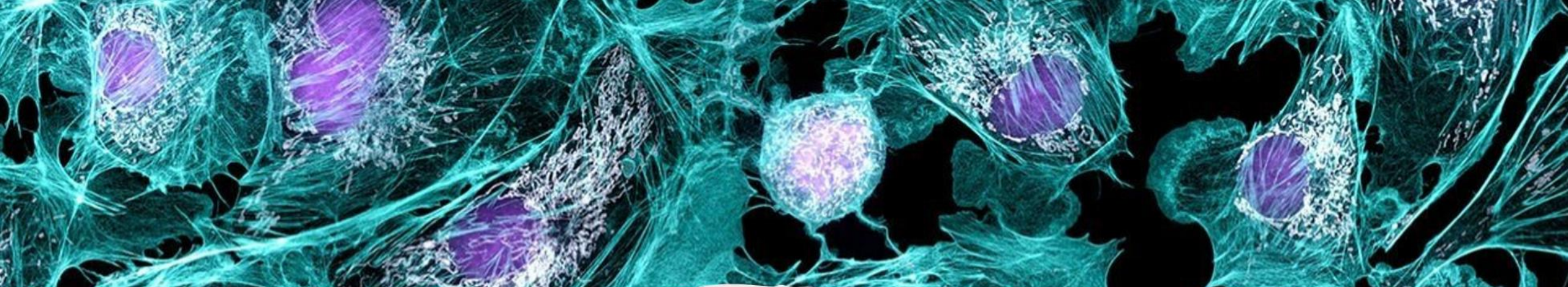
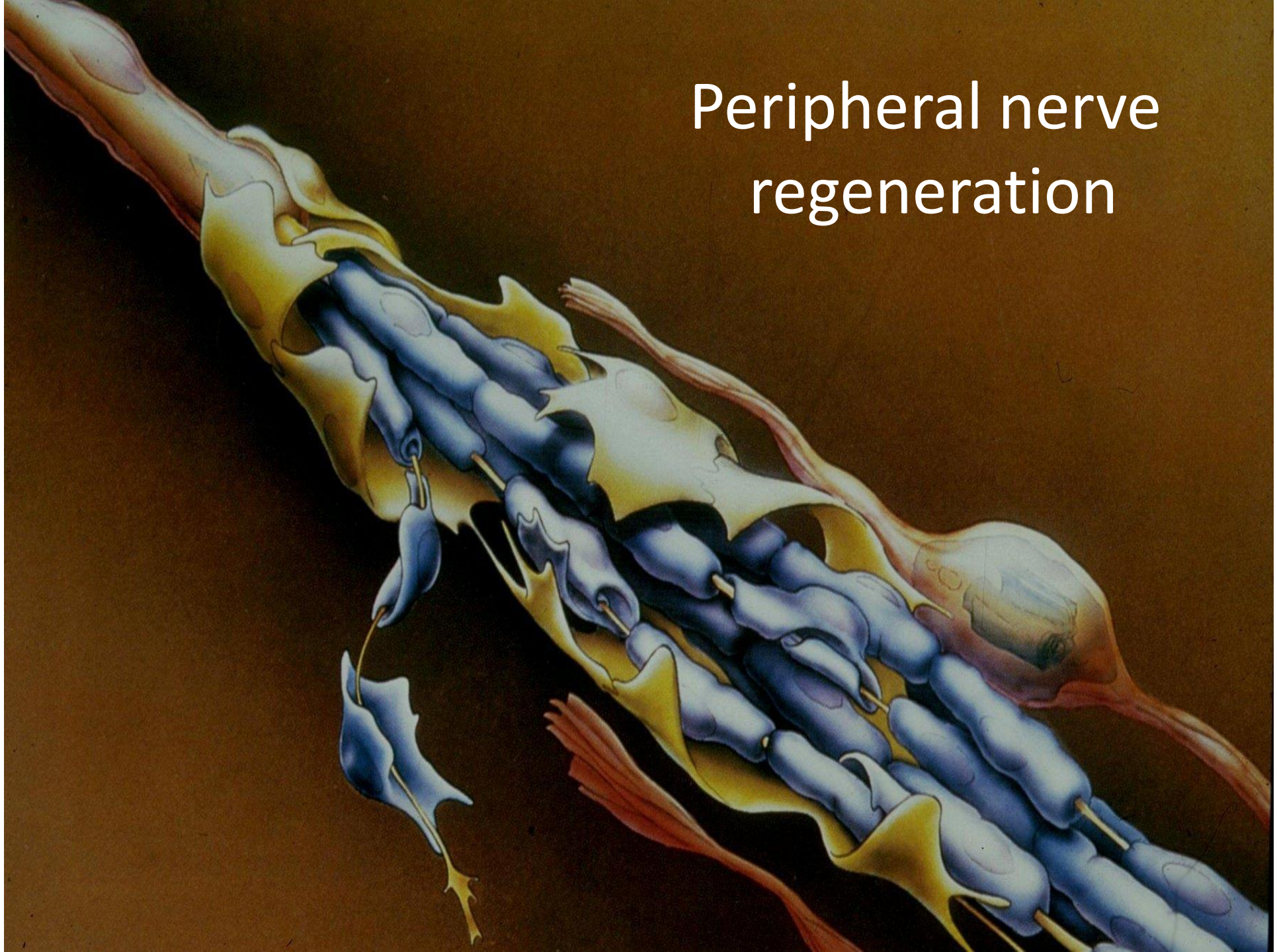


Advanced Cell Biology and Biotechnology

ACBB 2021/22

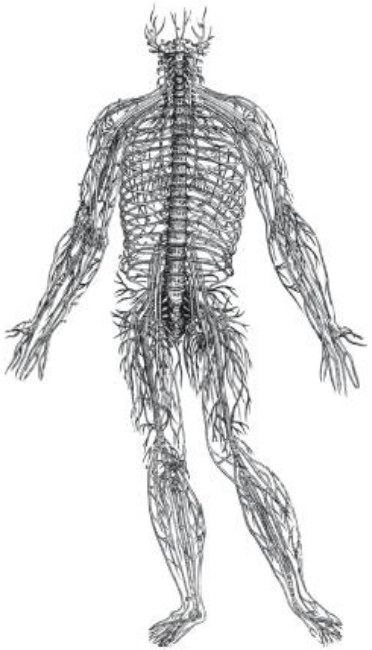
…the lecture of December 20th is about to begin…

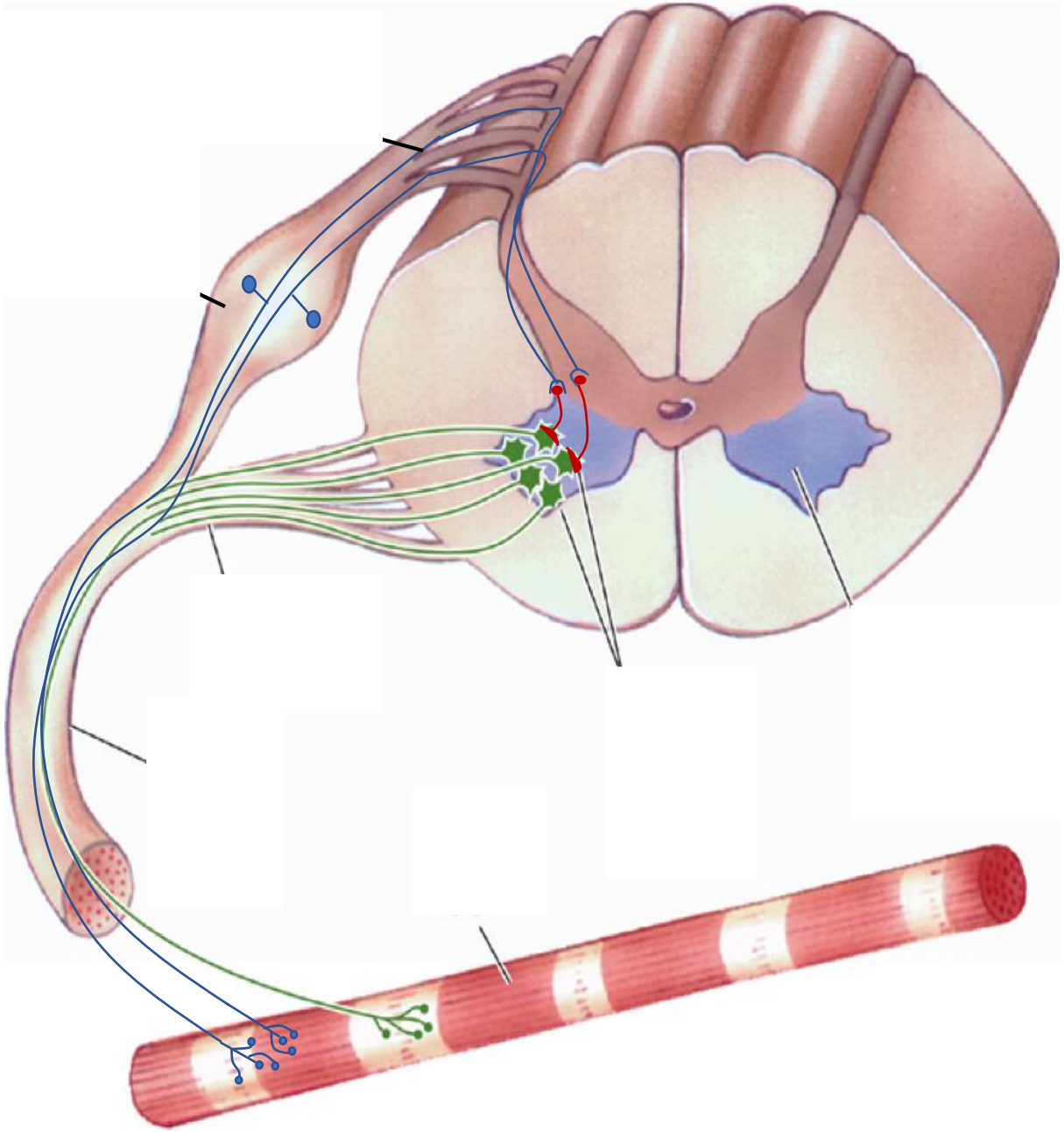

Peripheral nerve
regeneration

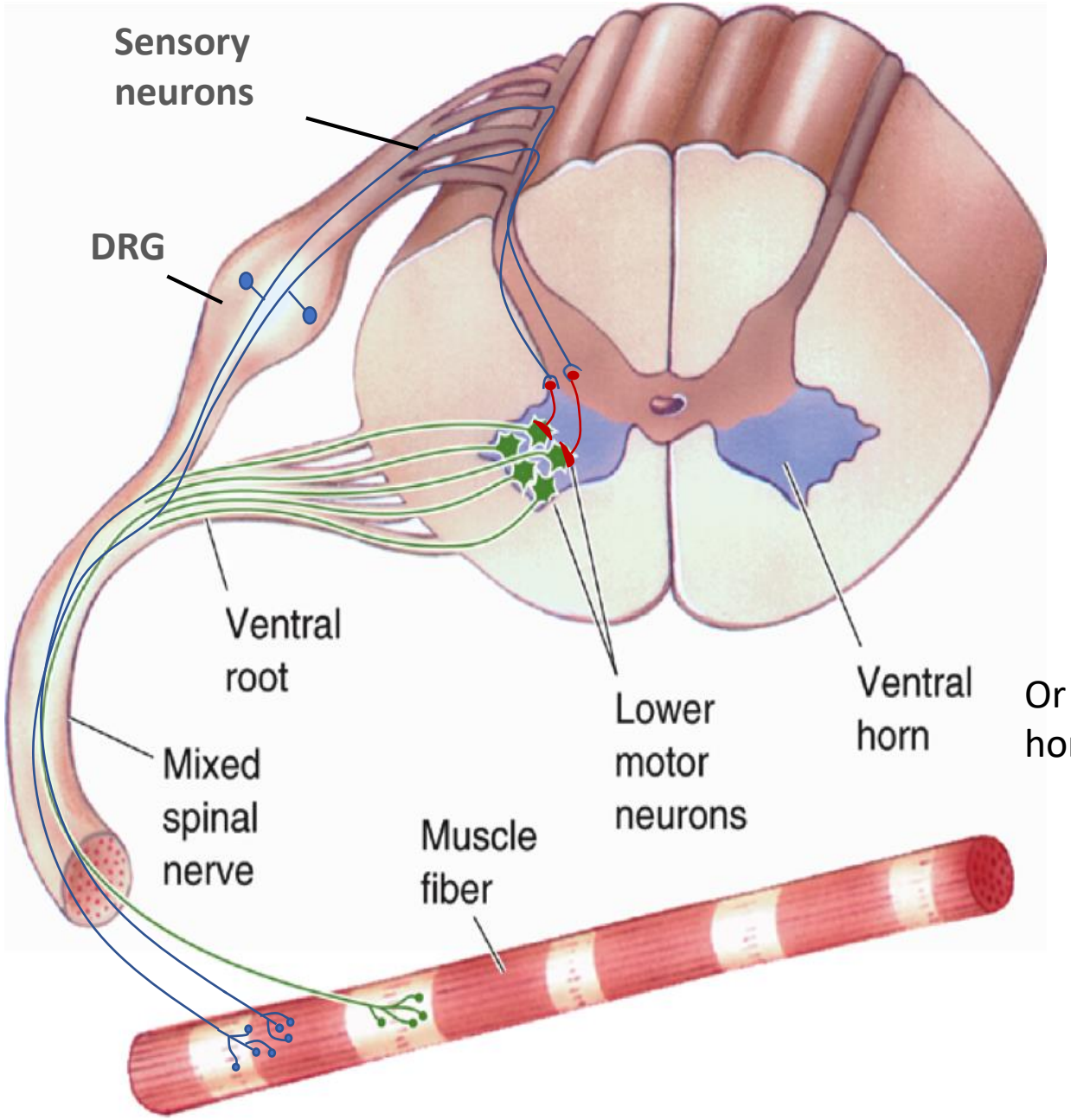
Peripheral nerve injuries

The **nerve fibers** in the Peripheral Nervous System, are **frequently subject to traumas and diseases**.

