateral funiculi. The noradrenergic fibers innervates the lumbar spinal cord bilaterally, although the ipsilateral contribution dominates (Kuypers 1981; Kuypers and Maisky 1977; Marshall 1983; Stevens et al. 1985). The serotoninergic axons originating in the raphe nuclei, such as raphe pallidus and obscuras, descend mainly in the ventral and ventrolateral funiculi, whereas axons from raphe magnus descend primarily through the DLF (Kuypers 1981; Martin et al. 1978).

According to these findings, most of the noradrenergic and the serotoninergic axons descending in the ventral and ventrolateral quadrants of cat EB7 probably were damaged. However, the noradrenergic and the serotoninergic axons descending in the left DLF mostly were preserved. In cat EB8, the existence of serotoninergic axons in left ventral quadrant cannot be excluded. Nevertheless, an extensive damage probably was done to serotoninergic and noradrenergic fibers descending in the lateral funiculi. The difference in the extent of the spinal lesions (less severe for cat EB8), which correlated with faster recovery and better locomotor performance (see Brustein and Rossignol 1998), may explain why generally larger doses of the tested drugs were needed to see effects in cat EB8 long-term postlesion (see Table 1). Yet, and most importantly, the applied drugs showed overall similar effects in both cats. Drugs such as clonidine had a detrimental effect in both cats, and drugs that caused a pronounced improvement in the walking of cat EB7 may have caused more subtle effects on the walking of cat EB8, but they were never detrimental.

**Noradrenergic drugs injection in the partial spinal cat**

**SUMMARY OF THE EFFECTS.** Injection of the neurotransmitter itself, NE, in the early period, highly improved speed performance and stepping regularity of both cats. It also improved the hindlimb joint angular excursion and the coupling between the homolateral fore- and hindlimb. These effects were more pronounced in cat EB7 than in cat EB8 most likely due to differences in their locomotor capacities, which related to the extent of the spinal lesions. In the early period, NE improved the stepping pattern considerably but not to the point that cats could walk on their own with full-weight support and lateral stability of the hindquarters. In the plateau period, however, the improvement in the walking regularity was accompanied by a pronounced increase in the weight support and lateral stability. The latter effects resembled the effects of \( \alpha_1 \)-agonist, methoxamine, and may be attributed to an increase in the extensor amplitude. Methoxamine, in contrast to NE, caused a general decrease in the joint angular excursion, which may explain why no improvement was observed in speed performance. Nevertheless, better stability, more regular hindlimb stepping, and interlimb coupling may account for more sustained walking at the same range of speeds. It is interesting to note that,

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**FIG. 9.** Histograms of EMG amplitude and burst duration after methoxamine injection (cats EB7 and EB8). Same format as Fig. 6. Time postdrug injection and their doses: A and B: cat EB7, 80 min after 4 mM/100 µl methoxamine 196 days postlesion. C and D: cat EB8, 50 min after 6 mM/100 µl methoxamine at day 104 postlesion (*\( P < 0.05, ** P < 0.01 \)).
after methoxamine, cat EB8 showed more regular walking and a decrease in joint angular excursion, as did cat EB7, but without major effects on the amplitude of the recorded muscles.

Contrary to the locomotor improvement seen after NE and the α1-agonist, methoxamine, the α2-agonist, clonidine, caused deterioration of the walking in both cats. There was a major decrease in weight support and in lateral stability of the hindquarters, which resulted in very deficient and inconsistent stepping at low treadmill speeds.

