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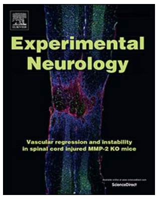

Research Paper

Enhanced axonal transport: A novel form of “plasticity” after primate and rodent spinal cord injury

J.H. Brock[a,b], E.S. Rosenzweig[b], H. Yang[b], M.H. Tuszynski[a,b,*]

[a] *Veterans Administration Medical Center, La Jolla, CA, United States*
[b] *Dept. of Neurosciences, University of California, San Diego, La Jolla, CA, United States*

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ABSTRACT

Deficient axonal transport after injury is believed to contribute to the failure of CNS regeneration. To better elucidate neural mechanisms associated with CNS responses to injury, we transected the dominant voluntary motor system, the corticospinal tract (CST), in the dorsolateral T10 spinal cord of rhesus monkeys. Three months later, a 4.5-fold increase in the number of CST axons located in the spared ventral corticospinal tract at both the lesion site and, surprisingly, remotely in the *cervical* spinal cord was observed. Additional studies of increases in corticospinal axon numbers in rat and primate models demonstrated that increases were transient and attributable to enhanced axonal transport rather than axonal sprouting. Accordingly, increases in axonal transport occur after CNS injury even in the longest projecting pathways of the non-human primate, likely representing an attempted adaptive response to injury as observed in the PNS.

1. Introduction

Recent advances in spinal cord injury (SCI) research have revealed mechanisms that limit CNS plasticity and regeneration, and several experimental approaches for enhancing axonal growth after injury have been reported. Most of these advances utilize methods of axonal tract tracing to reveal the effects of experimental manipulations. The most common method of axonal labeling injects anterogradely transported tracers, including dextran-containing particles. Other studies use retrograde tracer injections within or below zones of injury to quantify the number of retrogradely labeled neurons in regions remote from the lesion site that have presumably extended axons that take up and transport the tracer. All of these methods depend on axonal transport for visualization of the experimental response.

Both anterograde and retrograde axonal transport are critical processes for sustaining neuronal function. Anterograde transport is mediated by kinesin motor proteins, while retrograde transport requires dynein proteins (Black and Lasek, 1980; Galbraith and Gallant, 2000; McEwen and Grafstein, 1968; Scheff et al., 2003). Fast anterograde transport occurs at a rate of 50–400 mm/day, and slow anterograde transport occurs at two rates: 1) component “a” rate of 0.25 mm/day, which transports tubulin and neurofilament proteins, and 2) component “b” rate of 2–3 mm/day, which transports a variety of proteins including, actin (Black and Lasek, 1980; Galbraith and Gallant, 2000; McEwen and Grafstein, 1968; Scheff et al., 2003). Similar mechanisms have been identified that regulate retrograde axonal transport.

It has been known for some time that injury can influence axonal transport. Grafstein and Murray reported in the 1960s that optic nerve crush causes an increase in slow component b, based on transport of radiolabeled amino acids (Grafstein, 1969; Grafstein and Murray, 1969). More recent studies confirm alterations in axonal transport that may serve the purpose of carrying and organizing structural proteins required for formation of growth cone-like structures (Konzack et al., 2007; Mar et al., 2014a; Mar et al., 2014b). However, effects of lesions on axonal transport have been less thoroughly studied in the brain and spinal cord; indeed, it has been conjectured that a lack of increased axonal transport after central lesions may contribute to the failure of central axonal regeneration (Forbes and Andrews, 2017).

In the present experiment, we examined whether axons of the ventral corticospinal tract in rhesus monkeys undergo compensatory collateral sprouting after lesions of the majority (90%) component of the dorsolateral corticospinal projection. Ventral CST sprouting occurs in rodent models and is associated with partial improvement in motor function (Bareyre et al., 2004; Steward et al., 2008; Weidner et al., 2001), representing a form of endogenous axonal structural plasticity that may be associated with partial functional recovery. Given the dominance of corticospinal systems in primate motor control, we sought to characterize responses of the ventral CST after dorsolateral injury in rhesus monkeys. Subjects underwent lateral corticospinal tract lesions at T10, and the corticospinal tract was anterogradely traced

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[*] Corresponding author at: Dept. Neurosciences, 0626, University of California – San Diego, La Jolla, CA 92093, United States.
E-mail address: mtuszynski@ucsd.edu (M.H. Tuszynski).

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during the same surgical session. Surprisingly, we observed a 4.5-fold increase in the number of CST axons in the ventral white matter not only at spinal cord levels caudal to the injury, but at remote cervical levels also. To confirm whether new axonal growth in host white matter actually accounted for this observation, we explored the alternative possibility that spared axons enhanced their uptake or transport of the biotinylated dextran-based tracer. Indeed, detailed examination of the timing, nature and rate of tracer transport indicated that alterations in tracer transport, rather than true new growth, accounted for our observations of increased numbers of axons in the lesioned primate and rodent spinal cord. These findings reveal the presence of metabolic "plasticity" of neuronal transport in the rodent and primate CNS that may prime the neuron for a reparative response, even if subsequent regeneration fails.

2. Materials and methods

2.1. Experimental design

This experiment was originally designed to examine the nature of spontaneous axonal adaptation to unilateral transection of the dorsolateral component of the primate corticospinal projection at the T10 level. Following initial findings in the primate model, additional studies were performed in rodents to gain insight into mechanisms underlying observations in the primate model. Primate methods will be described first, followed by rodent methods.

