XXI World Congress of Neurology
Vienna, Austria

Teaching Course: Neurotrauma

Challenges to nerve regeneration in humans: The Long Term Denervated Stump

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Disclosure

None relevant to this presentation

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Learning Objectives

- To understand cellular mechanisms of poor recovery with proximal nerve injuries in humans
- To compare and contrast regenerative therapy strategies in experimental models versus human nerve regeneration

Challenges in PNS Regeneration

- Intrinsic determinants of axon regeneration
  - Slow rate of axonal elongation during regeneration
- Extrinsic determinants of axon regeneration
  - Chronic denervation
    - Changes in the pathway (i.e.. Schwann cells)
    - Changes in the target (muscle or skin)
- Common issues
  - Specificity of target reinnervation
    - Determined through a combination of intrinsic neuronal characteristics, extrinsic pathway properties and finally target

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Acute events after injury: CNS vs PNS Intrinsic Factors

CNS

NEURONS

- Direct damage to neurons at injury site (death)
- Axotomy causes neuronal death depending on proximity to cell body

AXONS

- CNS axons fail to form growth cone and form dystrophic end bulbs (microtubule depolymerization)
- ~1/3 of axons sprout for ~1mm
- Distal end undergoes inefficient Wallerian degeneration (myelin debris)

PNS

NEURONS

- Very little neuronal death
- Axotomy causes neuronal death only when it is very close to cell body

AXONS

- PNS axons form growth cone within hours (microtubules retain integrity and can bundle)
- PNS axons start to regenerate shortly after injury
- Retrograde injury signal
- Distal end undergoes efficient Wallerian degeneration (myelin debris)

Acute events after injury: CNS vs PNS Extrinsic Factors

CNS

CELLULAR

- Quick invasion of epicenter by fibroblast, vascular endothelial cells, and macrophages
- Surviving host cells (astrocytes, OPC, and microglia) surround the epicenter and form a glial scar

ECM

- CSPG (NG2, neurocan), fibronectin, laminin
- Myelin derived inhibitors (MAG, Nogo-A, OMgp)
- Guidance molecules (netrin, semaphorin, ephrin and slit families); expression changes after injury

PNS

CELLULAR

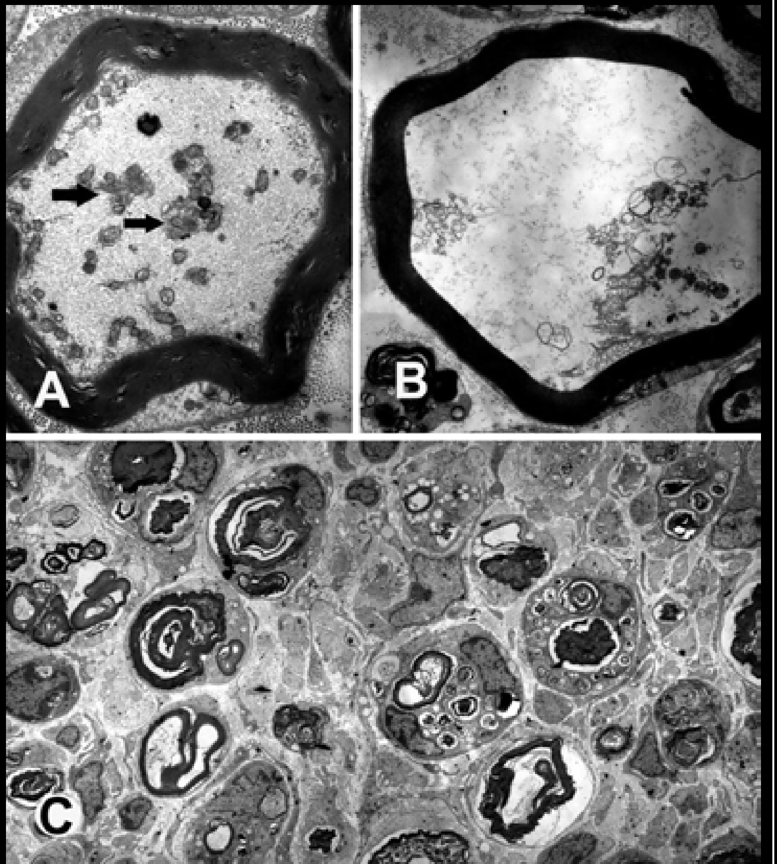
- Activation of Schwann cells and intrinsic macrophages
- Invasion of blood-borne macrophages
- Proliferation of blood vessels

ECM

- Basal lamina is supportive of regeneration (laminin, COLa4)
- Myelin inhibitors are cleared

Wallerian Degeneration

- Accumulation of intra-axonal organelles
- Granular disintegration of cytoskeleton
- BNB/BBB breakdown
- Glial changes (SC, Oligos)
  - Proliferation vs apoptosis
- Recruitment of macrophages
- Clearance of myelin ovoids



Wallerian Degeneration

Accumulation of intra-axonal organelles

Granular disintegration of cytoskeleton

BNB/BBB breakdown

Glial changes (SC, Oligos)

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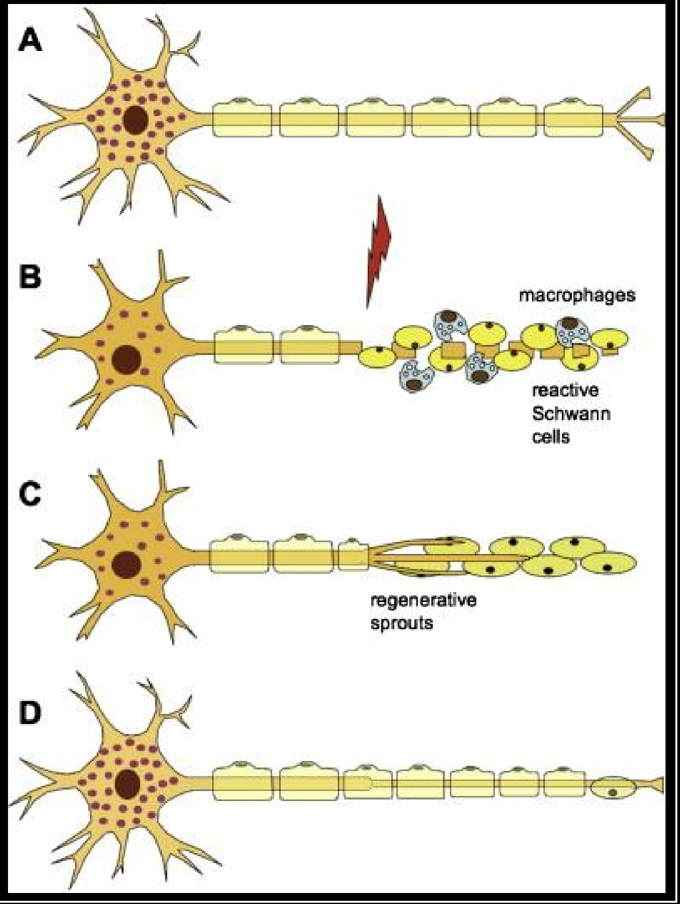
Clearance of myelin ovoids

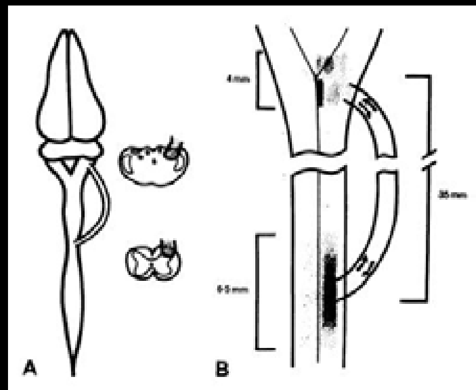


Axonal regeneration in CNS vs PNS

Unlike CNS, axons in the PNS can regenerate

Failure to regenerate in the CNS is NOT due to an innate inability of CNS axons to regenerate

- PNS environment supports regeneration
- (Aguayo's experiments)

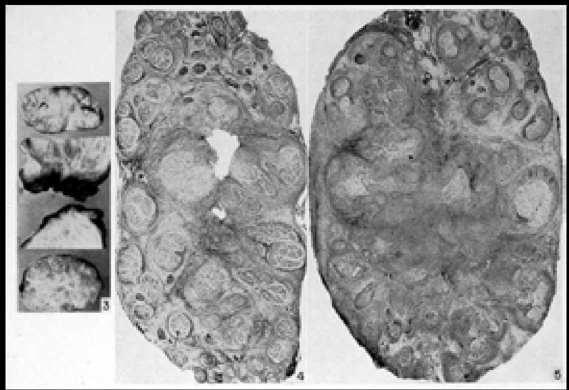


However, these observations do not correlate with clinical experience in humans

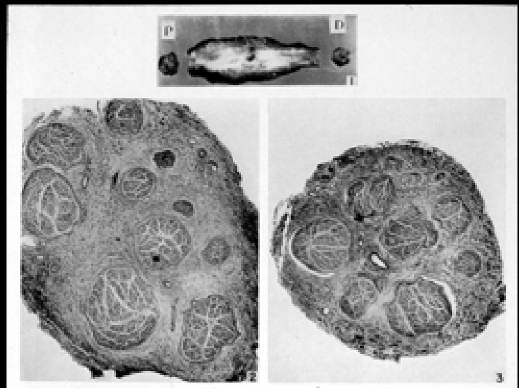
- Clinical recovery after nerve trauma is often suboptimal
- This is primarily due to "chronic denervation" (glial milieu)

