Lasting paraplegia caused by loss of lumbar spinal cord interneurons in rats: no direct correlation with motor neuron loss

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Object. The aims of this study were to investigate further the role played by lumbar spinal cord interneurons in the generation of locomotor activity and to develop a model of spinal cord injury suitable for testing neuron replacement strategies.

Methods. Adult rats received intraspinal injections of kainic acid (KA). Locomotion was assessed weekly for 4 weeks by using the Basso, Beattie, and Bresnahan (BBB) 21-point locomotor scale, and transcranial magnetic motor evoked potentials (MMEPs) were recorded in gastrocnemius and quadriceps muscles at 1 and 4 weeks. No changes in transcranial MMEP latency were noted following KA injection, indicating that the descending motor pathways responsible for these responses, including the alpha motor neurons, were not compromised. Rats in which KA injections included much of the L-2 segment (10 animals) showed severe locomotor deficits, with a mean BBB score of 4.5 ± 3.6 (± standard deviation). Rats that received lesions rostral to the L-2 segment (four animals) were able to locomote and had a mean BBB score of 14.6 ± 2.6. Three rats that received only one injection bilaterally centered at L-2 (three animals) had a mean BBB score of 3.2 ± 2. Histological examination revealed variable loss of motor neurons limited to the injection site. There was no correlation between motor neuron loss and BBB score.

Conclusions. Interneuron loss centered on the L-2 segment induces lasting paraplegia independent of motor neuron loss and white matter damage, supporting earlier suggestions that circuitry critical to the generator of locomotor activity (the central pattern generator) resides in this area. This injury model may prove ideal for studies of neuron replacement strategies.

KEY WORDS • spinal cord injury • kainic acid • central pattern generator • excitotoxicity • rat
Paraplegia secondary to lumbar spinal cord interneuron loss

Kainic acid is an excitotoxic agonist that acts on the non-NMDA class of glutamate receptors in the central nervous system. When injected into the brain or spinal cord, KA inflicts excitotoxic injury on neurons while sparing nearby white matter in a dose-dependent fashion. The experiments reported here were designed to test the hypothesis that discrete KA-induced injuries to the intermediate gray matter of the lower-thoracic and upper-lumbar enlargement in rats would render the animals unable to locomote because of damage to critical CPG neuronal circuitry, without causing significant loss of motor neurons or descending motor control pathways. We suggest that the model described here would be ideal for studies focused on transplantation to replace neurons lost after SCI. Furthermore, our results demonstrate that paraplegia can result from damage to only modest amounts of gray matter in the spinal cord, even in the absence of white matter loss.

Materials and Methods

Spinal Cord Injury

All procedures involving experimental animals were performed according to the guidelines of the University of Louisville Institutional Animal Care and Use Committee. Nineteen adult male Sprague–Dawley rats were used. Preoperative transcranial MMEPs and BBB scores were obtained in each animal. Anesthesia was induced in the rats by intraperitoneal administration of 0.25 to 0.4 ml pentobarbital (50-mg/ml concentration); in addition, each animal received intramuscular injections of 0.2 ml prophylactic gentamycin (50-mg/ml concentration). A dorsal midline incision was made over the T12–13 vertebrae. The location of the T-13 vertebra was determined independently by two investigators by using spinoanatomy and rib counts. A T12–13 or T13 laminectomy was performed, and the dura was opened and reflected with a 30-gauge needle. Bleeding was controlled with cotton swabs and/or electrocautery. Clamps were applied to the spinal processes above and below the laminectomy site to immobilize the spinal column. A 1 mM solution of KA in normal saline was made fresh daily; 1 N NaOH was added to achieve a pH of 6 to 7. As previously described, a low-pressure microinjector was used to deliver 1 μl of KA into the intermediate gray matter at predetermined points. In these experiments, injections were made 1.2 mm from the dorsal surface, approximately 0.5 to 0.8 mm from the midline. Injection micropipettes were pulled from glass capillaries on a micropipette puller. The micropipette tip was beveled to an inside diameter of 25 to 30 μm on a micropipette beveler and rinsed with alcohol. Injections were administered in 0.25-μl increments each over a 5-minute period for a total dose of 1 μl. The fascia was then closed with No. 4-0 silk thread and the skin was closed with clips. In the 10 animals that received two injections bilaterally, the injection sites were separated by 1.5 to 2 mm rostrocaudally. To examine deficits resulting from smaller KA-induced lesions, four animals received single injections bilaterally. One of these animals (Animal 2) received an injection at T-13, whereas the other three (Animals 10–12) received injections at L-2 (Table 1). Four control animals received two injections of normal saline bilaterally at T-13. 1 day postoperatively, and at weekly intervals thereafter for a period of 4 weeks. These scores were assigned independently by two investigators, and discrepancies were fully discussed. Transcranial MMEP monitoring was used to evaluate the function of descending motor pathways at 1 and 4 weeks postoperatively.