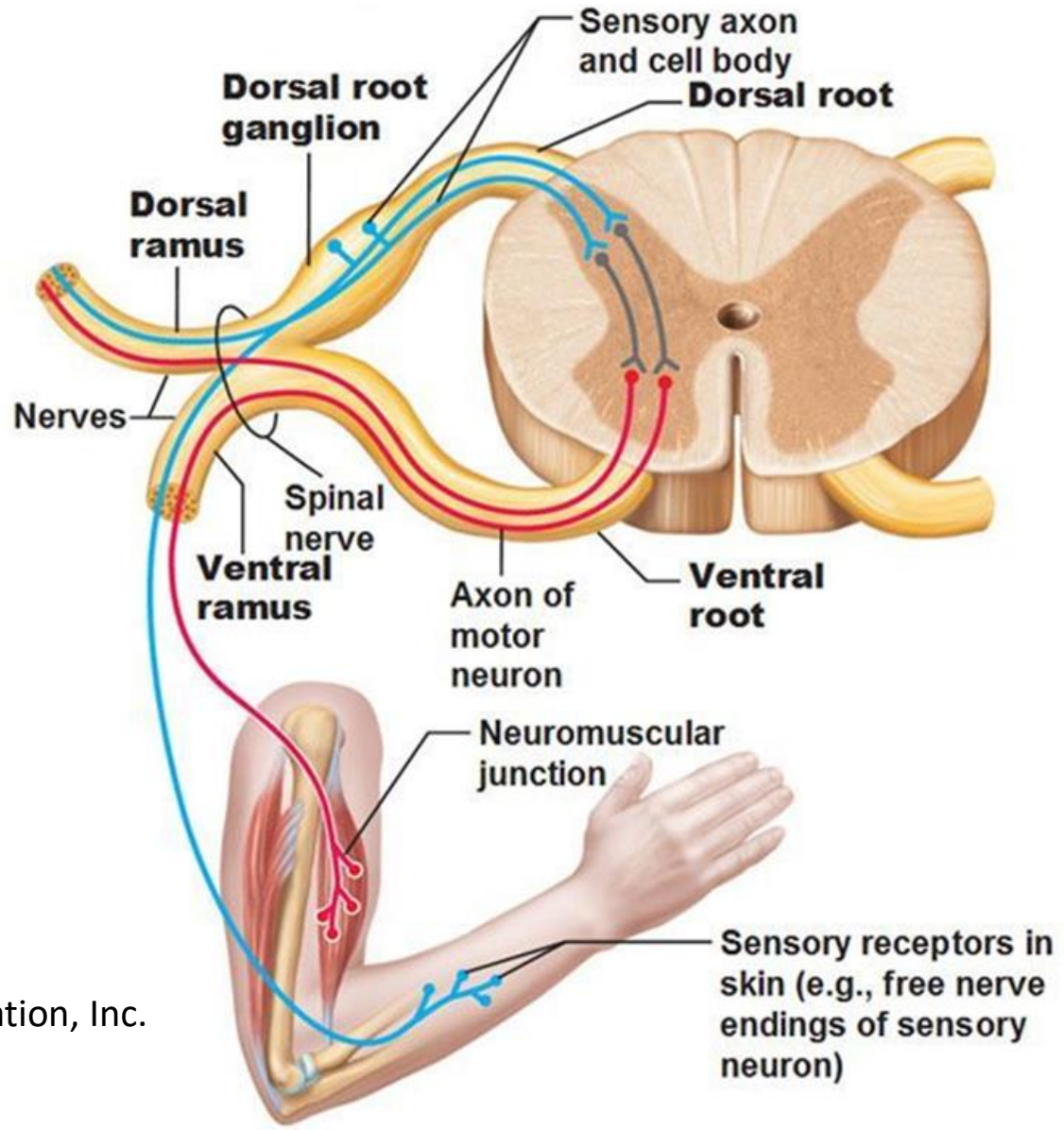
Peripheral nerve lesions are 5 times more frequent than spinal cord lesions and **lead to a decreased or a complete loss of sensitivity and/or motor activity**.

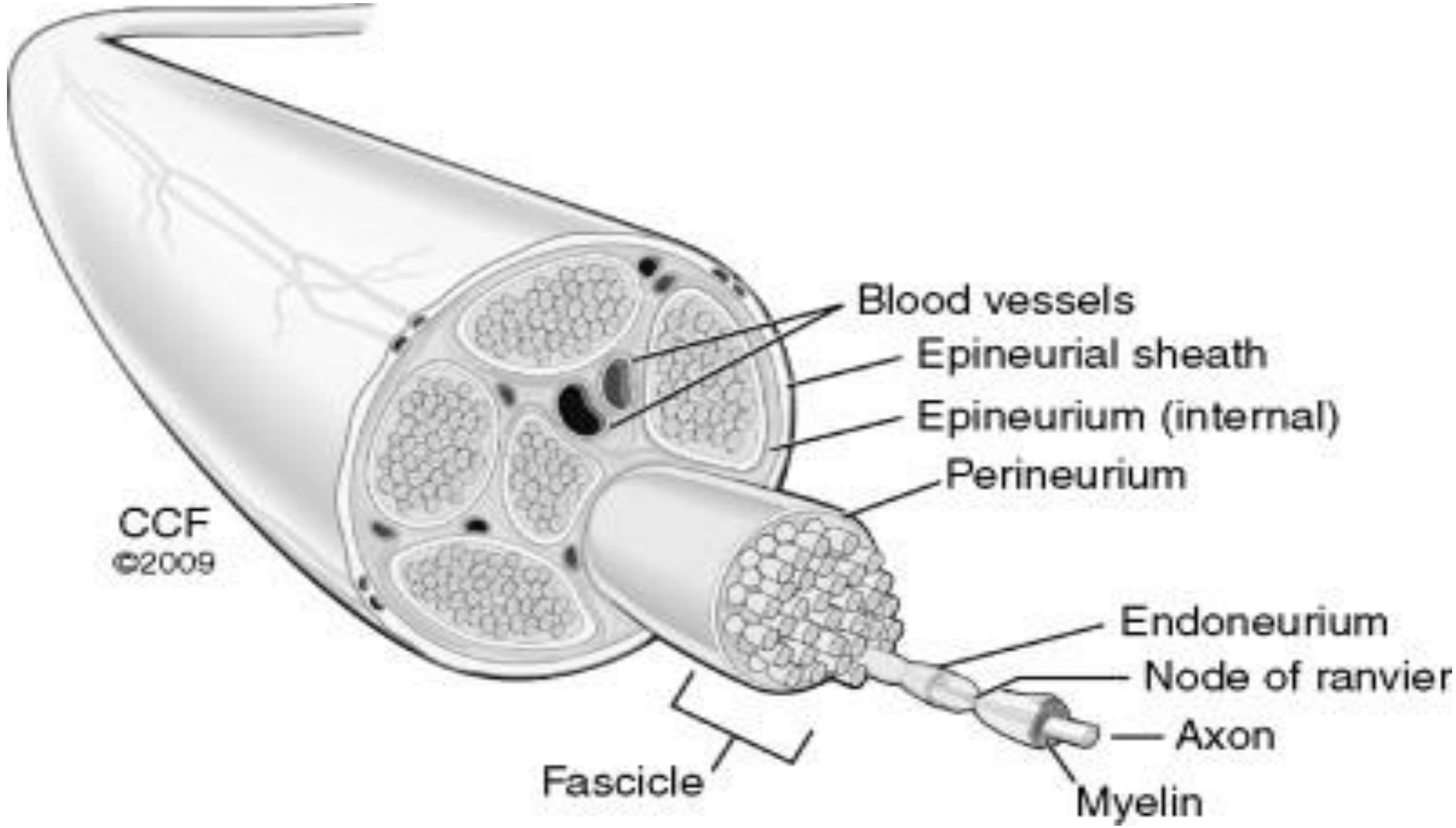





Sensory neurons
DRG
Ventral root
Mixed spinal nerve
Muscle fiber
Lower motor neurons
Ventral horn
spinal cord
Or Anterior horn
Copyright © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins

Peripheral nerves




Blood vessels
Epineurial sheath
Epineurium (internal)
Perineurium
CCF
©2009
Endoneurium
Node of ranvier
Axon
Fascicle
Myelin

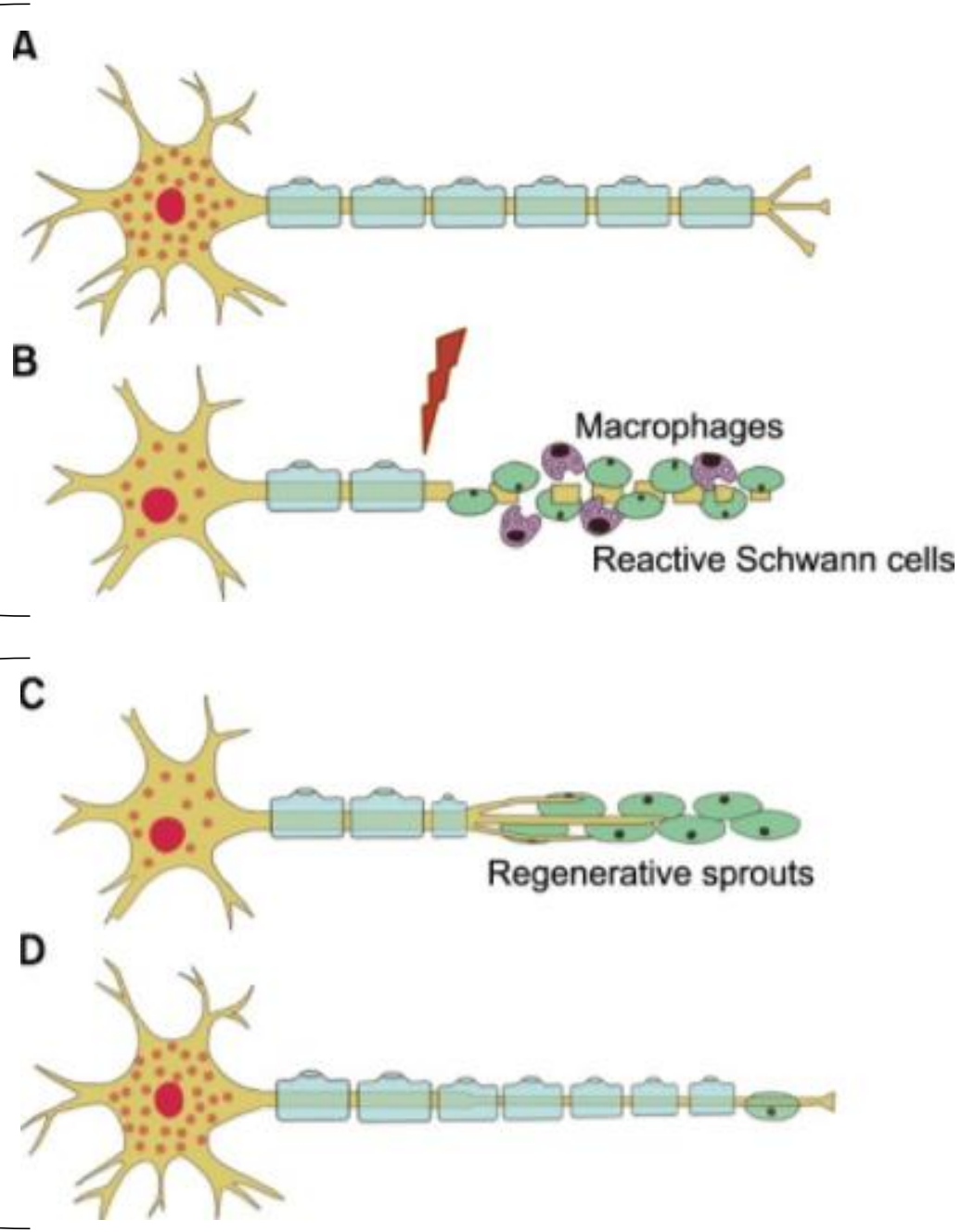
Injury and immediate regeneration

1 - DEGENERATION

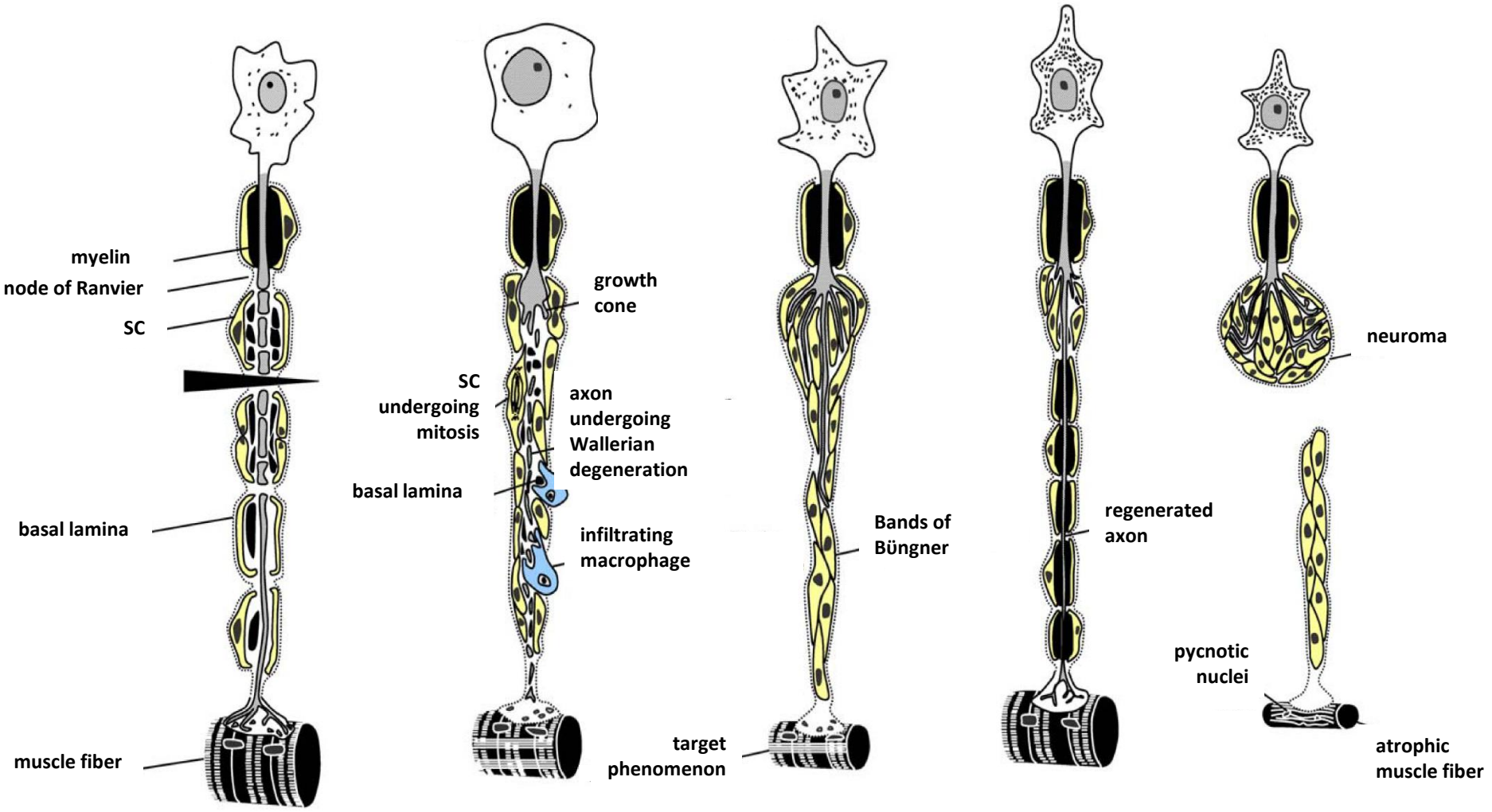
- Axonal and myelin degeneration,
- Schwann cell and macrophage activation distal to the injury

