

● PERSPECTIVE

Electrical stimulation of cortical neurons promotes oligodendrocyte development and remyelination in the injured spinal cord

Background and early studies: Endogenous tri-potential neural stem cells (NSCs) exist in the adult mammalian central nervous system (CNS). In the spinal cord, NSCs distribute throughout the entire cord, but exist predominately in white matter tracts. The phenotypic fate of these cells in white matter is glial, largely oligodendrocyte, but not neuronal. Proliferating oligodendrocyte progenitor cells (OPCs) contribute to the low-rate turnover of oligodendrocytes and the maintenance of myelination of intact white matter, and possess remyelination potential in response to pathological demyelination events including spinal cord injury (SCI). Myelination in the CNS is a dynamic process that includes the proliferation of OPCs, their differentiation into mature oligodendrocytes, and the ensheathment of axons. Endogenous, neuronal activity-dependent myelin remodeling in the adult CNS is emerging as a mechanism of CNS plasticity. The dependence of OPC proliferation on neuronal electrical activity in neighboring axons was first demonstrated in the developing optic nerve. Unilateral sectioning of rat optic nerves just behind the eyeballs at postnatal day 8 (P8), or unilateral injection of the specific Na^+ channel blocker tetrodotoxin (TTX) at P15 to eliminate the electrical activity of retinal ganglion cell axons, dramatically (86–90%) decreased the number of mitotic OPCs in the optic nerve (Barres and Raff, 1993). Meanwhile, activity-dependent oligodendrocyte development and myelination were also reported in *in vitro* studies. After an intraocular TTX injection, optic nerves were dissected from rats and cultured for several hours. A reduction in the number of cells dual-labelled with the mitotic indicator 5-bromo-2'-deoxyuridine (BrdU) and the OPC marker A2B5 was observed compared to control (Barres and Raff, 1993). In co-cultures of neurons and OPCs established from the cerebral hemispheres of 15-day-old mouse fetuses, administration of TTX significantly attenuated, while a highly selective Na^+ channel activator $\alpha\text{-ScTX}$ enhanced, myelin formation. Consistent with these studies was our finding that decreasing neuronal activity after administration of the GABA-B receptor agonist Baclofen, which is extensively used in clinic for treatment of spasticity and reducing neuropathic pain, decreased the number of proliferating OPCs and regenerative oligodendrocytes in an animal model of SCI (Li and McDonald, unpublished).

Effect of electrical stimulation on oligodendrocyte development: Augmentation of oligodendrocyte development and myelin remodeling by modulation of neuronal activity, which was induced through electrical stimulation, was first shown in *in vitro* models. In a three-compartment chamber equipped with stimulating electrodes, dorsal root ganglion (DRG) neurons and oligodendrocytes were co-cultured in side chambers, while axons growing into the central compartment beneath high-resistant barriers were electrically stimulated at 10 Hz. The electrical stimulation increased oligodendrocyte maturation and myelination through a mechanism requiring activation of

sodium-dependent action potentials (Ishibashi et al., 2006). Recently, other electrical stimulation modalities have been applied in neurodevelopmental studies. For instance, cultured human OPCs stimulated with a moderate intensity (0.3 T) static magnetic field (SMF) for a period of two weeks (two hours/day) displayed enhanced development and myelination capacity in SMF-stimulated oligodendrocytes compared to control (Prasad et al., 2017). Additionally, *in vivo* optogenetic activation of cortical projecting neurons in layer V promoted oligodendrogenesis and increased myelin sheath thickness in subcortical white matter tracts in mice (Gibson et al., 2014).

Functional and cognitive correlates of oligodendrocyte development: Activity-dependent oligodendrocyte development and myelination have been shown to regulate locomotive and cognitive behaviors. Selective neuronal stimulation is correlated with improved motor function of the corresponding limb, and induced neuronal activity modulates motor behaviors in uninjured animals. Myelination by oligodendrocytes appeared necessary for the observed functional improvement, as pharmacological and epigenetic blockage of oligodendrocyte differentiation and myelin change prevented the activity-regulated behavioral improvement (Gibson et al., 2014). Motor skill learning is hindered when OPC differentiation into oligodendrocytes is genetically blocked suggesting important roles of neuronal activity-dependent new-born oligodendrocytes and myelin remodeling for motor learning (McKenzie et al., 2014). Additionally, lack of activity caused by social isolation impairs myelin formation in the prefrontal cortex (Liu et al., 2012).

