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Restoring axonal localization and transport to promote repair within the injured CNS; a critical step in CNS regeneration

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Corresponding Author:	Melissa Renee Andrews, PhD University of St Andrews St Andrews, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of St Andrews
Corresponding Author's Secondary Institution:	University of Southampton
First Author:	Lindsey H Forbes, BSc
First Author Secondary Information:	
Order of Authors:	Lindsey H Forbes, BSc
	Melissa Renee Andrews, PhD
Order of Authors Secondary Information:	
Suggested Reviewers:	Patricia Phelps, PhD Professor, University of California Los Angeles pphelps@physci.ucla.edu Professor Phelps has vast experience with many aspects of CNS regeneration from cell transplantation to neuronal signalling and development.
	Rejji Kuruvilla, PhD Associate Professor, Johns Hopkins University rkuruvilla@jhu.edu Dr. Kuruvilla is a leading expert in neuronal trafficking and signalling.

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Perspective article

As neurons mature, proteins that were once key regulators of axon guidance and elongation are downregulated resulting in a reduced capacity for axonal repair after injury. By recapitulating neuronal expression of growth-promoting proteins, such as integrins, transmembrane receptors involved in mediating cell-cell and cell-matrix interactions, neurite outgrowth and axon regeneration can be significantly enhanced. The $\alpha 9\beta 1$ integrin heterodimer, for example, is highly expressed during CNS development aiding growth cone formation and axonal elongation but is downregulated in mature CNS axons. It binds the main extracellular matrix (ECM) glycoprotein of the CNS, tenascin-C, which is highly upregulated after injury and has been a recent target of axonal regeneration research (Andrews *et al*., 2009; Cheah *et al*., 2016). Despite these promising findings, recent data reveals region-specific and age-specific differences exist which result in variations in integrin trafficking into the axonal compartment (Andrews *et al.,* 2016; Franssen *et al.,* 2015) creating yet another hurdle for regeneration.

In newly published work, Andrews *et al.* highlight differences in integrin localisation in distinct neuronal subtypes, following viral vector-based expression (Andrews *et al.,* 2016). Their findings show that in mature corticospinal tract (CST) and rubrospinal tract (RST) axons, exogenously-expressed integrins are not localised or transported into the axonal compartment, remaining instead in the somatodendritic compartment (Andrews *et al.,* 2016). On the other hand, exogenously-expressed integrins in early postnatal CST neurons are localised within the axonal compartment of developing CST axons. Furthermore, exogenously-expressed integrins successfully localise in mature optic nerve axons as well as mature dorsal root axons following intravitreal or dorsal root ganglia injections, respectively (Andrews *et al.,* 2016). These data establish a differential ability of transmembrane receptors to localise in distinct areas of the nervous system. Previous research in cultured cortical neurons suggests this is due to the axon initial segment acting as a filter and barrier for integrin entry into the axonal compartment (Franssen *et al.,* 2015). Therefore, region-specific and age-specific axon transport mechanisms are likely to play a role in modulating intrinsic CNS repair. In this review we consider the difficulties of enhancing intrinsic-mediated repair of neurons including the delivery of growth-promoting proteins necessary for regrowth of adult axons in addition to considering extrinsic targets and therapies for enhancing CNS repair.

A number of experimental avenues have been pursued with the hope of finding a robust treatment to promote axonal regeneration within the CNS (see Figure 1). These include modification of ECM components, such as chondroitin sulphate proteoglycans (CSPGs), to stimulate neuronal plasticity, and removal of inhibitory proteins, such as Nogo, to alleviate degradation and apoptotic pathways, cellular replacement therapies, and application of biomaterials to stabilise lesion architecture. In addition, a number of signalling pathways involved in maintaining axonal growth, guidance, trafficking, receptor turnover and apoptosis, including neurotrophic factors and other growth-promoting receptors such as integrins, have provided several targets for viral vector-based gene therapy to promote intrinsic regeneration following axonal injury.