Regeneration after nerve trauma

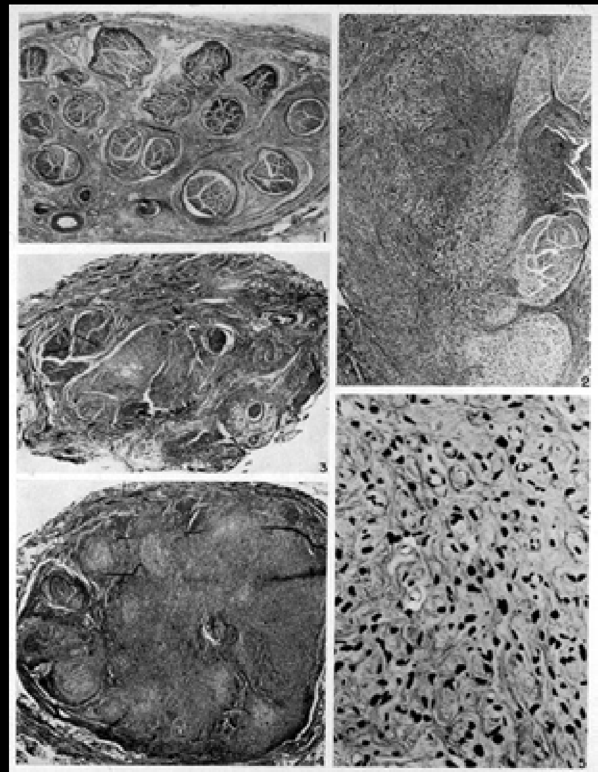
Sciatic nerve injury: no repair



Ulnar nerve injury:
Immediate repair at day 2



Laceration to median nerve:
Delayed repair at 10 mo



Lyons & Woodhall 1949

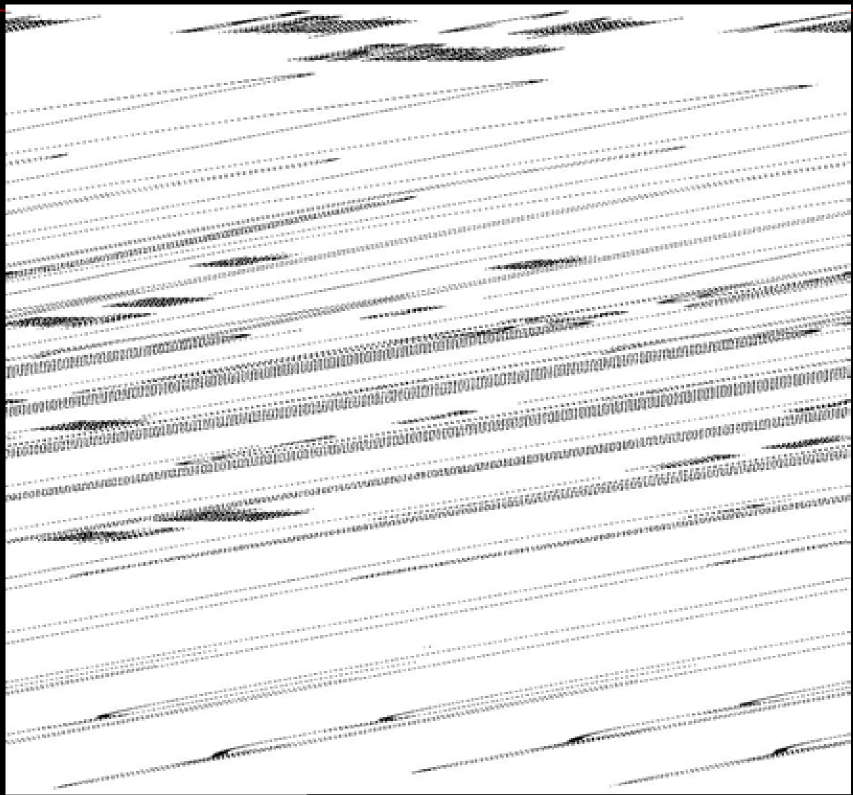
Chronically denervated nerves do not support regeneration

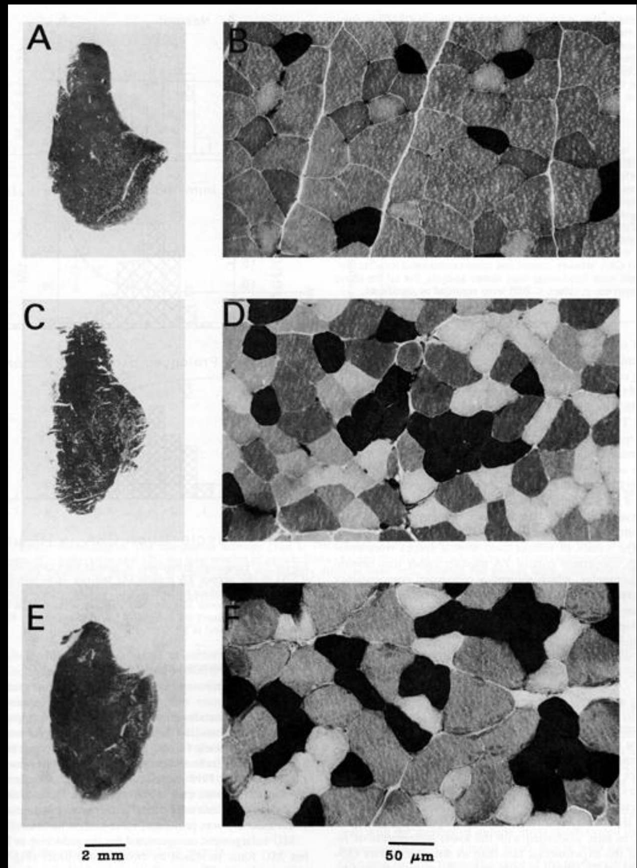
Is this a neuron/axon problem?

or

Is there a problem with the Schwann cells?

