Neuroscience 272 (2014) 65–75

VECTOR-INDUCED NT-3 EXPRESSION IN RATS PROMOTES COLLATERAL GROWTH OF INJURED CORTICOSPINAL TRACT AXONS FAR ROSTRAL TO A SPINAL CORD INJURY

N. WEISHAUPT, [a*] **A. L. O. MASON,** [b†] **C. HURD,** [b‡] **Z. MAY,** [c††] **D. C. ZMYSLOWSKI,** [b‡‡] **D. GALLEGUILLOS,** [c,d†††] **S. SIPIONE** [c,d‡‡‡] **AND K. FOUAD** [c,e††††]

[a] *Department of Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, MSB 453, Canada*

[b] *Department of Rehabilitation Medicine, University of Alberta, 3-88 Corbett Hall, Edmonton, AB T6E 2G4, Canada*

[c] *Centre for Neuroscience, University of Alberta, 3-88 Corbett Hall, Edmonton, AB T6E 2G4, Canada*

[d] *Department of Pharmacology, University of Alberta, 9-21 Medical Sciences, Edmonton, AB T6G 2H7, Canada*

[e] *Faculty of Rehabilitation Medicine, University of Alberta, 3-87 Corbett Hall, Edmonton, AB T6G 2G4, Canada*

Abstract—Rewiring the injured corticospinal tract (CST) by promoting connections between CST axons and spared neurons is a strategy being explored experimentally to achieve improved recovery of motor function after spinal cord injury (SCI). Reliable interventions to promote and direct growth of collaterals from injured CST axons are in high demand to promote functionally relevant detour pathways. A promising tool is neurotrophin-3 (NT-3), which has shown growth-stimulating and chemo-attractive effects for spared CST axons caudal to a CST lesion. Yet, efforts to promote growth of injured CST axons rostral to a SCI with NT-3 have been less successful to date. Evidence indicates that immune activation in the local growth environment, either intrinsic or induced by the endotoxin lipopolysaccharide (LPS), can play a decisive role in the CST's responsiveness to NT-3. Here, we test the potential of NT-3 as a tool to enhance and direct collateral growth from the injured CST rostral to a SCI (1) using long-term expression of NT-3 by adeno-associated viral vectors, (2) with and without stimulating the immune system with LPS. Our results indicate that inducing a growth response from injured CST axons into a region of vector-mediated NT-3 expression is possible in the environment of the spinal cord rostral to a SCI, but seems dependent on the distance between the responding axon and the source of NT-3. Our findings also suggest that injured CST axons do not increase their growth response to NT-3 after immune activation with LPS in this environment. In conclusion, this is to our knowledge the first demonstration that NT-3 can be effective at promoting growth of injured CST collaterals far rostral to a SCI. Making NT-3 available in close proximity to CST target axons may be the key to success when using NT-3 to rewire the injured CST in future investigations.

Key words: neurotrophic factor, plasticity, lipopolysaccharide, axonal growth, viral vector.

INTRODUCTION

After spinal cord injury (SCI), long and short distance connectivity between neurons is permanently lost in an environment where meaningful regeneration remains an unmet challenge. Plasticity, the ability of the central nervous system to adapt and re-arrange even in adulthood, is thought to account for most of the spontaneous recovery that is frequently observed after SCI (Edgerton et al., 2004; Rossignol et al., 2011). Unfortunately, both the extent of such plasticity as well as the degree of the resulting recovery are fairly limited. Interventions to enhance and modify plastic responses of the nervous system could potentially help to rewire injured fibers to meaningful targets. A great majority of SCIs involve anatomically incomplete lesions where some fibers surrounding a lesion site remain intact, so that a number of connections that span across the injured spinal level are spared (NSCISC, 2012). These spared neurons can potentially be useful to build 'detour' pathways around the lesion for other neurons whose axons are injured (Courtine et al., 2008). Motor signals for example may

*Corresponding author. Address: Schulich School of Medicine and Dentistry, Department of Anatomy and Cell Biology, MSB 453, University of Western Ontario, Canada. Tel: +1-519-661-2111x86822; fax: +1-519-661-3936.
E-mail addresses: nweishau@uwo.ca (N. Weishaupt), almason@ualberta.ca (A. L. O. Mason), clhurd@ualberta.ca (C. Hurd), zacnicte@ualberta.ca (Z. May), zmyslowskid@mymail.macewan.ca (D. C. Zmyslowski), dgalleguillos@ualberta.ca (D. Galleguillos), ssipione@ualberta.ca (S. Sipione), karim.fouad@ualberta.ca (K. Fouad).
† Tel: +1-780-492-3217; fax: +1-780-492-1626.
‡ Tel: +1-780-492-3217; fax: +1-780-492-1626.
†† Tel: +1-780-492-3217; fax: +1-780-492-1626.
‡‡ Tel: +1-780-492-3217; fax: +1-780-492-1626.
††† Tel: +1-780-492-5885; fax: +1-780-492-4325.
‡‡‡ Tel: +1-780-492-5885; fax: +1-780-492-4325.
†††† Tel: +1-780-492-5971; fax: +1-780-492-1626.

