

# Regeneration of Corticospinal Axons into Neural Progenitor Cell Grafts After Spinal Cord Injury

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**ABSTRACT:** Spinal cord injuries leave patients with lifelong paralysis. To date, there are no therapies that promote the critical step required for the recovery of voluntary motor function: corticospinal axon regeneration. Spinal cord-derived neural progenitor cell (NPC) grafts integrate into the injured host spinal cord, enable robust corticospinal axon regeneration, and restore forelimb function following spinal cord injury in rodents. Consequently, engineered stem cell differentiation and transplantation techniques harbor promising potential for the design and implementation of therapies promoting corticospinal axon regeneration. However, in order to optimize the outcome of clinical trials, it is critical to fully understand the cellular and molecular mechanisms underlying this regeneration. Our recent study highlights the unexpected intrinsic potential of corticospinal neurons to regenerate and allows us to investigate new hypotheses exploiting this newly discovered potential.

**KEYWORDS:** Neural progenitor cells, corticospinal tract, transcriptomics, axon regeneration

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The corticospinal tract (CST) transmits movement signals from the motor cortex to the spinal cord. Since the CST is the predominant nerve fiber tract for voluntary motor function in humans, traumatic injuries to the CST leave patients with life long paralysis. Historically the CST has been very refractory to regenerating into a spinal cord lesion site; in the absence of a growth-supportive NPC graft, regeneration has only been reported through thin residual astroglial remnants<sup>1</sup> (Figure 1A). Even with the supply of a cellular graft such as mesenchymal stem cells (MSCs) cells, no CST growth is supported (Figure 1B). These findings were interpreted to suggest that corticospinal neurons lack the *intrinsic* transcriptomic and molecular mechanisms needed to adequately respond to injury and regenerate. This stands in stark contrast to neurons of the peripheral nervous system, which undergo extensive transcriptional changes in response to injury to activate the expression of Regeneration Associate Genes (RAGs). RAGs, such as the growth associated protein 43 (GAP-43), that are associated with successful periphery axon regeneration over long distances, which ultimately reconnect to their targets and lead to functional recovery.<sup>2</sup>

We reported in 2016 that corticospinal axons regenerate extensively into embryonic spinal cord-derived NPC grafts placed into a spinal cord lesion site,<sup>4</sup> indicating that when an appropriate target is provided, regenerative growth can occur (Figure 1C). Since then, we demonstrated that CST regeneration is triggered by embryonic spinal cord derived NPCs in mouse,<sup>3,5</sup> rat,<sup>4,6</sup> and non-human primate<sup>7</sup> models of spinal cord injury. These results could indicate that extrinsic signals (NPC-graft),<sup>8,9</sup> rather than intrinsic signals, trigger CST regeneration. To test this hypothesis, we investigated the transcriptomic