Activity-dependent oligodendrocyte development in the injured spinal cord: In SCI, demyelination occurs as the result of oligodendrocyte death. Spontaneous OPC proliferation and differentiation, as a regenerative response, occurs even in chronic stages of SCI, but is insufficient to prevent long-term neurological disability. Therefore, augmenting the intrinsic remyelination response by inducing neuronal electrical activity remains an important therapeutic direction for treatment of SCI and other myelin-related disorders. In our studies, we determined if induced neural activity of cortical neurons by electrical stimulation promotes oligodendrocyte development and myelination in the intact and injured spinal cord (Li et al., 2010, 2017). In the intact rat spinal cord, the cortical neurons projecting into the spinal cord were electrically stimulated at the medullary pyramid unilaterally, and then BrdU labeled NSCs were analyzed in the dorsal corticospinal tract (dCST). Phenotypes of proliferating cells were identified by BrdU with a panel of cell markers including NG2, APC-CC1, Nkx2.2, glial fibrillary acidic protein (GFAP), and glucose transporter 1 (Glut-1). Electrical stimulation increased the number of BrdU labeled NSCs, BrdU and NG2 double-labeled OPCs, and BrdU⁺ & APC-CC1⁺ or BrdU⁺ & Nkx2.2⁺ oligodendrocytes in the contralateral dCST. These BrdU⁺ proliferating cells contact, or are in close proximity to, neighboring stimulated axons which were labeled by the anterograde tracer biotinylated dextran amine (BDA). However, the stimulation did not affect the proliferation of astrocytes (BrdU⁺ & GFAP⁺) or endothelial cells (BrdU⁺ & Glut-1⁺) (Li et al., 2010). We also used a mild contusive SCI model at T10 in rats, which demyelinate surviving axons within the dCST, to investigate the effects of induced neuronal activity on oligodendrocyte development, remyelination, and motor function recovery after

SCI (Figures 1 and 2). We induced neuronal activity (4 hours daily for 3 weeks) in the primary motor (M1) cortex using epidural electrodes, which increased the number of proliferating OPCs in the contralateral dCST. Additionally, induced neuronal activity in sub-chronic stages of SCI increased the number of mature oligodendrocytes, and enhanced myelin basic protein (MBP) expression and myelin sheath formation in the dCST. The oligodendroglia regenerative response could be mediated by axon-OPC synapses, the number of which increased after electrical stimulation. Furthermore, induced neuronal activation promoted recovery of hindlimb motor function after SCI. Considering M1 cortical neuronal stimulation mainly elicits activity in CST axons and the main function of pyramidal tract axons is to control the precision and speed of skilled movement, the grid-walk assay was performed to analyze the recovery of fine movement of hind-paws in sub-chronic SCI. Unilateral

stimulation of M1 significantly increased the correct-step rate for the contralateral hind paws by more than 20% compared to control groups. Hind-paw motor function was assessed while the animals were not being stimulated, affirming that this mode of clinically applicable stimulation is therapeutic rather than simply neuroprosthetic (Li et al., 2017). Our findings provide the first *in vivo* demonstration that electrical stimulation of the cortex can selectively enhance the proliferation and differentiation of oligodendrocytes and promote remyelination in the damaged spinal cord.

Potential mechanisms for activity-dependent oligodendrocyte development and myelination: Mechanisms through which neuronal activity modulates oligodendrogenesis and remyelination are under investigation (all associated references for this paragraph are available in the “Discussion” of studies by Li et al. (2010, 2017). This electrical stimulation-caused development of myelinating glial cells is partially regulated through axonal vesicular release of glutamate, and its action at α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on OPCs. Stimulation of cortical neurons increases the numbers of spikes conducted into the dCST and could increase glutamate release from white matter tract axons, thereby providing an excitatory signal to drive glial cell birth and differentiation. In our SCI model, induced neuronal activation also increases axon-OPC synapses in the dCST, a white matter tract that is devoid of neuronal spines, dendrites, and neuronal somata. Axon-OPC synapses, which exhibit similar plasticity compared to neuronal synapses, could mediate the effects of glutamate in OPCs. By acting through AMPA/kainate receptors expressed on OPCs, either axon-released or synapse-released glutamate may increase the downstream phosphorylation of the cAMP response element binding protein (CREB), thereby promoting OPC proliferation and differentiation into mature oligodendrocytes. The vesicular release of glutamate at non-synaptic axon-OPC junctions may also induce myelination through sodium dependent glutamate transporters. Recently, it was reported that signaling through AMPA receptors on OPCs promoted myelination by enhancing oligodendrocyte survival (Kougioumtzidou et al., 2017). Furthermore, in the presence of neuregulin and brain-derived neurotrophic factor (BDNF), NMDA receptors on OPCs may also mediate activity-dependent effects of glutamate on enhancing remyelination. In addition to excitatory neurotransmission, the mitogen of OPCs platelet-derived growth factor (PDGF) also mediated effects on

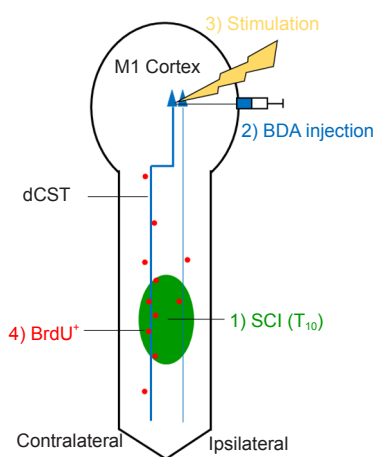


Figure 1 Schematic diagram of our spinal cord injury (SCI) study (Li et al., 2017).