Abbreviations: AAV-2, adeno-associated viral vectors of serotype 2; ANOVA, analysis of variance; BDA, biotinylated dextran amine; CST, corticospinal tract; DAB, diaminobenzidine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFP, green fluorescent protein; LPS, lipopolysaccharide; NDS, normal donkey serum; NT-3, neurotrophin 3; PBQ, *p*-benzoquinone; PCR, polymerase chain reaction; PFA, paraformaldehyde; RT, room temperature; SCI, spinal cord injury; TBS, Tris-buffered saline; WD, Wallerian degeneration.

http://dx.doi.org/10.1016/j.neuroscience.2014.04.041
0306-4522/

reach denervated areas below the injury via a relay circuit (Hunanyan et al., 2013), mediating substantial functional improvements (Courtine et al., 2008; van den Brand et al., 2012). To achieve such rewiring, injured axons have to make new collateral connections to spared neurons, for example spinal interneurons (Fig. 1 A). For the corticospinal tract (CST), the major motor pathway involved in voluntary motor function, such collateral-interneuronal connections have been described (Bareyre et al., 2004; Vavrek et al., 2006). Establishing reliable strategies to enhance CST collateral growth and to promote functional connections with spared neurons is a goal with great potential.

In order to promote new connections between the *injured* CST and spared neurons, an intervention that allows us not only to stimulate but also to direct CST collateral growth toward a meaningful target, for example spared spinal interneurons, would be extremely valuable. A promising candidate tool is neurotrophin-3 (NT-3), which has growth-promoting as well as chemoattractive properties for a variety of axons (Alto et al., 2009), including those that constitute the CST (Zhou et al., 2003). Unfortunately, experimental successes of NT-3-induced CST collateral growth have been limited so far to the lesion environment (Schnell et al., 1994; Hunanyan et al., 2013) and regions caudal to the lesion in the acute phase after SCI (Zhou et al., 2003). In contrast, the use of NT-3 in regions further rostral to a spinal lesion has been unsuccessful at stimulating collateral projections from *injured* CST axons in previous reports by our group (Vavrek et al., 2006; Weishaupt et al., 2013). Stimulating growth from injured CST axons at a distance from the lesion will be necessary so as not to limit NT-3-mediated axonal outgrowth to a small region neighboring the injury site in future therapeutic endeavors. Adding to the reported inconsistencies of NT-3's effects on CST outgrowth are results from NT-3 infusion into denervated areas of the spinal cord after partial pyramidal lesions (Hagg et al., 2005). Hagg and colleagues' data suggest that NT-3 may even inhibit growth of spared CST axons. Together, these reports indicate that NT-3 promotes growth of CST axons only in certain contexts and environments (Table 1).

In an effort to establish what factors may influence a growth-promoting effect of NT-3 on *injured* CST axons, we designed the present study as a proof-of-principle experiment with no intent to treat. First, we address proximity between *injured* CST axons and the source of NT-3 as a potential factor for successful stimulation of collateral growth. We investigate whether long-term expression of NT-3 by viral vectors far rostral to a SCI can stimulate collateral growth from ipsilateral and/or contralateral *injured* CST axons. Second, we examine whether immune stimulation may foster an environment more conducive to NT-3-induced growth of *injured* CST axons, similar to what has been reported for spared CST axons (Chen et al., 2008). To achieve this, we address the question whether a growth-promoting/

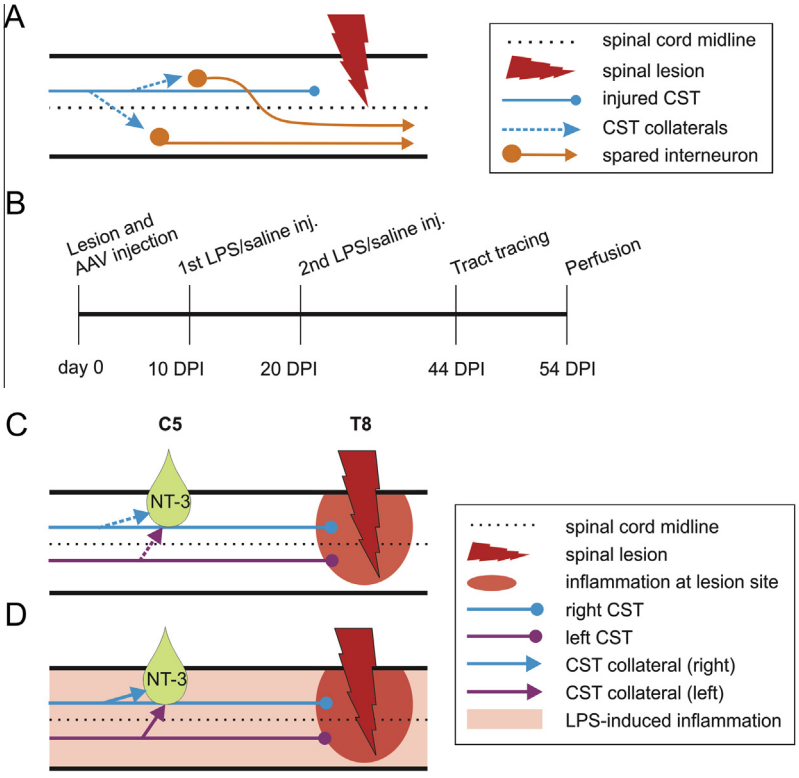

Fig. 1. Rationale and experimental design. (A) Graphic illustrates the concept of rewiring CST output via spared interneurons. (B) Experimental timeline demonstrates sequence of events. DPI = days post injury/AAV injection. Inj. = injection. (C) Schematic illustrates the concept of the non-inflammatory experimental condition. C5 = 5th cervical spinal segment. T8 = 8th thoracic spinal segment. (D) Schematic illustrates the concept of the experimental condition following LPS administration.