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ORIGINAL ARTICLE

Spinogenesis and Plastic Changes in the Dendritic Spines of Spinal Cord Motoneurons After Traumatic Injury in Rats

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Background. Spinal cord injury (SCI) is highly incapacitating, and the neurobiological factors involved in an eventual functional recovery remain uncertain. Plastic changes to dendritic spines are closely related with the functional modifications of behavior.

Aim of the Study. To explore the plastic response of dendritic spines in motoneurons after SCI.

Methods. Female rats were assigned to either of three groups: Intact (no manipulations), Sham (T9 laminectomy), and SCI (T9 laminectomy and spinal cord contusion).

Results. Motor function according to a BBBscale was progressively recovered from 2 week through 8 week postinjury, reaching a plateau through week 16. Dendritic spine density was greater in SCI vs. control groups, rostral as well as caudal to the lesion, at 8 and 16 weeks postinjury. Thin and stubby/wide spines were more abundant at both locations and time points, whereas mushroom spines predominated at 2 and 4 months in rostral to the lesion. Filopodia and atypical structures resembling dendritic spines were observed. Synaptophysin expression was lower in SCI at the caudal portion at 8 weeks, and was higher at week 16.

Conclusion. Spinogenesis in spinal motoneurons may be a crucial plastic response to favor spontaneous recovery after SCI.

Key Words: Spinal cord injury, Motoneuron, Dendritic spines, Spinogenesis, Synaptophysin, Neural plasticity.

Introduction

Spinal cord injury (SCI) leads to significant neurological impairment and reduced quality of life. SCI has a complex and poorly understood pathophysiology, and the treatments available do not yield adequate functional results (1).

Although SCI produces impairment of hindlimbs-dependent motor function, it may recover partially over time (2–4). This has been attributed to the survival of several descending axons, which contribute to functional recovery mediated by local plastic processes (5,6).

Intermediate interneurons in the lumbar spine constitute the postsynaptic site of the descending motor systems that survive after SCI (7) and are responsible for locomotive

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function modulation (8). Injured nerve fibers may produce changes in their connections to distal neurons, while surviving fibers may establish adaptive reconnections, all of which impacts locomotive recovery.

As observed in other neural systems, contact with the dendritic spines of spinal neurons represents a highly efficient mechanism of excitatory information transmission (9), including the motoneurons associated with voluntary movement (10), even with low dendritic spine density.

Under normal conditions, dendritic spine density varies with motor activity patterns (11). Moreover, under pathological conditions, dendritic spines show plastic changes associated with injury and the subsequent restitution of a previously lost motor activity at different regions of the CNS (12), including the spinal cord (13); however, such changes do not always translate into normal function (10). Density has been usually associated with the plastic capacity of spines, and it is now clear that their different geometric conformations as well as interconversions between them in terms of their geometrical structures are closely related to the functional consequences of such plasticity (14).

These phenomena have not been fully characterized after SCI, in spite of they could explain in part the gradual appearance of spontaneous motor recovery after incomplete SCI. Hence, the purpose of this study was to describe the plastic response of dendritic spines in motoneurons after SCI. The finding that there is indeed dendritic spine formation, may guide to further investigations aimed to therapeutic strategies.

Material and Methods

Experimental Design

Female (250−300 g) Long−Evans rats (Harlan Laboratories, IN) were kept in the local animal facility. Animals were assigned to either of three study groups: a) Intact, b) Sham, and c) SCI. The motor function of the pelvic limbs was assessed 24 h after sham operation or SCI, and then weekly for the following 2 or 4 months (n = 15 rats/group/age after lesion).

Spinal Cord Injury

As trauma to the spinal cord causes sensory and motor impairments associated to the distal portion to the point of injury (15), T9 level was selected with the aim to affect only the hindlimbs functionality of SCI animals.

Rats were anesthetized (xylazine, 25 mg/kg i.m.; ketamine, 75 mg/kg i.m.) and the dorsal thoracic region was prepared. After a sagittal incision (T9−T10) the paravertebral muscles were retracted, followed by a laminectomy at T9. A moderate contusive SCI was deliveredto the spinal cord using the NYU Impactor, controlled bythe Impactor Software v.7. A 10 g metallic cylinder was released directly against the spinal cord at 25 g/cm. After corroborating the presence of a hematoma, the muscles and skin were sutured. Animals received penicillin benzathine (1,200,000 IU, i.m.) immediately after the SCI, and acetaminophen (5 mL/L drinking water/3 d).

Assessment of Motor Function

One day after surgery, and weekly afterwards, the motor function was evaluated using the Basso–Bresnahan–Beattie (BBB) test (2) in a 1.20 × 1.20 m open field. The BBB scale measures incremental scores, where 0 represents complete absence of spontaneous movement and 21 a normal gait. It considers movement in hip, knee, and ankle joints, as well as the ability to stand on both legs, the plantar position on the floor, taking steps, tail posture, and coordination. Rats were video-recorded weekly in a single session over 4 min and assessed afterwards by two independent blinded observers.

Sampling

Two or four months after SCI, according to the study group, animals were anesthetized (50 mg/kg i.p. pentobarbital) and a spinal cord segment 1 cm rostral to 1 cm caudal to the lesion was collected and prepared for histopathological, Golgi, or Western blotting studies.

Histology

Animals received pentobarbital (50 mg/kg i.p.) and were perfused using a peristaltic pump at 30 mL/min through a cardiac cannula with 200 mL of phosphate-buffered solution (pH 7.4, 0.1 M) with heparin (1000 IU/L) and procaine (1 g/L), followed by 200 mL of 4% buffered formaldehyde (pH 7.4; 0.1 M). Immediately after perfusion, 2 cm of the spinal cord were obtained, including the injured zone and the rostral/caudal neighboring areas. Tissues were postfixed in 4% buffered formaldehyde for at least 24 h. Spinal cord pieces (3 mm thick and 5 mm rostral and caudal to the lesion) were fixed in 10% buffered formalin. Specimens were embedding in paraffin, and then serial longitudinal sections of 10 μm thick were obtained and stained with hematoxylin and eosin for histological and morphometric analysis. Photomicrographs were obtained by a digital camera mounted on an optical microscope.

Morphometric Analysis

The Images obtained were digitized and analyzed (ImageJ software). Then, the damaged area was evaluated regarded to the preserved tissue in 25 mm^2 from one section where the injury epicenter was more evident. Ependyma was taken as reference point, in order to comparable evaluations were possible.

Dendritic Spines

Six animals from each group were used for these studies. Horizontal spinal cord blocks 3 mm thick and 5 mm rostral and