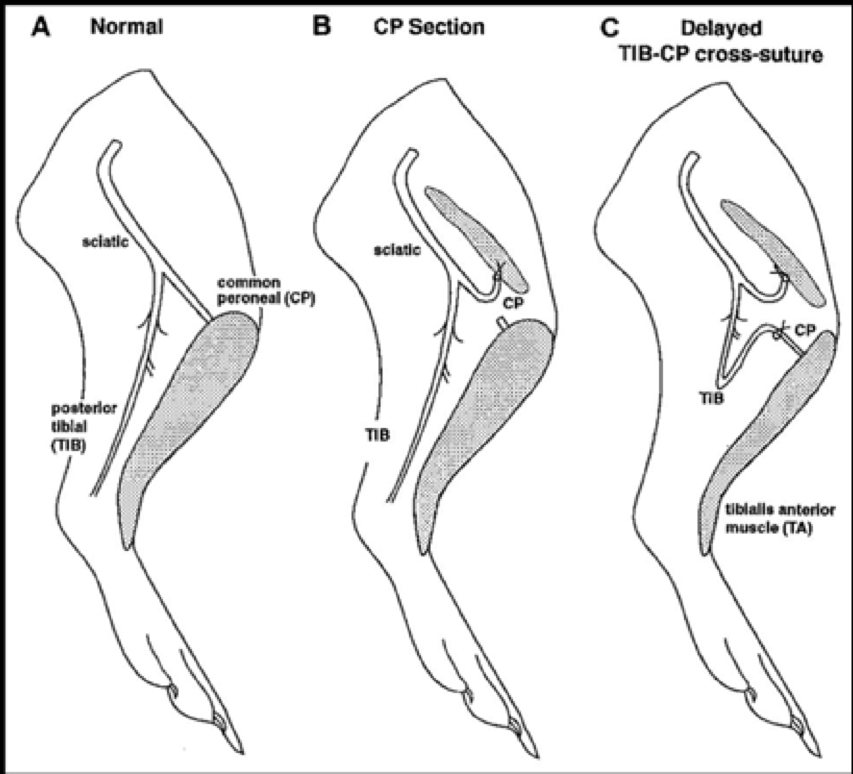
Effects of Prolonged Axotomy

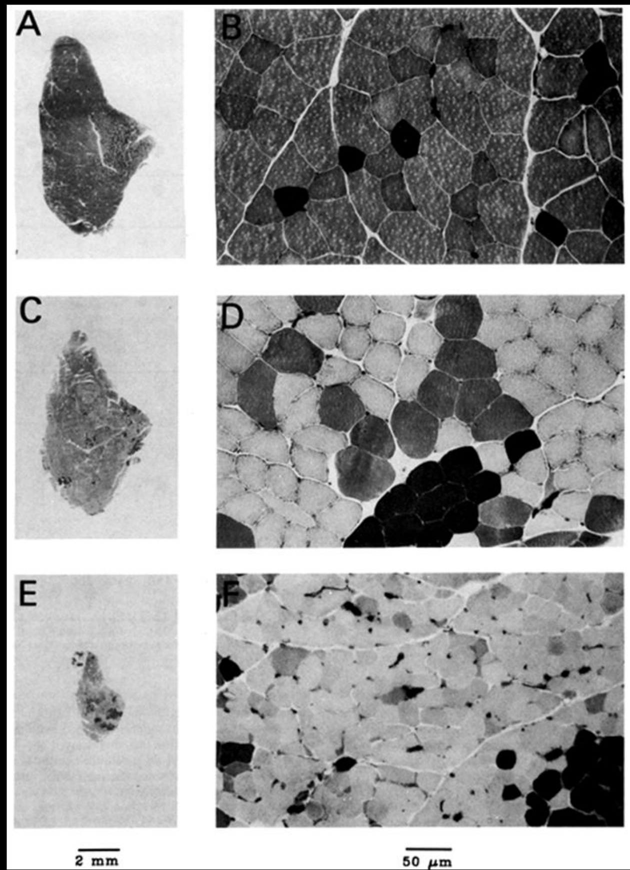




Fu & Gordon 1995

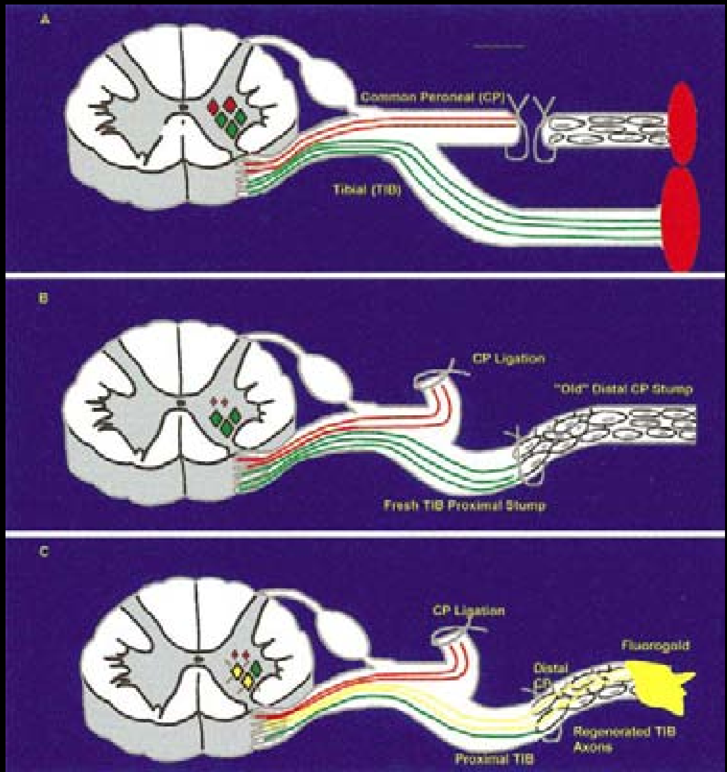
Effects of Prolonged Denervation

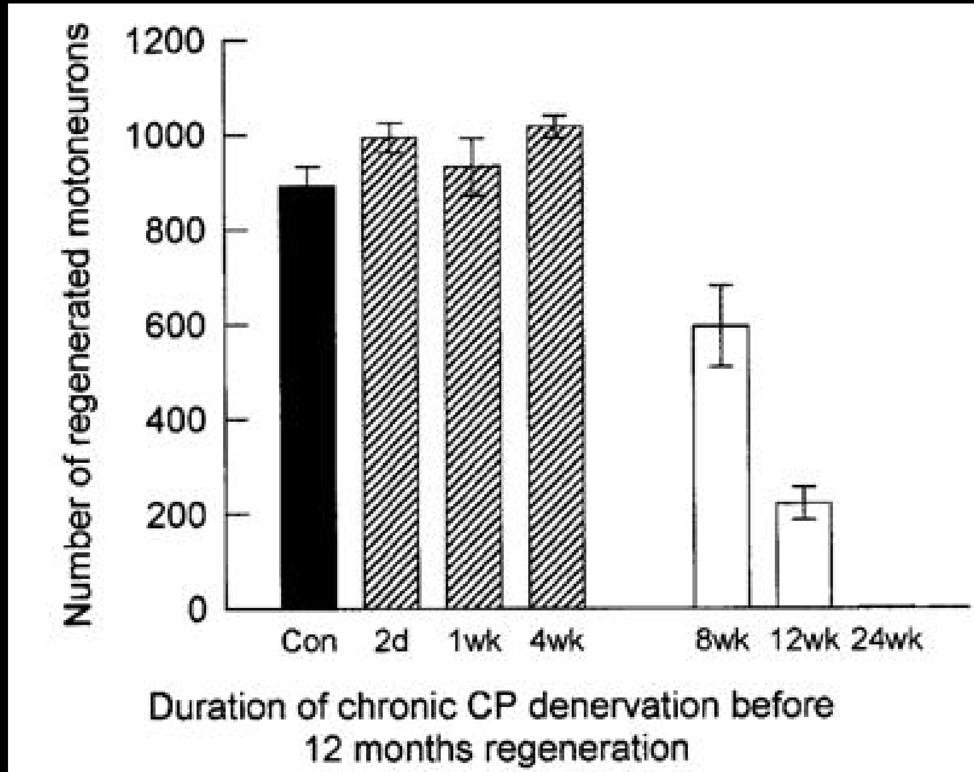




Fu & Gordon 1995

Impaired motor regeneration following chronic denervation

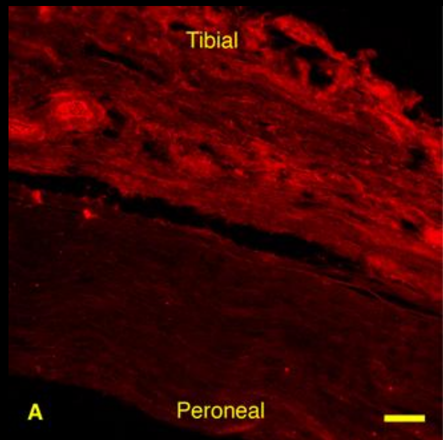




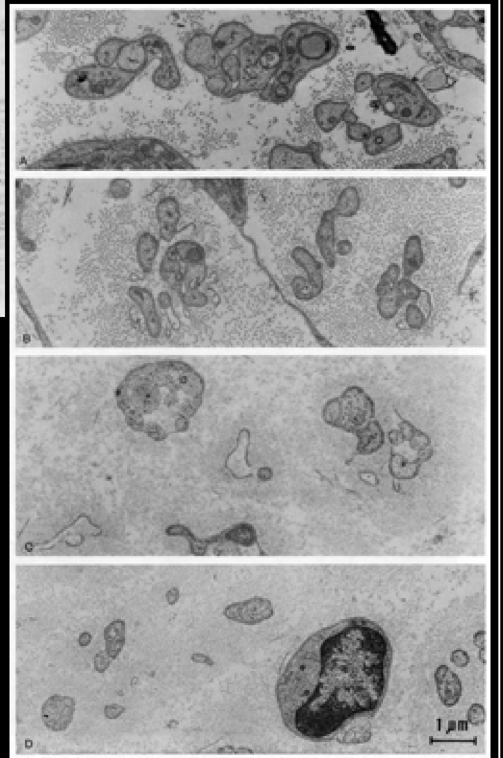
Sulaiman & Gordon 1997

Chronically denervated SCs lose their ability to support regeneration of axons

- Atrophy of SCs and disappearance of Bands of Büngner
- Decline in expression of growth associated molecules (p75, erbB3) *(Hall and Gordon labs)* and neurotrophic factors (GDNF) *(Hoke lab)*
- Increased expression of CSPGs (Muir and *Hoke labs*)

