

Reaction to Injury & Regeneration

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The adult mammalian central nervous system has the lowest regenerative capacity of all organ systems.

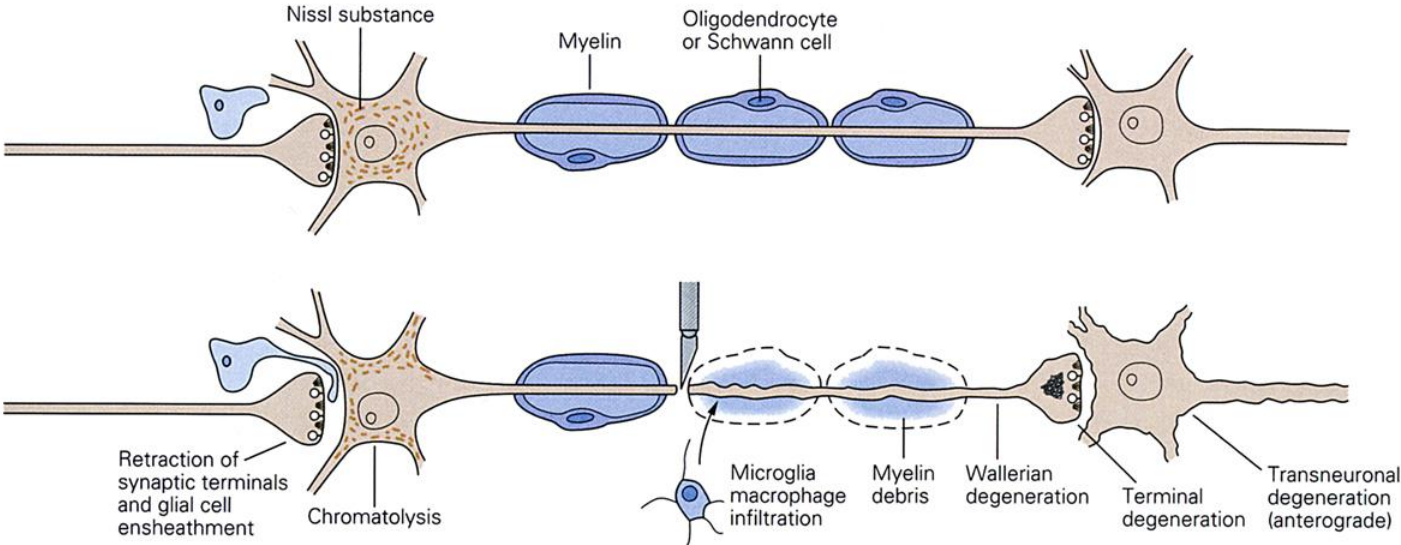
neuron loss



axon damage



Reaction to Axotomy



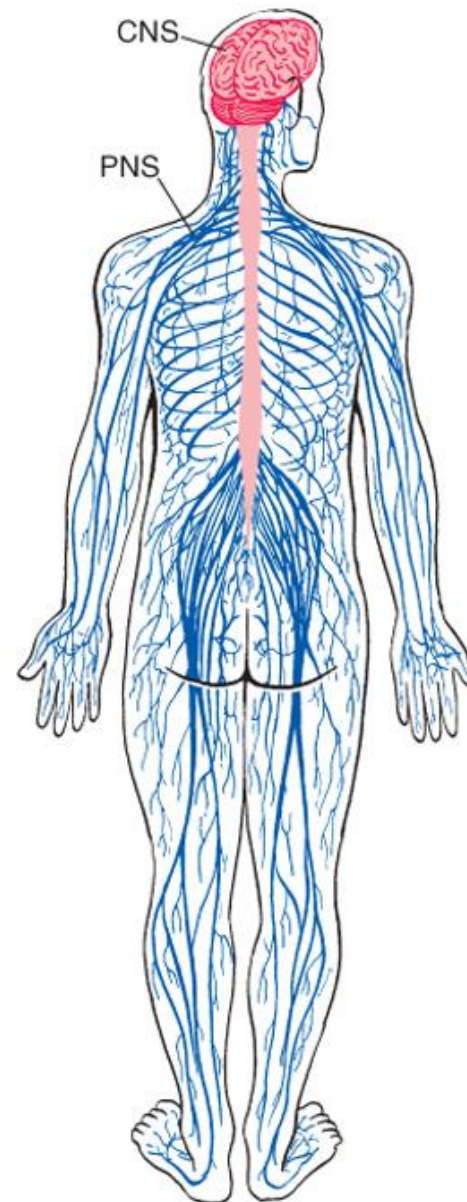
- Function distal to the axon cut is lost. (immediate)

Reaction to Axotomy

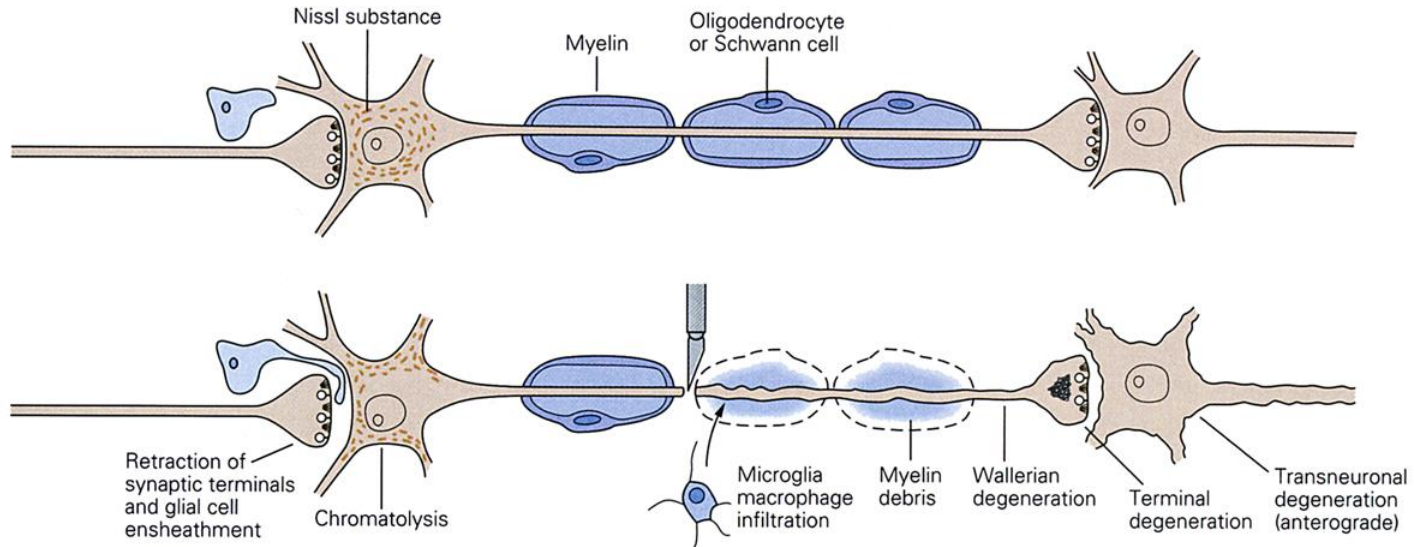
- Spinal cord injury results in an immediate loss of sensation and muscle paralysis below the level of the injury.

Spinal cord injury can be partial or complete, and the sensory/motor loss depends on which axons are injured.

- Peripheral nerve injury results in an immediate loss of sensation and muscle paralysis in the areas served by the injured nerve distal to the site of injury.

