Effect of acute spinal cord injury on axonal counts in the pyramidal tract of rats

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The concentration of axons in the pyramidal tract of normal and spinal cord-injured rats was determined by counting axons in sections of spinal cord stained by the Holmes technique. In the normal rat the axon concentration was uniform in the cervical, thoracic, and lumbar regions, although the size of the tract diminished progressively with its descent in the cord. After acute cord transection or compression injury, the axon concentration distal to the injury site diminished markedly. However, an appreciable number of distal axons persisted after injury, due to either delayed degeneration or to the presence of an admixture of afferent fibers. The axonal counting technique developed in this study should be helpful in experiments on spinal cord injury and regeneration.

KEY WORDS — spinal cord injury — axonal count — pyramidal tract — axons — silver stain

M AJOR injury to the mammalian spinal cord producing complete axonal interruption causes permanent loss of neurological function because of the failure of functionally significant axonal regeneration to occur. It is highly likely that recovery would be improved if methods were available for promoting regeneration of cord axons. Unfortunately, there is very little information available about central axonal regeneration in the spinal cord after trauma. Some recent studies suggest that central cord axons are capable of regeneration, but detailed quantitative data are limited. It would be very helpful to have an accurate method of obtaining axon counts at the light microscopic level for assessing the degree of regeneration in experimental studies of acute cord injury. To our knowledge, the only report of axon counts in the injured spinal cord was by Eidelberg, et al., who counted the axons in the cords of ferrets after experimental compression injury of the cord. Indeed, in the field of spinal cord regeneration, histological assessment has often been done in a qualitative way with investigators attempting to identify regenerated axons crossing the injury site. The purpose of the present study was to devise, at the light microscopic level, an objective quantitative method of assessing the degree of axonal injury, and any possible axonal regeneration, after injury to the pyramidal tract. To do this, we have developed a histological method of counting axons in silver-stained sections of the spinal cord of normal and injured rats. The pyramidal tract was chosen for study because it is easily identifiable in the rat, and its axons are quite uniform in terms of size and myelination. In normal rats pyramidal tract axons were counted in the cervical, thoracic, and lumbar regions, and in injured rats axonal counts were made in the pyramidal tract proximal and distal to cord transection or cord compression at T-6.

Materials and Methods

Experimental Protocol

Female albino rats of the Wistar strain, weighing between 250 and 350 gm each, were used in these experiments. The rats were divided into two groups. One group of three rats was used to study the histology of the cervical, thoracic, and lumbar areas of the normal pyramidal tract. Another group of six rats was used to study the results of compression injury or transection on axonal counts in the pyramidal tract.

Normal Pyramidal Tract. In the group used to study the normal pyramidal tract, the three animals were sacrificed and the entire spinal cord was removed and fixed in 10% neutral formalin. Blocks were taken

* Rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.
Pyramidal tract axons after spinal cord injury from C-2, C-5, T-2, T-5, T-7, T-10, and L-1, embedded in paraffin, sectioned at 6 μm, and stained as described below.

Injured Pyramidal Tract. The animals used in the study of the injured pyramidal tract were divided in two groups of three animals each. In one group, the cord was completely transected at T-6 with a scalpel and covered by a free fat graft to minimize epidural scarring. In the second group, the cord was injured at T-6 by the extradural clip compression technique previously developed in this laboratory. The force of clip closure was 174 gm, and the clip was left compressing the cord for 5 minutes. This degree of compression injury would be expected to render the animals completely and permanently paraplegic as shown by previous studies in our laboratory. Two weeks after cord transection or compression the animals were killed, and the thoracic spinal cord was removed and prepared as described below.