**PROPOSED MECHANISMS FOR THE ACTIONS OF THE DRUGS.** The improvement in locomotion of the partial spinal cat after NE and methoxamine injections can be attributed to global changes in spinal neurons excitability (Grillner 1981). According to this suggestion, the slight hyperpolarization caused by NE, a result of a decrease in membrane conductance, will cause a general potentiation of 25–50% of other non-NE synaptic input. The possible implication of noradrenergic drugs in mechanisms of gain amplification was suggested as well by Hounsgaard et al. (1988) and by Conway et al. (1988). L-3-4-Dihydroxyphenylalanine (L-DOPA) and clonidine induce plateau potentials in spinal motorneurons of acute complete spinal cats (Conway et al. 1988; Schomburg and Steffens 1996), so does methoxamine in the decerebrate cat preparation (Lee and Heckman 1996, 1997). Plateau potentials were proposed as a mechanism that reduces the need for sustained synaptic drive from the rhythm generator during locomotion. Therefore by maintaining excitability at a constant level, only short-lasting excitation or terminating inhibition is needed to start or stop the activity in the motorneurons (Conway et al. 1988; Hounsgaard et al. 1988). This may account for the major improvement in the walking regularity after methoxamine and NE in both cats even without major changes in EMG amplitudes as observed in cat EB8. Hence NE and methoxamine probably provided a constant background excitability on which the signals from the rhythm generator could be expressed reliably and consistently.

In contrast to NE and methoxamine, application of clonidine in the partially spinal cat had a detrimental effect on the walking. These results are also in contrast to those observed in early chronic spinal cats in which clonidine initiates hindlimb locomotion (Barbeau and Rossignol 1991; Barbeau et al. 1993; Chau et al. 1998b). In the late complete spinal cat (Barbeau et al. 1993; Chau et al. 1998b), small doses of clonidine given intrathecally (1 μg/100 μl) modulated the stabilized spinal hindlimb walking, increase of step length, joint angular displacement, and the amplitude and duration of flexors. However, larger dose of clonidine (10 μg/100 μl) it, decreased the amplitude of the extensors, reduced the weight support of the hindlimbs, and induced a pronounced foot drag, much like in the partial spinal cat. For the partially spinal cat, these effects were much more severe because the cats hardly could continue walking quadrupedally.

The detrimental effect of clonidine on the walking of partial spinal cats, its beneficial effects in the early spinal cats, and its dose-dependent effects in late complete spinal cats could be explained by a difference in the α2-receptor population remaining in each condition. After a complete spinal transection, there is a removal of the central presynaptic α2 receptors and the
The effect of the drugs is mainly due to activation of the remaining \( \alpha_2 \) postsynaptic receptors (Langer 1977; Marshall 1983). In the partially spinal cats, some presynaptic \( \alpha_2 \) receptors must be spared on remaining descending adrenergic terminals, mainly in the dorsal horn where they are mostly concentrated (Giroux et al. 1995) and also on primary sensory afferents (Howe et al. 1987). The latter may be even larger in number after the partial lesion, a result of sprouting of primary afferents (Helgren and Goldberger 1993). Thus in the partial spinal cat, in contrast to the complete spinal cat, application of clonidine also could largely affect presynaptic receptors to cause a decrease in NE release.

There are some evidences that indeed, \( L-DOPA \), the noradrenergic precursor, depressed transmission of activity from primary afferents but not of group I (Anden et al. 1966). Further, the local application of NE and NE agonists also was found to depress transmission in interneuronal pathways from group II afferents (Bras et al. 1989, 1990; Jordan et al. 1977), an effect that was observed in populations of neurons at \( L_4-5 \), where the tip of the intrathecal cannula is located in both cats. It is interesting to note that the depressive effect was primarily exerted by \( \alpha_2 \)-agonists such as tizanidine. The depressive effects of descending noradrenergic system on interneuronal pathways involved in mediating the reflex action of group II afferents on motorneurons was demonstrated further by stimulation of the locus coeruleus and subcoeruleus of decerebrated cats (Jankowska et al. 1993). Further, it was reported that \( L-DOPA \) reduced spasticity in paraplegic patients (Eriksson et al. 1996).

The aforementioned effects of noradrenergic drugs may not be beneficial for the partial spinal cat, as a reduction in the transmission from the periphery may interfere with the potential compensatory mechanisms from sensory feedback that the cats may develop after the spinal lesion to maintain weight support and locomotion (Brustein and Rossignol 1998; Grillner 1972; Guertin et al. 1995).