Results

Lesion Description and BBB Scores

Fifteen adult Sprague–Dawley rats were injected intraspinal with KA. One animal died before postinjection Week 2 of an identified abscess and accompanying sepsis. Localization of injections was based initially on spinous process, rib counts, and other anatomical landmarks (vertebral level in Table 1). Definitive descriptions of the lesioned areas were made with reference to dorsal root and spinal cord anatomy assessed during dissection after fixation (spinal level in Table 1). Table 1 also shows the BBB scores obtained for each hindlimb at Weeks 1 and 4 as well as the mean 4-week BBB scores (right and left hindlimbs).
shows photomicrographs of Nissl-stained 50-

Figure 1 which involved the regions once occupied by the dorsalities had developed at the injury epicenter (injection sites), average 4-week right- and left-hindlimb BBB score of 4.5.

Two KA injections bilaterally; the rats had received an

These sections demonstrate that at a distance of 1.5 mm from the injection site the intermediate gray matter (Fig. 1). As can be seen in the photomicrographs in Fig. 1 appear at higher magnification in Fig. 2. These sections having collapsed onto the neuron-depleted gray

Histological Studies

In the two groups. Each of the four control animals was subjected to a laminectomy combined with single saline injections bilaterally. All four control animals were assigned BBB scores of 21 (normal locomotion) throughout the experiments and are not included in Table 1.

Gross examination of spinal cord sections obtained in the KA-injected rats revealed regions of discoloration and degeneration (shrinkage) that extended up to 7 mm in length. In the majority of spinal cords the regions of degeneration spanned slightly more than one vertebral level and were 3 to 4 mm in length; these are designated as normal in Table 1. Many of the injuries that included the L-2 segment did spread to L-1; however, none included any portion of the T-13 segment. Similarly, injuries that included the T-13 segment also spread to L-1 but not L-2. (The T-13, L-1, and L-2 segments are each approximately 3 mm long in the adult Sprague–Dawley rat.) The lesioned area of one spinal cord was slightly less than 3 mm long and is designated as normal in Table 1. Four KA-injected spinal cords showed regions of degeneration that extended to 5 mm or more, and these are designated as large in Table 1. No pathological changes were grossly evident in the four spinal cords obtained in saline-injected control animals.

Histological examination revealed that small cystic cavities had developed at the injury epicenter (injection sites), which involved the regions once occupied by the dorsal horns and much of the intermediate gray matter. Figure 1 shows photomicrographs of Nissl-stained 50-µm transverse sections of the spinal cord in an animal that received two KA injections bilaterally; the rats had received an average 4-week right- and left-hindlimb BBB score of 4.5. (The corresponding motor neuron counts for this animal are shown in Fig. 5D.) The regions shown in the upper frames in Fig. 1 appear at higher magnification in Fig. 2. These sections demonstrate that at a distance of 1.5 mm rostral and caudal to the injury epicenter, cavitation had diminished, and the dorsal half of the spinal cord appeared shrunken, with the dorsolateral and dorsolateral white matter columns having collapsed onto the neuron-depleted gray matter (Fig. 1). As can be seen in the photomicrographs in Fig. 2, the intermediate gray matter ± 1.5 mm from the injection site was devoid of neurons and filled with many small darkly stained cells indicative of gliosis. At ± 4.5 mm and ± 7.5 mm from the injection site the intermediate gray matter was filled with medium-to-large interneurons, with mild gliosis also visible (Fig. 2). In Fig. 3 similar high-magnification photomicrographs (obtained from the lower frames in Fig. 1) show that in motor neurons in sections obtained ± 1.5 mm from the site of KA injection and in sections obtained ± 4.5 mm from the epicenter, the motor neurons were large and had a robust appearance. As expected, cystic cavities and regions of gliosis were absent in the spinal cords of the saline-injected control animals.