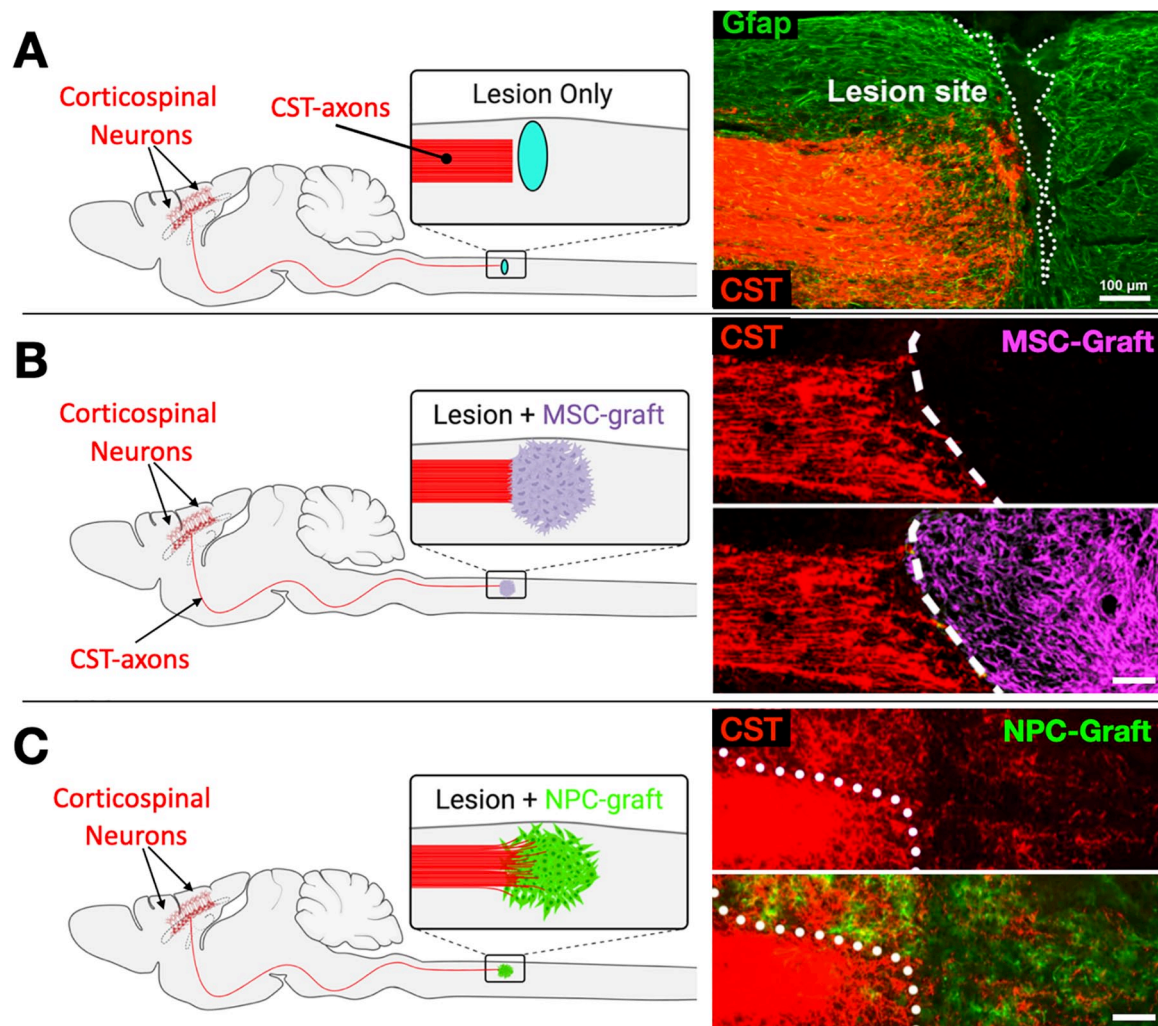
changes in corticospinal neurons in response to lesion alone (non-regenerating CST, Figure 1A) and during NPC-graft supported regeneration (regenerating CST, Figure 1C). We compared transcriptomic profiles of corticospinal neurons in both conditions to an intact animal to be able to differentiate the injury signal from the regeneration signal.<sup>3</sup>

## Injury-Induced Transcriptional Changes are Essential for CST Regeneration

As expected, injury to the CST axons drastically altered gene expression in corticospinal neurons (~4000 significantly differently expressed genes (DEG) at 10 days post lesion using a significance criterion of False Discovery Rate  $\leq$  10%). Over the time course of 3 weeks, these changes diminished almost completely and gene expression reverted back to the intact state. Interestingly, the regenerating cohort that received a NPC-graft displayed a similar pattern of gene expression at 10 days post lesion, indicating that the transcriptomic profile at this early timepoint is dominated by the injury signal. Notably, in the presence of a graft, this pro-regenerative transcriptional profile did not diminish but was sustained after 3 weeks post injury. This demonstrated that intrinsic transcriptional mechanisms necessary for regeneration of corticospinal axons are activated by injury alone. This finding fundamentally changes our view on the intrinsic regenerative capacity of the corticospinal system.