FIG. 11. Interlimb coupling after quipazine injection (cat EB7). Data used to illustrate the coupling relations are taken from the walking sections of Fig. 10 and correspond to predrug (A) and 75 min after intrathecal injection (B) of 1.2 mM/100 \( \mu \)l quipazine (for details see Fig. 8). Notice, however, that in A, 3 average foot fall diagrams are illustrated to show the variability in the coupling pattern used by the cat in the predrug situation. L, left; R, right; H, hindlimb; F, forelimb.
The important effect of NE and methoxamine on the coupling between the fore- and hindlimbs may be, as discussed earlier, due to an improved background excitability level, on which the signals from the rhythm generator can be expressed more consistently. Stabilization of the hindlimb walking pattern will result in a generally more organized quadrupedal coupling. It is also possible that the observed improvement in the hindlimb locomotion results in a more consistent information flow between the two girdles through the remaining ascending and descending spinal pathways to better integrate their activity. Such a modulatory action (facilitatory and inhibitory) of NE on ascending information from muscle and skin afferents was suggested by Jankowska et al. (1997).

Serotoninergic drugs injection in the partial spinal cat

SUMMARY OF THE EFFECTS. 5HT, the neurotransmitter itself, its precursor, 5HTP, and quipazine, a 5HT1,2,3 agonist, improved the lateral stability and the weight support of the hindlimbs, which resulted in a more regular quadrupedal walking with better interlimb coupling pattern, especially in the most lesioned cat, EB7. In addition, the serotoninergic drugs prolong the step cycle duration in both cats. In cat EB8, this prolongation was accompanied by an increase in flexors and by a slight increase in extensor burst duration, whereas in cat EB7, a significant increase in extensor burst duration was observed with a slight decrease in the normalized EMG amplitude. These effects were different from those observed after application of NE or methoxamine, which decreased the step cycle duration, joint angular excursion, and increased the amplitude of the EMGs.

The 5HT1A agonist, DPAT, had a detrimental effect on the locomotion of cat EB7, which was manifested as a severe foot drag in both hindlimbs, causing the cat to stumble often. However, compared with clonidine, this effect was short-lasting and was not accompanied by an apparent decrease of the weight support of the hindlimbs or an increased wobbliness.

PROPOSED MECHANISMS FOR THE ACTIONS OF THE DRUGS. In contrast to NE and clonidine, the 5HT precursor, 5HTP, was not found to evoke locomotor rhythm in either the low spinal and decerebrate cat (Grillner and Shik 1973) or in the complete chronic spinal cat during the first week after spinalization, whereas clonidine or L-DOPA caused a dramatic change in kinematics and EMG pattern (Barbeau and Rossignol 1990, 1991; Barbeau et al. 1993). However, 5HTP markedly increased the tonic activity in all muscles in both preparations (Barbeau and Rossignol 1991; Grillner and Shik 1973). An increase in extensor and flexor muscles activity (amplitude and duration) also was observed in the chronic late spinal cat after intraperitoneal injection of different serotoninergic drugs (Barbeau and Rossignol 1990, 1991).