Histological Techniques

Two methods of axon staining were used: the Holmes technique for silver staining of sections, and the Ranson technique for silver staining of tissue blocks. The Holmes technique was found to give very consistent and reliable results, and all the data presented in this paper are derived from sections stained by the Holmes method. Although the Ranson technique is easier to use, it produced inconsistent staining with frequent failure of penetration of the stain into the deeper portion of the blocks, especially in injured cord tissue. With the Holmes technique, axonal staining was consistent, and the black-stained axons stood out sharply against the pale background. Fresh silver solutions were used, and the solutions were frequently filtered to remove the fine precipitate that occurs with aging.

Fresh specimens of spinal cord were removed at autopsy and fixed in 10% neutral formalin. The paraffin-embedded spinal cord blocks were cut in cross sections 6 μm thick and stained with the Holmes stain. The area chosen for counting was the pyramidal tract, since in the rat this tract is distinctive both in appearance and in location. It contains a high proportion of unmyelinated fibers, packed very closely together, and is located in the ventral aspect of the dorsal columns. An eyepiece grid and a ×100 oil-immersion lens were used to count all the axons in 25 squares, the 25 squares comprising a total area of 625 sq μm. The mean concentration of axons was obtained by averaging the counts in 625-sq μm areas from both the left and right sides of the cord.

Results

Normal Pyramidal Tract

Even at low magnification, the pyramidal tract was readily identifiable in the silver-stained sections because of its closely packed unmyelinated axons. In contrast, in all the other white matter areas of the cord, the axons were farther apart, mainly due to the presence of a much higher proportion of myelinated fibers. In all levels of the cord the pyramidal tract lay in the ventral aspect of the dorsal columns immediately adjacent to the gray matter. However, as shown in Fig. 1, both the

![Fig. 1. Sections of normal rat spinal cord in the cervical, thoracic, and lumbar regions (left), and corresponding diagrams in which the pyramidal tract is indicated by heavy stippling (right). The shape and size of the tract changed at the various levels. In the cervical region it is circular or hexagonal in shape, progressing to diamond-, kidney-, and heart-shaped in the thoracic region, and then crescentic in the lumbar region. The size of the tract gradually diminishes from above downward. Holmes silver stain, × 17.](image-url)
FIG. 2. Photomicrograph of the normal pyramidal tract in the thoracic region. The axons are closely packed, and mainly unmyelinated. Most have been cut in cross section and appear as round dark circles. Holmes silver stain, × 405.

The shape and size of the tract varied markedly with the cord level. In the cervical region, it was large and circular or hexagonal in shape. In the upper thoracic region, it was smaller and diamond-shaped progressing to kidney-shaped in the lower thoracic area. In the lumbar region, it was smaller and heart-shaped progressing to crescent-shaped. Table 1 shows the diminishing area of the pyramidal tract with descent in the cord. Indeed, its area at C-2 was approximately five times greater than at L-1.

Figure 2 shows the densely packed axons in the pyramidal tract. Most of the fibers in the tract are small in caliber and unmyelinated. The concentration of axons obtained by counting the number of axons in the 625-sq μm areas as described above is shown in Table 1. The concentration of axons in the tract was remarkably similar in the various areas of the cord, with only a slight tendency to increase as the tract descended in the cord.

**Pyramidal Tract After Cord Transection**

The silver-stained sections of the cord obtained 2 weeks after complete transection of the cord at T-6 showed marked changes cephalad and caudad to the site of transection. There was marked destruction and hemorrhagic necrosis of the cord immediately adjacent to the transection site. These effects were less marked 4 to 7 mm in either direction. Thus, axonal counts could be made in all three animals at the sites 4 and 7 mm cephalad, but were possible in only two animals at the 4-mm site and in only one animal at the 7-mm site due to marked necrosis and destruction (Table 2). In these animals the destructive changes emanating from the site of transection had spread farther along the cord than usual. In several animals discrete areas of the cord were infarcted, especially in or near the pyramidal tract and adjacent dorsal columns.