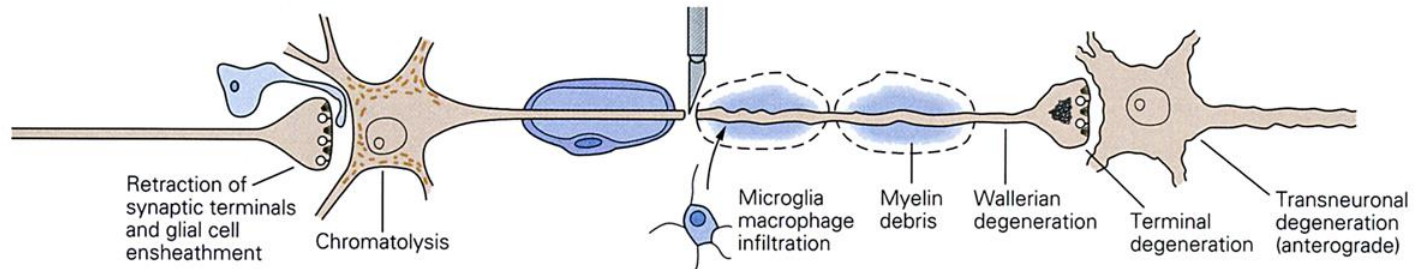


Reaction to Axotomy



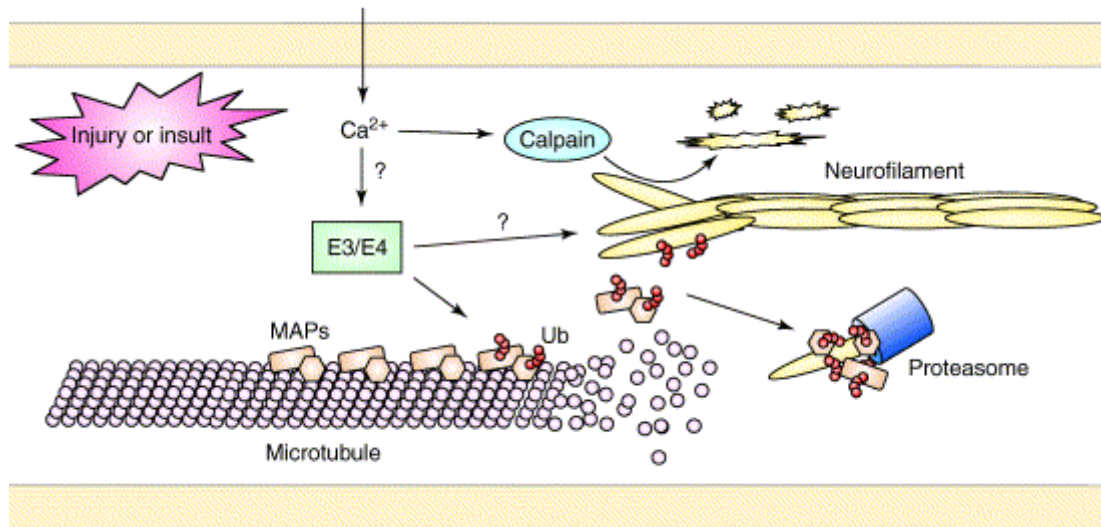
- K^+ leaks out of the cell and Na^+/Ca^{++} leak into the cell. (seconds)
- Proximal and distal segments of the axon reseal slightly away from the cut ends. (~2 hrs)
- Subsequent anterograde & retrograde effects ...

Anterograde Effects (Wallerian Degeneration)



- Axon swells. (within 12 hrs)
- Axolemma and mitochondria begin to fragment. (within 3 days)
- Myelin not associated with a viable axon begins to fragment. (within 1 wk)
- Astrocytes or Schwann cells proliferate (within 1 wk), which can continue for over a month. Results in >10x the original number of cells.
- Microglia (or macrophages in the PNS) invade the area.
- Glia and microglia phagocytize debris. (1 month in PNS; >3 months in CNS)

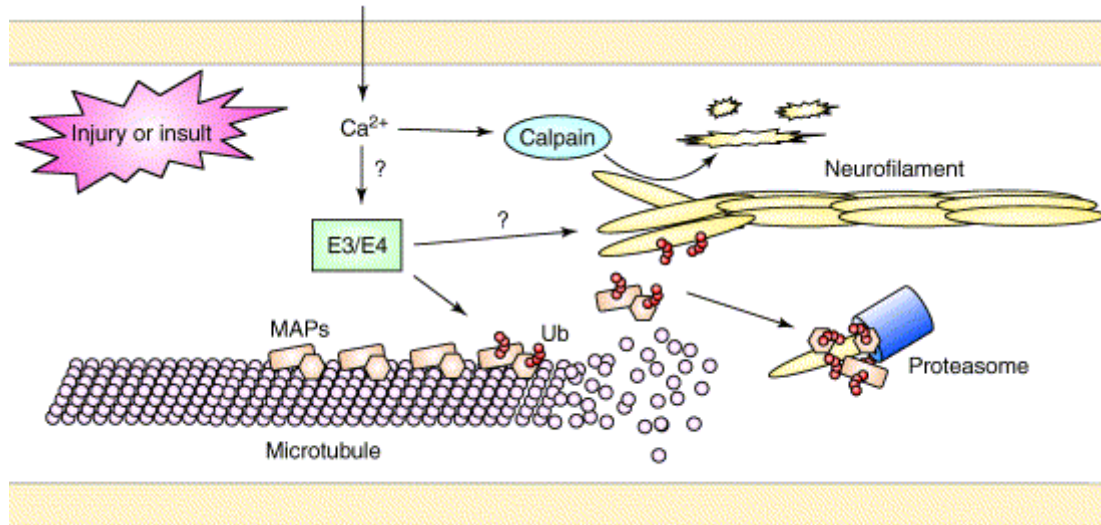
Anterograde Effects (Wallerian Degeneration)



Degradation of the axon involves self proteolysis:

- In the 'Wallerian degeneration slow' (*Wld^s*) mouse mutation, the distal portion of severed axons are slow to degenerate; the dominant mutation involves a ubiquitin regulatory enzyme.
- Inhibitors of the ubiquitin-proteasome system slow Wallerian degeneration.
- Several enzymes are involved, including ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligases (E3 & E4).

Anterograde Effects (Wallerian Degeneration)

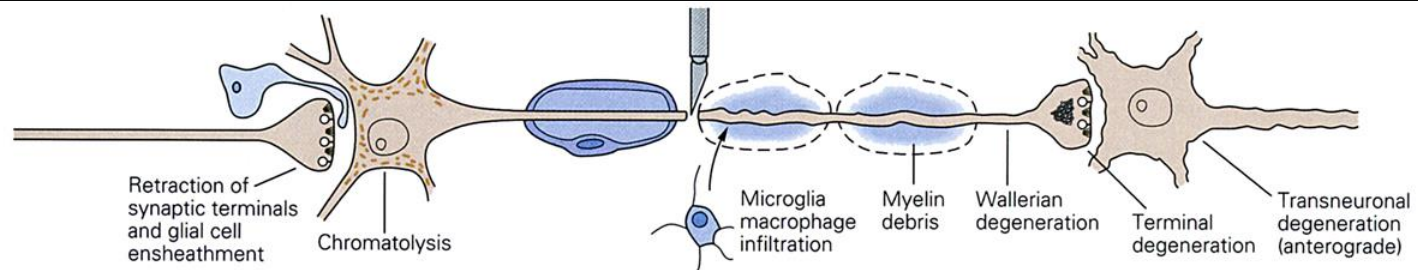


Degradation of the axon involves self proteolysis:

- Nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) is a labile axon survival factor that appears to counter Wlds in healthy axons.