2 - REGENERATION

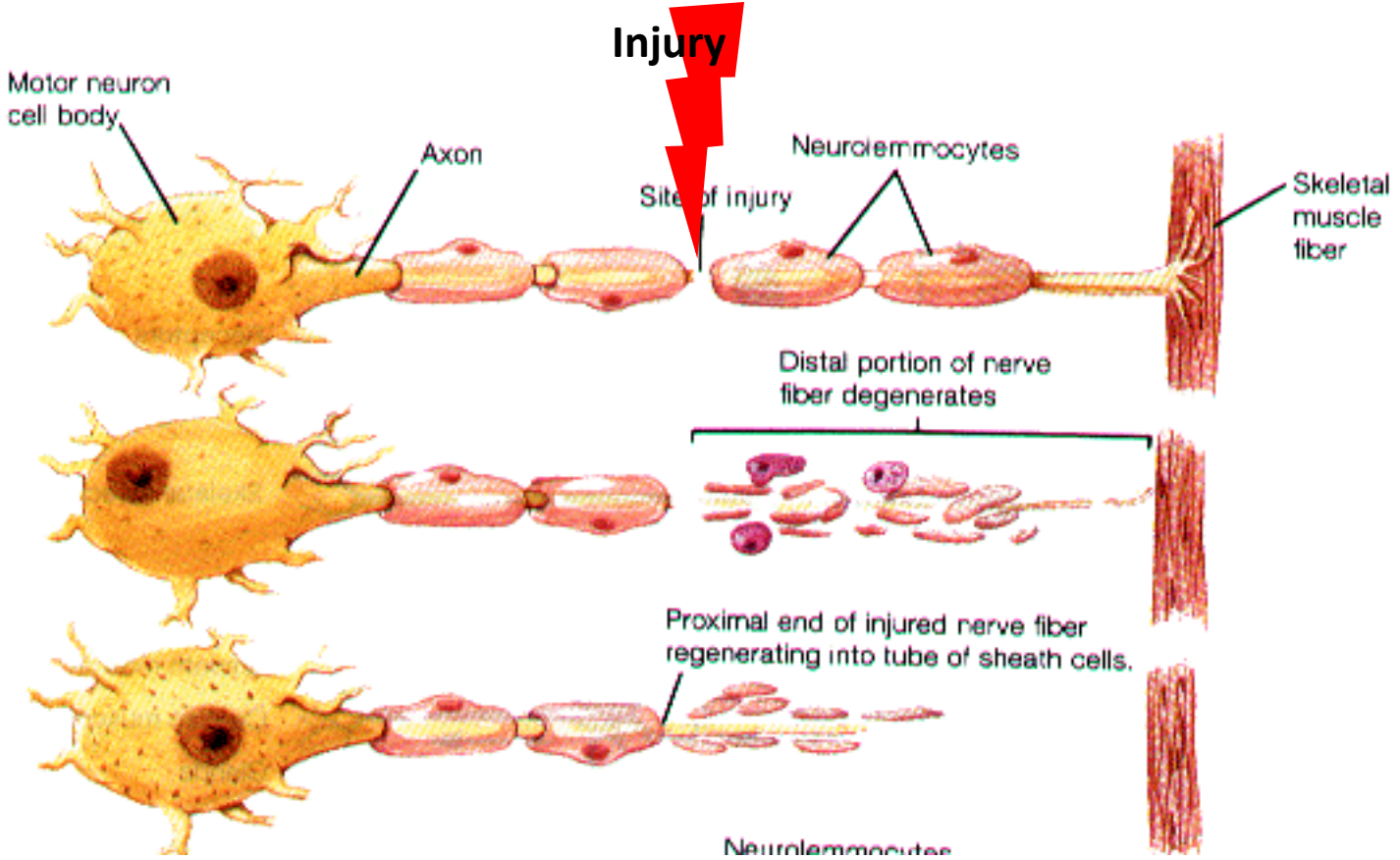
- Schwann cell alignment (bands of Bungner) to guide axonal regeneration and myelin reconstitution



Degenerative & regenerative events following peripheral nerve lesion



(Deumens, Bozkurt et al. 2010)

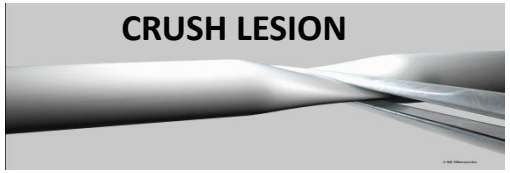


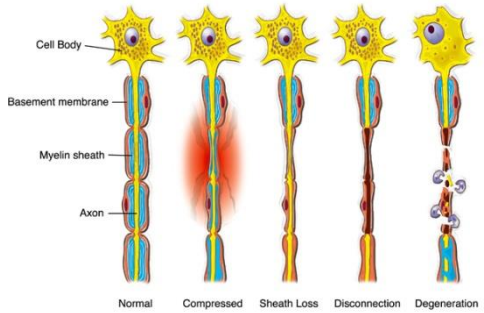
- even if peripheral nerve fibres retain a considerable **regeneration potential** also in the adulthood, **recovery** can be **poor**

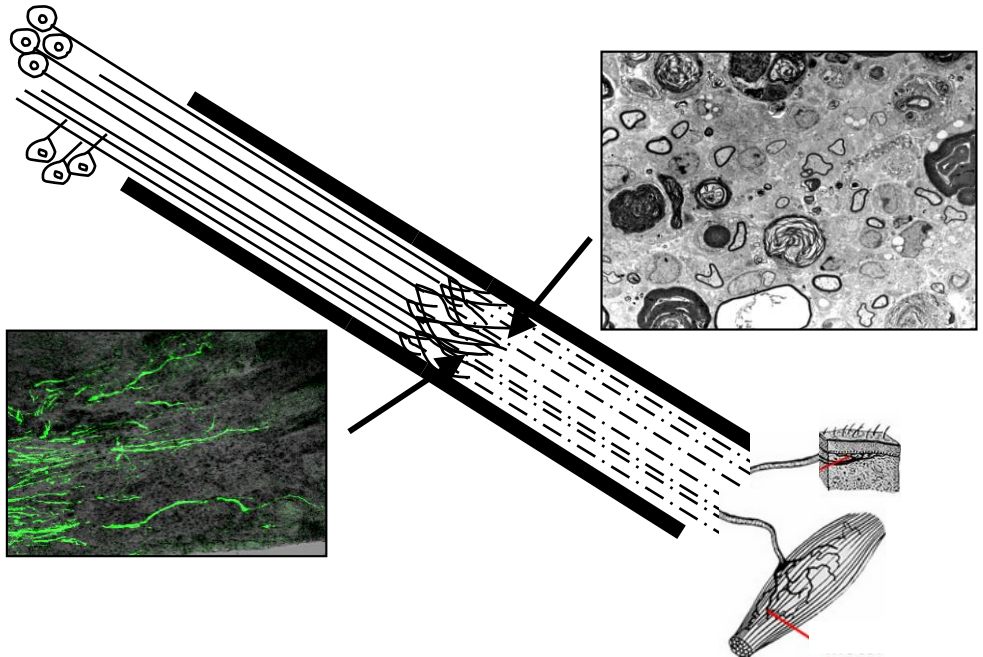
- recovery after peripheral nerve injury depends on a variety of **critical factors**

CRITICAL FACTORS: 1-severity of injury

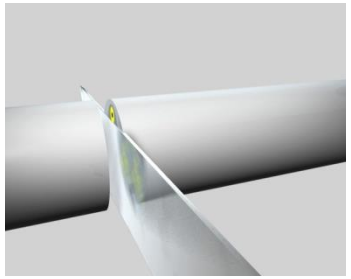
AXONOTMESIS

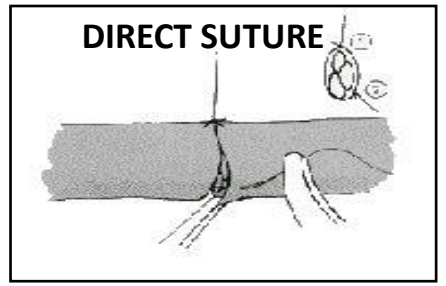






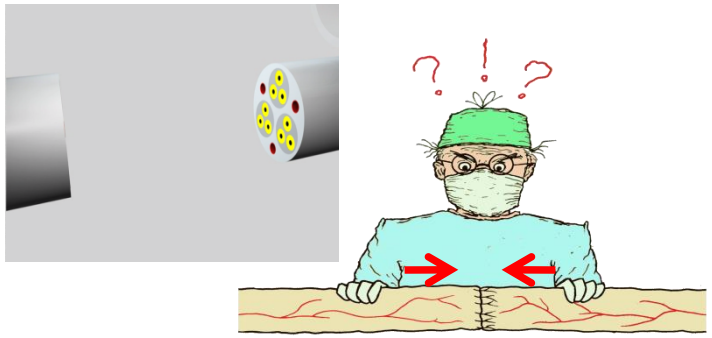
NEUROTMESIS

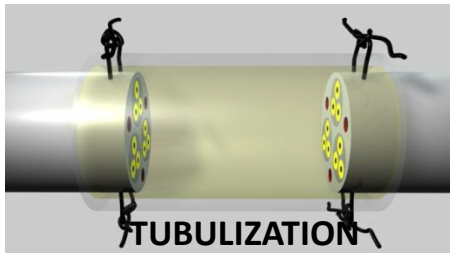




LESION WITHOUT

OR WITH SUBTANCE LOSS



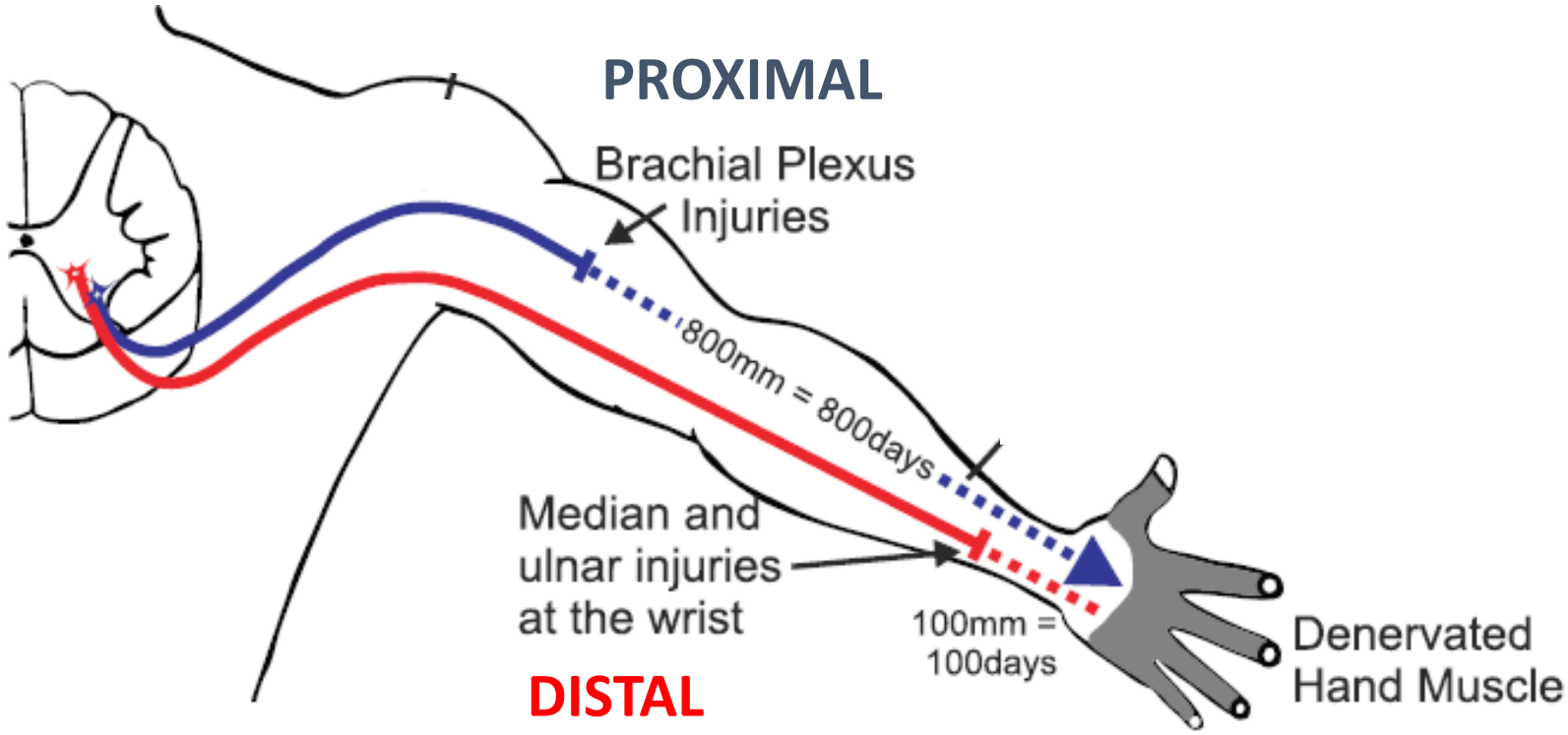


Longer is the gap more difficult is the regeneration and poorer the recovery.