Figure 3 shows diagrammatically the effect of transection on the pyramidal tract and the adjacent segment of the dorsal columns. Cephalad to the transection there was degeneration of the dorsal columns, and caudad to the transection there was degeneration of the majority of the axons in the pyramidal tract. Figures 4 and 5 show these changes at low and high power, respectively. Indeed, the pyramidal tract became even more sharply defined cephalad to the transection because of the degenerative changes in the adjacent dorsal columns (Fig. 4 left). Figure 5 shows that caudad to the transection there were many silver-stained axons in the pyramidal tract, which was a surprising finding. Indeed, the cau-

![FIG. 3. Diagrammatic representation of axons in the pyramidal tract, and adjacent dorsal columns cephalad and caudad to the transection site. Not all the axons in the tract caudad to the transection site had degenerated.](image)

### TABLE 1

<table>
<thead>
<tr>
<th>Spinal Cord Level</th>
<th>Axonal Concentration</th>
<th>Area (sq mm)</th>
</tr>
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<tbody>
<tr>
<td>C-2</td>
<td>212 ± 34</td>
<td>0.216 ± 0.026</td>
</tr>
<tr>
<td>C-5</td>
<td>223 ± 49</td>
<td>0.185 ± 0.035†</td>
</tr>
<tr>
<td>T-2</td>
<td>240 ± 21</td>
<td>0.105 ± 0.030</td>
</tr>
<tr>
<td>T-7</td>
<td>233 ± 42</td>
<td>0.070 ± 0.011</td>
</tr>
<tr>
<td>T-10</td>
<td>276 ± 36</td>
<td>0.046 ± 0.013</td>
</tr>
<tr>
<td>L-1</td>
<td>254 ± 21</td>
<td>0.041 ± 0.011</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviations for three rats. Axonal concentration was determined by counting the number of axons in a 625-sq μm area.

† Values are derived from two rats.
Pyramidal tract axons after spinal cord injury

Two weeks after the clip compression injury at T-6, the effects on the cord 4 to 7 mm cephalad and caudad to the injury site were similar to those described above for the transection injury. The degenerative changes in the cephalad dorsal columns and in the caudad pyramidal tract were as severe as after complete transection. Table 3 shows that there was a marked reduction in the axonal concentration in the tract 4 and 7 mm caudad to the compression injury.

Discussion

It is almost 100 years since Spitzka first described the unusual location of the pyramidal tract in rats, and this was confirmed later by Barron and Ranson. Ranson also showed its changing shape at various levels of the spinal cord. Linowiecki's study in 1914 of the comparative anatomy of the tract showed that, in the
rout, the tract is composed primarily of small unmyelinated fibers. To our knowledge, axonal concentrations have not been reported for the spinal segments of the tract, although Lassek and Rasmussen\(^8\) reported area measurements and axonal counts in the medullary portion of the tract in several species including the rat. In the rat medulla just rostral to the decussation, the tract area was 0.30 sq mm, somewhat larger than we found it to be at T-2 (Table 1). They found that, in the medullary pyramid, the tract contained 243,100 axons/sq mm, whereas in the present study the tract had a somewhat higher concentration of axons in the cord than in the medulla. In terms of axons per sq mm, the range in the cord was from 339,200 at C-2 to 441,600 at T-10 (Table 1). Thus, as the size of the tract decreases with descent in the brain and cord, the concentration of axons increases.

There have been no previous reports of the effect of spinal cord trauma on axon counts in the pyramidal tract. Eidelberg, et al.,\(^6\) performed axon counts on plastic-embedded sections of ferret spinal cord, and related axon density in entire cross sections of the cord to spinal cord function in animals subjected to cord compression injury. However, specific counts in the pyramidal tract were not performed.

In the present experiments, the Holmes silver staining technique has proven to be a reliable, consistent method of staining the pyramidal tract axons in the normal or traumatized cord. Most of the normal axons appeared as round, closely packed, darkly stained circles or ellipses, although some were obviously coursing obliquely and appeared as cylinders. In one normal animal, the entire tract coursed obliquely at T-5, which made axon counting difficult at that level.

