Sensitivity of the short-range spinal interneurons of the cat to experimental spinal cord trauma

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The technique of retrograde axoplasmic transport was used to demonstrate the effect of experimental spinal cord injury on the spinal interneurons in the upper lumbar and lower thoracic segments of cats. Force of varied intensity was applied to the dorsal surface of the spinal cord and horseradish peroxidase (HRP) was injected into the next caudal segment. A large impact (250 to 350 gm-cm) inducing permanent paraplegia of the hind legs blocked the axoplasmic transport instantaneously in both cranial and caudal directions. If 1 week elapsed between the trauma and injection, neurons cranial to the trauma did not show any evidence for retrograde axoplasmic transport, while few neurons in the caudal direction were labeled with HRP. A moderate impact (150 gm-cm) which rendered the animals only transiently paraplegic spared the axoplasmic transport in some neurons both cranially and caudally to the injection. No obvious recovery or additional loss in the number of HRP-labeled neurons could be found in the cats if the injection followed the trauma by 1 week. The loss of spinal cord neurons following the injury seems to be the immediate mechanical consequence of the trauma.

Key Words - spinal cord injury - spinal interneurons - axoplasmic transport - horseradish peroxidase technique

Movement of the limbs is the result of the coordinated interaction of at least three neuronal systems: descending tracts, spinal interneurons, and primary sensory neurons. The ensuing impulse patterns are then transmitted to the alpha motoneurons which, in turn, innervate the limb musculature.

Blunt trauma on the spinal cord, either in the everyday practice or in the case of laboratory experiments, causes paraplegia caudal to its site. The paraplegia is due partly to the impairment of the descending tract axons since they cease to conduct impulses as an immediate reaction to the experimental trauma. The sensory evoked potentials can also be monitored in order to assess the impulse-conduction ability of the long ascending pathways in experimental trauma. The sensory evoked potentials can also be monitored in order to assess the impulse-conduction ability of the long ascending pathways in experimental trauma.

On the other hand, the role of the spinal interneurons might be as important as that of the descending tract fibers in the elaboration of proper movements (see the recent review of Grillner). It is common to classify spinal interneurons into long- and short-range categories. The distribution of the perikarya of short-range spinal interneurons was found mostly in the intermediate zone both in cats and monkeys, confined to a cylindrical area called the central core of the spinal gray matter. The first experimental histological study on the axon trajectory of these neurons was given by Szentágothai. Several later studies using a more recent modification of histological techniques to visualize degenerated fibers and terminals, and the combination of experimental lesions and the horseradish peroxidase (HRP) technique, substantiated and extended Szentágothai's pioneering observations. Now it is generally accepted that the axons of the short-range interneurons ascend and/or descend to six to eight segments, terminating ipsi- and/or contralaterally mainly in the intermediate zone and in the ventral horn. The significance of the spinal interneurons is underlined by the fact that all but one kind of primary sensory
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neurons terminate on them;\textsuperscript{40,42} the only exception (Group Ia muscle afferents) has several collaterals in the intermediate zone in addition to the direct terminals to motoneurons.\textsuperscript{3,35,46} Fibers of the descending tracts terminate almost exclusively on spinal interneurons in lower vertebrates.\textsuperscript{33,47} Direct descending connections to motoneurons occur only in monkeys\textsuperscript{29,48} and probably also in man.

In a series of experiments on cats we attempted to analyze the effect of experimentally induced spinal cord trauma on the retrograde axoplasmic transport of short-range spinal cord interneurons of the lower thoracic and upper lumbar segments. The spinal cord was traumatized by the method described by Allen,\textsuperscript{2} and horseradish peroxidase (HRP) was injected into the caudal segment adjacent to the trauma. The presence or absence of labeled neuronal perikarya cranially and caudally to the site of the trauma was correlated with the intensity of the impact and with the clinical symptoms of the animals.

**Materials and Methods**

Experiments were performed on 18 cats. Six of these cats were used in the course of the pilot experiments to test the long-term clinical effects of the experimental trauma. Twelve other animals were traumatized and subsequently injected with HRP.

**Surgery**

The animals were anesthetized with Nembutal (pentobarbital sodium, 50 mg/kg body weight). The vertebral canal was opened in the upper lumbar region under aseptic conditions. With the dura mater intact, the spinal cord was traumatized by Allen's weight-dropping technique\textsuperscript{2} using an impactor with a circular surface, 5 mm in diameter and 10 gm in weight. The metal cylinder moved freely in a glass tube.

After the injury 1.0 to 3.0 mg of 30\% HRP (Sigma Type VI or Boehringer) dissolved in saline was injected into the spinal cord by means of a rigidly mounted 10 \( \mu l \) Hamilton syringe. The solution was injected in four to five places across the entire width of the spinal segment caudally adjacent to the trauma site. In six animals (acute cases) the HRP was given right after injury. In another six animals 1 week elapsed between the trauma and injection (chronic cases). In the latter group the animals were anesthetized again and the spinal canal was reopened. On completion of the surgery the muscles and skin were sutured and under careful postoperative inspection the animals were allowed to recover from anesthesia.