CRITICAL FACTORS: 2-Location of the injury

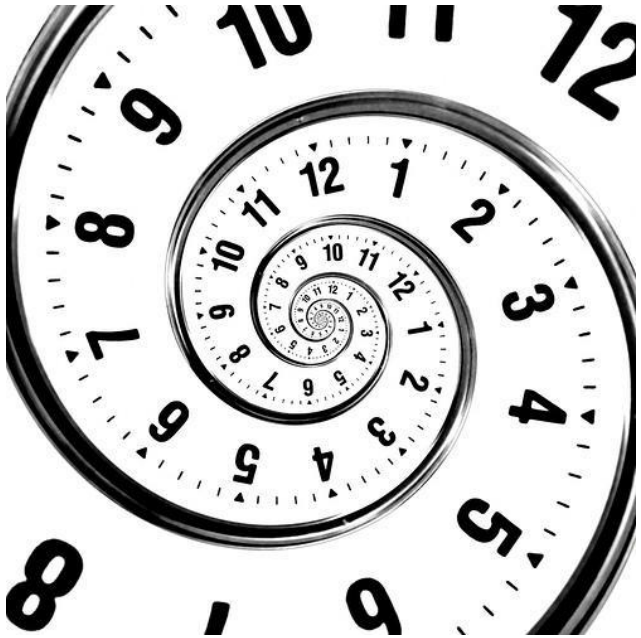
Distance to the target organs:

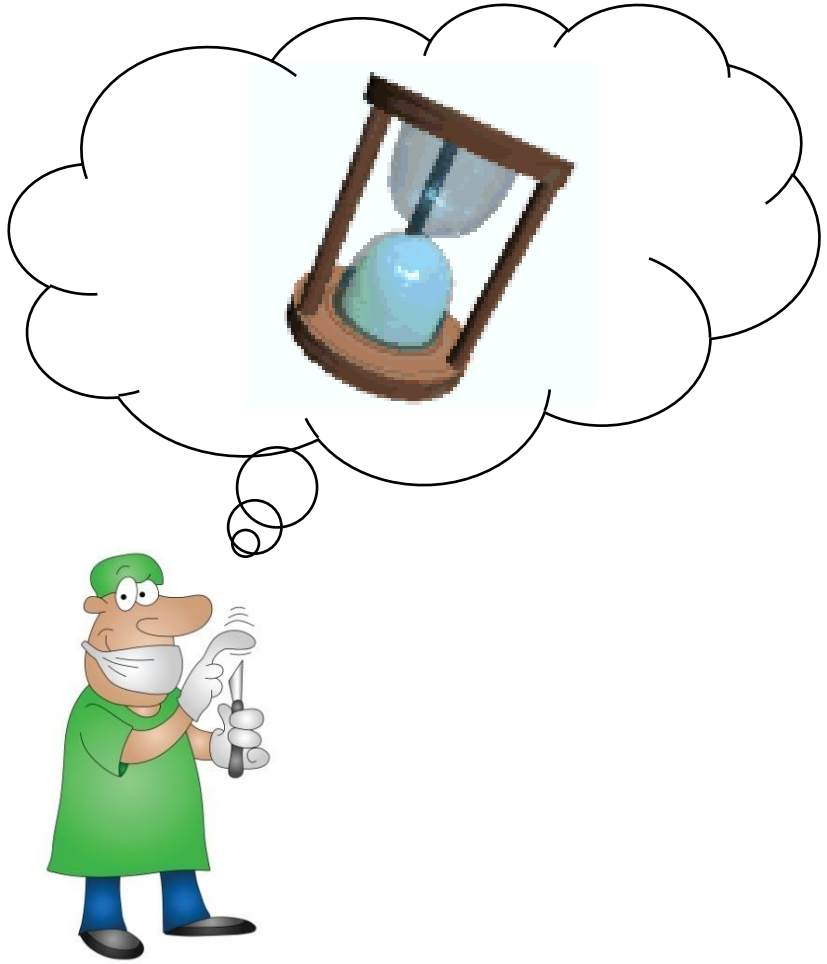
Nerve fibres reach the target organs before if the lesion is more distal.



CRITICAL FACTORS: 3-Delay of the reconstruction

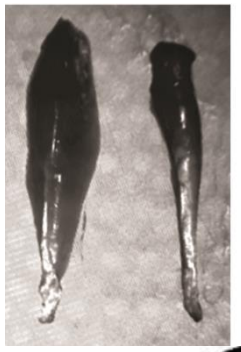
Length of time that passes before surgical repair is performed.





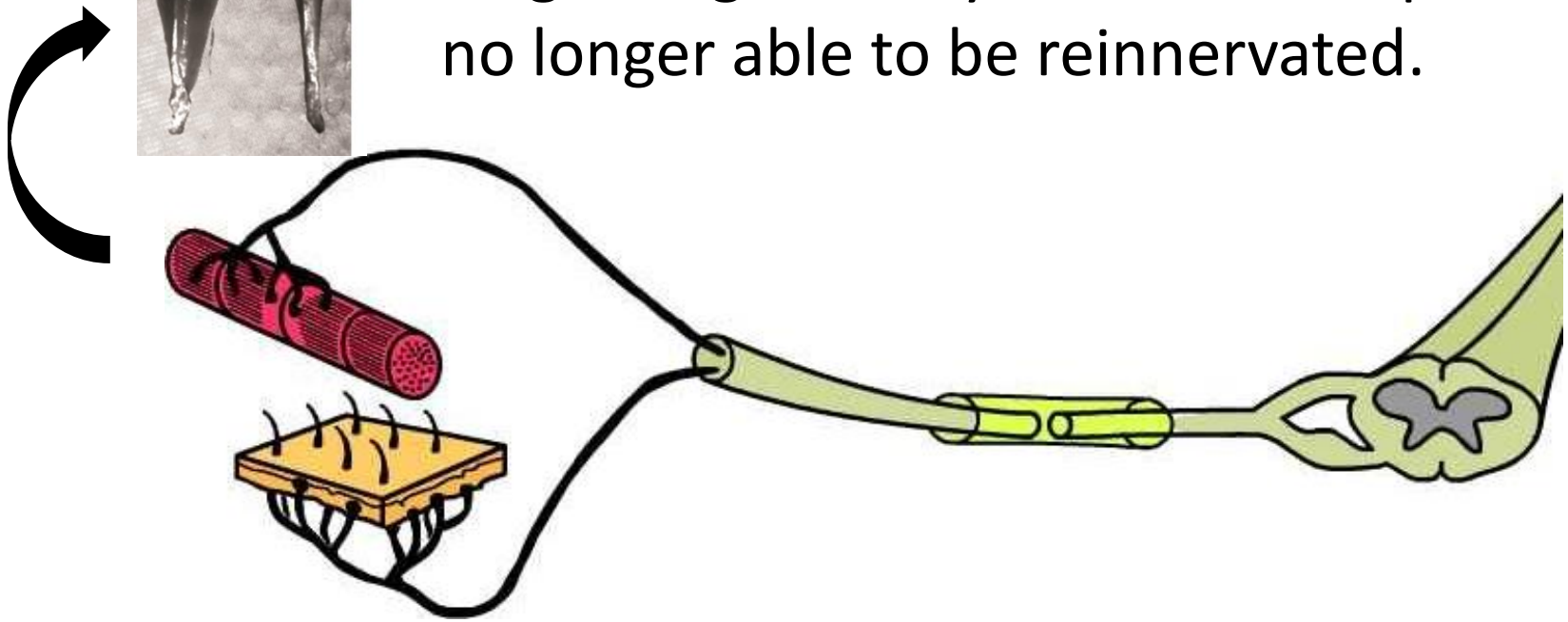
CRITICAL FACTORS: 4-Atrophy of the target organ

Both proximal lesions (that need a longer time of regeneration) and delay of the reconstruction can affect the ability of target organs to recover after nerve injury.



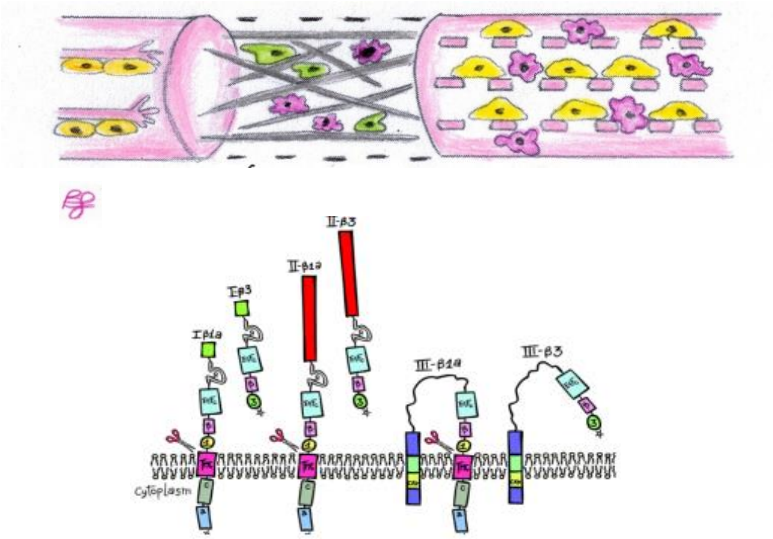
MUSCLES

Target organs may become atrophic and no longer able to be reinnervated.



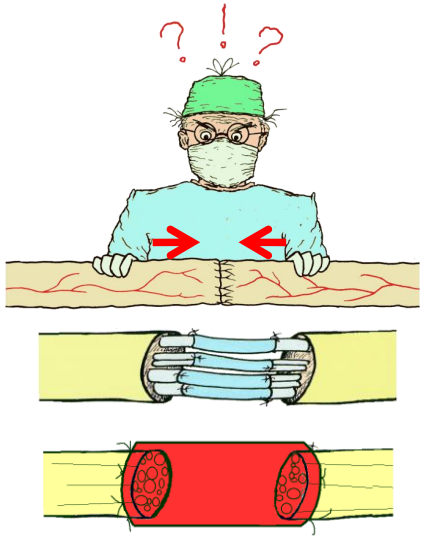
OUR RESEARCH

BASIC RESEARCH



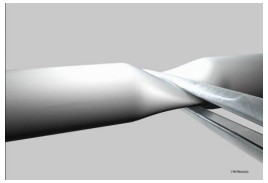
Study of the biological mechanisms involved in peripheral nerve injuries and regeneration.

APPLIED RESEARCH



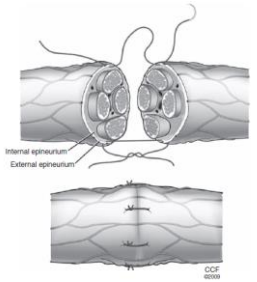
Study of peripheral nerve repair techniques and strategies for the improvement of peripheral nerve regeneration.

Axonotmesis (crush)
- mild nerve lesion -

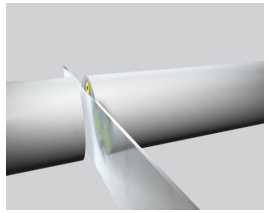


Nerve injury

gap < 2 mm — end-to-end repair



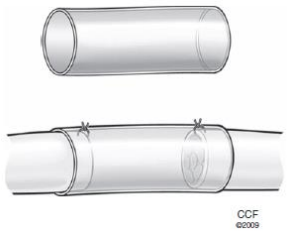
Neurotmesis (cut)
- severe nerve lesion -