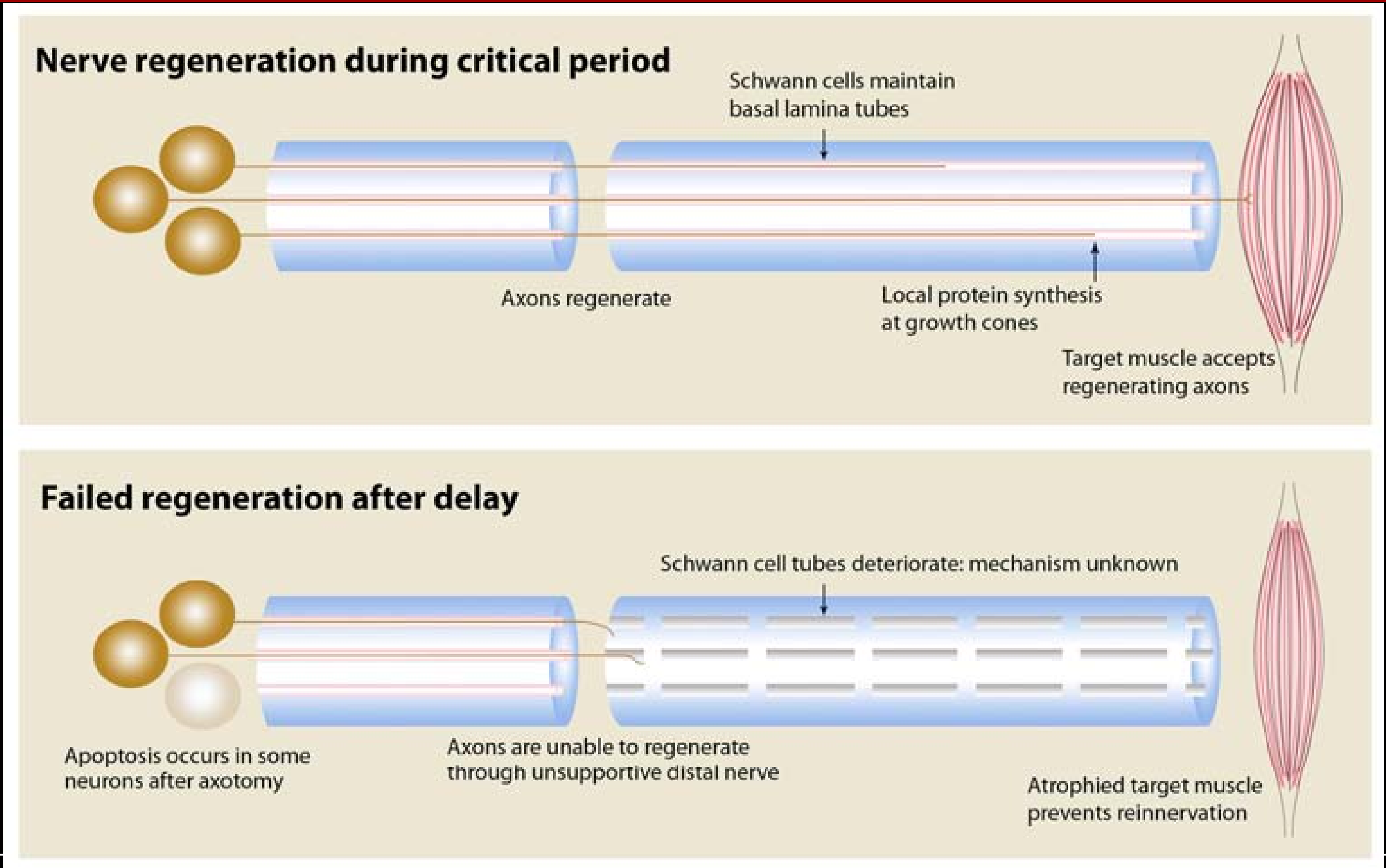


Heine et al 2003



Peripheral Neuropathy Dyck ed 2nd ed

Chronically denervated SCs lose their ability to support regeneration of axons

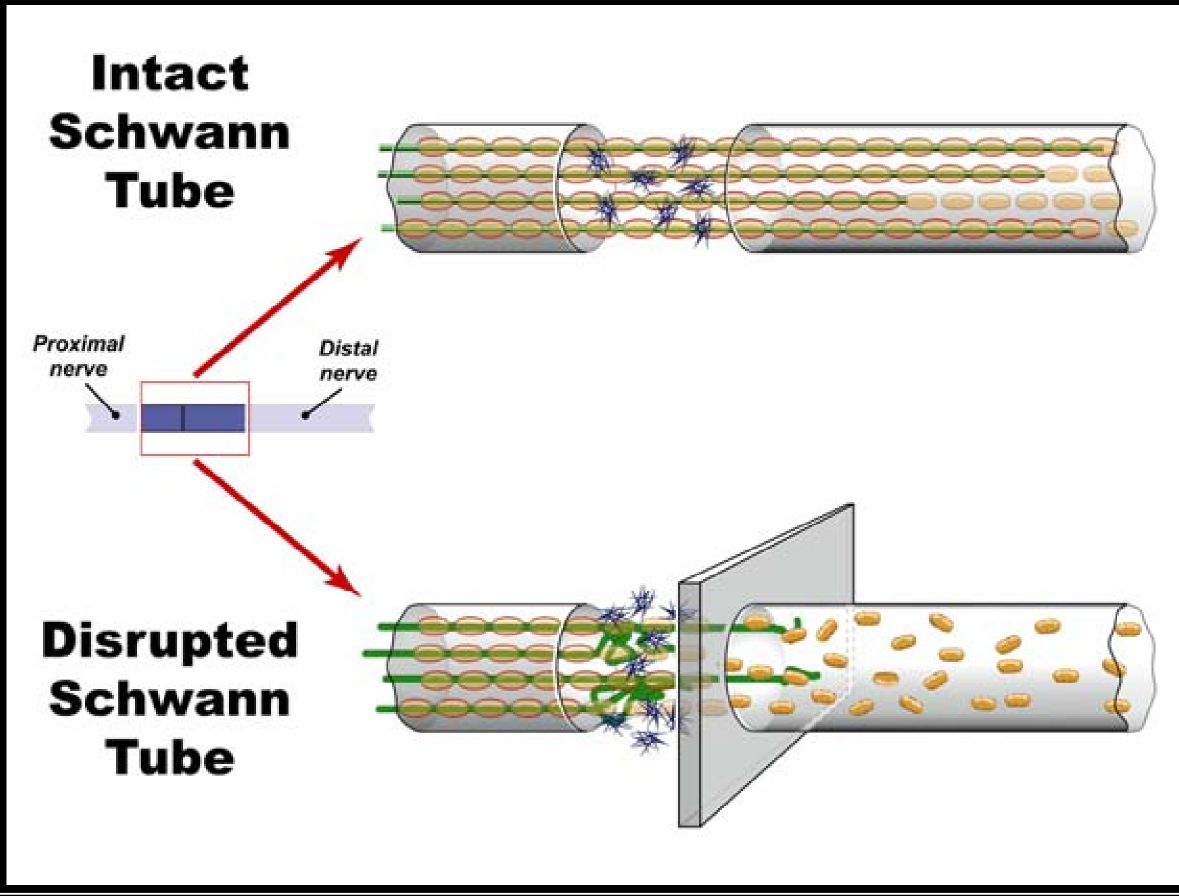


Nature Neurol Reviews 2013

Chronically denervated SCs lose their ability to support regeneration of axons

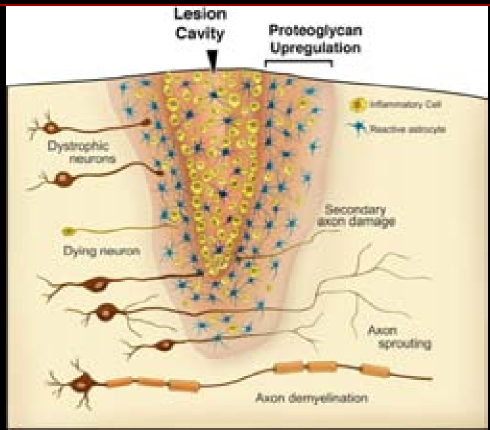
Acute denervation and regeneration

Chronic denervation and regeneration



Hoke Nature Neurol Clin Prac 2006

Regenerative challenges in CNS and PNS



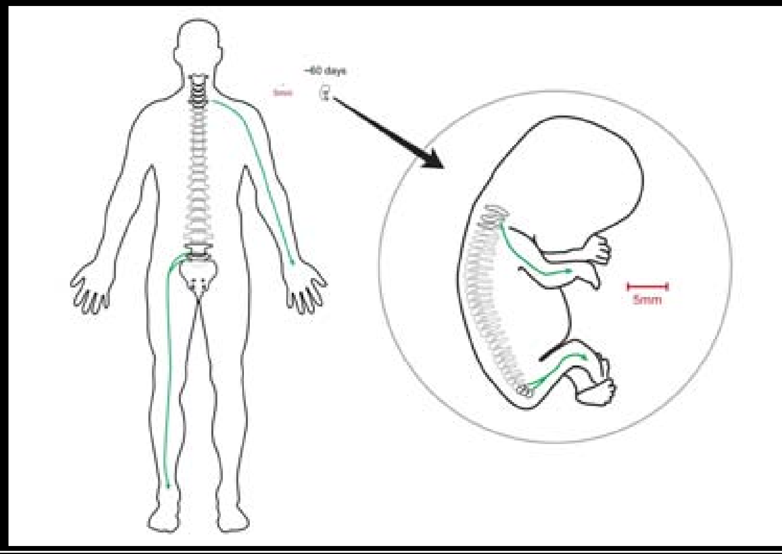
Glial scar

Neuronal death

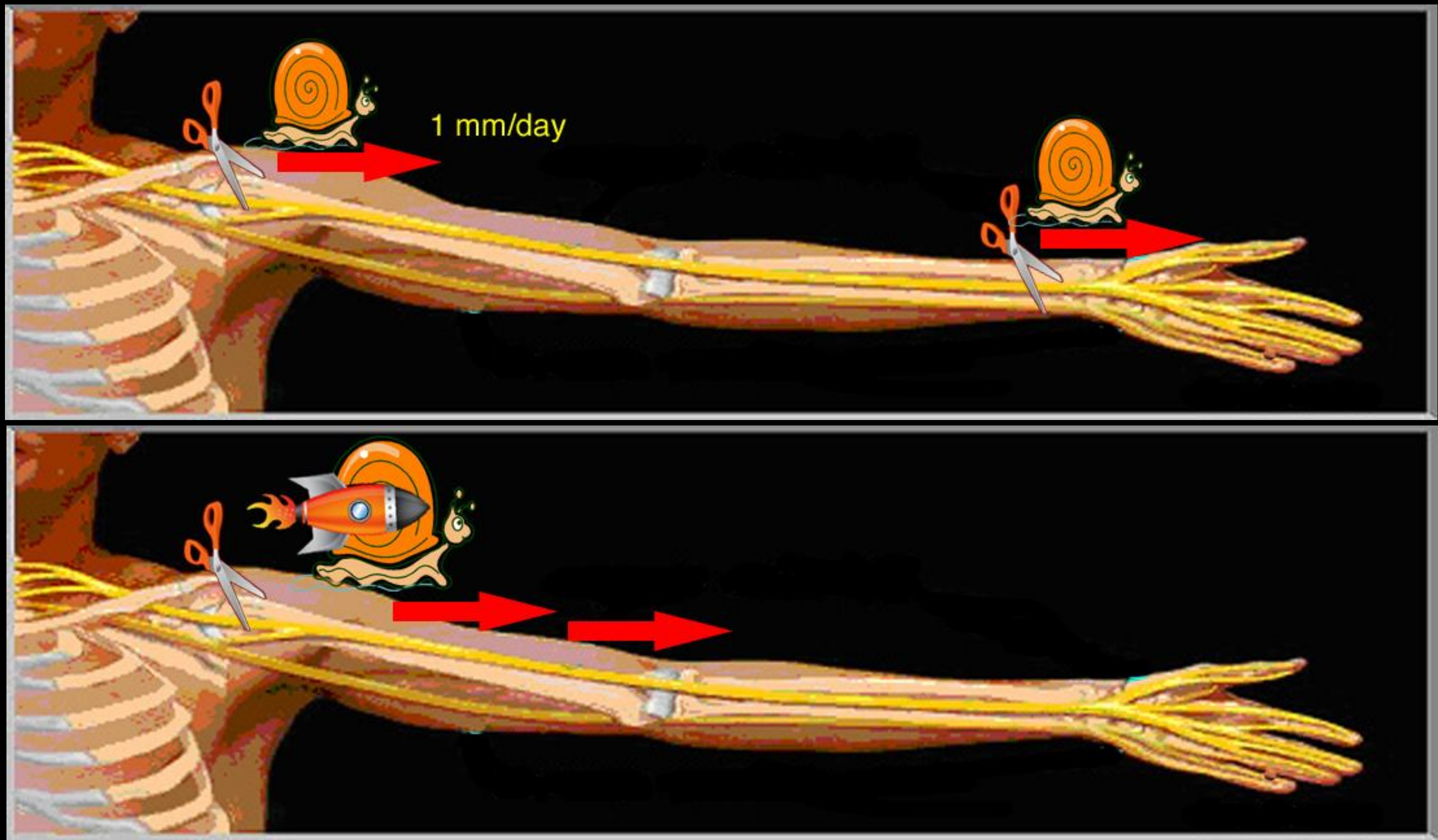
Demyelination of spared axons

Regeneration is not the same as developmental growth:

- Distance to regenerate – embryo vs adult



Impact of slow rate of axonal elongation on nerve regeneration in humans



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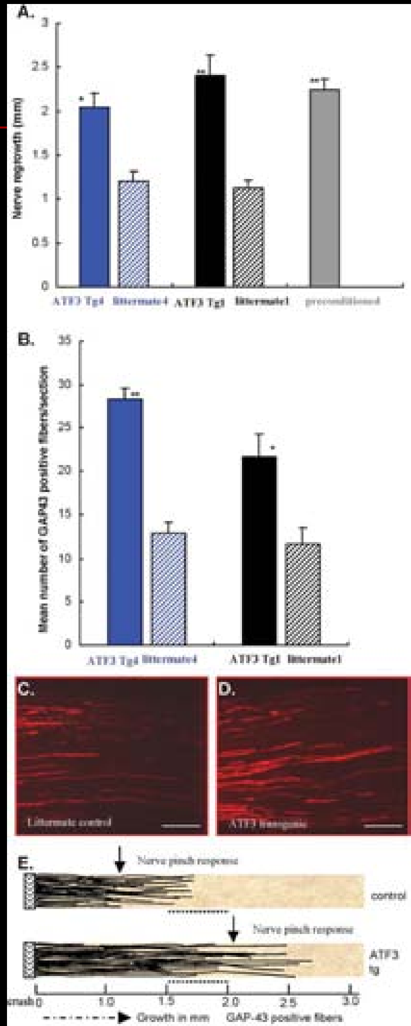
Can we alter the rate of axonal elongation? - PNS

- Conditioning lesion
  - Impact on peripheral regeneration
  - Impact on central regeneration
- Electrical stimulation (Gordon & Brushart)
  - Provide pathway specificity without enhancing rate of axonal elongation
- ATF-3 over expression (Woolf)
  - Effect on peripheral but not central regeneration
- Hsp27 over expression (Woolf)
  - Effect on sensory and motor regeneration



(Seiffert etal 2007)

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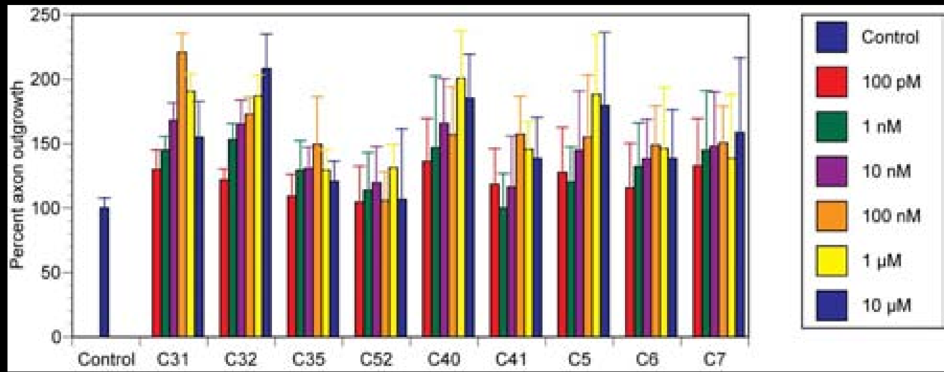
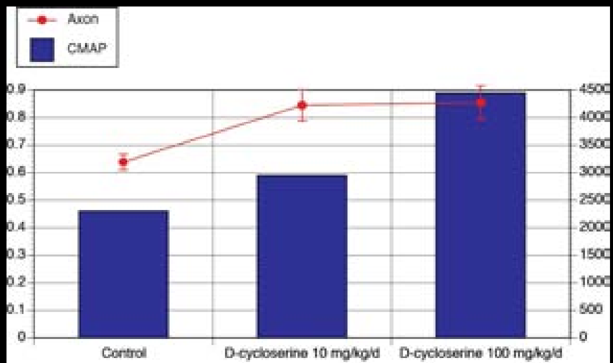
Can we alter the rate of axonal elongation? - PNS

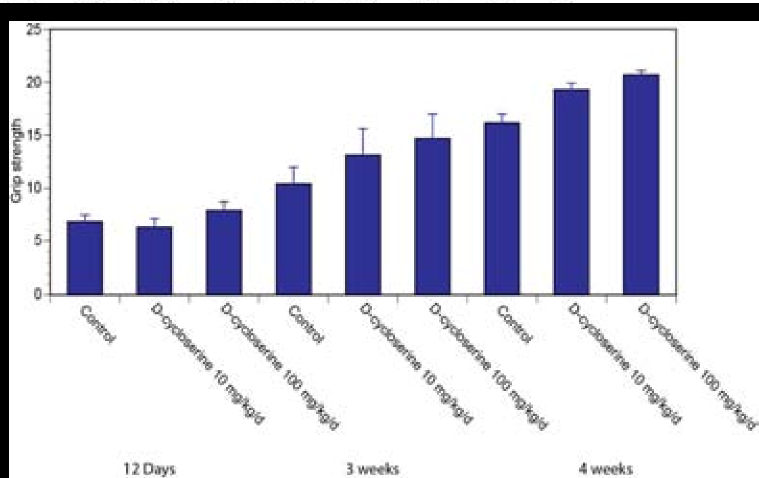
Unbiased screen for compounds that increased outgrowth in SC explants and DRG neurons



D-cycloserine

A weak NMDA agonist

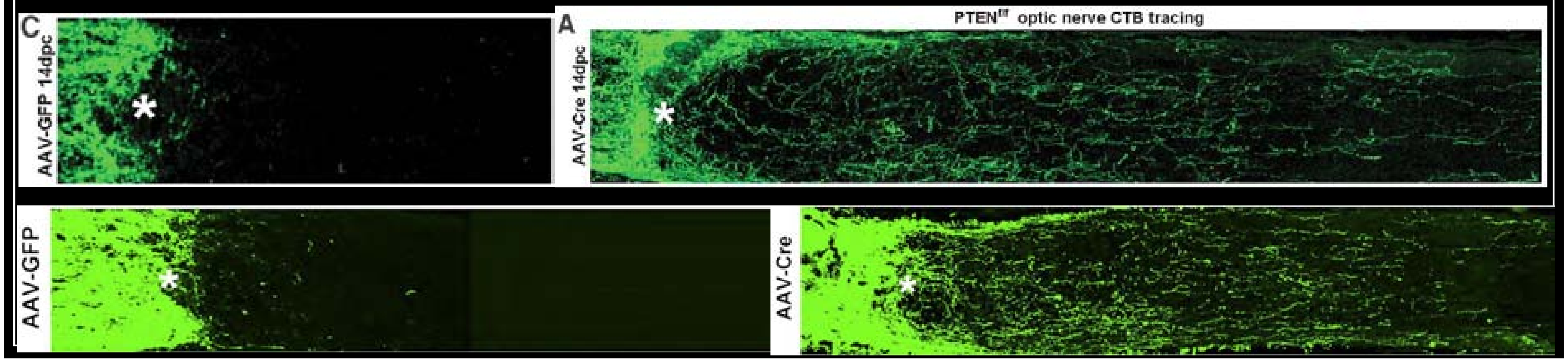




(Wang etal Unpublished)

Can we alter the rate of axonal elongation? - CNS

- Phosphatase and tensin homologue (PTEN)
  - Mammalian target of rapamycin (mTOR)
- Suppressor of cytokine signaling 3 (SOCS3)
  - Janus kinase/signal transducers and activators of transcription (JAK/STAT)
- Optic nerve model of CNS regeneration:
  - Adult retinal ganglion cells (RGCs)

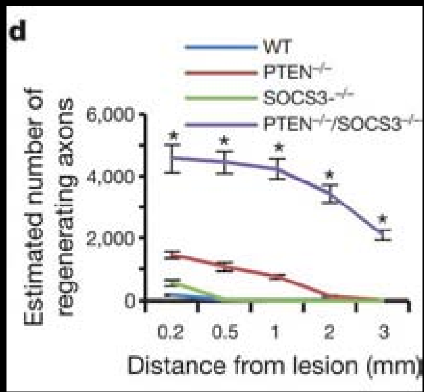


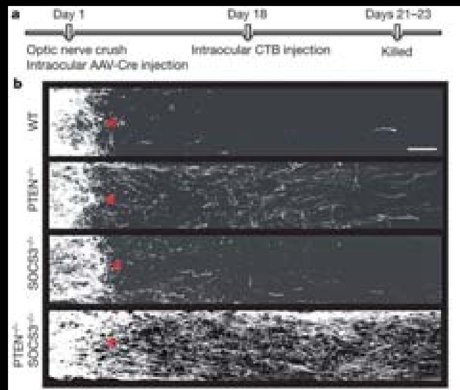
(Park etal 2008 and Smith etal 2009)

Can we alter the rate of axonal elongation? – CNS vs PNS

Combined PTEN/SOCS3 deletion

Concurrent activation of mTOR and JAK/STAT pathways in neurons





(Sun etal 2011)

But no effect on PNS regeneration!