The spinal cord represented in Figs. 1 through 3 was obtained in an animal that received two bilateral 1-µl injections of 1.0 mM KA, which resulted in loss of motor neurons from approximately 2 to 2.5 mm of lumbar spinal cord (Figs. 1 and 5D). This was the most severe injury observed in the study in terms of motor neuron loss; however, less severe injuries with little or no noticeable motor neuron loss also resulted in severe locomotor deficits. Figure 4 shows sections from an animal that received single 1-µl injections of 1 mM KA bilaterally. This animal had a 4-week BBB score of 2; the corresponding motor neuron counts can be seen in Fig. 5B. The cross sections

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Vertebral Level</th>
<th>Spinal Level</th>
<th>Lesion Size (lt) (rt) (lt) (rt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cT-12, rT-13</td>
<td>T-13</td>
<td>6 15 6 20</td>
</tr>
<tr>
<td>2</td>
<td>T-13</td>
<td>S</td>
<td>19 20 17 20</td>
</tr>
<tr>
<td>3</td>
<td>T12–13</td>
<td>L</td>
<td>7 7 14 14</td>
</tr>
<tr>
<td>4</td>
<td>T12–13</td>
<td>L</td>
<td>0 12 12 14</td>
</tr>
<tr>
<td>5</td>
<td>rT-13</td>
<td>L1–2</td>
<td>N 1 3 1 2</td>
</tr>
<tr>
<td>6</td>
<td>L1–2</td>
<td>N</td>
<td>1 14 2 15</td>
</tr>
<tr>
<td>7</td>
<td>L1–2</td>
<td>N</td>
<td>0 6 1 6</td>
</tr>
<tr>
<td>8‡</td>
<td>L2–3</td>
<td>N</td>
<td>0 6 6 3</td>
</tr>
<tr>
<td>9</td>
<td>L2</td>
<td>L</td>
<td>0 1 1 2</td>
</tr>
<tr>
<td>10</td>
<td>mT-13</td>
<td>L2–3</td>
<td>N 9 6 5 6</td>
</tr>
<tr>
<td>11</td>
<td>L2–3</td>
<td>N</td>
<td>1 3 1 3</td>
</tr>
<tr>
<td>12‡</td>
<td>L2–3</td>
<td>N</td>
<td>5 8 2 2</td>
</tr>
<tr>
<td>13</td>
<td>cT-13</td>
<td>L1–2</td>
<td>N 0 10 0 9</td>
</tr>
<tr>
<td>14</td>
<td>L2–3</td>
<td>N</td>
<td>0 10 1 3</td>
</tr>
</tbody>
</table>

* Mean hindlimb (right and left) BBB score at 4 weeks (Animals 1–4) = 14.6 ± 2.6; mean hindlimb (right and left) BBB score at 4 weeks (Animals 5–14) = 4.5 ± 3.6. Mean hindlimb BBB scores for animals with injuries rostral to L-2 were significantly different from those for animals with injuries that included L-2 (p < 0.05; one-way analysis of variance and post hoc t-tests).
† Histological findings in this animal are represented in Fig. 4.
‡ Histological findings in this animal are represented in Figs. 1 through 3.