(Gilley J and Coleman MP, 2010)

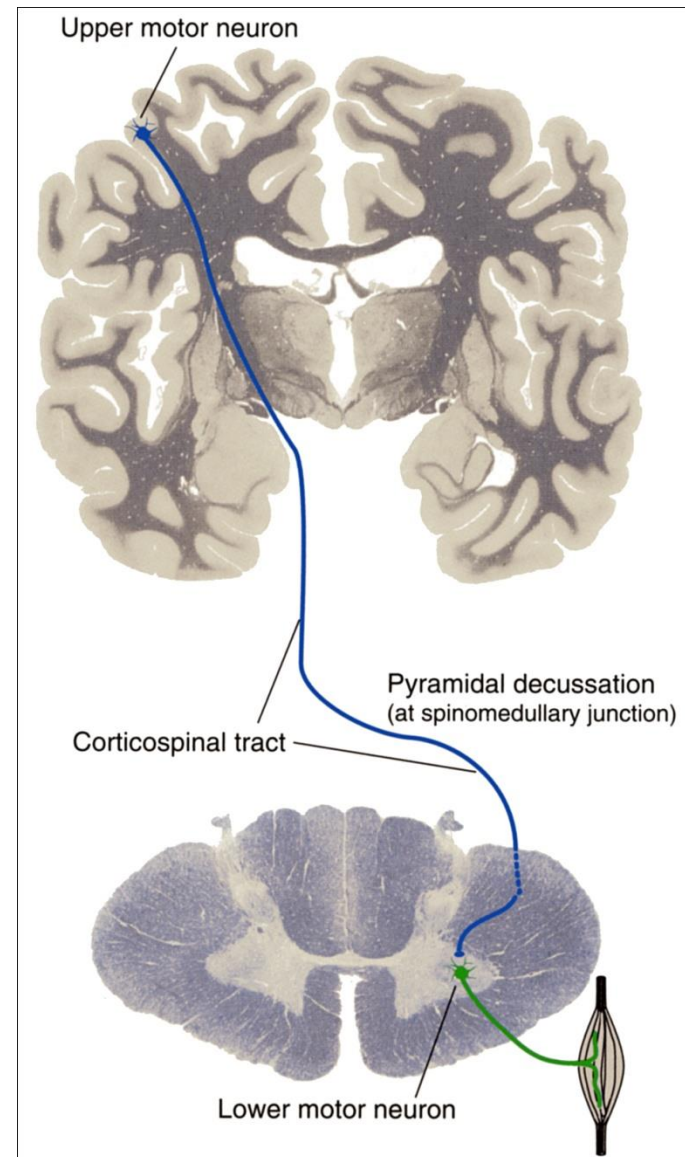
Transneuronal Effects



- In the absence of presynaptic innervation, some neurons die; effect varies depending on the presence of other connections and age.
- Muscle exhibits changes with loss of neuronal innervation:
 - As injured motor axons degenerate, action potentials are spontaneously initiated causing fasciculations (visible contractions of the muscle).
 - Once the axon has degenerated, muscle undergoes denervation atrophy.

Effects of Upper vrs. Lower Motor Neuron Loss on Muscle

Owing to the loss of reflex activity and of 'trophic support' from the nerve, denervation atrophy is more rapid and more pronounced than the atrophy associated with disuse or with loss of innervation to the spinal motor neuron from upper motor neurons.



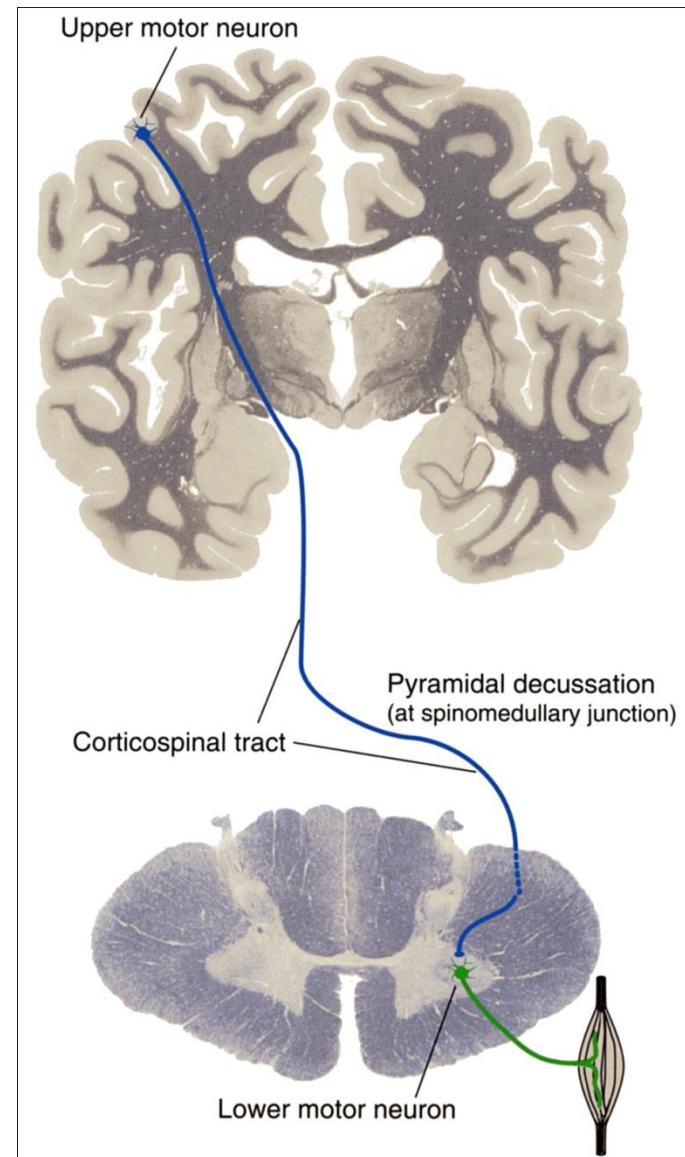
Effects of Upper vrs. Lower Motor Neuron Loss on Muscle

upper motor neuron loss (CNS):

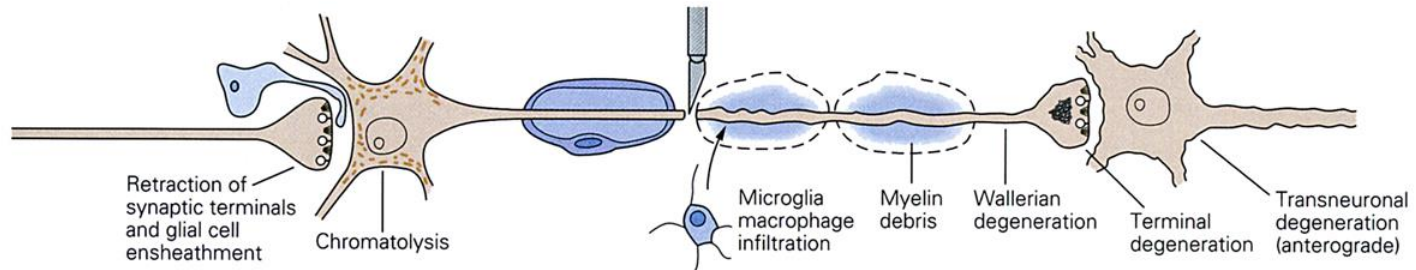
- hyper-reflexive
- rigidity
- spasticity
- slow & mild atrophy

lower motor neuron loss (PNS):