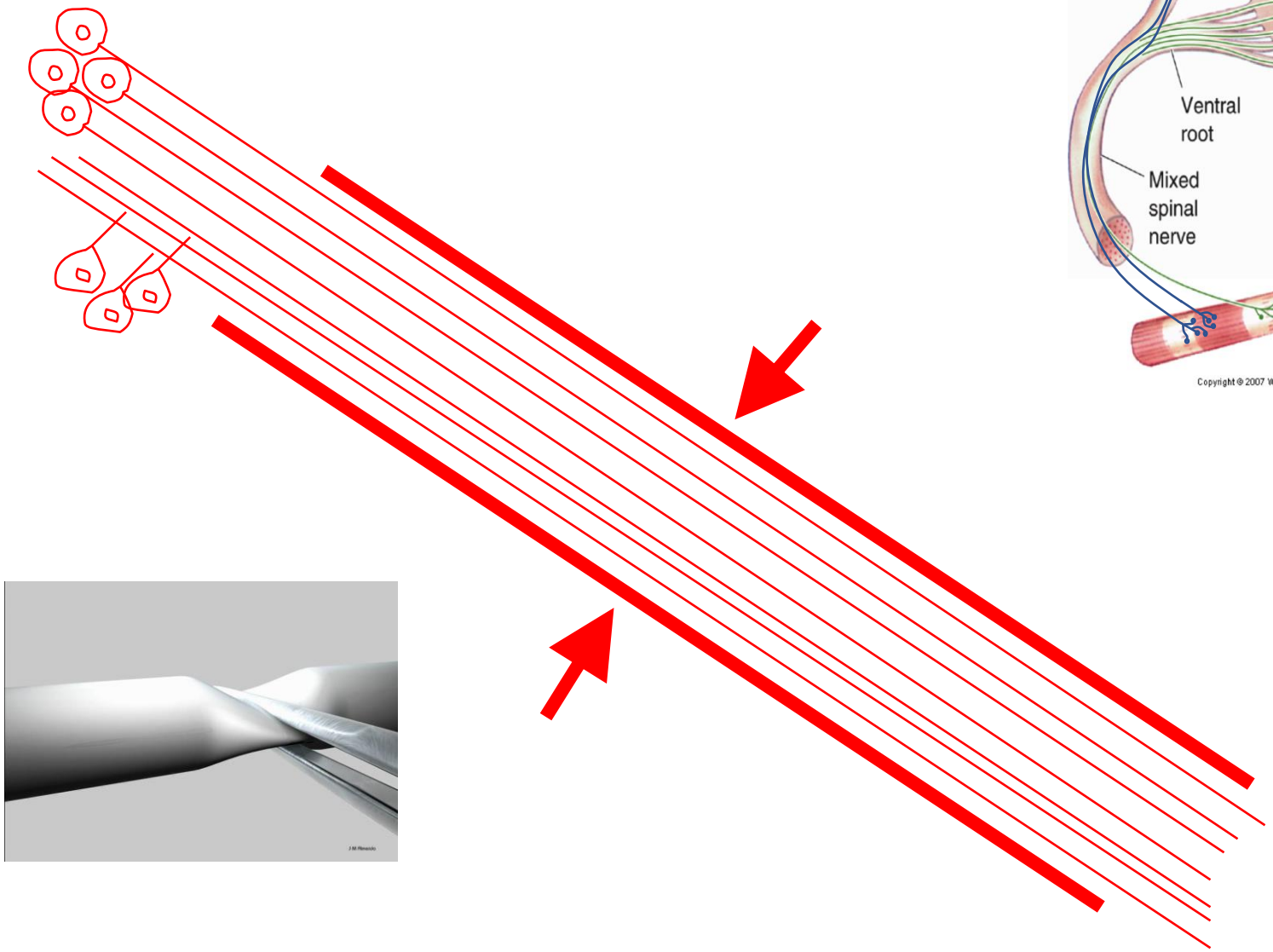
gap > 2 mm

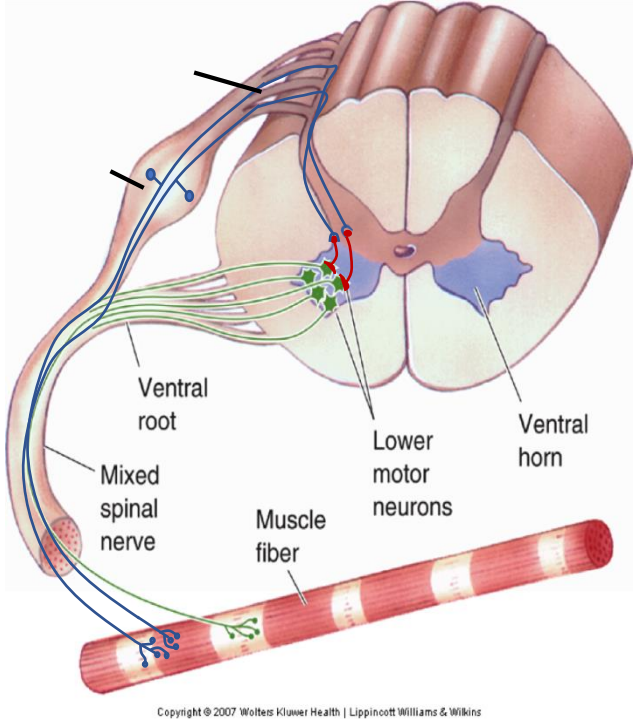
autologous graft

artificial graft

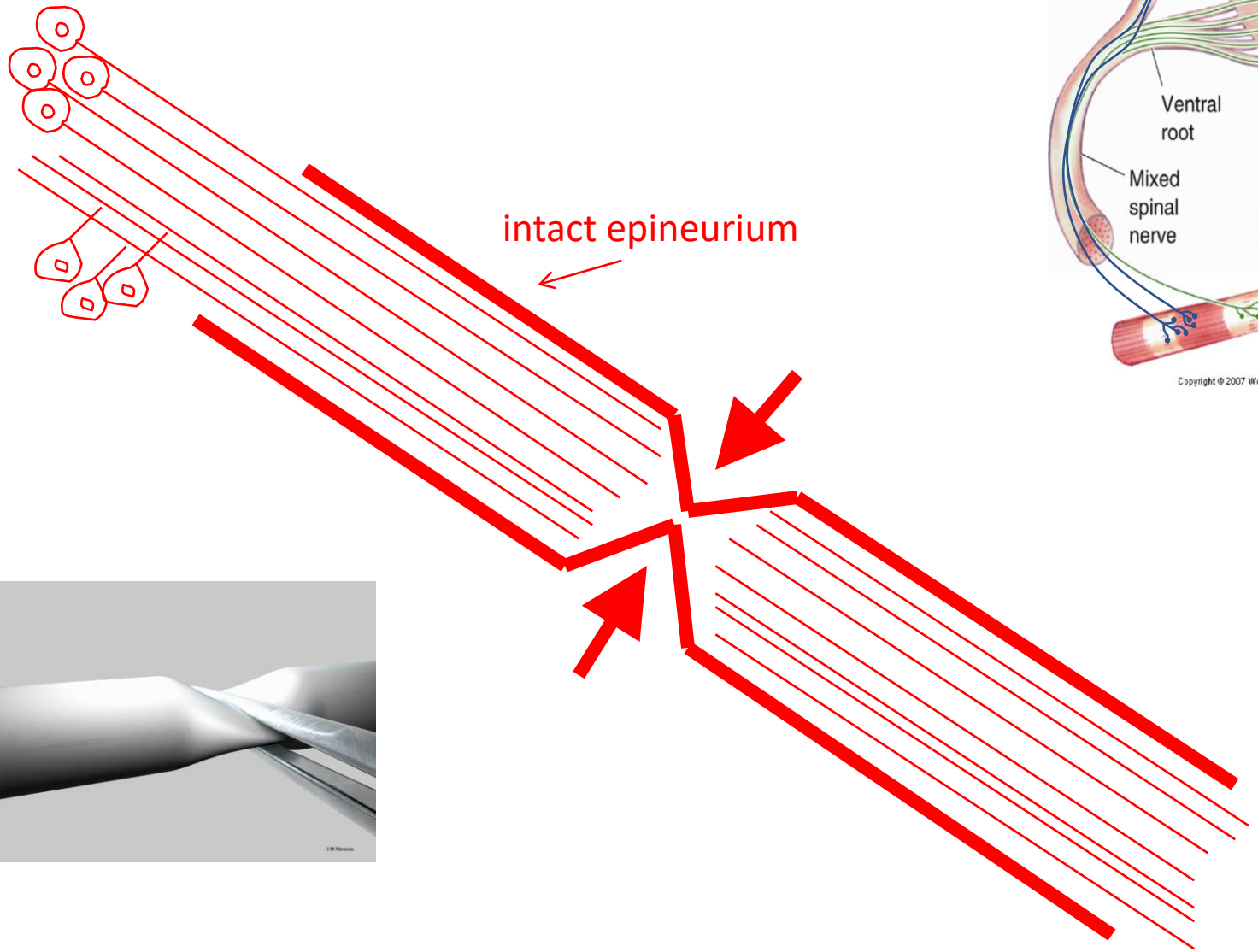


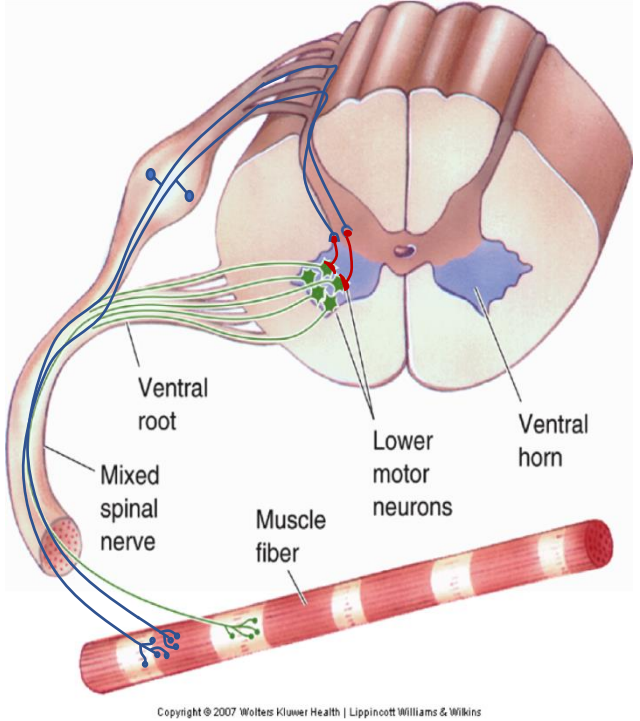
Axonotmesis (crush)
- mild nerve lesion -



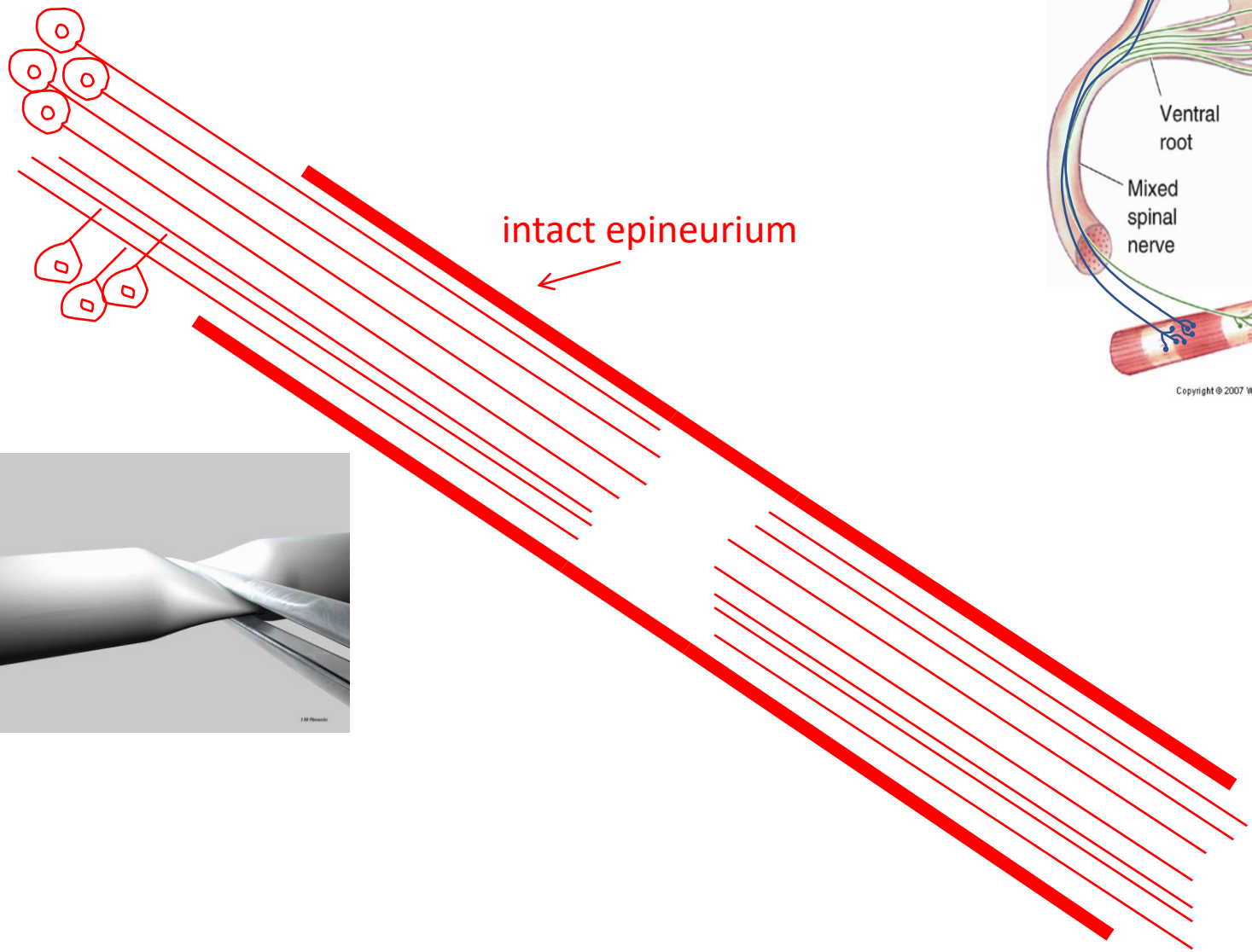


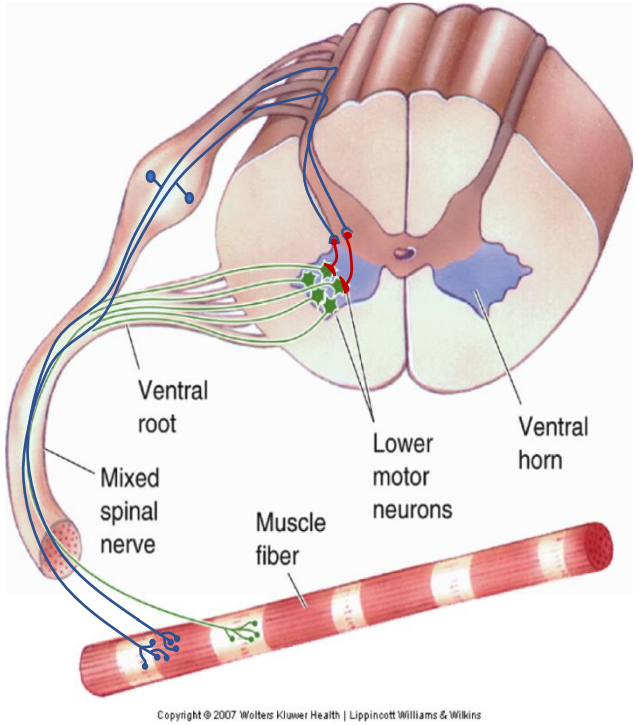
Axonotmesis (crush)