Abbreviations: c = caudal; DREZ = dorsal root entry zone; L = large (> 4 mm in length); m = middle; N = normal size (3–4 mm in length); r = rostral; S = small (< 3 mm in length).
shown in Fig. 4 demonstrate that although much of the dorsal horn and intermediate gray matter is damaged at the injury epicenter, motor neurons are readily visible in the ventral horn. This can also be seen in the high-magnification photomicrographs taken from the boxed areas in the corresponding sections. In this animal’s spinal cord, both motor neurons and intermediate lamina interneurons were readily visible at 1.5 mm rostral and caudal to the epicenter of the KA-induced injury.

Locomotor Function

Individual BBB scores for each hindlimb at Weeks 1 and 4 are shown in Table 1. The mean BBB scores obtained in rats with lesions that included much of the L-2 segment were significantly different from those in animals with lesions rostral to L-2. The terminal (4-week) BBB scores were 4.5 ± 3.6 (mean ± standard deviation) in the 10 animals injured at L-2 and 14.6 ± 2.6 in the four animals injured at T12–13.

In Table 2, hindlimb BBB scores are divided into categories that have been used previously to illustrate three different stages of functional recovery following contusion SCI. A BBB score of 0 indicates complete plegia of the limb; 7, movement of all three joints, but with the limb unable to support body weight; 8, some sweeping movements or plantar placement of the foot without weight support, except while standing; 13, inconsistent weight support with some stepping movements and frequent forelimb–hindlimb coordination; 14, coordinated stepping with plantar weight support, but with incorrect foot placement, body posture, and tail position; and 21 indicates normal locomotion. Table 2 shows that hindlimb BBB scores in animals with KA-induced excitotoxic injuries, including L-2, were much more likely to be 0 to 7 or 8 to 13 than those in animals in which the injuries did not include this spinal cord segment.

Motor Neuron Counts

Figure 5 displays graphs of motor neuron counts from four representative rats. Each graph also shows the approximate segmental location of the injury epicenter and region where motor neurons were counted based on both gross and histological examination. In addition, based on the work of Nicolopoulos-Stournaras and Iles, we have indicated in the graphs the approximate relative locations of motor neuron pools supplying the quadriceps and gastrocnemius muscles from which transcranial MMEP responses were recorded. Motor neurons were counted from every third section cut from the lower thoracic and upper lumbar spinal cord by using a semiautomated system. Criteria used by the image analysis software to select motor neurons for counting included intensity of staining, area, diameter (length/width) ratio, and minimum radius. The BBB scores for the representative spinal cord section ranged from 21 (Fig. 5A, control spinal cord) to 2 (Fig. 5B, single injections bilaterally). The spinal cord represented in Fig. 5D is the same one represented in Figs. 1 through 3, and the motor neuron counts for the spinal cord represented in Fig. 4 are shown in Fig. 5B. The mean motor neuron counts in injured spinal cords were sometimes higher than in the con-
trol rat spinal cords because of the shrinkage of cord tissue following KA injection and also because of the tendency of neurons to stain more darkly in injured spinal cords. When a Pearson correlation analysis was performed in which mean right- and left-sided BBB scores were compared with motor neuron counts at the injury epicenter (±1 mm, Fig. 5E), no correlation was found (r = 0.26, p < 0.05). These data indicate that, overall, the BBB scores were not correlated with motor neuron loss. However, we cannot rule out the possibility that in rats in which motor neuron loss occurred (Fig. 5C and D), this loss could account for some of the decrease in BBB scores.