Intrinsic mechanisms of CNS vs PNS regeneration are likely to be different

Nevertheless, local inhibition of PTEN at site of regeneration accelerates PNS regeneration *(Christie etal 2010)*

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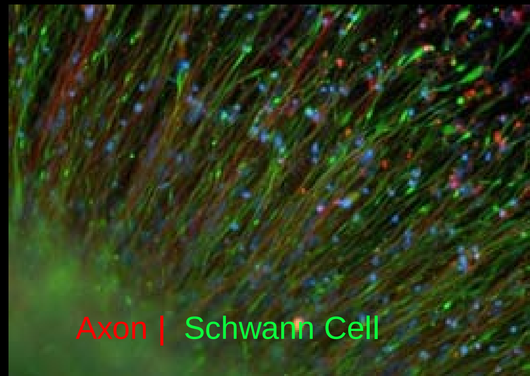
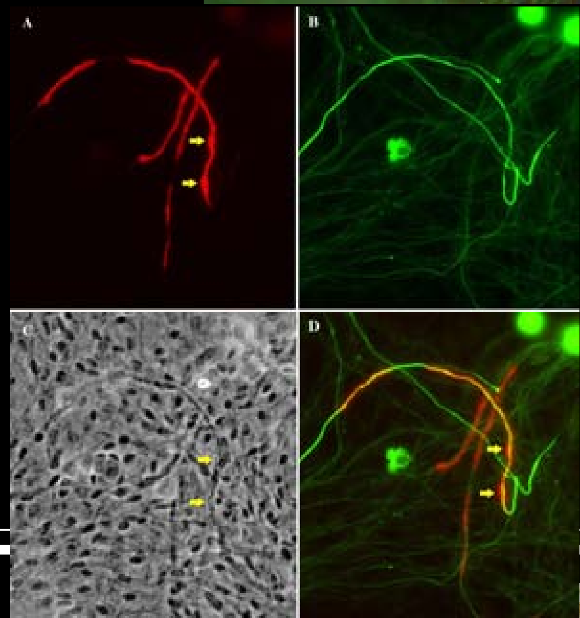
Gaps in our knowledge and future directions – ongoing studies

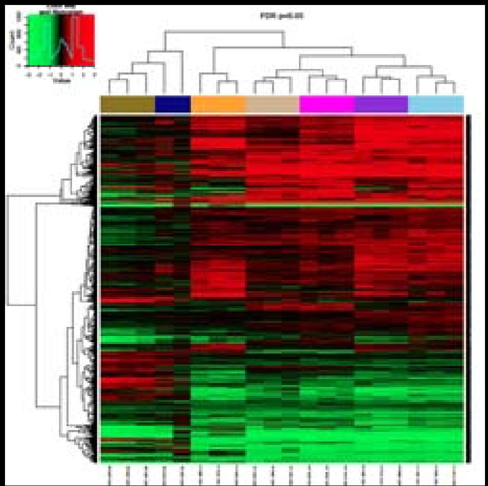
- Chronic denervation in the:
  - Pathway: Schwann cells
  - Target: muscle, skin
- Can we reactivate atrophied SCs or prevent atrophy?
  - Role of neuregulin-1 type III
- Do we need to replace atrophied SCs? Can we do it?
  - Human ESC or iPS generated Neural Crest Stem Cells as a source