With the exception of an intramuscular injection of 15 mg Depersolon (21-desoxy-21-N-(N'-methylpiperazinyl)-prednisolone HCl), given immediately preceding the trauma, and antibiotics in the initial postoperative period, the animals did not receive any kind of therapy. These six cats in the pilot experiments survived 6 months and eventually were killed with an overdose of Nembutal. The 12 animals with HRP injection were kept alive 48 hours after the injection.

**Histological Procedure**

Under deep Nembutal anesthesia, the HRP-injected animals were perfused by a solution containing 0.5\% paraformaldehyde and 2.5\% glutaraldehyde in 0.2 M phosphate buffer (pH = 7.2), preceded by a quick rinse with saline. The lower thoracic and lumbar spinal cord was dissected and kept in the same fixative for 4 more hours at 4\textdegree C temperature. Later the fixative was replaced by 0.2 M phosphate buffer with 5\% sucrose. Frozen sections 25 \( \mu m \) thick were prepared in the transverse plane from the segment of the injection and from five segments caudally and rostrally. The sections were incubated either in diaminobenzidine\textsuperscript{17} or in Hanker-Yates medium\textsuperscript{19} and counterstained lightly with cresyl violet. Figure 1 gives a schematic view of the location of the trauma and injection, and shows the two types of interneurons involved in this study. It is emphasized that, although the axons of both neurons pass the site of injury, they are clearly in a different position with respect to the site of injection. The injured segment is interposed between the injection and location of the

![Figure 1](image_url)

*Fig. 1. Diagram of the experimental arrangement. The spinal cord is represented as an elongated transverse band, the segmental boundaries are marked by vertical broken lines. Sites of trauma and horseradish peroxidase (HRP) injection are located in immediately adjacent segments in craniocaudal sequence. The traumatized spinal segment lies between the perikaryon and HRP injection in the case of neuron No. 1 (left). Since the impairment of the axon blocks the retrograde axoplasmic transport in a direct way, these neurons were called "experimental neurons." In the case of neuron No. 2 (right), the traumatized spinal segment lies distant from the HRP injection with respect to the perikaryon ("test neuron"); therefore, the trauma affects the retrograde axoplasmic transport indirectly.*
perikaryon of neuron No. 1 (the neuron of directly affected axoplasmic transport, "experimental neuron"), whereas the injury affects the distal axon arborization of neuron No. 2 (the neuron with indirectly affected axoplasmic transport, "test neuron").

Control experiments (HRP injection without trauma) were done on nine cats. The distribution of HRP-labeled perikarya of spinal interneurons was found to be similar to that described in a recent publication.31

**Results**

**Pilot Experiments**

The spinal cord of three cats was traumatized with 250 to 350 gm-cm (10-gm weight dropped from 25 and 35 cm respectively). These animals became paraplegic immediately after trauma and the paraplegia did not show any sign of reversibility throughout the 6-month observation period. The spinal cord of three other animals was traumatized with 150 gm-cm (10-gm weight dropped from 15 cm). Although these animals became paraplegic, isolated small movements appeared in the musculature of the hind legs on the third to fifth day following the trauma. During the second week of the observation period they were able to change position by using their still paretic hind legs. The neurological symptoms showed a continuous process of recovery in the course of the first 2 months. By the end of this time the paraparetic hind legs, still with some spasticity in the muscles, were used in locomotion. The sphincter musculature functioned adequately in these animals.

Based on the results of the pilot experiments, in the course of the main experimental series, cats suffering experimental spinal trauma of 250 to 350 gm-cm intensity were considered permanently paraplegic, while those suffering less intense trauma (150 gm-cm) were considered as transiently paraplegic.

**Experiments with HRP Injection**

On the basis of the intensity of trauma and time interval between the injury and HRP injection, the 12 animals could be subdivided into four groups, three cats in each group: Group I: major trauma (250 to 350 gm-cm), immediate injection; Group II: major trauma, delayed injection; Group III: minor trauma (150 gm-cm), immediate injection; Group IV: minor trauma, delayed injection.

Sections prepared from the injected spinal cord segment were entirely brown due to the multiple HRP injections. No acceptable frozen sections could be prepared from the segment of injury because of the hemorrhagic necrosis in the gray matter. Sections prepared from the adjacent spinal cord segments did or did not contain HRP-labeled perikarya, depending on the force exerted on the surface of the spinal cord. These latter findings were considered as signs of intact or damaged axoplasmic transport in the affected interneurons.