**Transcranial MMEPs**

In this study transcranial MMEP responses were interpreted to indicate that the ventral white matter tracts were functional (that is, presence or absence of response) and that the action potential conduction velocity of the descending axons had not been affected (that is, latency). Table 3 shows the transcranial MMEP latencies for gas-
trocnemius and quadriceps muscles for the experimental (L-2 and T12–13 lesions) and control groups. Latencies were determined as the time in milliseconds from the onset of the transcranial magnetic stimulation artifact until the first deviation from baseline. In all the animals in the study, transcranial MMEP responses were detected before and after KA-injection injury, and no significant differences were observed in transcranial MMEP latencies at any time point. Figure 6 shows examples of transcranial MMEP responses recorded in the left gastrocnemius and quadriceps muscles in two different animals preinjury and at 1 week and 4 weeks after KA-induced L-2 injuries. The gastrocnemius responses were recorded in the animal represented in Fig. 5B and the quadriceps responses in the animal in Fig. 5C. The responses represented in Fig. 6 illustrate that although the latencies did not change following KA-induced injury, transcranial MMEP responses appeared to be larger in amplitude and longer in duration postinjury, suggesting a loss of inhibitory control of motor circuitry below the site of KA-induced SCI.

Discussion

Adult rats in which the excitotoxin KA was injected into the lumbar spinal cord developed long-lasting locomotor deficits and significantly decreased BBB scores. Animals with lesions that included the intermediate L-2 gray matter were unable to locomote and were functionally paraplegic. Animals that sustained more rostral lesions illustrate that although the latencies did not change following KA-induced injury, transcranial MMEP responses appeared to be larger in amplitude and longer in duration postinjury, suggesting a loss of inhibitory control of motor circuitry below the site of KA-induced SCI.

<table>
<thead>
<tr>
<th>BBB Score Range</th>
<th>BBB Category</th>
<th>Control (L1–2)</th>
<th>T12–13</th>
<th>L1–L3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–7</td>
<td>frank plegia, movement of three joints</td>
<td>0</td>
<td>2</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>8–13</td>
<td>standing weight support only, some stepping movements</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>14–21</td>
<td>coordinated stepping, completely normal</td>
<td>32</td>
<td>17</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>32</td>
<td>20</td>
<td>76</td>
<td>128</td>
</tr>
</tbody>
</table>

* Some data were not obtained for T12–13 and L1–3 groups; total number of hind limbs for T12–13 and L1–3 should be 32 and 80 respectively. The BBB scores are divided into functional categories based on a previous study by Basso, et al. A Pearson chi-square nonparametric test shows that hindlimb BBB scores for animals with injuries that include L-2 are more likely to range from 0–7 than are hindlimb scores for animals with T12–13 injuries or for control animals ($\chi^2 = 101.8$, df = 4, p < 0.001).
not extending into the L-2 segment regained the ability to ambulate by 4 weeks postinjury.

Excitotoxically Induced Lesions

Injury involving intraspinal KA injections was specifically designed to damage the interneurons in the intermediate gray matter while preserving the white matter tracts and alpha motor neurons. The excitatory and toxic properties of KA are well documented: it is structurally related to glutamate, and systemic injections are known to induce immobility and “wet dog shakes” via the AMPA (amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/KA class of non-NMDA receptors. Several investigators have examined the effects of KA injection compared with acromelic acid, another neuroexcitatory compound. Injected systemically, acromelic acid selectively damages the interneurons of the spinal cord, causing spastic paraplegia without inducing wet dog shakes. Systemic injection of KA to induce paraplegia necessitates a dosage 30 times greater than that of acromelic acid; permanent flaccid paralysis is the primary deficit. This observation brings into question whe-

Fig. 5. A–D: Graphs depicting results of motor neuron counts made in cresyl violet–stained 50-μm transverse sections representative of spinal cords from saline-injected control animals (A) and animals that received intraspinal injections of KA (B–D). The spinal cord shown in Graph B received a single 1 μl × 1 mM injection of KA bilaterally at L-2, whereas those represented in Graphs C and D each received two injections of 1 μl × 1 mM KA bilaterally 2 mm apart. The 4-week postinjury mean BBB scores are indicated above the x axis. E: Graph representing the mean number of motor neurons (~ standard error of the mean) counted per cresyl violet–stained 50-μm section in a 2-mm zone at the injury epicenter in all spinal cords in which motor neurons were counted. The spinal cords represented in Graphs A through D are indicated in Graph E by arrows. The spinal cord sections counted in Graphs A to D for Graph E are indicated by horizontal brackets. The approximate relative locations of the spinal cord segments and motor neuron pools for quadriceps (Q) and gastrocnemius (G) muscles are indicated along the top of the graph.
Paraplegia secondary to lumbar spinal cord interneuron loss