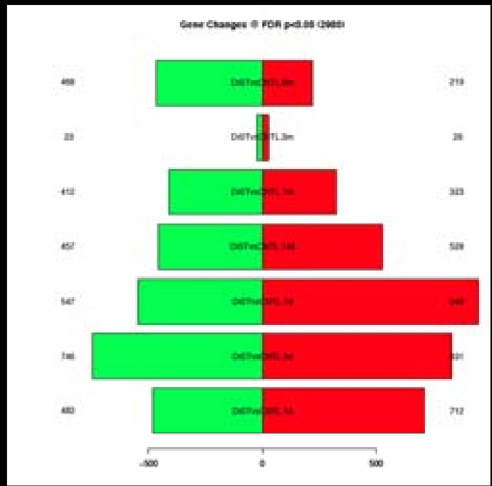




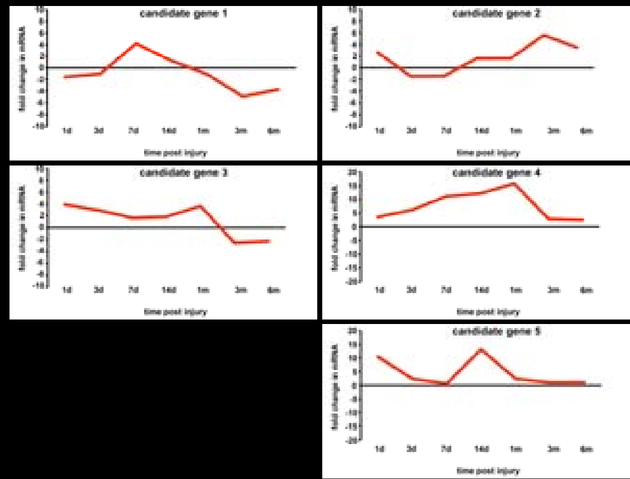
Chronic denervation in the SCs

Rat sciatic nerve denervation 1d – 6 mo

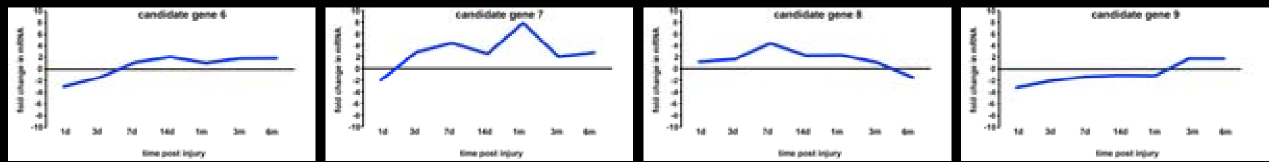




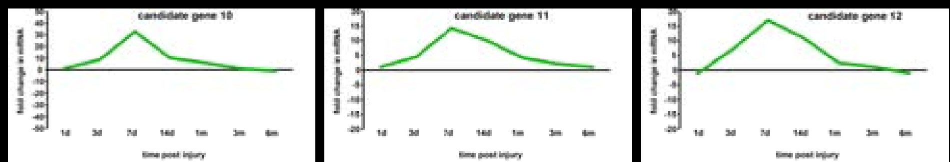
Transcription Factors



Apoptosis Related

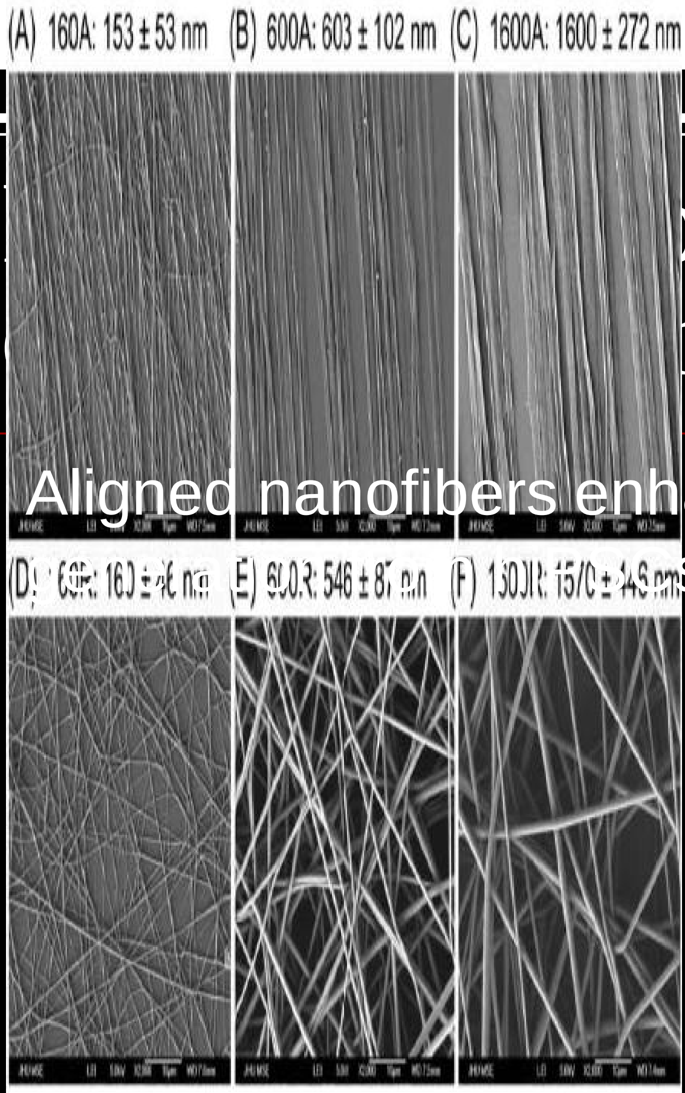


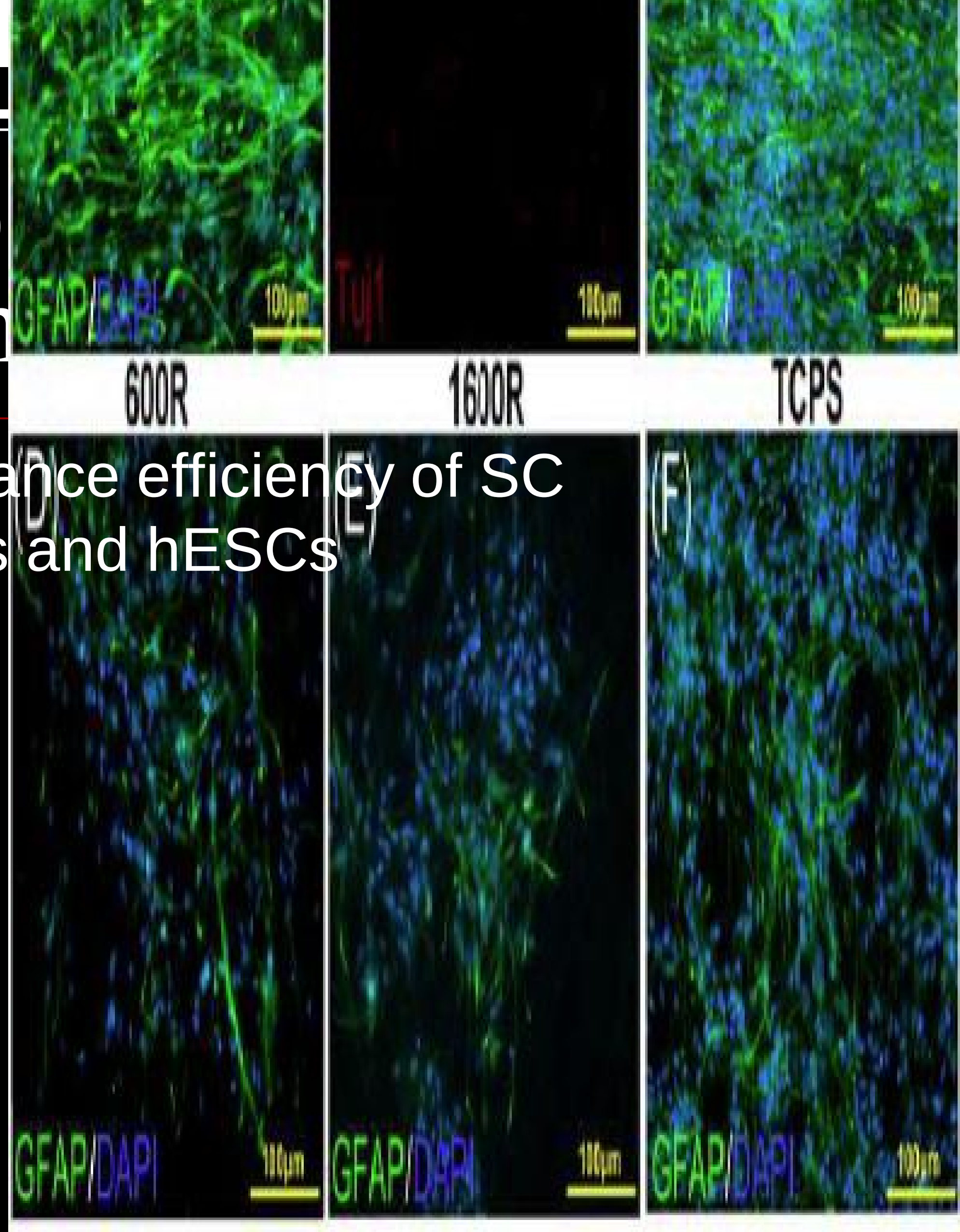
Cell-cycle proteins



(Wright etal Unpublished)

Aligned nanofibers enhance efficiency of SC
s and hESCs

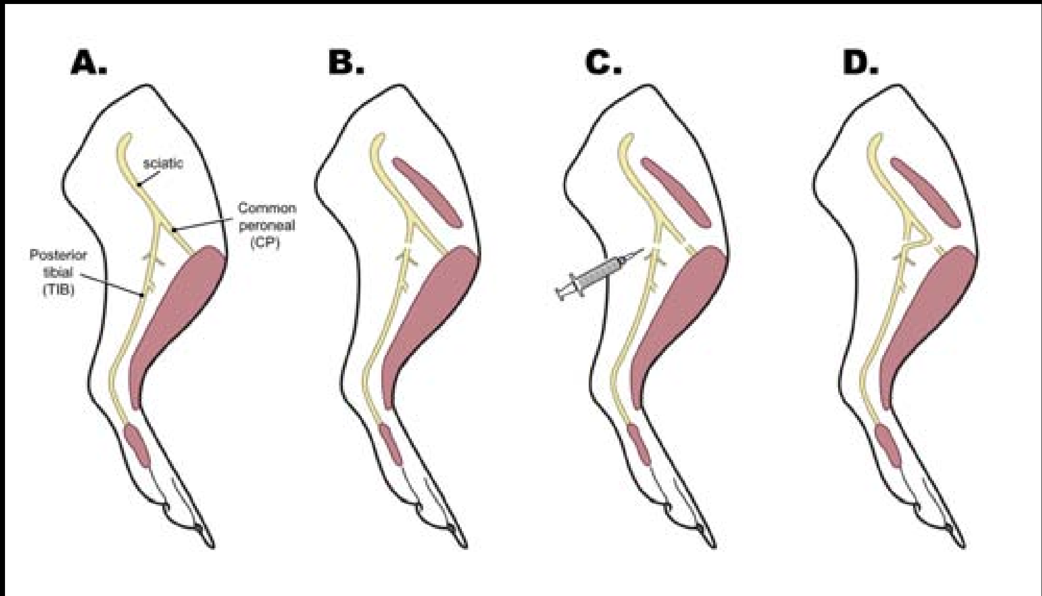


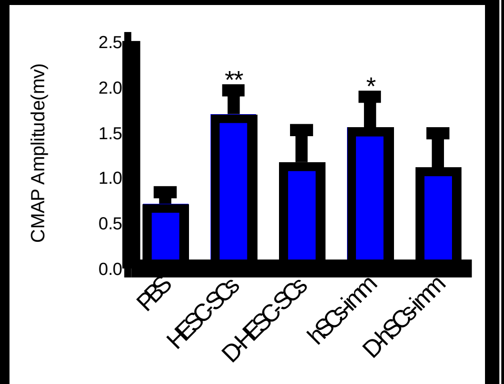


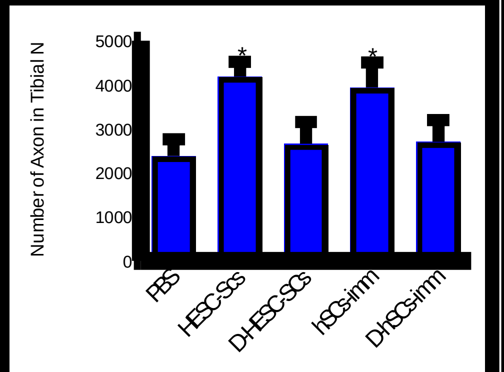
(Ren etal In Press)

Replacement of chronically denervated Schwann cells

- hESC-derived Schwann cells enhance regeneration in chronically denervated nerves







- But they do not survive

(Mi etal Unpublished)

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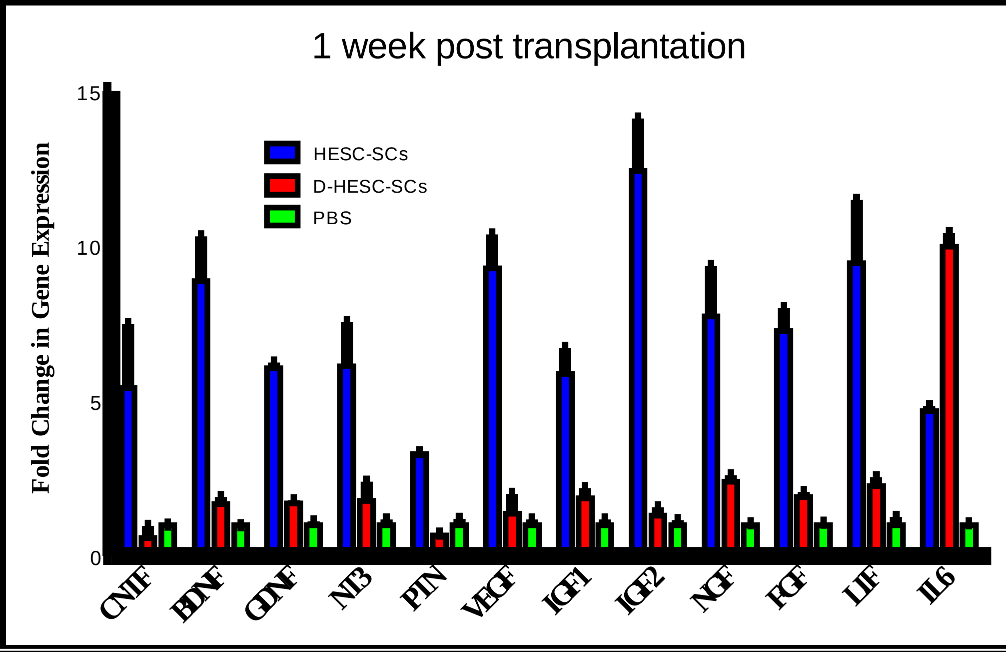
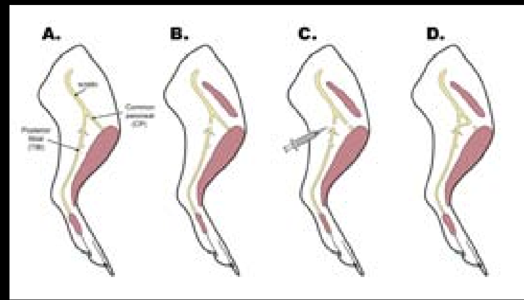
Replacement of chronically denervated Schwann cells

- hESC-derived Schwann cells enhance regeneration in chronically denervated nerves
- Increased secretion of NTFs