Current research has focused on targeting and reinstating regenerative signalling pathways associated with neurite outgrowth and survival, such as tropomyosin-related kinase (Trk) receptors, insulin-like growth factor 1 (IGF-I) receptors and integrin receptors following injury. Advancements in viral vector research have made gene therapy a commonly used experimental tool to study regenerative signalling pathways together with axonal repair. Research suggests expression levels of endogenous levels of TrkB in injured CST axons are not sufficient to promote neurite outgrowth and repair (Lu *et al.,* 2001), however forced expression of TrkB using lentiviral vectors following CST lesioning has resulted in enhanced regeneration (Hollis *et al.,* 2009). In that study, however, Hollis *et al* demonstrated that exogenously expressed TrkB was only localised as far as the proximal part of CST axons (subcortical white matter) suggesting that intrinsic transport mechanisms within these axons are compromised in the adult nervous system (Hollis *et al.,* 2009). Axonal transport and localisation of growth-promoting molecules involved in neuronal signalling are necessary and essential for the development of the nervous system as well as for axon growth and homeostasis. Long-distance transport is driven by kinesin and dynein motors which are regulated by Rab GTPases, kinases and a number of scaffolding proteins (Maday *et al.,* 2014). Failure of axon transport can have a detrimental effect on axon growth and function and has been linked to disease and injury, such as in motor neuron disease (MND), Alzheimer's disease (AD), and spinal cord injury (SCI). Research now suggests that axon transport and localisation in certain neuronal regions is inherently downregulated as the normal uninjured CNS matures (Andrews *et al.,* 2016). Furthermore, evidence suggests growth-promoting receptors are excluded from mature CST axons, including tropomyosin-

related kinase (Trk) receptor TrkB, the receptor for brain-derived neurotrophic factor (BDNF) (Lu *et al.*, 2001) and integrins (Franssen *at al.*, 2015; Andrews *et al.*, 2016).

Not only is necessary to consider localisation of virally-expressed proteins in neurons, viral vectors themselves may have limited temporal expression, although more treatments currently utilise standard adeno-associated virus or lentivirus which have been shown to have long-term sustained expression. The literature suggests however that by combining gene therapy with biomaterials, better long-term expression may result (Thomas *et al.,* 2014). Scaffold bridges made from poly(lactide-co-glycolide) (PLG) have been used to deliver lentiviral vectors carrying sonic hedgehog (Shh) and Neurotrophin-3 (NT3) to T9/T10 region of mice spinal cords following lateral hemisection (Thomas *et al.,* 2014). Results demonstrate that expression of both proteins was sustained at the injury site, promoting axonal regrowth through the bridge architecture as well as facilitating recruitment of endogenous oligodendrocyte precursors and Schwann cells. This also resulted in increased axonal myelination 8 weeks post-transplant. Use of biodegradable bridges that contain a channel network for axonal guidance allows for localised delivery of multiple transgenes to the injury site, however, delivery of scaffolds can be invasive and does not overcome the inhibitory milieu created after injury. In addition, there are cases where sustained expression of virally-expressed proteins is unnecessary. In these cases, tamoxifen-inducible systems have been shown to be effective for regulating expression.

Restoring intracellular signalling pathways is one approach to tackle the failure in axon repair however we can also target removal of inhibitory proteins. The lesion environment consists of proteins that are known to prevent axonal regeneration including CSPGs and myelin-derived inhibitory proteins, such as Nogo, myelin associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp). Removing or inactivating inhibitory molecules by blocking their action has shown efficacy in promoting regeneration. These strategies include application of the bacterial enzyme, chondroitinase ABC (ChABC) to digest glycosaminoglycan side chains of CSPGs and monoclonal antibodies against Nogo-A to inhibit Nogo activity. Research indicates a combination of treatments may provide the best recovery after axonal injury. Indeed, administration of anti-Nogo-A antibodies by intrathecal infusion, and ChABC by intraspinal injections and intrathecal infusion, alongside rehabilitation was shown to promote axonal regeneration and functional recovery following SCI, more than either treatment alone (Zhao *et al.,* 2013). Unfortunately, administration of

both molecules is invasive with ChABC requiring multiple applications to maintain the level of enzymatic activity required for axonal regeneration and sprouting.