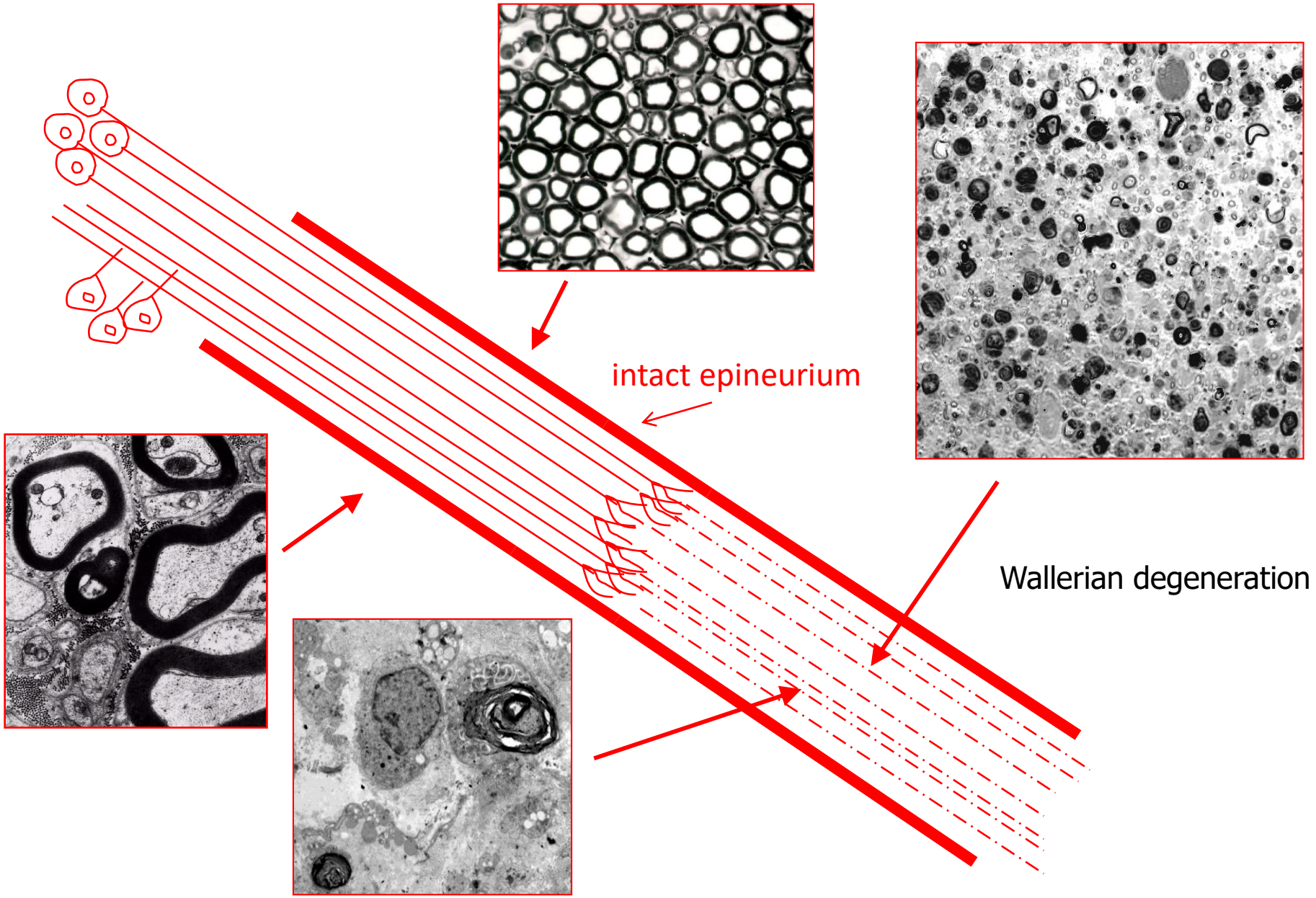


Axonotmesis (crush)

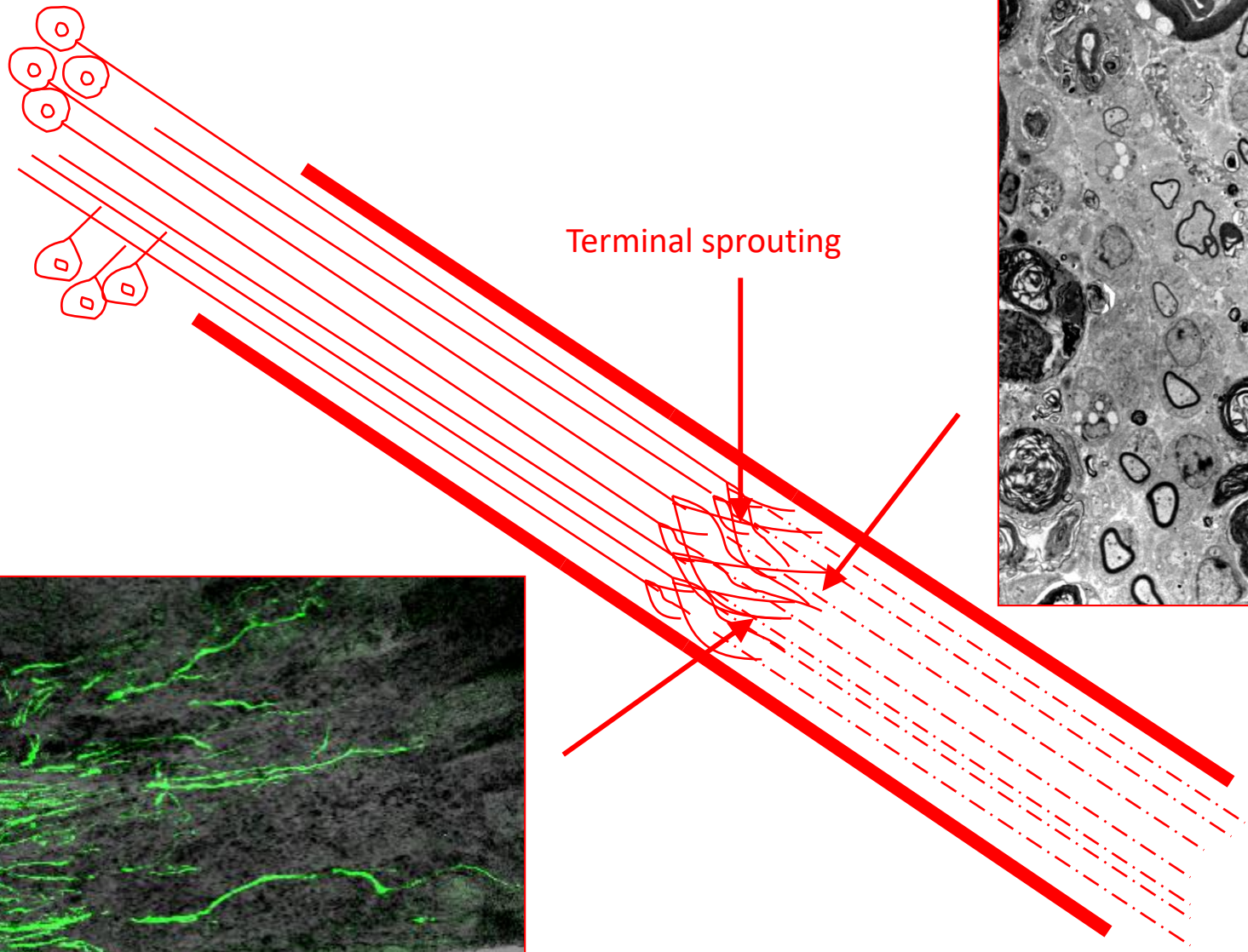


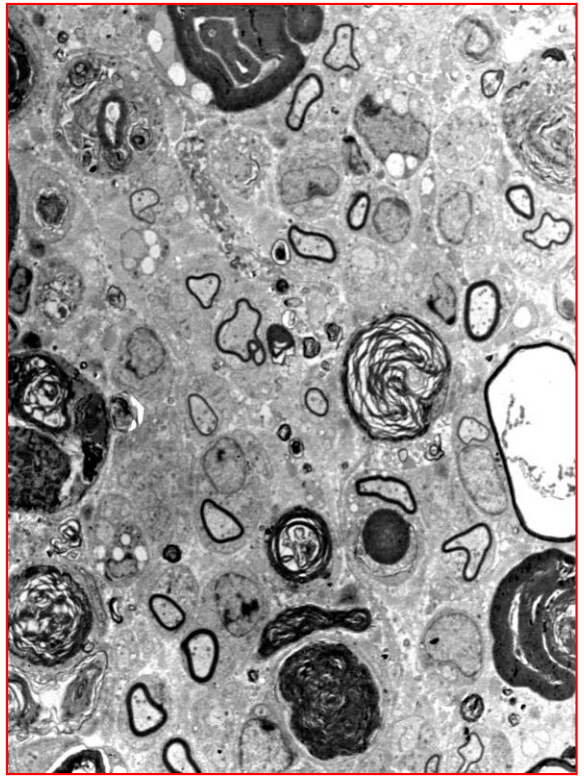


Axonotmesis (crush)

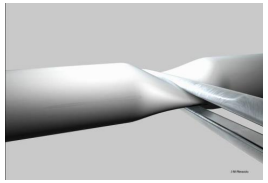


Axonotmesis (crush)



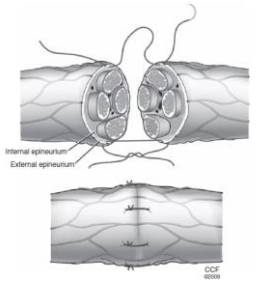


Axonotmesis (crush)
- mild nerve lesion -

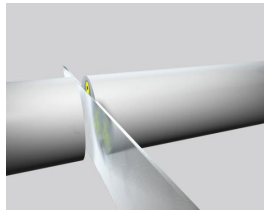


Nerve injury

gap < 2 mm — end-to-end repair



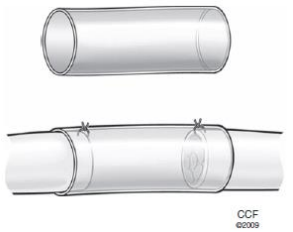
Neurotmesis (cut)
- severe nerve lesion -