- flaccid paralysis
- fasciculations (twitching)
- rapid & severe atrophy

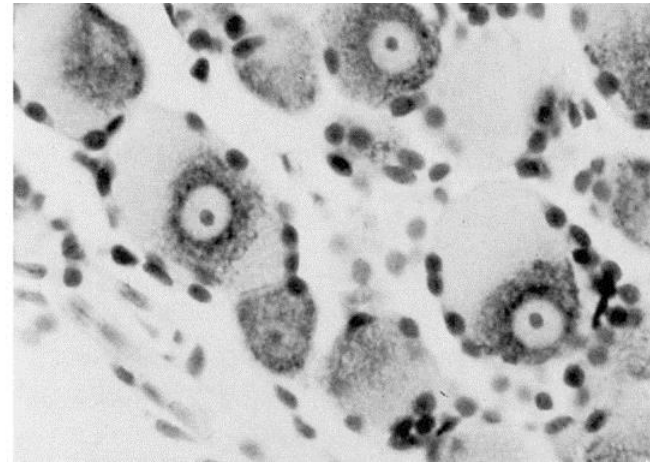
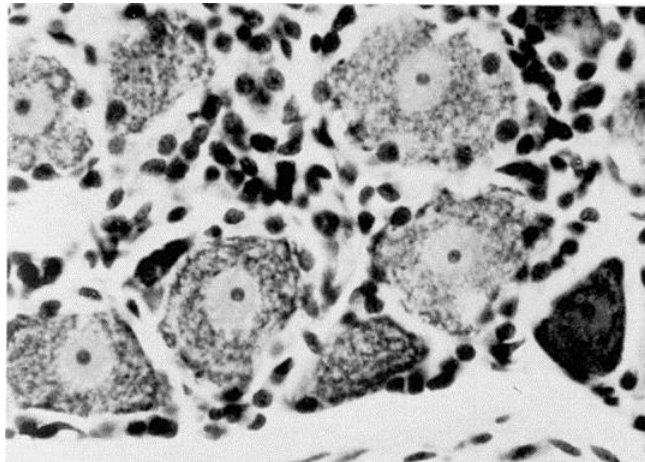


Retrograde Response to Axotomy (Axon Reaction)



- Loss of neurotrophin supply from the target initiates changes in the soma. (beginning within 2-3 days depending on distance to soma)
- Presynaptic terminals pull off the neuron. (within 3 days)

Retrograde Response to Axotomy (Axon Reaction)



- Soma undergoes chromatolysis: loss of rER, soma swells, nucleus moves eccentric, nucleolus enlarges. (within 3 days)
- Cell down-regulates expression of molecules required for neuronal communication (e.g. neuro-transmitters) and up-regulates synthesis of molecules needed for axon growth (e.g. GAP43, actin & tubulin).
- Axon begins to regrow (regenerate) from its cut end. (1-2 wks)

[Administration of the appropriate neurotrophin can largely prevent the axon reaction.]

Axons in the PNS regenerate.

- Axons grow 2-4 mm/day; 1.5 mm/day used clinically to estimate time to recovery of function.
- Axons grow within the endoneurium (connective tissue sheath) along channels formed by Schwann cells.
- Optimal regeneration requires nerve sheath to be intact; ends of cut nerve can be reapposed surgically with sutures in the epineurium.
- Axons that grow outside of the sheath can form neuromas.

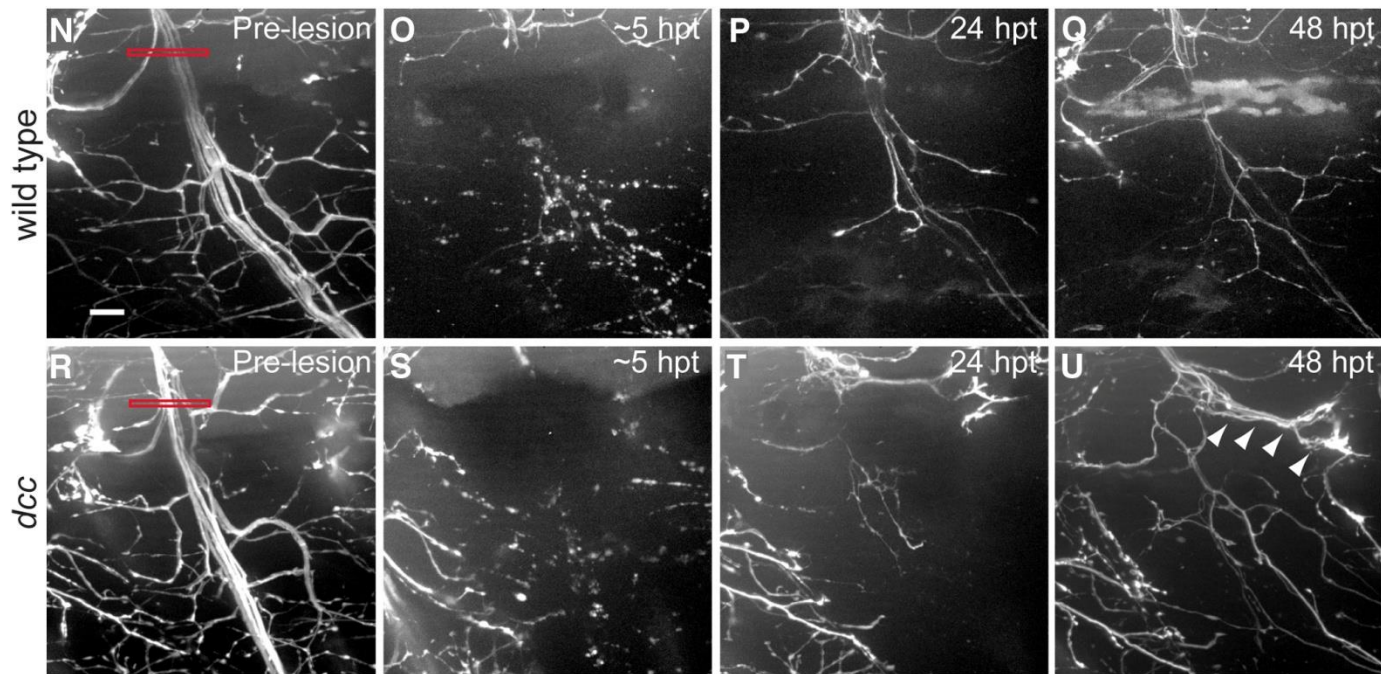
Axons in the PNS regenerate.

- Schwann cells along the path increase expression of adhesive glycoproteins (e.g. laminin).
- In response to nerve injury, Schwann cells express neurotrophins, particularly NGF and glial derived neurotrophic factor (GDNF).
- Schwann cells also increase expression of the p75 neurotrophin receptor. p75^{NTR} may bind neurotrophin and present it to regenerating axons.

Axons in the PNS regenerate.

- Schwann cells are required for the directed growth of PNS axons in the denervated nerve.
- This requires the netrin receptor, DCC, to be expressed by regenerating axons.

Rosenberg AF, et al. (2014) J. Neurosci. 34



Axons in the PNS regenerate.

- Somatic motor & autonomic axons will reinnervate muscle, glands, blood vessels & viscera at original synaptic sites specified by molecules in the basal lamina.
- Sensory axons will reinnervate original territory and may displace axons that had invaded its territory.
- Once synapses are reestablished, gene expression switches to a mode needed for neurochemical communication.
- The myelin sheath is eventually reestablished.