The tract was easily recognizable at all levels of the cord and, as described above, it changed its shape and size as it descended in the cord. It was of major interest to find that the axon concentration remained almost constant throughout the normal cord. This constancy suggests that axonal concentration in the pyramidal tract may be a useful, objective index for assessing the degree of experimental cord injury or recovery. Indeed, in the presence of the degenerative axonal changes induced by trauma, the tract was even more sharply defined cephalad and caudal to the injury site.

After transection or compression of the cord, there was marked hemorrhagic necrosis of the cord which extended cephalad and caudal to the injury site for at least 2 to 3 mm in most animals. Accordingly, axonal counts could only be performed beginning at approximately 4 mm cephalad and caudal. Indeed, in some animals, discrete infarcts were seen in various locations of the cord, especially in the posterior columns, which prevented axonal counts at even this proximity to the injury site. These ischemic changes have been described previously in animals subjected to acute cord compression injury.\(^14\)

Two weeks after either transection or compression injury, there was a marked reduction in the caudal axonal counts, even though the reduction was not as marked as anticipated. There are several possible explanations for the persistence of these caudal axons. Two weeks may have been insufficient time for some of the destroyed axons to have lost their ability to take up the stain. The macrophages may not have had sufficient time to reabsorb the debris. In his studies of Wallerian degeneration, Cajal\(^4\) showed that the finer unmyelinated or lightly myelinated fibers persisted the longest after central axonal interruption. Indeed, some were "preserved perfectly" for up to 10 days, and only showed marked changes at "a much more advanced date" than the larger fibers. As noted above, the pyramidal tract in rats is distinguished by the high density of these small fibers. It is also possible that some of the persisting axons may have emanated locally from interneurons caudal to the injury site, or alternatively may have been due to an admixture of afferent or sensory fibers within the pyramidal tract.

It is concluded that axonal counts of the pyramidal tract may be useful as an objective index in experiments on spinal cord injury. The counts are easily performed at the light microscopic level and thus may have a wide application in many laboratories for the objective quantitative evaluation of the degree of cord injury or the extent of cord recovery. Other tracts in the cord, such as the dorsal columns, could be studied in a similar fashion. With a knowledge of the size and axonal concentration of any tract, the total number of axons remaining in the tract after injury could be determined.

**Acknowledgments**

The technical assistance of Mrs. L. Marmash and Miss Bev Michel is gratefully acknowledged.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Distance from Lesion</th>
<th>No. of Rats</th>
<th>Axonal Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 mm cephalad</td>
<td>3</td>
<td>216 ± 57</td>
</tr>
<tr>
<td>4 mm cephalad</td>
<td>3</td>
<td>202 ± 58</td>
</tr>
<tr>
<td>4 mm caudal</td>
<td>2</td>
<td>103 ± 6</td>
</tr>
<tr>
<td>7 mm caudal</td>
<td>1</td>
<td>112</td>
</tr>
</tbody>
</table>

*Axonal concentration was determined by counting the number of axons in a 625-sq μm area.

**TABLE 3**

<table>
<thead>
<tr>
<th>Distance From Lesion</th>
<th>No. of Rats</th>
<th>Axonal Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 mm cephalad</td>
<td>3</td>
<td>201 ± 61</td>
</tr>
<tr>
<td>4 mm cephalad</td>
<td>2</td>
<td>208 ± 44</td>
</tr>
<tr>
<td>4 mm caudal</td>
<td>3</td>
<td>81 ± 18</td>
</tr>
<tr>
<td>7 mm caudal</td>
<td>3</td>
<td>137 ± 12</td>
</tr>
</tbody>
</table>

*Axonal concentration was determined by counting the number of axons in a 625-sq μm area.
Pyramidal tract axons after spinal cord injury

References

2. Bernstein JJ, Bernstein ME: Axonal regeneration and formation of synapses proximal to the site of lesion following hemisection of the rat spinal cord. Exp Neurol 30:336-351, 1971

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