Please cite this article in press as: Takiguchi M et al. Compensatory projections of primary sensory fibers in lumbar spinal cord after neonatal thoracic spinal transection in rats. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.07.046

Neuroscience xxx (2015) xxx–xxx

COMPENSATORY PROJECTIONS OF PRIMARY SENSORY FIBERS IN LUMBAR SPINAL CORD AFTER NEONATAL THORACIC SPINAL TRANSECTION IN RATS

M. TAKIGUCHI, Y. ATOBE, T. KADOTA AND K. FUNAKOSHI *

Neuroanatomy, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Abstract—Complete spinal transection in adult rats results in poor recovery of hind limb function, whereas significant spontaneous recovery can occur following spinal cord transection in rat neonates. The mechanisms underlying the recovery, however, are poorly understood. Recent studies in rodents suggested that the recovery is not due to axonal regeneration, but rather due to reorganization of the neural circuits in the spinal cord below the injury site, including central pattern generators. Few studies have reported histological evidence for changes in the primary sensory fibers or terminals. Thus, in the present study, we transected spinal cords of rats at thoracic level 8 at postnatal day 5. Four weeks after the injury, biotinylated-dextran amine (BDA), an anterograde tracer, was injected into the dorsal root ganglion of the lumbar spinal cord to examine the localization of sensory fibers and their terminal buttons in the spinal cord. BDA-positive axons in the rat spinal cord following neonatal spinal transection (neo ST) were longer than those in sham-operated or normal rats. The number of terminal buttons was also higher in spinal cords of neo ST rats compared with sham-operated or normal rats. These findings suggest that sensory fibers project more strongly and make more synapses following neo ST to compensate for the lack of supraspinal projections.

Key words: neonatal spinal injury, primary sensory fiber, dextran amine, dorsal root ganglion, hind limb locomotion, spinal cord.

INTRODUCTION

Thoracic spinal cord transection in adult rats leads to refractory paraplegia at spinal levels below the injury site. On the other hand, in neonatal rats with thoracic spinal cord transection, hind limb function partially recovers despite separation of the spinal cord below the injury site from upper spinal cord. (Stelzner et al., 1975). It was recently reported that neonatally transected (neonatal spinal transection, neo ST) rats had significantly higher scores on the Basso, Beattie, Bresnahan (BBB) Locomotor Rating scale (Basso et al., 1995) than rats transected as adults (Yuan et al., 2013).

Although the difference in functional outcomes has long been known, the mechanisms of recovery after neonatal spinal cord injury have not been elucidated. Previous studies of neonatal complete spinal transection revealed that descending axons did not regenerate beyond the lesion site (Tillakaratne et al., 2010).

On the other hand, rehabilitative approaches to spinal cord injury have attracted recent attention based on the findings that treadmill training in spinalized rodents had ameliorative effects on hind limb dysfunction (reviewed by Morawietz and Moffat, 2013). Thus, sensory information is expected to activate a spinal network called locomotor central pattern generator (CPG) after complete spinal injury (Edgerton et al., 2008; Harkema et al., 2012). The CPG is thought to comprise a specialized network of interneurons in the lumbar and sacral spinal cord that contributes to generate locomotor patterns, such as alternating activity between flexors and extensors on one side, without supraspinal tracts and is coupled with the CPG on the other side (for review, see Rossignol and Frigon, 2011). Neonatal animals have extensive capacities for remodeling the fiber connections in response to the central nervous system injury (Yoshikawa et al., 2011; Umeda and Isa, 2011). Therefore, we hypothesized that central projections and synaptic terminals of primary sensory fibers may change after complete spinal transection in neonates, and that primary sensory afferents may trigger the activation of CPG and motoneurons leading to partial recovery of hind limb function.

In the present study, we administrated biotinylated-dextran amine (BDA), an anterograde tracer, into dorsal root ganglion (DRG) to label primary sensory fibers and observed their localization and number of synaptic terminal buttons after complete spinal transection in neonatal rats.

*Corresponding author.
E-mail addresses: takiguchi.masa@gmail.com (M. Takiguchi), atoyan@med.yokohama-cu.ac.jp (Y. Atobe), kadotat@med.yokohama-cu.ac.jp (T. Kadota), funako@med.yokohama-cu.ac.jp (K. Funakoshi).

Abbreviations: 4% PFA, 4% paraformaldehyde; BBB, Basso, Beattie, Bresnahan; BDA, biotinylated-dextran amine; CPG, central pattern generator; DRG, dorsal root ganglion; IZ, intermediate zone; neo ST, neonatal spinal transection; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; VHl, lateral part of ventral horn; VHm, medial part of ventral horn.

http://dx.doi.org/10.1016/j.neuroscience.2015.07.046
0306-4522/

EXPERIMENTAL PROCEDURES

Rats

Neonatal Wistar rats (Japan SLC, Hamamatsu, Japan) were used in the present study (n = 26). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Animal Research Center, Yokohama City University Graduate School of Medicine.