Axons in the PNS regenerate.

- Regeneration is never perfect:
 - strength & dexterity is reduced
 - sensory discrimination is poor
 - motor units are larger than normal
 - topography is not perfect
 - conduction velocity is 80% of normal
- Regeneration is probably most important for continually making repairs due to minor traumas.

Axons in the CNS fail to regenerate.

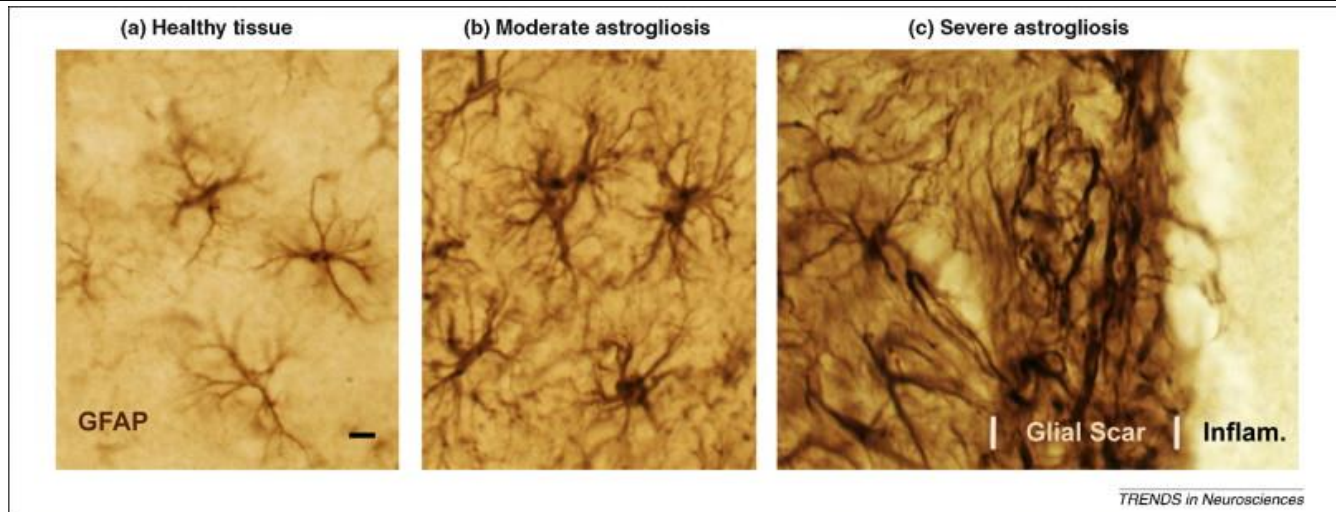
- Axons begin to regenerate in the injured CNS, but make little progress and abort further growth after a month or more.

Axons in the CNS fail to regenerate.

- Research on the failure of axons to regenerate in the adult CNS has focused on four main issues:
 - Glial scar
 - Myelin inhibitory molecules
 - Intrinsic inability of mature neurons to grow axons
 - Lack of neurotrophic support

Axons in the CNS fail to regenerate.

Glial Scar



- Reactive astrocytes form a 'glial scar' near the injury site that could block axon regeneration.
 - A glial scar includes a thick layer of parallel astrocytic processes and deposits of chondroitin sulfate proteoglycans (CSPGs) in the ECM.
 - CSPGs collapse growth cones in tissue culture.
 - Treatment of CSPGs with chondroitinase eliminates the growth inhibitory activity.
 - Injection of chondroitinase into injured rodent spinal cord resulted in limited improvement in axon regeneration.

Axons in the CNS fail to regenerate.

Glial Scar

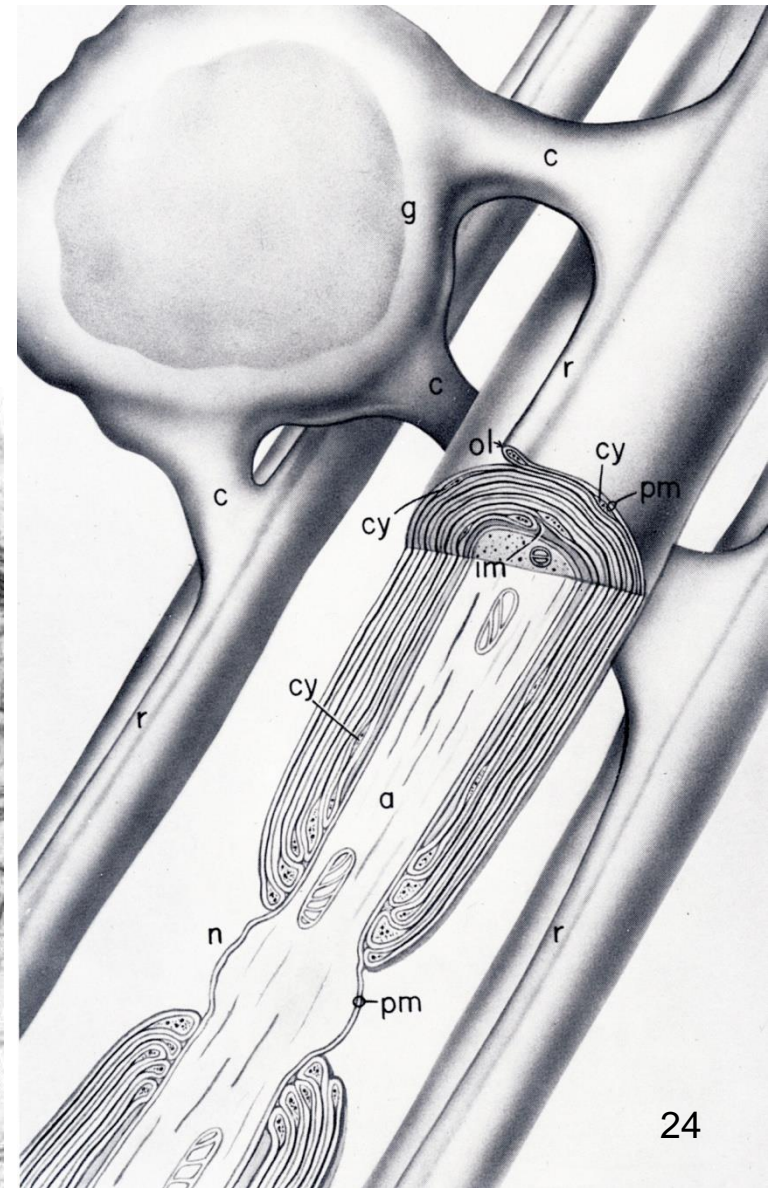
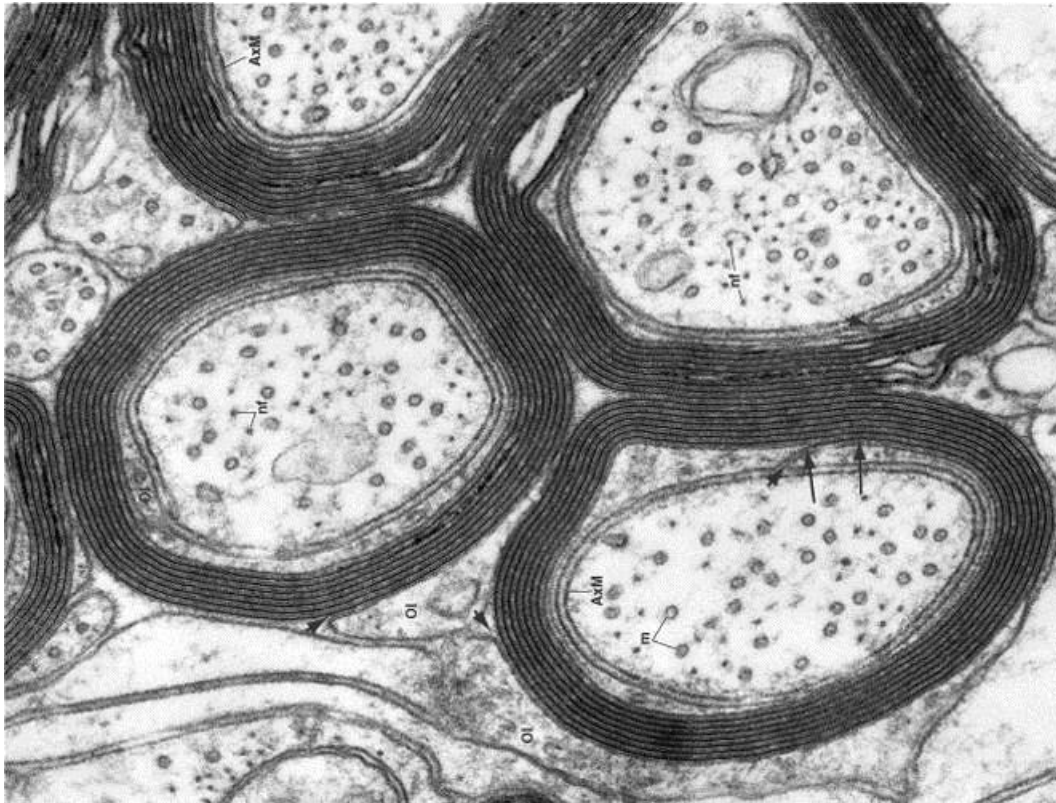
- A recent study knocked out several genes in mice, which prevented glial scar formation. Not only did injured spinal cord axons not regenerate in these mice, but they showed that molecules released by reactive astrocytes help promote axon growth.