The involvement of serotonin in the modulation of the membrane properties of motorneurons in the decerebrate cat was demonstrated by Hounsgaard et al. (1988). It was shown that the bistable properties, mainly of extensor motorneurons, which disappeared after acute spinalization, could be restored after intravenous application of large doses of 5HTP. The
effect of 5HTP on the bistable properties of the motorneurons have many similarities to the effect of L-DOPA (Conway et al. 1988). However, they differ in their targets and strength of action. 5HTP strongly affected extensor motorneurons, whereas L-DOPA affected extensors and flexors but to a lesser extent. This may explain why after 5HT we observed a tendency to increase step cycle duration and bursts duration, especially in extensors (see cat EB7), whereas after application of NE, early after the lesion, a general increase was noted in the amplitude of all muscles, especially in flexors. During the plateau period, however, the effects of NE and methoxamine on extensors were more pronounced compared their effects during the early period, probably a result of different excitability levels. In the early period, extensor activity was almost absent, a result of elimination of strong excitatory input from descending pathways. At that stage, a very strong excitatory input is needed to increase their weak activity. In contrast, long-term postlesion, when some adaptive processes already have taken place (see Brustein and Rossignol 1998 for discussion), the baseline activity of these muscles is much better and less input is needed to increase their action. This possible explanation is supported by observations that the level of the bias current applied to extensor motoneurons in the decerebrate cat had an important effect on expression of the bistable properties (Hounsgaard et al. 1988).

The involvement of serotoninergic drugs in restoring tonic background activity in extensors was further demonstrated by Miller et al. (1996). 5HT2 agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI), restored the tonic background activity in GM and soleus and facilitated the GM stretch reflex, both abolished after acute spinalization of the decerebrate cats. Such a tonic excitation was suggested by Mori (1987) to be the main action of serotoninergic system.

5HT, like NE, also was found to have a specific modulatory effect on transmission from peripheral afferents (Bras et al. 1989, 1990; Jankowska et al. 1993, 1997). 5HT was found to depress transmission from group II afferents but not of group I afferents. However, its effectiveness was different than seen after NE. NE had more pronounced effects in depressing potentials in the ventral horn, and its action appeared sooner and was faster relative to 5HT (Bras et al. 1989). It further was suggested that this depression may involve different membrane receptors at different locations. α2-agonists were found to be effective primarily in the intermediate zone and in the ventral horn, whereas 5HT1A was effective in the dorsal horn (Bras et al. 1990). Such influences were observed as well after stimulation of the raphe nucleus (Jankowska et al. 1993). 5HT also was found to affect the ascending transmission from group II afferents (Jankowska et al. 1997). These modulatory effects may explain why in cat EB8, which had better weight support, 5HT drugs improved the regularity of the locomotion without a major effect on EMG activity.

Both quipazine and methoxamine given separately had beneficial effects on locomotion. Their combination was most efficient in improving the locomotion of the cats, and only half the dose of each drug used individually was sufficient to give a pronounced effect. The combination of both drugs induced the most regular and maintained locomotion, with elegant and smooth movements, probably because they complete each other’s actions.

Taken together it seems that the serotoninergic and the noradrenergic systems can modulate the pattern of locomotion by changing the neuronal excitability. In the absence of, or after a decrease in, these descending inputs, signals for generation of the walking rhythm cannot be expressed consistently, resulting in irregular walking. A lesion to these descending pathways effect as well the coupling between the fore- and the hindlimbs. It also seems that serotoninergic and noradrenergic neurotransmitters contribute to continual stabilization of the fore- and hindlimb coupling by modulating the transmission of ascending information from the muscle and skin afferents.

Our results have important clinical implications, showing that the effects of some drugs such as clonidine depend on
whether or not the spinal lesion is complete. In the complete spinal cat, clonidine may exert beneficial effects; however, in the partial spinal cats, its effects may interfere with compensatory mechanisms developed after the lesion. This may indicate that different classes of drugs may be used in patients with different types of spinal cord injuries. Indeed, results of intrathecal injection of clonidine in patients showed an important variability of effects (Barbeau et al. 1998; Dietz et al. 1995). In addition, a most important finding is that these drugs effects can be integrated into the residual voluntary locomotor control to improve locomotion and its postural aspects. It is then probable that such changes in neuronal excitability could be used beneficially by patients who still have residual capabilities to induce the rhythm of walking but are unable to sustain it.

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REFERENCES


HOUNSGAARD, J., HULTBORN, H., JESPersen, J., AND KIEHN, O. Bistability of locomotion following low thoracic hemisection in adult cats involves com-