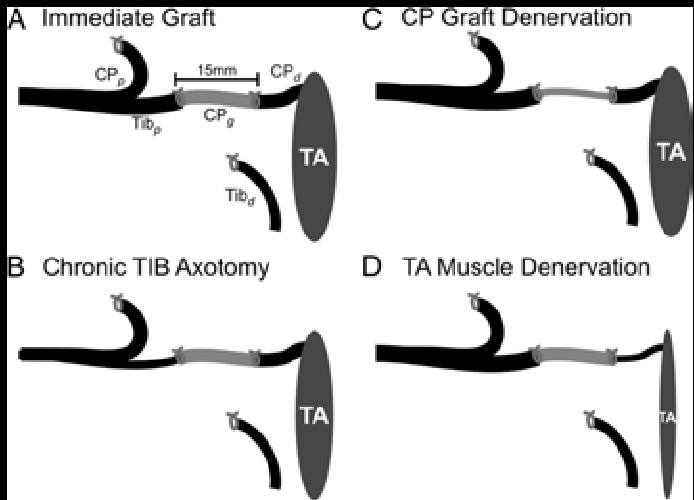


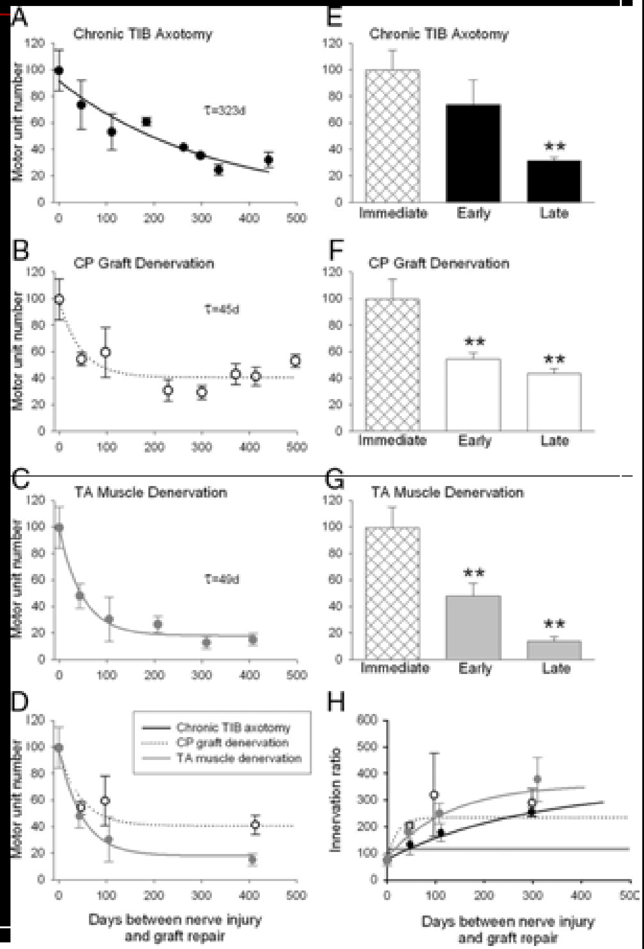
(Mi etal Unpublished)

Chronically denervated target: muscle

Even if we solve the issue of chronic denervation in Schwann cells, we still need to focus on changes at the NMJ and muscle

100-500 days of denervation



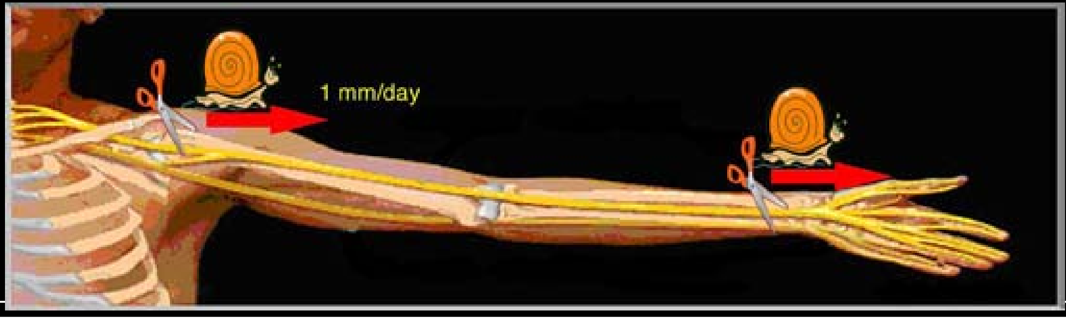


(Gordon etal J Neurosci 2011)

Challenges in translating to humans

Do we have the appropriate biomarkers, tools to assess regeneration?

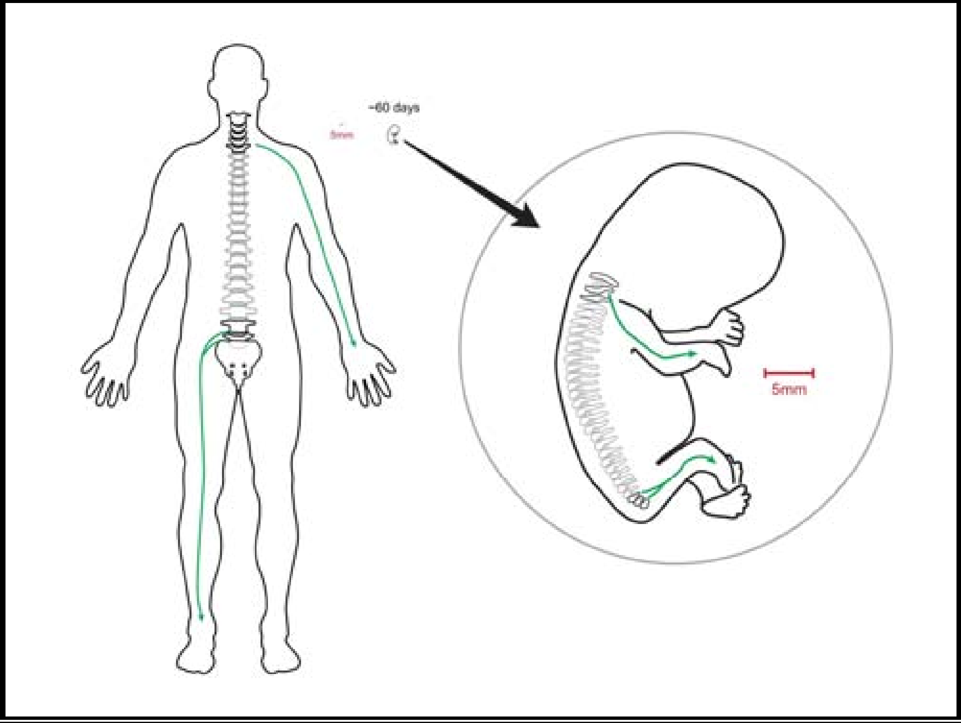
- Nerve regeneration in humans is slow!!!
  - Issue of distance and rate of nerve regeneration in humans
- We need validated biomarkers that can predict successful outcome before full recovery takes place
  - Novel human experimental assays
  - Imaging?



Challenges in translating to humans

Regeneration is not same as development

We need to make it faster and better



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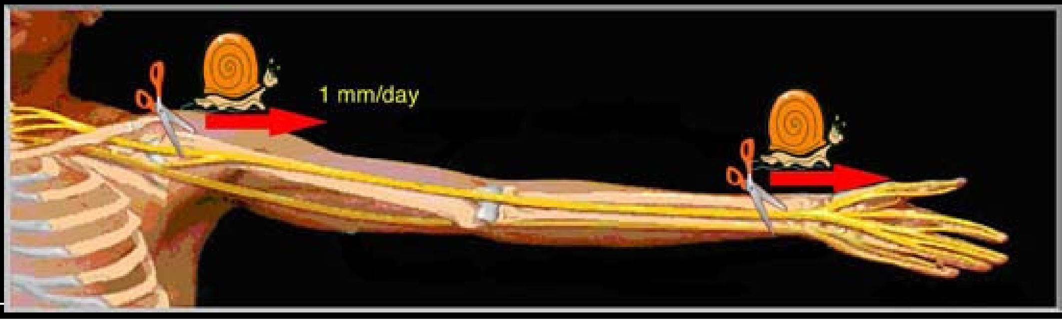
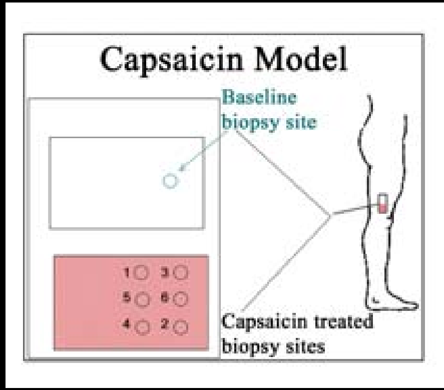
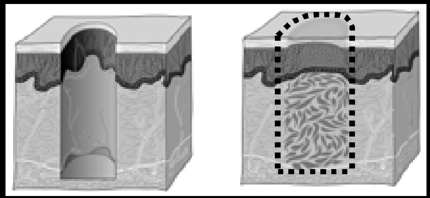
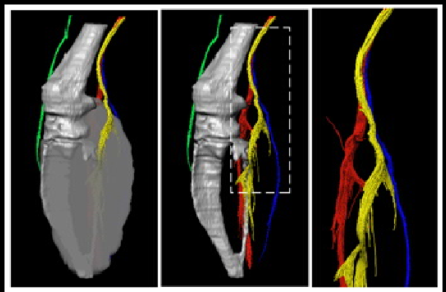


Biomarkers and tools to assess nerve regeneration

- Clinical trials in peripheral nerve injuries are costly:
  - Nerve regeneration in humans is slow
- Novel models of assessing nerve regeneration in humans
- Novel imaging techniques?
  - Diffusion Tensor Imaging







Polydefkis etal 2004, Rajan etal 2003, Zhang etal 2008

Thanks

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