In the rat model, mild (40 kdyn) contusive injury at T₁₀ level demyelinated surviving axons of dorsal corticospinal tract (dCST). The 4 hour daily electrical stimulation (1.0–1.5 mA; 200 Hz) for three weeks (from day 28 to day 49) was applied through epidural electrodes that were implanted over the ipsilateral primary motor (M1) cortex. Meantime, anterograde tracer biotinylated dextran amine (BDA) was injected into M1 cortex to trace contralateral corticospinal tract (CST) axons. 5-Bromo-2'-deoxyuridine (BrdU) labeled progenitor cells and double-labeling of BrdU with a panel of oligodendrocyte lineage markers were counted in dCST. Comparisons were performed between contralateral and ipsilateral dCST, and between stimulated and sham animals.

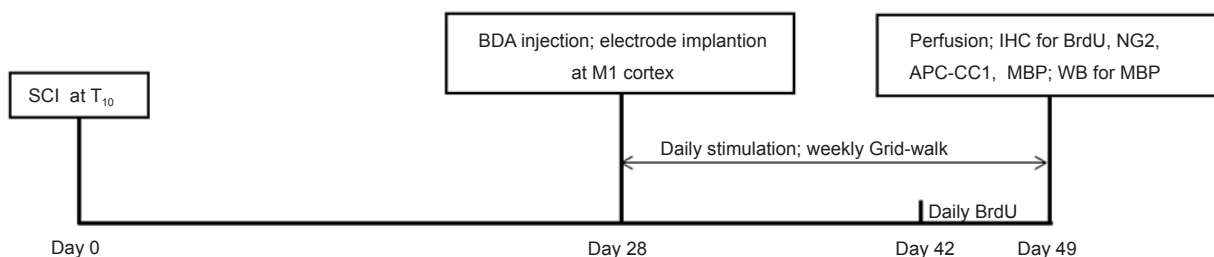


Figure 2 Experimental timeline.

1) Animals received spinal cord injury (SCI) on day 0. 2) Biotinylated dextran amine (BDA) was injected in, and electrodes were implanted over primary motor (M1) cortex on day 28. 3) Daily electrical stimulation and weekly Grid-walk assay were performed from day 28 to day 49. 4) During the last week of the experiment (days 42–49), daily 5-bromo-2'-deoxyuridine (BrdU) (50 mg/kg) was administered intraperitoneally. 5) All animals were sacrificed at day 49 and tissue was processed for immunohistochemistry (IHC) and western blot (WB) assay. MBP: Myelin basic protein.

myelination. Activated axons release PDGF directly, or produce a signal that stimulates PDGF release by nearby astrocytes. The AA homodimeric form of PDGF induces a class of bi-potential glial progenitors (O-2A progenitor cells) to differentiate into OPCs by asymmetric division. PDGF- α receptors are mainly expressed in OPCs that generate myelinating cells in the CNS. The findings of Ishibashi et al. (2006) provide another mechanism by which axonal activity promotes nearby myelination by glial cells. In the co-culture of DRG neurons with oligodendrocytes in multi-compartment chambers, ATP was released from both DRG cell bodies and axonal compartments after electrical axon stimulation. ATP, along with its extracellular breakdown product adenosine, may induce the release of calcium from intracellular stores as a result of action at purinergic and adenosine receptors which are selectively expressed on OPCs, thus leading to the differentiation of OPCs into mature oligodendrocytes. Notably, calcium, as a secondary message in signal transduction and modulation of gene expression, is involved in synthesis of important proteins such as MBP and proteolipid protein (PLP).

