Internship Research Paper

Locomotion and Motor Neuron Plasticity after Incomplete Spinal Cord Injury

Completed at St. Joseph's Hospital

Barrow Neurological Institute

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By

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Introduction

Throughout scientific history, spinal cord injury has been considered irreversible and untreatable. As understanding of the nervous system and its function advanced, research has shown that even though there are obstacles in the regeneration of the central nervous system, some forms of plasticity and new connections can occur, which gives hope of at least partial recovery after spinal cord injury. For example, Bareyre et al., 2004 found that axonal sprouting and neuron regrowth can occur along a new intraspinal circuit to allow for potential recovery. The questions of our study are the following: How do motor and spinal cord neurons change after an injury? Do these changes help with recovery or limit recovery? Our research analyzes how an incomplete injury to the spinal cord can alter the function and organization spinal cord and motor neurons, as well as the behavioral and physiological aspects of recovery after this incomplete injury.

Dr. Thomas Hamm, the principal investigator, Dr. Vladimir Turkin, the post-doctoral research associate, and Derek O'Neill, the senior research assistant, have been working towards finding what occurs after spinal cord injury at the neuronal level. A fundamental aspect of the motor system is the organization of spinal motor neurons according to their size and the properties of the muscle fibers they innervate. One question is how the physiological properties of motor neurons and the synaptic connections to them from other nerve cells change after spinal cord injury. A second question is how movement ability recovers after injury. We also wish to understand how changes in motor neurons affect recovery of movement. The second question is the focus of this paper.

Experimental Procedure

In this experiment, two types of surgeries were performed: survival surgery and terminal surgery. In the survival surgeries, a force-controlled impact of 170 kdynes was given to the anesthetized rat, which resulted in a moderate contusion on the T9 region of the spinal cord. Three experimental groups were set: an uninjured control rat, an injured rat in a standard cage, and an injured rat in a cage with an exercise wheel.

Over several weeks, behavioral tests were performed through open-field walking, grid walk, and horizontal ladder walk in order to monitor the recovery process. The first experimental test is open field walking, in which we focused on the three joints of the hind limb – the hip, the knee, and the ankle – in order to analyze coordination, clearance, placement, and extent of movement for an overall measurement of locomotion (see Figure 1). The Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale divides the recovery process into three general phases. The early phase is characterized by little to no hind limb joint movement, the intermediate phase has uncoordinated steps, and the late phase focuses on details such as dragging of toes and tail as well as paw rotation. The BBB scale has a maximum of 21 points, in which the early phase ranges from 0 to 7, the intermediate phase ranges from 8 to 13, and the late phase ranges from 14 to 21. The early phase includes signs of little to no hind limb movement or uneven, extensive movement in one or two joints of the hind limb. The intermediate phase consists of varying weight support in plantar steps and little to no frontlimb-hindlimb coordination. For a score in the late phase, coordination, weight supported steps, toe clearance, and parallel paw positioning is consistent to frequent (Basso et al., 1995).

Although the open-field test determines ordinary locomotion, the grid walk and ladder walk tests analyze the coordination through accuracy of placement. Successful performance in these tests of accurate foot placement indicate significant recovery in pathways from the brain to the spinal cord, like the corticospinal tract. The grid walk allows the rats' steps to be analyzed by watching video recordings of each. Each step is categorized as a slip, miss, adjustment, or correct step (Figures 2 and 3). These numbers are then compiled to compare the number of correct steps versus number of incorrect steps within 30 seconds (Kunkel-Bagden et al., 1993).

Figure 1. Locomotion in open-field test (1st day post injury – DPI 1)

Figure 2.Correct step in grid walk test (DPI 35)

Figure 3.Miss or slip in grid walk test—characterized as a miss if the paw went straight through center of grid; characterized as a slip if the paw slipped off the edge of grid (DPI 35)

The terminal surgery was performed 8 to 10 weeks after the injury in order to measure electrophysiological responses of motor neurons to injection of current through a microelectrode and synaptic input from muscle spindles, movement sensors in muscle.

Data Analysis and Conclusion

Although full recovery did not occur, the rats showed signs of marked recovery. After giving moderate contusion injuries to the spinal cord, locomotor performance recovered substantially as shown through the following behavioral data. The open-field scores from the BBB Locomotor Rating Scale plateau at approximately 14 or 15 for the injured rats in both the standard cage and the activity cage (Figure 4). The misses per step in the grid walk also decreased substantially as the injured exercise rat lowered from a mean of 0.35 to 0.25 misses per step and the injured standard rat lowered from 0.31 to 0.23 misses per step (Figure 5). Although performance improved in this skilled task of walking, performance was still substantially impaired compared to sham-operated animals. We also found that housing injured animals in exercise cages had little effect on basic or skilled walking.