We have further shown that this intrinsic change in gene expression represented a shift to a more immature transcriptional state of the corticospinal neuron, allowing for the recapitulation of the temporal progression of distinct aspects of CST development. Gene expression starts with the activation





**Figure 1.** NPC-grafts support corticospinal tract (CST) axon regeneration. Sagittal spinal cord sections (40  $\mu\text{m}$ ) from non-regenerating conditions: (A) lesion without graft,<sup>3</sup> (B) lesion + mesenchymal stem cell graft (MSC-Graft),<sup>4</sup> and (C) lesion + NPC graft. Sections were labeled for corticospinal tract (CST) axons and in (A) the glial scar marker (GFAP). Drawings were Created with BioRender.com. Lesion margins are indicated with white striped/dotted lines. Scale bars: 100  $\mu\text{m}$ .

of cell survival and cellular growth genes, followed by the activation of axon regeneration and axon guidance pathways, to finally genes involved in synapse formation and synaptic plasticity. Hence, within a 3-week time frame the anatomical and transcriptional changes mimicked developmental processes of corticospinal neurons. And these changes seem to be necessary for successful axon regeneration.

### Huntingtin is Essential for CST Regeneration

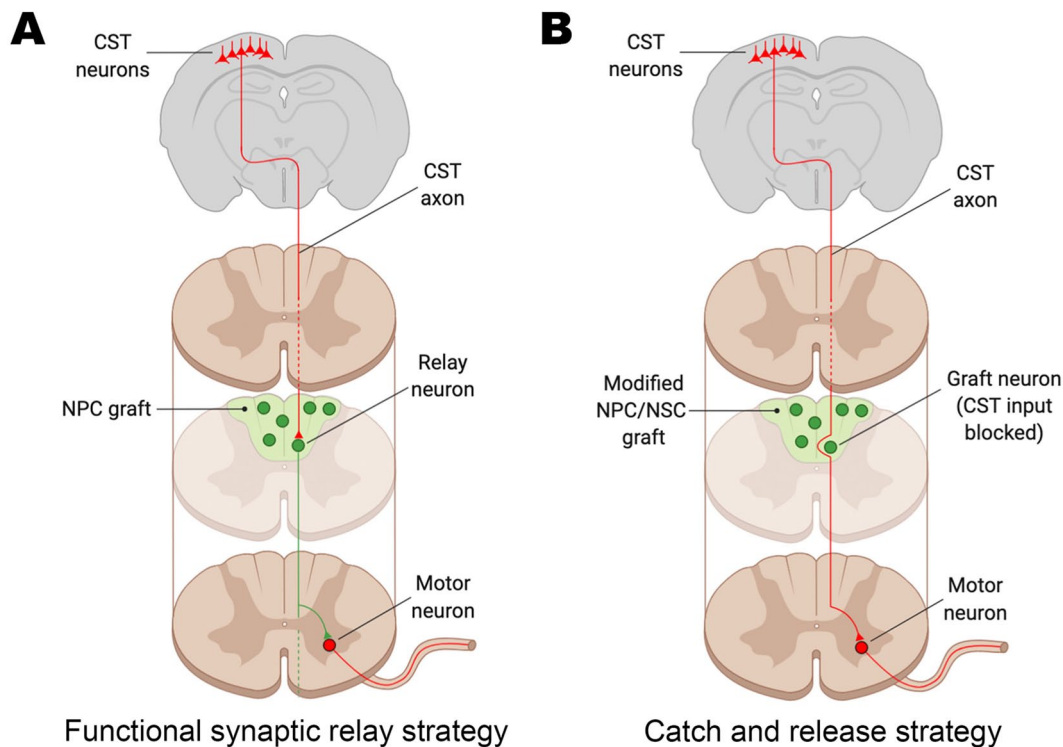
We utilized bioinformatic datamining to identify transcriptional regulators that modulate the regenerative response and showed a critical role for huntingtin (HTT) in corticospinal axon regeneration. It is interesting that HTT mRNA expression was not altered during CST injury and regeneration, but it was identified as a central hub of a network of differentially expressed genes that constituted the regeneration state. The identification of HTT as a potential candidate involved in CST regeneration was based purely on unbiased bioinformatic analysis. Indeed, HTT knockout resulted in a significant reduction of corticospinal regeneration. Whether HTT