Demyelination contributes to functional impairments in neurological disorders including SCI. Restoring myelination is a major therapeutic goal in patients with these conditions. Proliferation and differentiation of endogenous OPCs, and their subsequent maturation into myelinating oligodendrocytes, are critical for significant remyelination after injury. Thus, augmenting this intrinsic remyelination response is a potentially important therapeutic direction. In our studies, we showed that electrical stimulation of motor cortical neurons promotes oligodendrocyte development and myelin formation in the dCST area of intact or injured spinal cord. Further, this neuronal activity-caused remyelination enhances functional recovery of fine movement in hind-paws. Our findings have important implications for devising therapies with the goal of improving outcomes after SCI. Clinically, the activity of extensive descending projection systems can be augmented non-invasively using repetitive transcranial magnetic stimulation (rTMS) or transcranial direct current stimulation (tDCS). In addition, closed loop epidural stimulation devices have been developed for this purpose as well. These stimulation techniques have been used for treatment of SCI patients and have demonstrated potential utility (Belci et al., 2004; Torregrosa and Koppes, 2016). Our findings provide significant explanations on potential mechanisms for these clinical observations, and may also provide a novel strategy for promoting remyelination in multiple sclerosis and other demyelination disorders as well.

Dan C. Li, Qun Li*

Department of Neuroscience, Emory University School of Medicine, Atlanta, GA, USA (Li DC)

Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA (Li Q)

*Correspondence to: Qun Li, M.D., Ph.D., qunli49@gmail.com.

orcid: 0000-0003-1560-2023 (Qun Li)

Accepted: 2017-08-14

doi: 10.4103/1673-5374.217330

How to cite this article: Li DC, Li Q (2017) Electrical stimulation of cortical neurons promotes oligodendrocyte development and remyelination in the injured spinal cord. *Neural Regen Res* 12(10):1613-1615.

Plagiarism check: Checked twice by iThenticate.

Peer review: Externally peer reviewed.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under identical terms.

Open peer review reports:

Reviewer 1: Wei Wu, Indiana University-Purdue University Indianapolis, USA.

Comments to authors: Activity-dependent CNS plasticity using electrical stimulation is an important strategy for promoting voluntary locomotor function recovery. This review focuses on the relationship between electrical stimulation and oligodendrocyte development and remyelination in injured spinal cord. Spinal cord injury causes demyelination, causing function deficit. Electrical stimulation increases the OPC proliferation and differentiation, axon-OPC synapses formation, and myelination, leading to function improvement. This review also introduced the mechanism of activity-dependent oligogenesis and myelination, from other's and their previous work. The manuscript is well written and organized.

Reviewer 2: Ping K. Yip, Queen Mary University of London, UK.

Comments to authors: The review discusses about how stimulating the neurons, in particular the corticospinal neurons can promote oligodendrocyte development and promote remyelination after spinal cord injury. It is based on recent studies using optogenetic and/or direct electrical stimulation techniques. This review will be of interest to researchers in neuroinjury, since this novel concept could open up a new field of therapy.

References

- Barres BA, Raff MC (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 361:258-260.
- Belci M, Catley M, Husain M, Frankel HL, Davey NJ (2004) Magnetic brain stimulation can improve clinical outcome in incomplete spinal cord injured patients. *Spinal Cord* 42:417-419.
- Gibson EM, Purger D, Mount CW, Goldstein AK, Lin GL, Wood LS, Inema I, Miller SE, Bieri G, Zuchero JB, Barres BA, Woo PJ, Vogel H, Monje M (2014) Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. *Science* 344:1252-304.
- Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD (2006) Astrocytes promote myelination in response to electrical impulses. *Neuron* 49:823-832.
- Kougoumtzidou E, Shimizu T, Hamilton NB, Tohyama K, Sprengel R, Monyer H, Attwell D, Richardson WD (2017) Signalling through AMPA receptors on oligodendrocyte precursors promotes myelination by enhancing oligodendrocyte survival. *Elife* 6:e28080.
- Li Q, Brus-Ramer M, Martin JH, McDonald JW (2010) Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. *Neurosci Lett* 479:128-133.
- Li Q, Houdayer T, Liu S, Belegu V (2017) Induced neural activity promotes an oligodendroglia regenerative response in the injured spinal cord and improves motor function after spinal cord injury. *J Neurotrauma* doi: 10.1089/neu.2016.4913.
- Liu J, Dietz K, DeLoyht JM, Pedre X, Kelkar D, Kaur J, Vialou V, Lobo MK, Dietz DM, Nestler EJ, Dupree J, Casaccia P (2012) Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nat Neurosci* 15:1621-1623.
- McKenzie IA, Ohayon D, Li H, de Faria JP, Emery B, Tohyama K, Richardson WD (2014) Motor skill learning requires active central myelination. *Science* 346:318-322.
- Prasad A, Teh DBL, Blasiak A, Chai C, Wu Y, Gharibani PM, Yang IH, Phan TT, Lim KL, Yang H, Liu X, All AH (2017) Static magnetic field stimulation enhances oligodendrocyte differentiation and secretion of neurotrophic factors. *Sci Rep* 7:6743.
- Torregrosa T, Koppes RA (2016) Bioelectric medicine and devices for the treatment of spinal cord injury. *Cells Tissues Organs* 202:6-22.