Bridging SCI lesion sites with cellular replacement therapies including oligodendrocytes and Schwann cells to encourage remyelination, as well as astrocytes and neural stem cells to replace lost cells is another avenue that has resulted in successful regeneration following damage. Research suggests however that an injured environment can affect transplanted cell survival, engraftment, migration, proliferation and the availability of differentiation and growth promoting cues (Sontag *et al.,* 2014). This study suggests that cell transplant treatments need to be combined with tandem therapies to utilise ECM environment to support and promote the grafted cells *in vivo*. Indeed, combining a NSC graft with ChABC enzyme to degrade CSPGs can promote graft survival, regeneration, axonal plasticity and functional recovery in a chronic animal model of SCI (Karimi-Abdolrezaee *et al.,* 2010).

Research indicates there are age-associated changed within CNS axonal transport, including a decrease in anterograde trafficking (Milde *et al*., 2015). Interestingly this decrease in transport in mature neurons can be partially reversed within peripheral nerves (Milde *et al.,* 2015). Notably age-associated changes in axonal transport are also region specific, with differing transport rates in different neuronal areas, such as optic nerve, sciatic nerve and areas of the hippocampus (Milde *et al.,* 2015). This brings us back to the current study. Andrews and colleagues showed developing motor and sensory neurons have the ability to traffic growth-promoting integrins within the axonal compartment but only sensory neurons of the PNS, specifically neurons of the DRGs and retinol ganglion cells (RGCs), retain this ability as they mature. In the case of mature CST and RST axons, integrins are excluded and instead are retained within the somatodendritic compartment. This work confirms Milde and colleagues' observations that there are region-specific differences in axonal transport, but also indicates there are age-related changes. This suggests that treatment for targeting CNS injury would not be the same for every injury and would likely have to be modified to target injuries in different neuronal regions.

Together this work emphasises promoting transport and expression of growth-promoting proteins can prompt axon repair however, a better awareness of the mechanisms required for targeting delivery into axons of the CNS remains. The answer to this problem may include a combination of several approaches such as biomaterials and nanoparticles, cell replacement,

modification of ECM, and gene therapy. Viral vectors allow for long-term gene expression however there is a risk of insertional mutagenesis which keeps these techniques currently out of clinical trials. As researchers we need to consider how these age-related and site-specific changes in axon transport can tailor our approaches for repair. This includes CNS delivery methods as research indicates different viral vectors transduce DRGs differently (Mason *et al.*, 2010). The question is not, do we need to re-establish axon transport, but rather how can it be done in a controlled site-specific, age-specific manner. Within this review we have discussed how reinstating age-associated changes in signalling pathways can lead to enhanced axon transport and hence better repair after injury, but when it comes to CNS injury this is only half the battle. A combination of treatments that target the milieu of injury-induced proteins to alleviate inhibitory signalling alongside region-specific modification of target signalling pathways together with physical rehabilitation is likely to offer the best hope for robust functional recovery after CNS injury.

Figure 1. Current approaches for promoting axonal repair following CNS injury. Schematic diagram highlighting an overview of axonal injury and degradation at the lesion site along with current approaches to enhance repair. After CNS injury, an inhibitory lesion site is created surrounded by reactive astrocytes and a number of growth-inhibiting proteins including CSPGs and Nogo. Transport within axons is mediated by both kinesin and dynein motors and allow cargo-carrying vesicles to travel in both anterograde and retrograde directions. However, within the mature CNS many growth-promoting proteins, such as TrkB, are trafficked back to cell body in the retrograde direction resulting in a reduced ability of axons to regenerate after injury. **(1)** Modification of the ECM using ChABC enzyme to degrade CSPGs or anti-Nogo A antibodies to inhibit Nogo activity can reduce the action and signalling of inhibitory components of the injury site to promote axonal regeneration after injury. **(2)** Biomaterials can be used to stabilise the lesion site as well as provide guidance channels for axon regrowth. Modified scaffolds have been used to deliver multiple neurotrophic factors to lesion site aiding axon repair. **(3)** Alternatively, delivery of viral vectors that are tissue or cell-specific can carry DNA encoding for growth-promoting proteins e.g. TrkB to the lesion site. Using gene therapy to reinstate proteins involved in axon

growth and guidance can prompt growth-promoting signalling pathways. **(4)** Cell therapy involves replacing lost or damaged cells at the lesion site. These can include OECs to promote remyelination of injured axons or transplantation of NSCs to replace injured neurons/axons.

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Figure

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