overexpression might improve CST regeneration will be investigated in future studies.

### CST Axon Regeneration Strategies Utilizing NPC/NSC Grafts

Hypothetically, two distinct mechanisms might be associated with NPC grafts to promote functional recovery following spinal cord injury: (1) The *functional synaptic relay* strategy and (2) the *catch and release* strategy.

In the functional synaptic relay strategy (Figure 2A), NPC-grafts receive direct corticospinal synaptic inputs that do not extend beyond the graft. The neural stem cells, in turn, extend axons into the caudal host white matter, eventually (through mono- or poly-synaptic relays) innervating motor neurons in the ventral horn of the distal host spinal cord. This would form novel relays across the injury site by “splicing the circuit.” We hypothesize that this relay formation might be further refined and optimized by rehabilitative training and possibly by electrical stimulation to rewire interrupted circuitry and restore lost motor function.<sup>10</sup>



**Figure 2.** CST axon regeneration strategies utilizing NPC/NSC grafts. Schematic overview of possible scenarios of NPC-graft triggered CST regeneration: (A) Functional synaptic relay strategy and (B) Catch and release strategy. NPC-graft derived neurons send out axons into host white and gray matter and reconnect with host motor neurons. In (A) grafted neurons can receive synaptic inputs from regenerating CST axons, while in (B), NPCs/NSCs have been genetically altered to not receive synaptic inputs. Drawings were Created with BioRender.com.

In an alternative hypothesis, the catch and release strategy, NPC grafts might trigger corticospinal axon regeneration into and entirely beyond the graft. We have observed corticospinal regeneration into and beyond grafts over short gap lengths of 1 mm, but we have not observed regeneration of corticospinal axons over greater distances.<sup>4</sup> To promote longer-distance regeneration, we hypothesize that synaptic connectivity with grafted neurons would need to be inhibited. This will be tested in future studies.

It might also be possible to promote host corticospinal regeneration across a lesion site by identifying specific molecules that are presented by the NPC grafts to the injured CST axons and that trigger regeneration. We have shown in 2016 that these molecules are not secreted by the NPC grafts since direct contact with the CST axons is necessary for regeneration.<sup>4</sup> Accordingly, we will apply multi-omic screens of the grafted NPCs as well as the regenerating CST axons to identify the extracellular proteins, molecules and lipids on the NPCs that trigger CST growth. Once the key molecular mechanisms that are necessary to stimulate CST regeneration are identified, cellular or molecular grafts can be engineered, expressing or carrying these growth promoting biomolecules.

### Summary

Injuries to the corticospinal tract following spinal cord injury leave patients with lifelong paralysis. We have demonstrated that corticospinal neurons activate intrinsic regenerative programs in response to injury alone. Successful regeneration is

dependent on the sustained activity of these regenerative transcriptomic profiles throughout the regenerative process.<sup>3</sup> This can be achieved via the application of neural progenitor or neural stem cell grafts into the lesion site. The complete molecular and cellular mechanisms that promote the extension of the active transcriptomic signature remain to be identified. Progenitor cell grafts provide an important tool to study the extrinsic and intrinsic mechanisms of successful CST regeneration and will be instrumental in the development of therapies promoting functional recovery following spinal cord injury.

### Author Contributions

Both authors wrote the manuscript.

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