Histological examination of the rat spinal cords revealed extensive interneuronal loss following intraspinal microinjections of KA. Injections had been directed at Rexed lamina 7 approximately 1.2 mm from the dorsal surface, a region that has previously been shown in vitro to contain rhythmically active cells in the neonatal rat spinal cord and that also contains neurons labeled in an activity-dependent manner following drug-induced “air stepping”.3,8 The most severe damage appeared to involve the dorsal horns and intermediate gray matter, with noticeable cystic cavities appearing in place of the dorsal horn (posterior) gray matter in some specimens. In other specimens, no noticeable cavities developed; however, the dorsal (posterior) columns and dorsolateral white matter appeared to have collapsed medially, giving the spinal cord a distinctive pear-shaped appearance in cross section. Authors of previous studies in which the effects of KA injections were examined (studies conducted both systemically and intrathecally) have reported pathological processes quite similar to those associated with administration of acromelic acid in the acute phase (<7 days), but then accompanied by a degeneration of alpha motor neurons beginning 2 to 3 weeks postinjury.15,19 Intraspinal injection of KA has also been used previously but at doses approximately 50 times greater than those used in the present study. Not surprisingly, both interneurons and motor neurons were eliminated in several lumbar segments.26 In that study two injections were made bilaterally into the lumbar enlargement, with each injection site separated by 3 mm. These authors reported spastic paralysis followed initially by flaccid paralysis, with muscle wasting beginning at 1 week.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Preop Baseline (no. of rats)</th>
<th>Week 1 (no. of rats)</th>
<th>Week 4 (no. of rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gastrocnemius control</td>
<td>6.23 ± 0.21 (4)</td>
<td>6.11 ± 0.10 (4)</td>
<td>6.25 ± 0.17 (4)</td>
</tr>
<tr>
<td>T12–13</td>
<td>6.31 ± 0.10 (4)</td>
<td>6.01 ± 0.11 (4)</td>
<td>6.48 ± 0.22 (4)</td>
</tr>
<tr>
<td>L1–2</td>
<td>6.21 ± 0.18 (10)</td>
<td>5.90 ± 0.46 (10)</td>
<td>6.04 ± 0.47 (10)</td>
</tr>
<tr>
<td>quadriceps control</td>
<td>5.19 ± 0.16 (4)</td>
<td>5.25 ± 0.16 (4)</td>
<td>5.33 ± 0.17 (4)</td>
</tr>
<tr>
<td>T12–13</td>
<td>5.33 ± 0.10 (4)</td>
<td>5.27 ± 0.16 (4)</td>
<td>5.39 ± 0.22 (4)</td>
</tr>
<tr>
<td>L1–2</td>
<td>5.30 ± 0.14 (10)</td>
<td>5.13 ± 0.29 (10)</td>
<td>5.34 ± 0.36 (10)</td>
</tr>
</tbody>
</table>

* Data are given as mean ± standard deviation. There were no significant differences in the mean latencies recorded preoperatively (baseline) and at Week 1 or 4 postinjury for any of the groups.

Preservation of Motor Neurons and White Matter

Morphological and quantitative examination of the KA-induced spinal cord lesions in our study showed in most samples a relative preservation of alpha motor neuron populations compared with control animals. The resulting spastic paraplegia may thus be secondary to an overall decrease in the number and/or proportion of glycinergic and/or γ-aminobutyric acidergic neurons compared with glutamatergic neurons, specific to the injury, as opposed to the flaccid paraplegia seen following alpha motor neuron damage. It is important to note that, despite the loss of motor neurons at the injection site in some animals, there was no correlation found between motor neuron loss and BBB score, further suggesting that interneuron loss was responsible for both the drop in BBB score and the accompanying spasticity. This theory is supported by the functional transcranial MEP responses, which were robust at 1 and 4 weeks postinjury, as well as by anecdotal evidence of enhanced Hoffman’s reflex and cutaneous reflex responses in KA lesion-induced animals (unpublished data).