For primate studies, the following groups were examined: **Group 1** (**Intact**, N = 4) consisted of intact animals without spinal cord lesions that underwent tracing of the corticospinal tract (CST), with quantification of axons at the L4 spinal cord segment. Tracing time was sufficient (3 months) to allow the tracer to reach the lumbar spinal cord segment (Lacroix et al., 2004). **Group 2** (**Lesion/No Delay Trace**, N = 5) consisted of animals that underwent T10 right dorsolateral CST transections. This lesion removes 90% of all CST axons originating from the left motor cortex that project down the right side of the spinal cord, together with 8% of CST axons originating from the right motor cortex that descend ipsilaterally down the spinal cord in the dorsolateral tract (Lacroix et al., 2004). Lesions spared the *ventral* CST on the right side, which contains 2% of CST projections that originate from the right motor cortex and travel down the spinal cord (Lacroix et al., 2004). CST axons were quantified at the L4 segment to assess responses of the spared right ventral CST after removal of the right dorsolateral CST. Tracers were injected during the same surgical session that CST lesions were placed. **Group 3** (**Intact Cervical**, N = 3) consisted of intact animals that underwent tracing of the CST, with quantification of axons at the C8 spinal cord segment. Tracing time was sufficient (6 weeks) to allow the tracer to reach the cervical spinal cord segment; tracing times longer than 6 weeks result in movement of most tracer down the spinal cord, away from the mid-cervical segment. **Group 4** (**Lesion/Delayed Trace**, N = 2) consisted of animals with right C7 dorsolateral CST transections that underwent tracer injections six weeks *after* spinal cord lesions were placed. Axons were quantified at the C8 spinal cord segment to assess responses of spared right ventral CST axons to removal of the dorsolateral CST. While we would have preferred to study a complete set of animals with lesions at a single spinal level (either all T10 or all C7), the lesion model was moved from T10 to C7 during the course of this primate research program to enable study of hand function in other experiments; we did not feel justified in subjecting additional monkeys to T10 lesion to address this specific experimental transport question. Instead, we opted to use data from right C7 dorsolateral quadrant lesions in monkeys and to perform additional studies in rat models to confirm findings form the primate model and gain insight into underlying mechanism, as described below. The monkey experiment design is summarized in Fig. 1 and the rat in Fig. 5.

2.1.1. Subjects

Fourteen adult male monkeys (*Macaca mulatta*; mean age 9.1 ± 5.3 years, range: 3.8–14.5 years) were studied. All surgical procedures were carried out using principles of the Laboratory Animal Care Act (National Institutes of Health Publication 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC).

2.1.2. Spinal cord lesions

Monkeys were housed individually. Induction of anesthesia was performed with ketamine HCl (10 mg/kg i.m.) followed by maintenance with isoflurane. To create CST lesions, the level for laminectomy was identified by palpating rostrally from the T2 (C7 spinal level lesion) or T12 (T10 spinal level lesion) spinous processes. A dorsal skin incision was placed over the identified C7 or T10 region and a dorsal laminectomy was performed. To place T10 spinal cord dorsolateral CST lesions, a microwire lesion knife (Kopf, General Valve, Fairfield, NJ) was positioned 0.5 mm lateral to the spinal cord midline on the right side, and lowered to a depth of 2 mm into the cord through a small dural incision. The wire knife was then extruded toward the right lateral aspect of the spinal cord, creating a final lesion dimension of 3 mm depth × 3 mm width (Fig. 1B). The wire was raised through the cord with the arc extended. Downward pressure was applied to the arc using a suction tip, thereby transecting all structures within the arc. Muscle and skin layers were sutured in layers. Post-operatively, the monkeys were provided analgesics routinely for 3–5 days and antibiotics for 5–7 days. All subjects retained bowel and bladder function following the lesion. This right-sided T10 spinal cord lesion transects approximately 90% of CST axons originating from the left motor cortex and 8% of CST axons originating from the right cortex (Fig. 1A) (Lacroix et al., 2004; Rosenzweig et al., 2009), but spares ventral CST axons on the right side of the spinal cord that originate from the right motor cortex. Nissl stained sections indicate that the lesion is contained entirely within the dorsal quadrant, sparing the ventral funiculus (Fig. 1). C7 cervical lesions were placed identically on the right side of the spinal cord in two monkeys, but at the C7 rather than T10 level (Rosenzweig et al., 2010).

2.1.3. CST tract tracing

Immediately or 2 months later, the corticospinal tract was anterogradely labeled as described previously (Lacroix et al., 2004; Rosenzweig et al., 2009; Rosenzweig et al., 2010). Biotinylated dextran amine (BDA; 10% in NaCl; 10,000 molecular weight, Molecular Probes, Eugene, OR) was injected into the right hemisphere to examine compensatory axonal responses to injury arising from neuronal cell bodies of the motor cortex primarily unaffected by the right-sided spinal cord lesion. 150 nl of the 10% BDA solution was injected at each site through a glass micropipette attached to a picospritzer. The medial/caudal boundary of the primary motor cortex was identified as the junction of the central sulcus with the inter-hemispheric fissure. The first tracer injection was placed 1 mm lateral to midline and 1 mm rostral to the central sulcus at 3 separate dorsal/ventral coordinates. The injections then proceeded linearly every 1 mm over 13 additional sites rostral to the central sulcus. Then a second and third row of injections were performed, each 1 mm lateral to each other. Three separate dorsal/ventral injections were made, which resulted in a total of 127 injection sites. These injection sites included areas of the motor cortex that innervate hand, trunk and foot areas. Each injection was made under microscopic guidance. Subjects survived an additional 6 weeks for cervical lesions or 3 months for thoracic lesions, to allow sufficient time for transport of BDA past the lesion into the cervical or lumbar enlargements, respectively (Lacroix et al., 2004; Rosenzweig et al., 2009; Rosenzweig et al., 2010).

2.1.4. Tissue processing and histology

Prior to perfusion, monkeys were sedated with ketamine (25 mg/kg;