Table 1 shows that the application of the major trauma promptly blocked the axoplasmic transport of practically all spinal neurons both cranially and caudally (Group I). If the injection followed the traumatization after a 1-week interval (Group II),
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neurons caudal to the injection showed weak partial recovery, while those located cranially did not show any substantial sign of recovery. The application of minor trauma (Groups III and IV) spared the integrity of some spinal interneurons with axons passing through the traumatized region irrespective of the time interval between the trauma and injection. The number of labeled neurons cranial to the trauma (experimental neurons) varied substantially from animal to animal. They were either fusiform or multipolar in the transverse plane (Fig. 2); their longest diameter varied from 20 to 40 μm. Labeled neurons were confined mostly to the dorsal part of the ventral horn; only a few of them were located dorsal to the level of the central canal, mostly in the lateral part of the intermediate zone (Fig. 3). No obvious difference could be found in the distribution of the labeled neurons between animals in Group III versus Group IV. Also the number of labeled neurons caudal to the trauma (test neurons) varied from animal to animal in these two groups. Their location was identical to those shown in Fig. 3.

Discussion

Methodological Considerations

The results of the pilot experiments indicated that in our laboratory less force was needed to produce both transient and permanent paraplegia than has been generally reported in the literature. This observation underlines "the need for testing one's particular device with chronic preparations before drawing broad conclusions from acute experiments." Furthermore, recent analysis of the weight-dropping technique has to be considered when results of various laboratories are to be compared.

The HRP technique used in these experiments shows that under certain circumstances the experimentally traumatized region of the spinal cord still contains axons with intact retrograde transport capacity. This sign was taken as evidence for the viability of the neurons. The great variety in numbers of labeled neurons found in the individual animals of Groups III and IV may argue for differences in the severity of the injury, but may be the result of
Fig. 3. Distribution of horseradish peroxidase-labeled perikarya of experimental neurons in four cats. The injected segment is indicated at the bottom of each drawing, the level of the segments in which the perikarya were plotted are shown at right together with a fraction indicating the number of labeled neurons per number of sections used for plotting.

technical artifacts; for instance, the injected HRP was taken up only by a few axon terminals. For the very same reason the exact quantitative evaluation of our results seemed to be of little value. Instead, a semi-quantitative estimation of the number of labeled neurons (none, few, or numerous) was adopted to signal differences between animals.

No emphasis was put on the histopathology of the injured segment because this question has been treated in several other papers. The instant development of paraplegia was used to measure the effect of the trauma.

Intricacy of Spinal Interneurons with Axons Passing Through Traumatized Segment

Numerous reports have pointed out the positive correlation between the force of the injury and the extent of the histological and vascular alterations in the spinal cord after experimental spinal cord trauma. Therefore, the most obvious conclusion of our results, that minor trauma spares the integrity of some interneurons while major trauma kills virtually all neurons, seems to be trivial. On a closer look, however, the present results might indicate some other aspects of the pathogenesis of paraplegia induced by blunt spinal cord trauma.

Number of Labeled Experimental Neurons. No consistent difference could be found in the number of labeled experimental neurons depending on the time interval between trauma and injection. In the groups with delayed injection the number of labeled neurons neither increased nor decreased with respect to the appropriate groups with immediate injection. This observation seems to suggest that the number of surviving neurons is grossly determined by the impact itself. No obvious recovery occurs in the number of functioning interneurons, and no obvious additional loss could be detected in the first week after the trauma, in spite of the fact that the hemorrhagic necrosis in the spinal cord tends to progress during the very same period. Since the rate of the retrograde transport is in the order of 2 to 3 mm/hr, the lack of occurrence of labeled neurons at the time of sacrifice truly reveals the state of neurons during the first 16 to 18 hours following the trauma. These findings are all the more important because, according to the current views, the neuronal degenerations are secondary to vascular changes and the subsequent hypoxia. Since the number of labeled neurons was reduced immediately after the trauma and no consistent further reduction could be found if the HRP was injected 1 week later, we favor the interpretation of Gelfan and Tarlov, Tarlov, Fairholm and Turnbull, and Kobrine, et al., that the loss of neurons as the consequence of a blunt spinal cord trauma is a primary process. The amount of loss seems to depend mainly, if not entirely, on the severity of the trauma and only to a lesser degree on the hemorrhagic necrosis. The inconsistent results of various therapeutic approaches lead to a similar conclusion.

Number of Experimental vs. Test Neurons. Contrary to our expectations, no consistent difference
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could be found in the number of experimental versus test neurons, although, as was emphasized earlier, their topographical relationship to the trauma and injection is not identical. The only weak sign that the test neurons were able to recover following the major trauma was found in Group II animals. These findings may suggest that the integrity of the spinal interneurons can be seriously impaired by mechanical injury applied not only to the perikaryon but also to the region of the relatively distant axon arborization.

The distribution of labeled experimental neurons in Group III and IV animals was identical to that of short-range spinal interneurons with descending axons, suggesting that spinal interneurons of different location in the gray matter are equally sensitive or resistant to mechanical injury.

Clinical Significance. A discrepancy was found between the clinical symptoms and the occurrence of functioning interneurons in Group III. At the time of the HRP injection these animals were paraplegic, yet in some of them (the best example is Cat TM-40) a fairly large number of interneurons survived the injury. This finding might explain the restitution of limb movements in some patients in clinical practice, in spite of serious initial clinical symptoms. Furthermore, it underlines the desirability of attempting therapeutic means in each clinical case, because the degree of restitution cannot be accurately predicted immediately following the injury.

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References


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