The dissociation of BBB scores and transcranial MEPs (drop in BBB score with no change in the transcranial MEP) is significant also in light of historical prognostic implications. In the thoracic contusion model of SCI, recovery of locomotor function in the rat is closely...
paralleled by the recovery of transcranial MMEPs; however, these parallels are not applicable to KA lesion–induced rats, indicating that the use of MMEPs as an indicator of ambulatory recovery may need to be viewed with caution. Thus, in the clinical setting, SCIs involving variable amounts of cervical or lumbar enlargement gray matter may entail long-lasting motor or locomotor deficits that are not readily predicted by evoked potentials, which are largely indicative of white matter continuity.

### Central Pattern Generator

The functional significance of damage to spinal cord gray matter varies according to the level damaged. It has been proposed that an oscillatory network capable of producing a local pattern of locomotor activity (a CPG) is located in the intermediate gray matter of the spinal cord lumbar enlargement in rats and other mammals, producing rhythmic locomotor output onto hindlimb motor neurons. This network is thought to contain extensive reciprocal inhibitory and excitatory connections characterized by commissural pathways that cross from one side of the spinal cord to the other, as well as rostrocaudal pathways connecting motor nuclei ipsilaterally, mediating the alternating right–left and flexor–extensor pattern characteristic of locomotion. The location of this CPG is still in question; however, a variety of in vitro studies have provided evidence that the rostral lumbar segments contain the bulk of CPG circuitry, which may be limited to the L-1 and L-2 segments. Results demonstrating that more caudal segments, when isolated in vitro, can generate a rhythm in the presence of 5-hydroxytryptamine and NMDA appear to dispute the idea of an anatomically circumscribed CPG and argue that the rhythm producing circuitry is in fact distributed throughout the entire lumbar cord. In our experiment, we found that lasting paraplegia was obtained most effectively in rats with bilateral lesions that included the L-2 spinal cord level. Transient paraplegia was induced after placing focal lesions throughout the lower-thoracic and upper-lumbar spinal cord, but permanent paraplegia occurred only when the lesion included the L-2 segment. The significance of the L-2 segment in relation to the CPG is still unclear, but its potential role as the core of the CPG for locomotor functions should not be dismissed. It is difficult to argue that the loss of locomotor ability occurs secondary to motor neuron loss, because two major hindlimb muscles (the gastrocnemius and quadriceps muscles) displayed intact transcranial MMEP responses following even the largest KA injection and also because the bulk of the motor neurons serving these muscles are located caudal to the injury site in the L-3, L-4, and L-5 segments. Thus, we suggest that loss of locomotor ability occurs secondary to the loss of interneurons residing in the intermediate gray matter of the rostral portion of the lumbar enlargement and that these interneurons form a necessary component of the CPG itself.

### Conclusions

A bilateral KA-induced lesion 3 to 4 mm long that included the intermediate gray matter of the L-2 segment in adult rats produced long-lasting paraplegia. The paraplegia was produced in the absence of damage to the primary descending motor pathways (white matter) and did not correlate with loss of alpha motor neurons as assessed by transcranial MMEPs and histological examination. Kainic acid–injured animals recovered locomotor function after sustaining bilateral lesions placed rostral to the L-2 segment; recovery was presumably due to the sparing of critical pattern-generating circuitry. The significance of the paraplegia resulting from damage to the intermediate (central) gray matter can easily be extended to the human model, in which both cervical and lumbar segments contain significant amounts of gray matter responsible for arm and leg movement and generation of patterns of locomotor activity. Thus, KA-injured animals may serve as a model of SCI in which successful incorporation of transplanted neural precursor–derived neurons could aid in the recovery of ambulatory function. Our findings also suggest that research into SCI must address both gray and white matter damage because recovery of locomotor function hinges on both of these anatomical components.

### Acknowledgments

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Paraplegia secondary to lumbar spinal cord interneuron loss