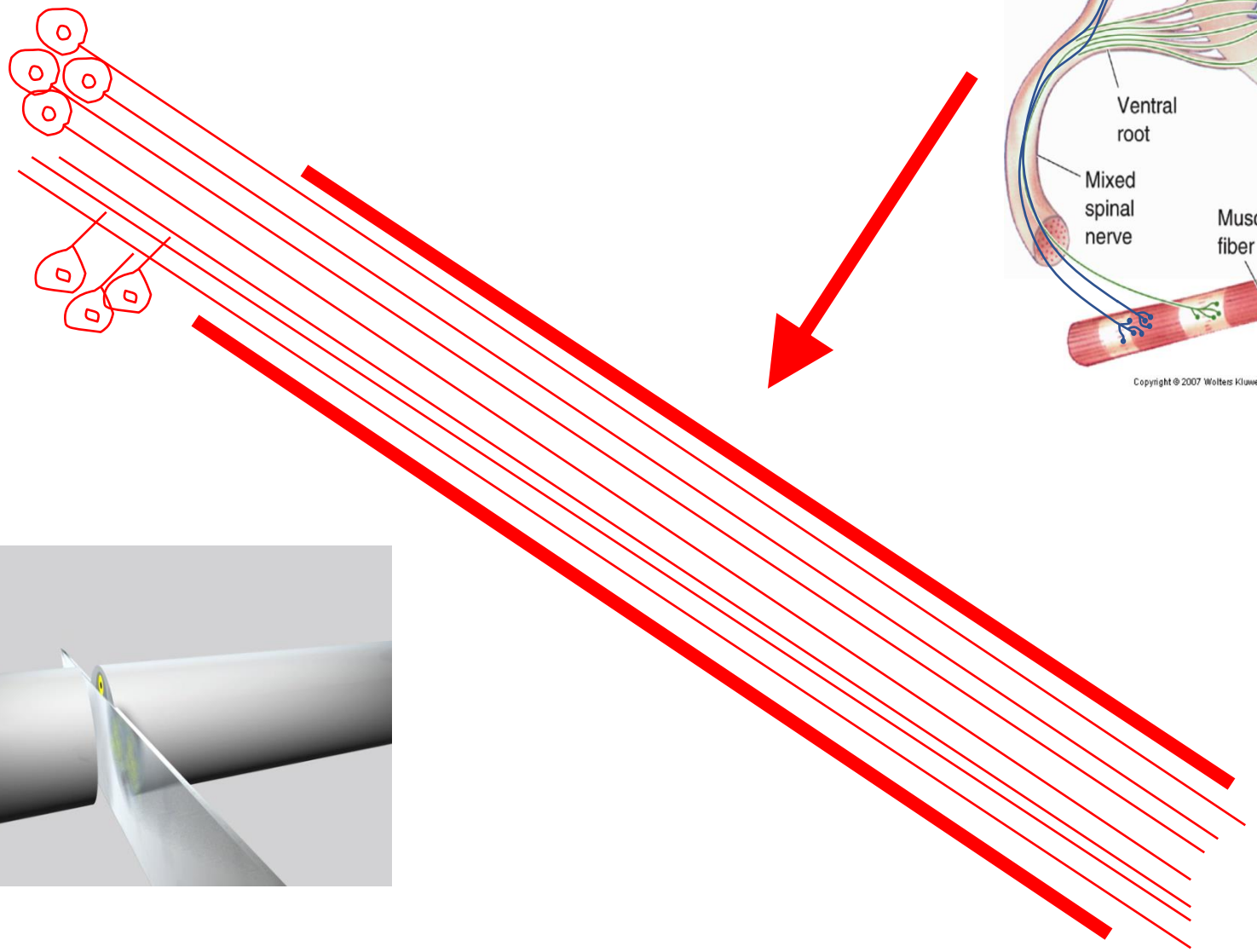
gap > 2 mm

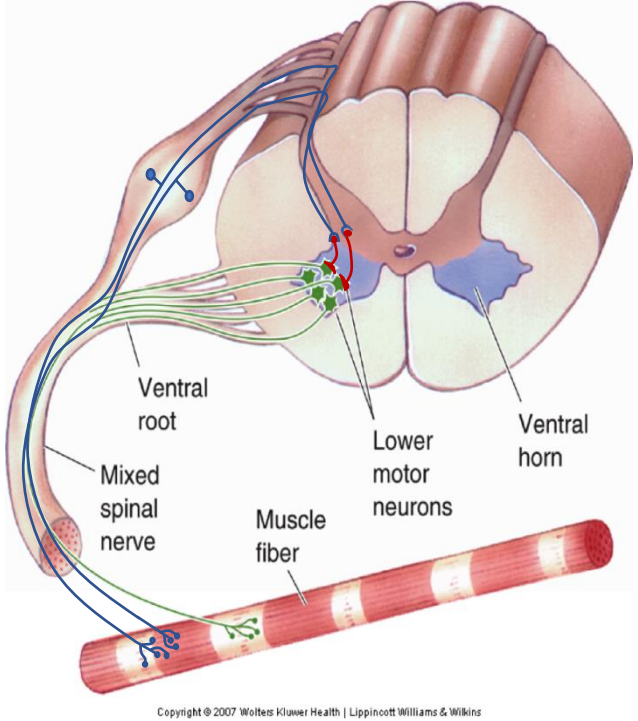
autologous graft

artificial graft

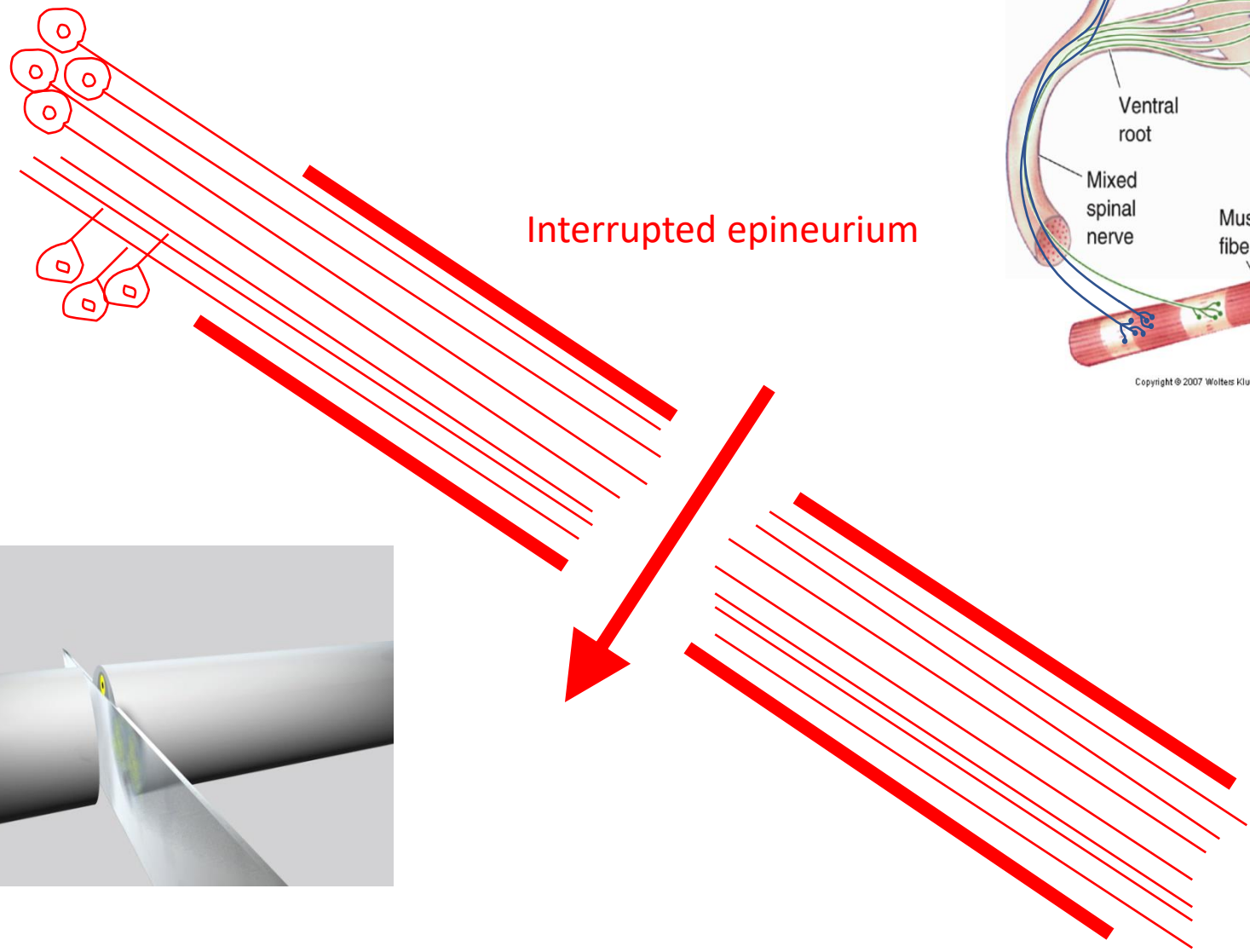


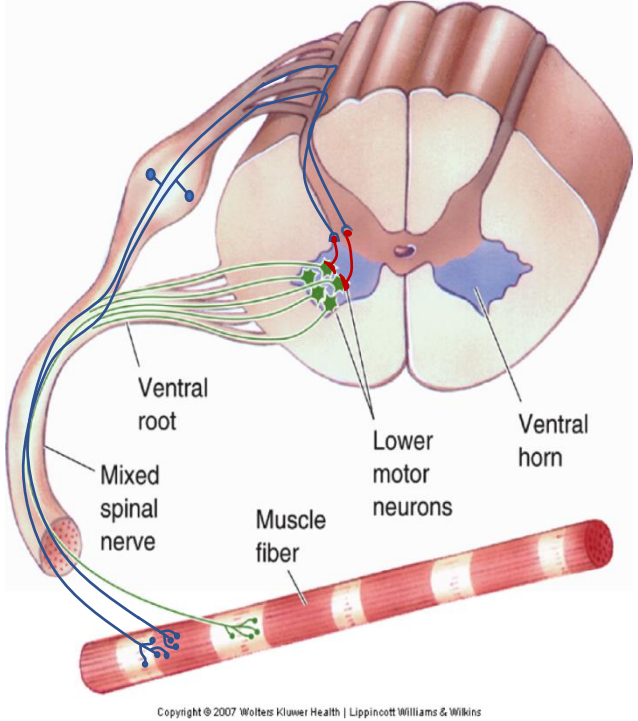
Neurotmesis (cut)
- SEVERE nerve lesion -



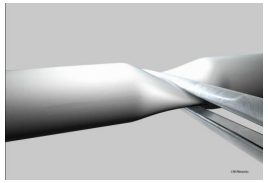


Neurotmesis (cut)
- SEVERE nerve lesion -



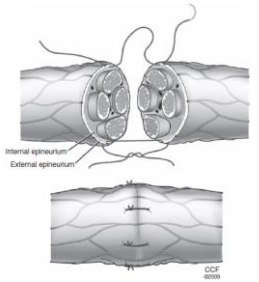


Axonotmesis (crush)
- mild nerve lesion -



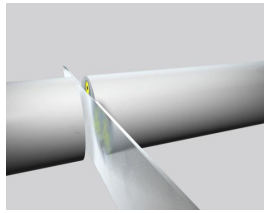
Nerve injury

bridge formation



gap < 2 mm — **end-to-end repair**

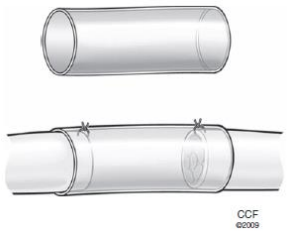
Neurotmesis (cut)
- severe nerve lesion -



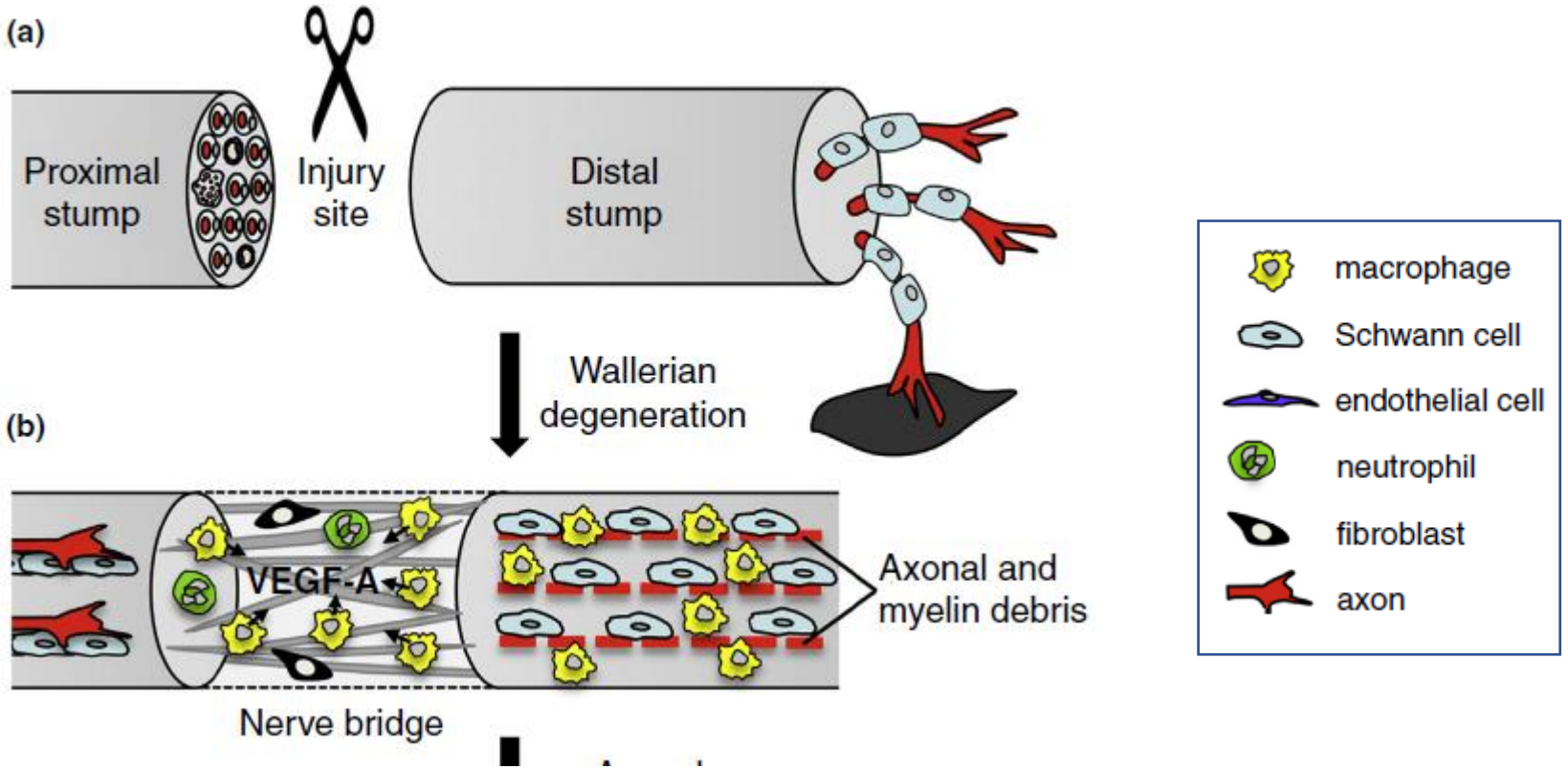
gap > 2 mm

autologous graft

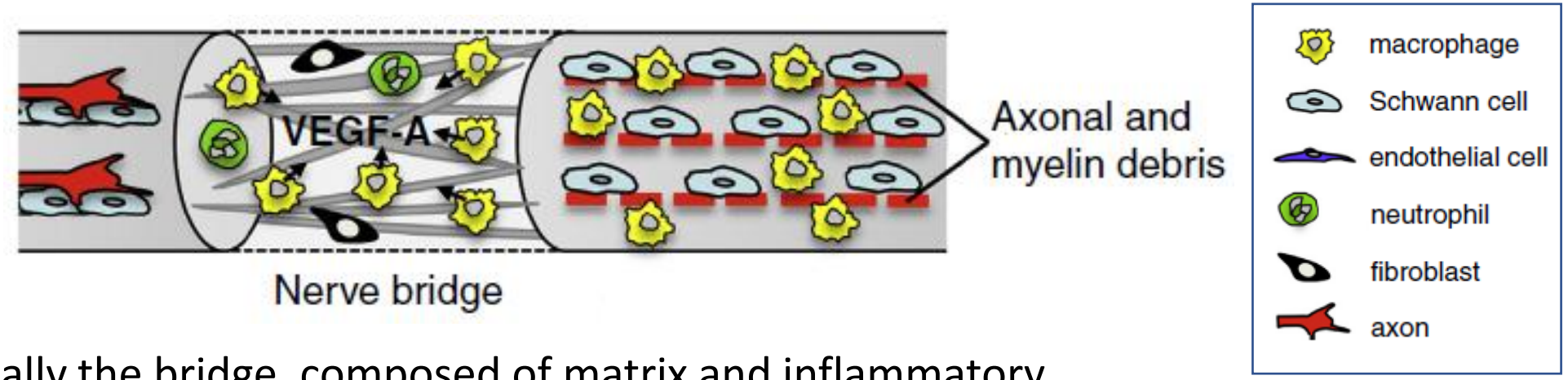
artificial graft



Nerve bridge formation

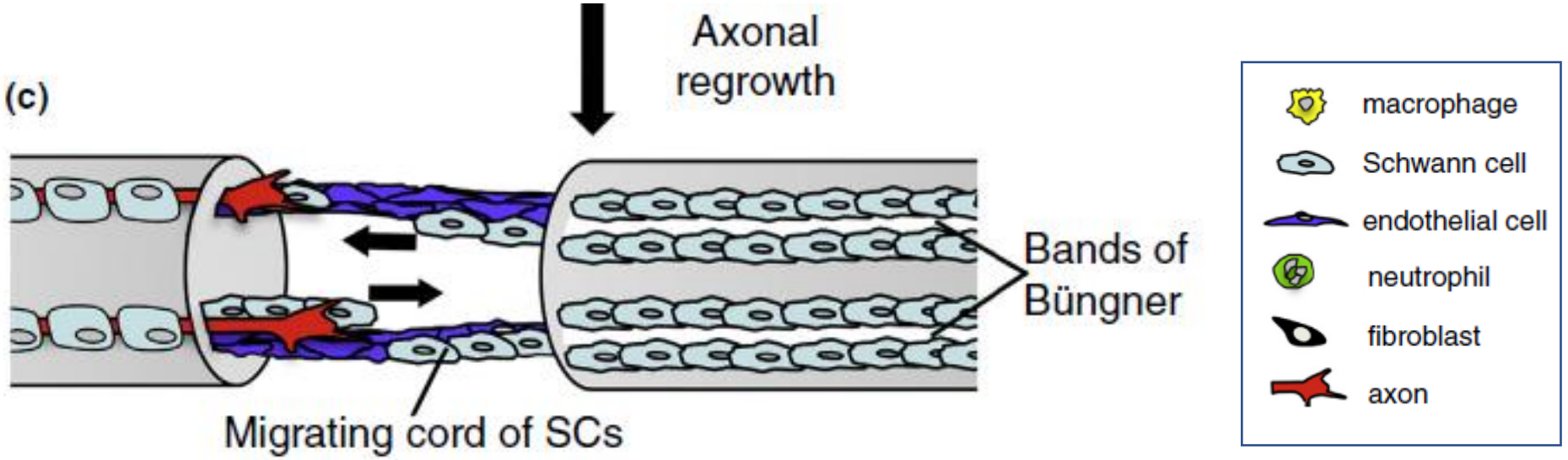


Following transection of a peripheral nerve, the stumps initially retract **(a)** but are reconnected by new tissue known as the **nerve bridge** (b).