Neonatal spinal cord transections

The spinal cords of postnatal day 5 (P5) rat pups (n = 10) were completely transected at the midthoracic level, as previously described (Kubasak et al., 2005; Tillakaratne et al., 2010). The pups were anesthetized with isoflurane gas (1.0–2.0%) via facemask. A dorsal midline skin incision was made from thoracic 6 (T6) to T10, and paravertebral muscles and fascia from T7 to T9 were partially retracted or removed with microforceps to expose the lamina of the vertebral arch. A partial laminectomy was performed at T7–T9 with microforceps, followed by complete spinal transection at T8 with small spring microscissors, and Spongel (Astellas Seiyaku Co., Ltd., Tokyo, Japan) was used to stop bleeding. After confirming the completeness of spinal transection and arrest of bleeding, we closed the muscles and fascia in the layers and skin with 6–0 nylon suturing. After surgery, all animals were placed in a warm incubator until fully recovered. Sham-operated rats (n = 8) underwent all the same procedures as neo ST rats, except for the spinal transection. All rat pups were returned to their mothers as a group. Normal rats (n = 8) received no surgical treatments during the neonatal period. One neo ST rat was killed 2 weeks after the surgery to confirm the completeness of transection (Fig. 1). Rat pups were weaned at 3 weeks after surgery (P26), and housed individually in polycarbonate cages in a room maintained at 25 ± 1 °C, with a 05:00 on/19:00 off light cycle.

Behavioral analysis

Four weeks after surgery (P33) before BDA administration, the hind limb locomotor functions of sham-operated and neo ST rats were measured according to the BBB scale (Basso et al., 1995), as described previously (Yuan et al., 2013).

BDA administration

Four weeks after surgery (P33), all rats (n = 25) were anesthetized with isoflurane as described above and the left DRG of the L5 was exposed by unilateral laminectomy. BDA (0.5 μL; MW 10,000, 10% in distilled water, D1956, Life Technologies, Carlsbad, CA, United States) was injected using a glass pipette attached to a manipulator (MNM-333, Narishige, Tokyo, Japan). The tip of the pipette was 50 μm in diameter, and the pipette was connected to a 1.0-μL Hamilton syringe by polyethylene tubing (size 5, Igarashi Ika Kogyo Co., Ltd., Tokyo, Japan).

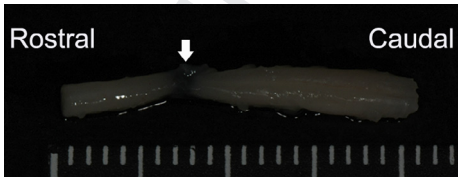

Fig. 1. The spinal cord harvested from neo ST rats at P19. The tissue images were similar beyond P19. White arrow indicates the transected site, which lacks gliosis, as described previously (Stelzner et al., 1975). Scale divisions = 1 mm.

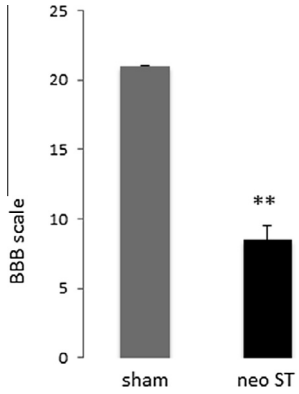

Fig. 2. BBB scores 4 weeks after spinal cord transection in neo ST rats (P33). Mean score of neo ST rats was 8.5. Each sham-operated rat scored 21 and exhibited no paralysis or motor dysfunction. Scores differed significantly between the two groups ($p < 0.01$). Data are the mean ± standard deviation (n = 4 in each group; $^{**}p < 0.01$, unpaired t-test).

Tissue preparation

One week after BDA injection, the rats were deeply anesthetized with isoflurane and perfused transcardially with 4% paraformaldehyde (4% PFA) in 0.1 M phosphate buffer (PB). The spinal cord and DRG were dissected and postfixed with 4% PFA overnight at 4 °C. The tissues were then cryoprotected in 25% sucrose for 2 days and embedded in O.C.T. compound via 2-methylbutane (isopentane) in liquid N_2. Sections of the DRGs and the spinal cords that were appropriate for analysis (three non-operated, four sham-operated, and

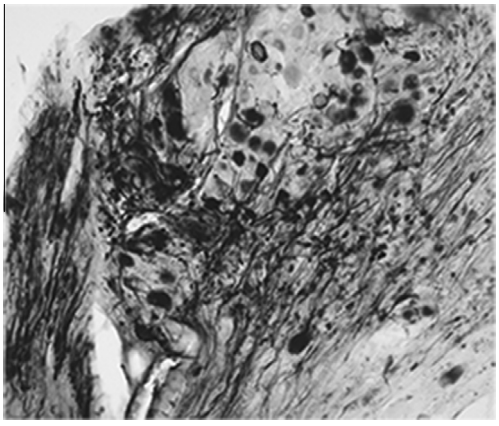

Fig. 3. An example of nickel-enhanced BDA staining in the DRG. Sensory neurons and fibers were stained in black. Scale bar = 100 μm.