Anderson MA et al. (2016) Nature 532:195

Axons in the CNS fail to regenerate. *Myelin Inhibitory Molecules*

Myelin is formed by glial cells wrapping their membranes around an axon:

- Schwann cells in the PNS.
- Oligodendrocytes in the CNS.



Axons in the CNS fail to regenerate.

Myelin Inhibitory Molecules

- Evidence that mammalian CNS myelin inhibits axon growth:
 - Neurites in culture avoid oligodendrocytes or regions of the dish coated with an extract of myelin.
 - Axons can regenerate in the CNS of fish and amphibians. Fish retinal ganglion cell axons grow in culture on a substrate of fish CNS myelin but not on mammalian myelin.
 - PNS and CNS axons can regenerate through a peripheral nerve in tissue culture and in vivo. PNS and CNS axons cannot regenerate through an optic nerve.
- Myelin debris takes >3 months to clear in the CNS, compared to a few weeks in the PNS.

Axons in the CNS fail to regenerate.

Myelin Inhibitory Molecules

- Mammalian CNS myelin includes several molecules that inhibit axon growth:
 - Nogo-A (Nogo-66)
 - Myelin-associated glycoprotein (MAG)
 - Oligodendrocyte-myelin glycoprotein (OMgp)

Axons in the CNS fail to regenerate.

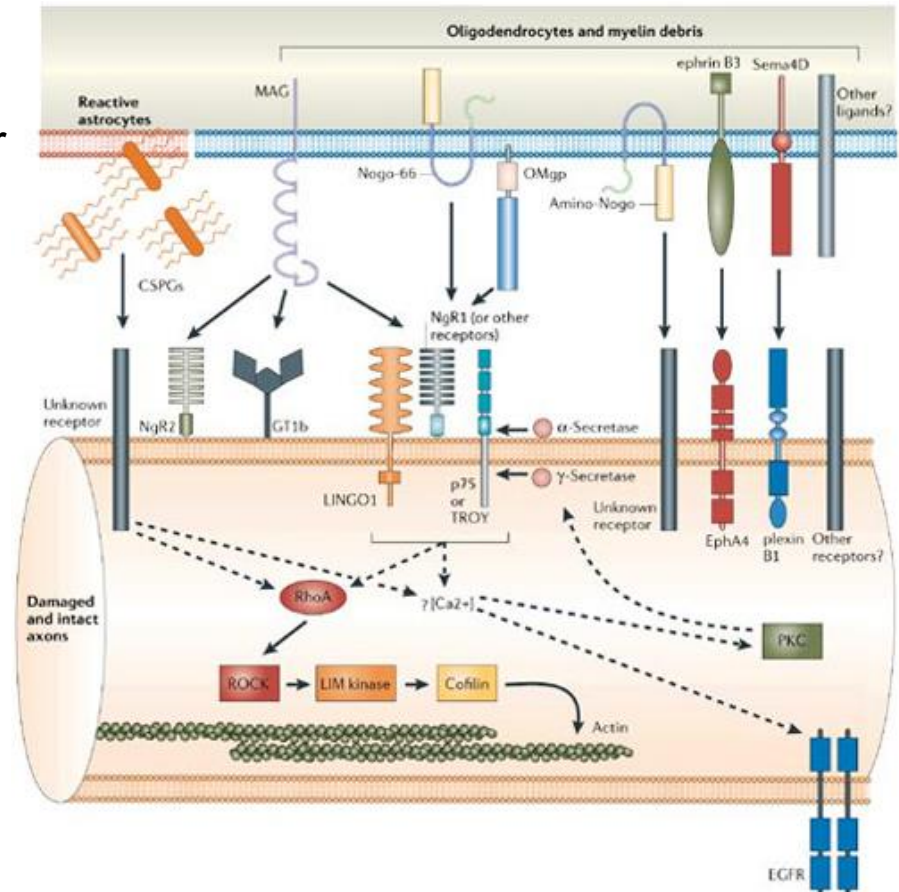
Myelin Inhibitory Molecules

- Nogo (Reticulon-4)
 - Three isoforms (splice variants): Nogo-A, -B & -C.
 - Nogo-A is expressed only in the CNS in the membrane of oligodendrocytes.
 - Nogo-A has a unique N-terminal cytoplasmic domain that is essential for inhibiting axon growth.

Axons in the CNS fail to regenerate. *Myelin Inhibitory Molecules*

- The Nogo receptor (NgR) is expressed by neurons.
- It is a GPI linked cell surface receptor with no transmembrane domain.
- NgR is the common receptor for MAG, OMgp and Nogo-A (even though they appear structurally unrelated).
- NgR trimerizes with the p75^{NTR} and LINGO1.