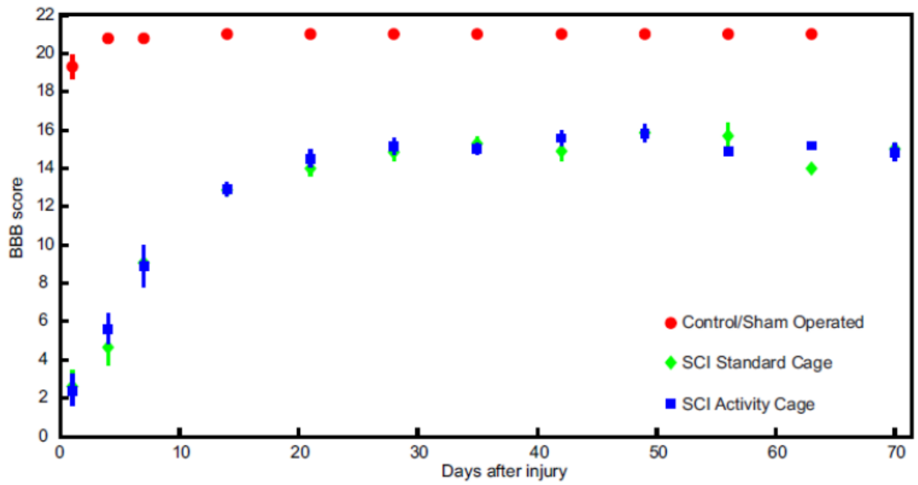

Figure 4. Mean BBB scores of uninjured control rats, injured standard cage rats, and injured activity cage rats analyzed in this study between 0 to 70 days post injury

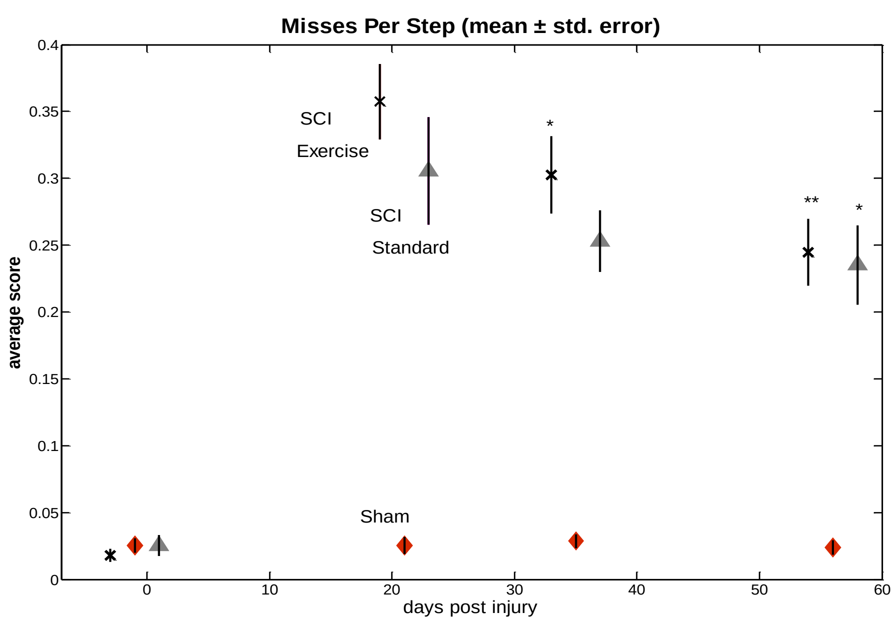

Figure 5.Average misses per step of uninjured control rats, injured standard cage rats, and injured activity cage rats between 0 to 60 days post injury. Asterisks indicate significant changes

compared to DPI 21.

This partial recovery of locomotion occurs while the properties of motor neurons and their synaptic inputs change after injury. Synaptic inputs to motor neurons from Ia spindle afferents are correlated with input resistance, an inverse measure of neuron size, in control animals. This relation weakens or is lost in injured animals (Turkin et al. 2015). Some motor neuron properties are also altered after injury (Turkin et al. 2016).

Even though full recovery is not attained, significant improvements in hind limb function occur within a few weeks of injury. Do changes in motor neurons or their synaptic inputs contribute to recovery? Or do such changes limit recovery? With further research, such knowledge can potentially be applied to physiotherapy for humans in recovering after a spinal cord injury.

References

Bareyre, F. M., Kerschensteiner, M., Raineteau, O., Mettenleiter, T. C., Weinmann, O. and Schwab, M. E. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nature Neuroscience 7: 269-277, 2004.

Basso, D. M., Beattie, M. S., Bresnahan, J. C.A sensitive and reliable locomotor rating scale for open field testing in rats. Journal of Neurotrauma 12: 1-21. 1995.

Kunkel-Bagden, E., Dai, H.-N., Bregman, B. S., Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. Experimental Neurology 119: 153-164, 1993.

Turkin, V. V., O'Neill, D., and Hamm, T. M.Changes in Ia EPSPs and effective synaptic currents following incomplete spinal cord injury. Soc. Neurosci. Abstr. 2015. Program Number 157.10.

Turkin, V.V., O'Neill, D. and Hamm, T. M.Changes in the afterhyperpolarizations of rat hindlimbmotoneurons following incomplete spinal cord injury and exercise. Soc. Neurosci.

Abstr. 2016. Program Number 612.13.

Acknowledgements

Barrow Neurological Institute

Dr. Thomas Hamm

Dr. Vladimir Turkin

Derek O'Neill

Students and Volunteers: Pareena Kaur, Sunna Khokhar, ShamilaShanmugam, LasyaAnantuni, Mira Marwah, Bethany Kasprzyk