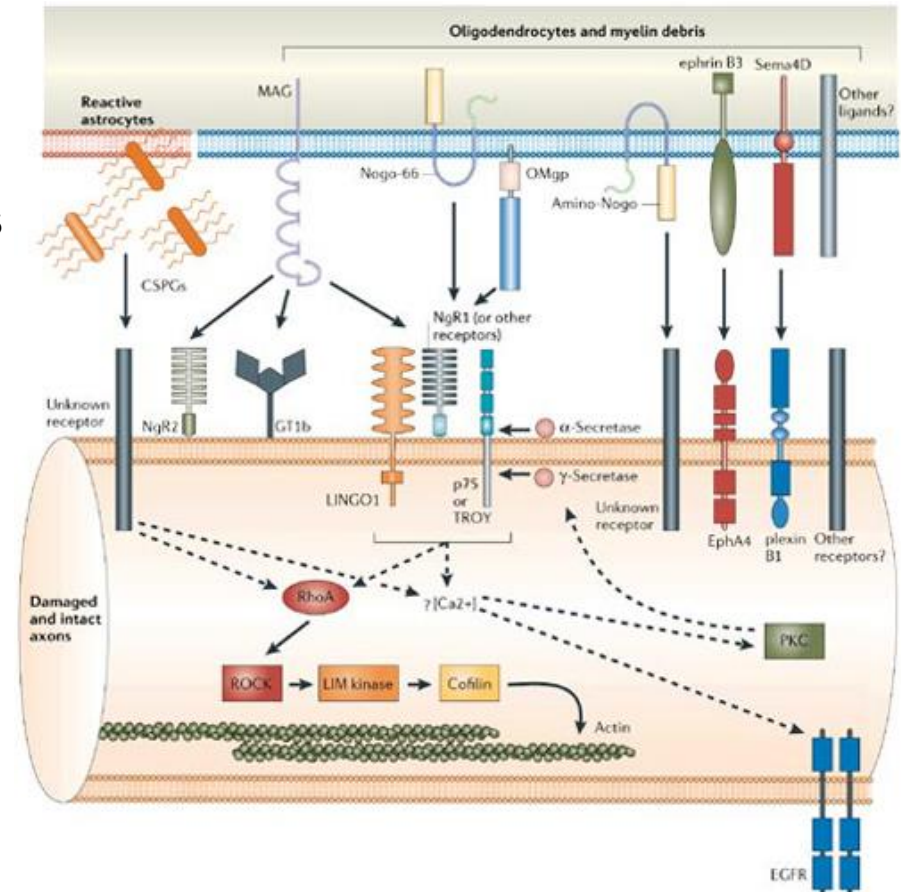
(TROY, a tumor necrosis factor receptor family member, can substitute for p75^{NTR}.)



Axons in the CNS fail to regenerate.

Myelin Inhibitory Molecules

- NgR1/LINGO1/p75 activates RhoA.
- The RhoA signaling cascade (RhoA→ROCK→LimK1→cofilin) reduces actin dynamics, which leads to collapse of the growth cone.

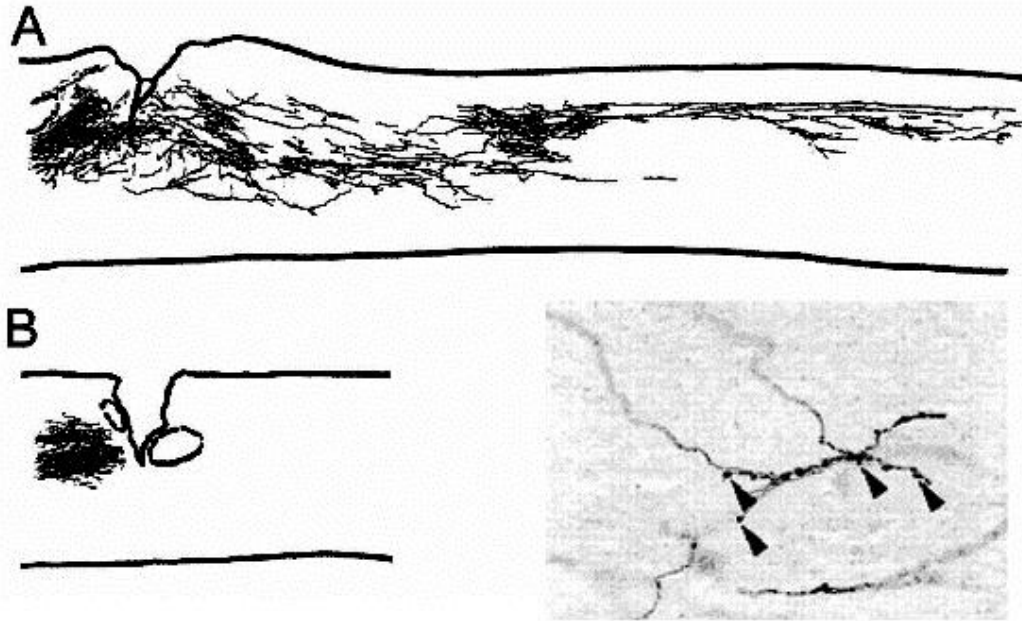


Axons in the CNS fail to regenerate.

Myelin Inhibitory Molecules

- Knockout of Nogo had no or minimal effect on CNS axon regeneration.
- Knockout of MAG showed no improvement in CNS axon regeneration.
- Knockout of NgR1, NgR2 and NgR3 showed no improvement in CNS axon regeneration.
- Knockout of p75^{NTR} showed reduced inhibition of axon growth by myelin in culture. However, there was no improvement in axon regeneration following spinal cord injury in vivo.

Axons in the CNS fail to regenerate. *Myelin Inhibitory Molecules*



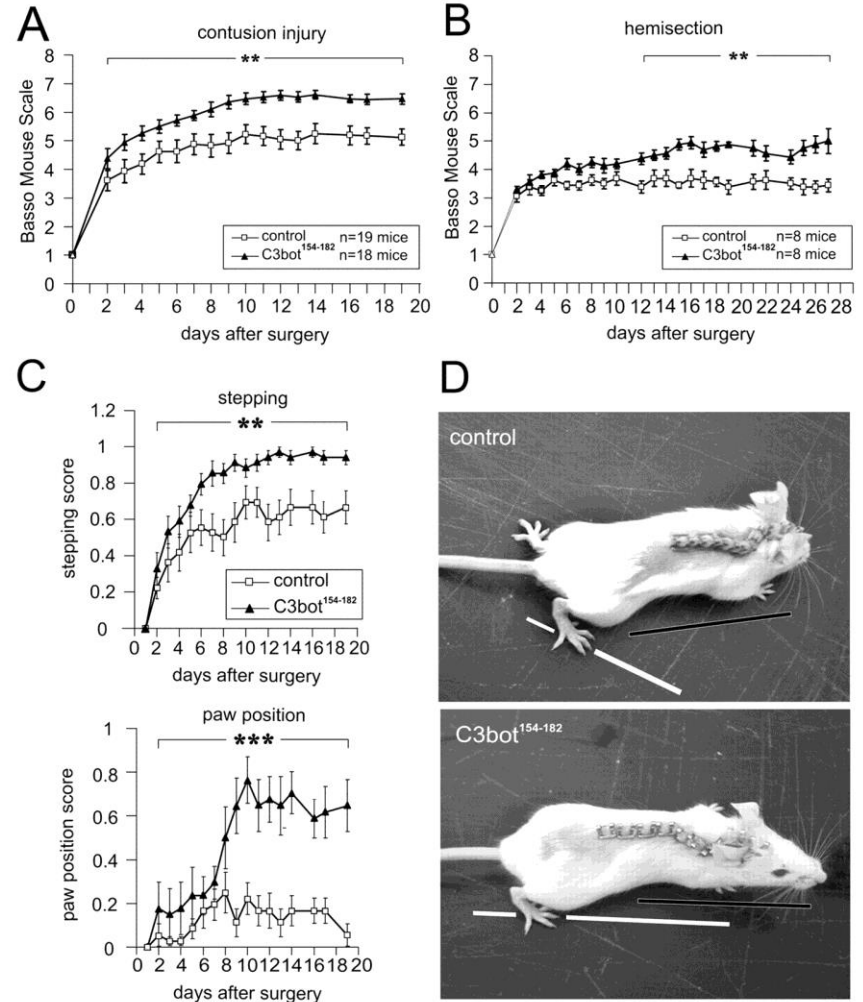
- Infusion of function blocking antibodies to Nogo-A into damaged rat spinal cord allowed regeneration of some corticospinal axons over 9mm beyond the site of injury.
[But why, since MAG and OMgp were still present?]
- 9mm is not very far, and very few axons regenerated.

Axons in the CNS fail to regenerate.
Myelin Inhibitory Molecules

- Clinical trials are underway testing antibodies to Nogo for treatment of spinal cord injury.

Axons in the CNS fail to regenerate. *Myelin Inhibitory Molecules*

- Rho appears to be common to all pathways for myelin inhibition of axon growth.
- Treatment with a Rho inhibitor, C3 or Y27632, resulted in some behavioral recovery after spinal cord injury in mice.

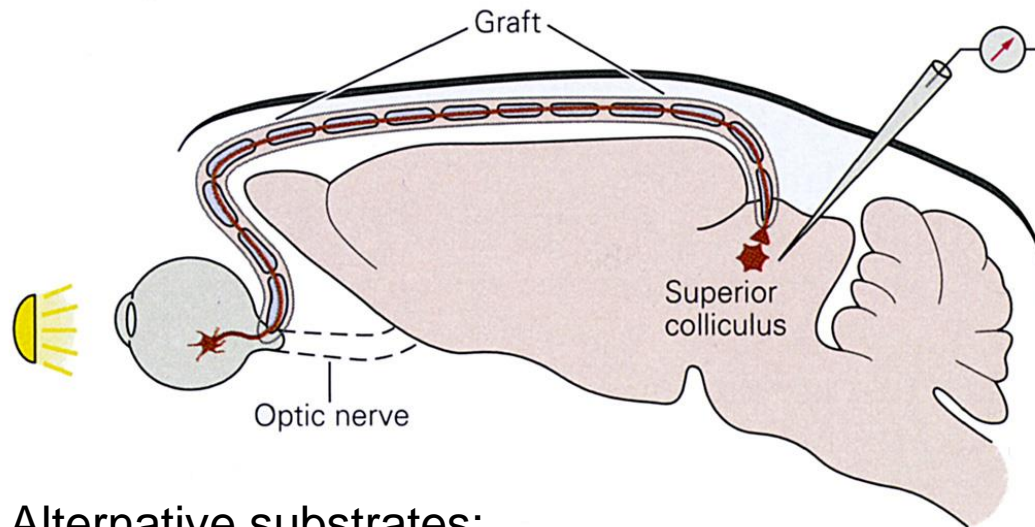


Axons in the CNS fail to regenerate.
Myelin Inhibitory Molecules

- Clinical trials are underway testing Cephirin, C3 attached to a peptide making it more cell permeable, for treatment of spinal cord injury.

Axons in the CNS fail to regenerate.

Myelin Inhibitory Molecules



- Alternative substrates:
 - A peripheral nerve bridge can serve as a substrate for axon regeneration. However, axons exhibit very limited growth once they reenter the CNS.
 - Transplanting Schwann cells into the spinal cord can promote axon regeneration following injury.
 - In the best cases, only 10% of the axons regenerate.

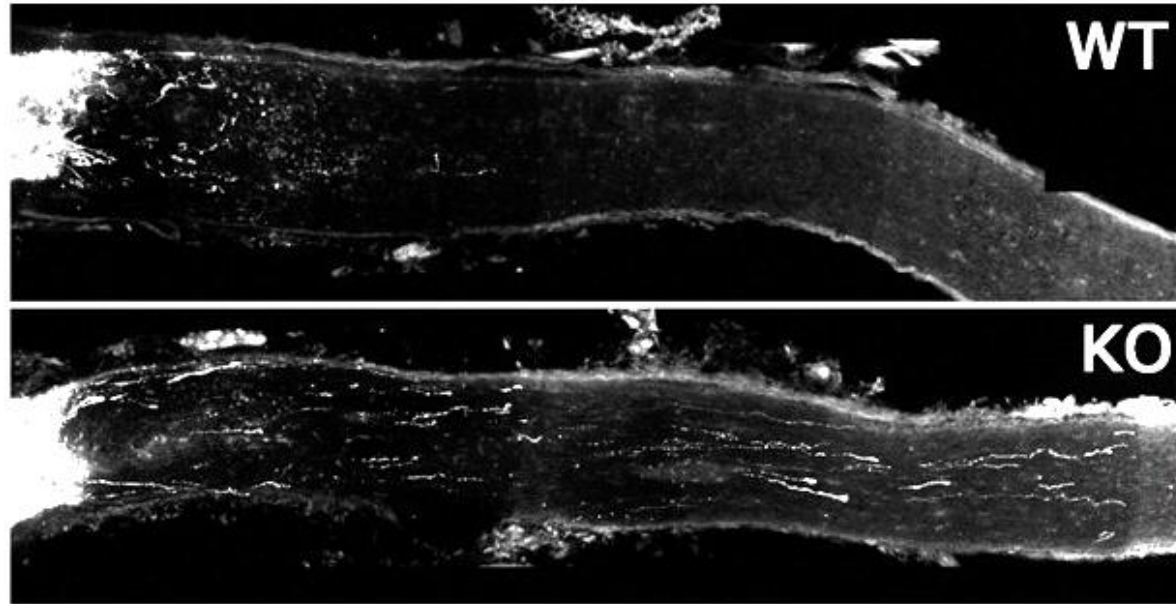
Axons in the CNS fail to regenerate. *Intrinsic Limitation*

- The intrinsic nature of mature CNS neurons limits their ability to regenerate an axon:
 - PNS axons regenerate better than CNS axons through a peripheral nerve.
 - CNS neurons lose the ability to regenerate an axon as they mature:

Retinal ganglion cells lose the ability to regenerate an axon in vitro and in vivo as they mature (even if cell death is blocked). This change does not happen when ganglion cells mature in low density culture. Addition of retinal culture conditioned medium or amacrine cells to the RGC cultures initiated the change in their ability to grow an axon.

- Retinal ganglion cells express Kruppel-like factor-4 (KLF4), a transcription repressor, which blocks their ability to regenerate an axon. [[see discussion paper](#)]

Axons in the CNS fail to regenerate. *Intrinsic Limitation*



- Adult retinal ganglion cells in which KLF4 was eliminated were able to regenerate axons in the optic nerve past the injury site.

[see discussion paper]

However, only a small number of axons regenerated.

Axons in the CNS fail to regenerate.

Lack of Neurotrophic Support

- Unlike in the PNS, there is no increase in neurotrophin expression in the CNS following axotomy.
- In experimental animals, infusion of neurotrophin (BDNF) into a CNS injury site made axons immune to inhibitory molecules and allowed limited axon regeneration.

Axons in the CNS fail to regenerate.

Conditioning Lesion of DRG Axons

- Conditioning lesion of peripheral DRG axons increased subsequent regenerative capacity of their central axons.
- Conditioning lesion resulted in upregulation of growth associated protein-43 (GAP43) and cAMP. This was not seen following section of the central axon alone.
- Injection of cell-permeable cAMP into a spinal cord injury improved axon regeneration.

Axotomy Induced Cell Death

- Axotomy near the soma is likely to result in cell death.
- Young neurons are more likely to undergo cell death in response to axotomy regardless of the position of the axotomy.
- In the adult, neurons may die but are more likely to atrophy if regeneration fails.