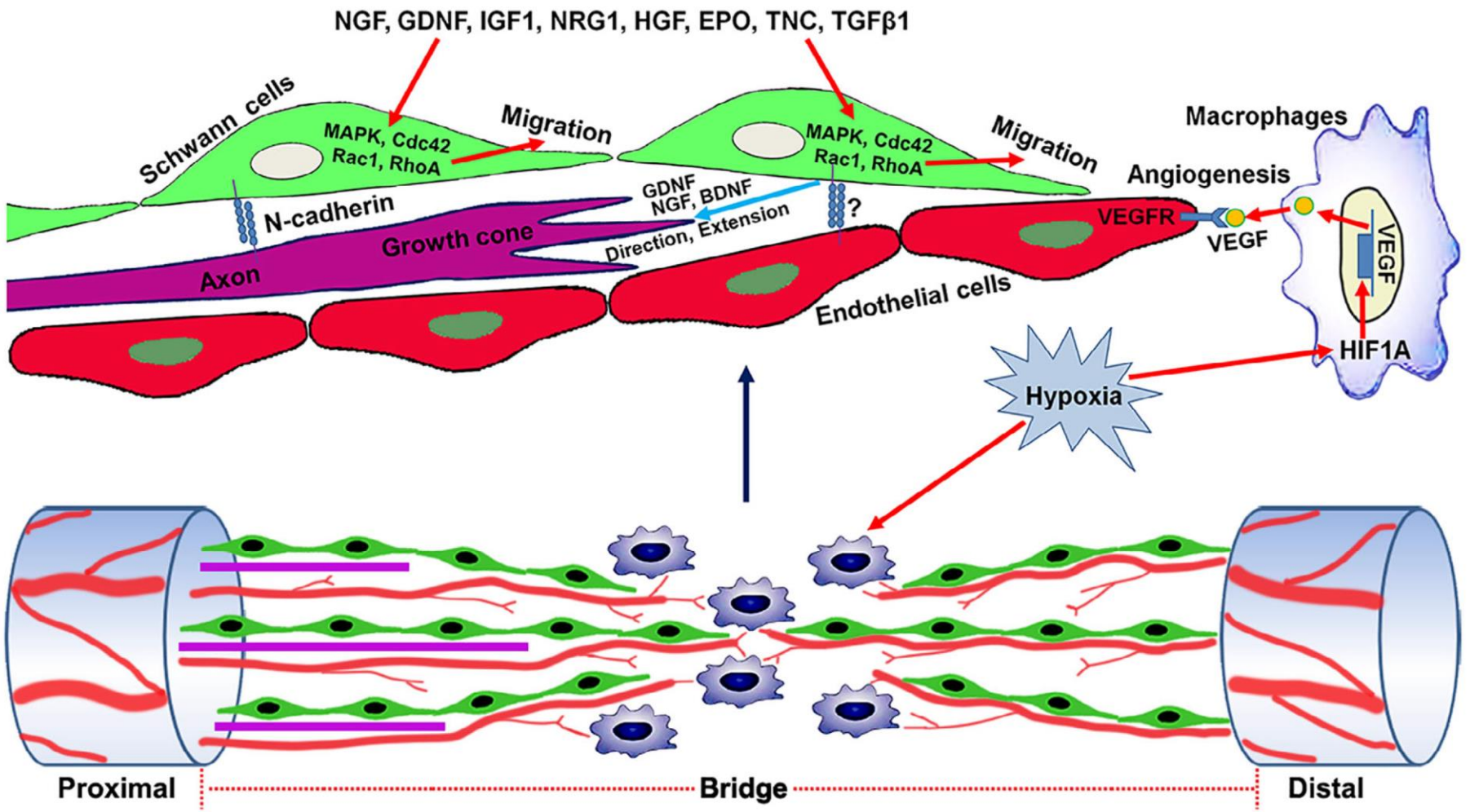
Initially the bridge, composed of matrix and inflammatory cells, is poorly vascularised and as a result becomes **hypoxic**.
This is sensed by the **macrophages**, the major cell type of the bridge, which secrete **VEGF** that promotes **angiogenesis**.
This sensitivity to hypoxia is an intrinsic property of the macrophages, as it can be reproduced in vitro.

Downstream of the transection in the distal stump, the axons degenerate in the process known as **Wallerian degeneration**. SCs, sensing the damage, disassociate from the degenerating axons and dedifferentiate to a progenitor-like state that orchestrates many aspects of the regenerative process. This includes recruitment of macrophages, which together with the SCs, clear axonal and myelin debris and remodel the environment to create a conducive environment for axonal regrowth.



SCs, sensing the damage, disassociate from the degenerating axons and dedifferentiate to a progenitor-like state that are involved in the formation of the **bands of Büngner (c)** that result from SCs elongating along the length of their original basement membrane to create directional tubes that provide a sustaining substrate for axonal regrowth back to their original targets. Meanwhile, the bridge has become **vascularised** in response to the macrophage-induced VEGF signal and **SCs migrate along the vasculature**, taking the regrowing axons across the bridge and into the distal stump **(c)**.

Signals activating Schwann cell migration and substrates for Schwann cell migration in the nerve bridge



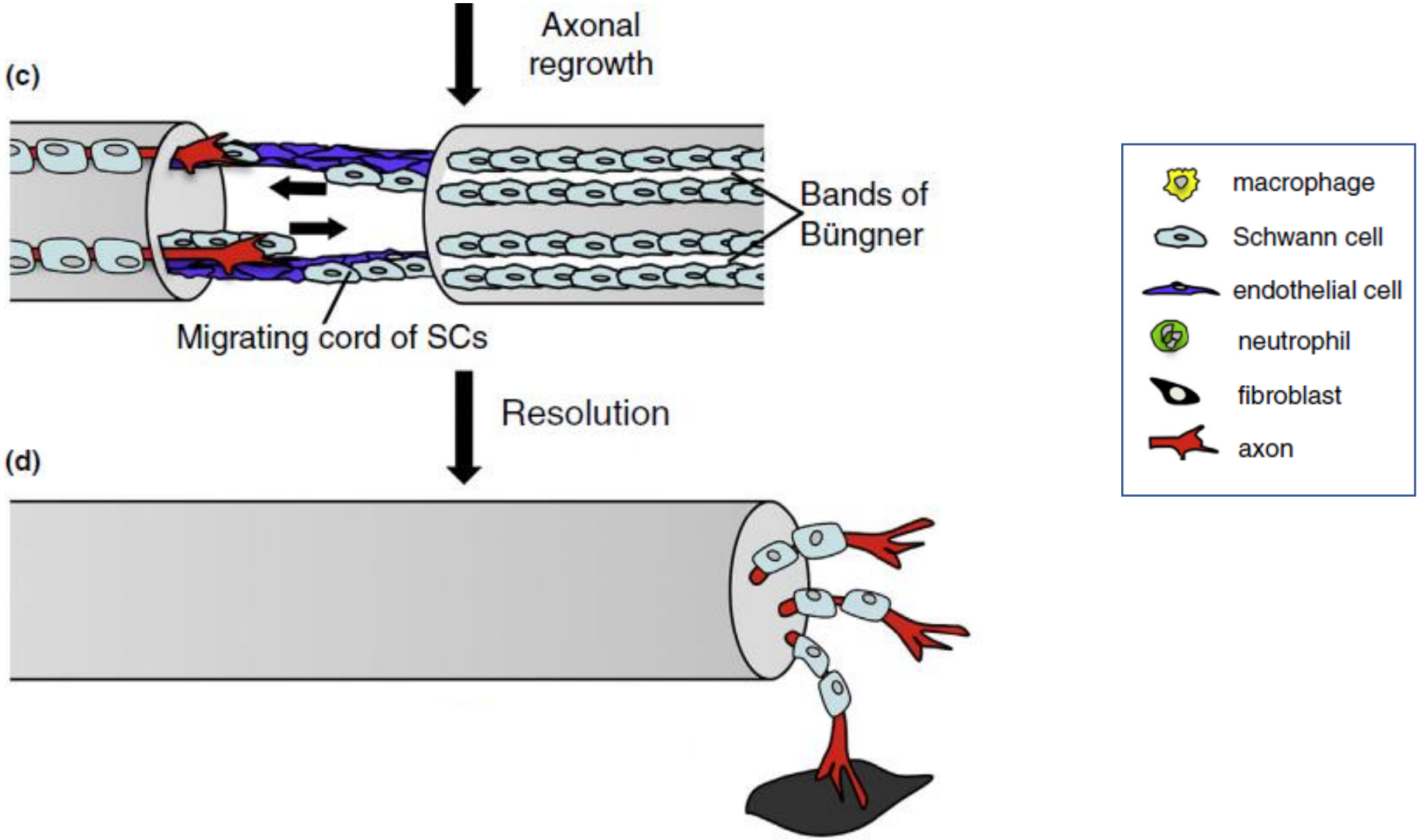
(Min et al., Glia, 2020)

Signals activating Schwann cell migration and substrates for Schwann cell migration in the nerve bridge

Upon nerve transection, macrophages in the nerve bridge sense hypoxia and upregulate vascular endothelial growth factor (VEGF) to induce blood vessel regeneration.

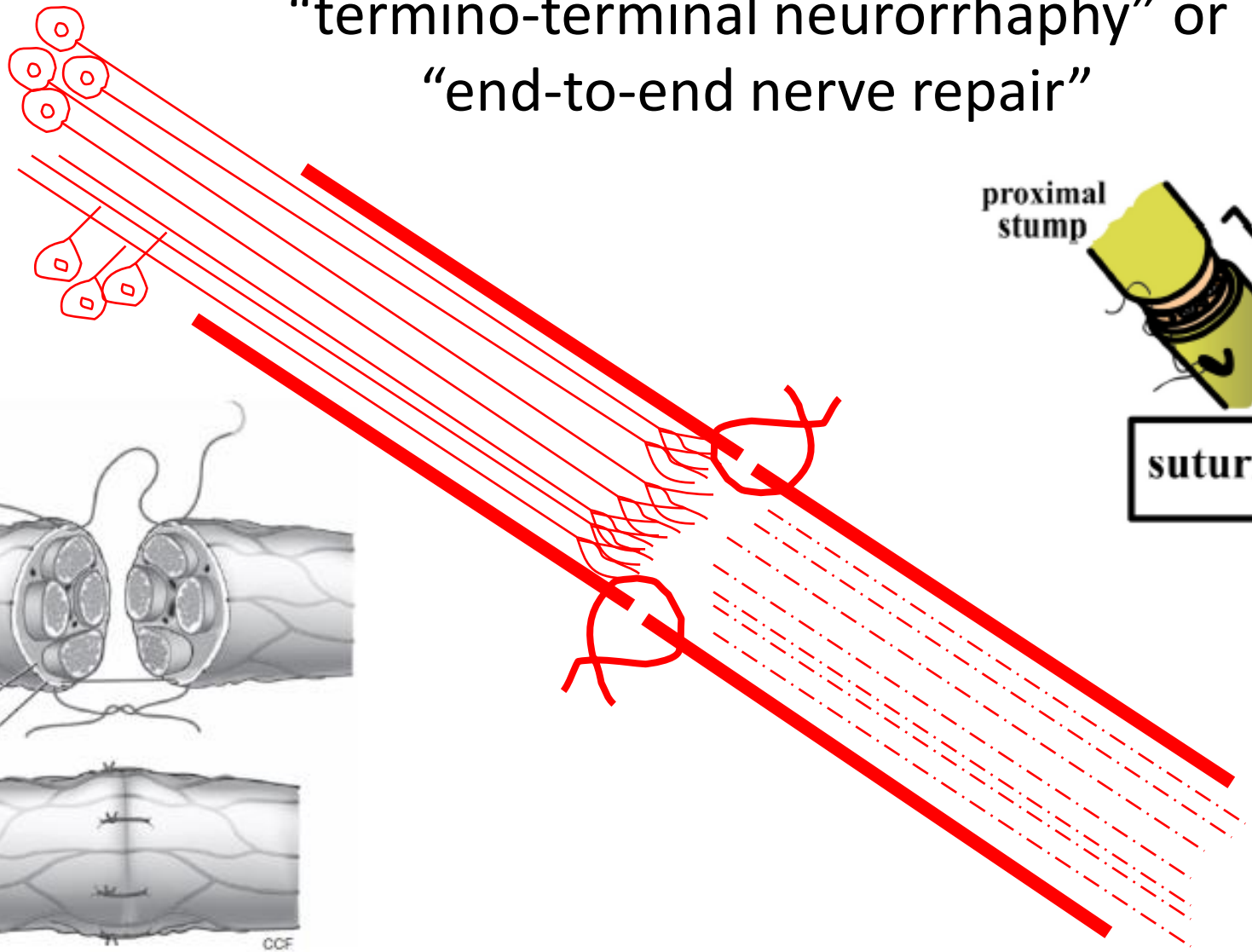
The Schwann cell intrinsic migration property is activated by a number of growth factors. Upon putative activation of mitogen activated protein kinase (MAPK), Cdc42, Rac1, and RhoA signaling, Schwann cells at both nerve ends actively search for a substrate and migrate.

In the proximal nerve stump, Schwann cells initially use regenerating axons as a substrate to migrate, but then use blood vessels as an alternative substrate once they overtake elongating axons. Schwann cells in the distal nerve stump also use blood vessels as substrate to migrate. Migrating Schwann cells from both nerve ends meet in the middle of the nerve bridge and form Schwann cells cords to direct axon regeneration. Nerve growth factor (NGF), BDNF, and glial cell line derived neurotrophic factor (GDNF) are critical signals that Schwann cells utilize to direct axons into the distal nerve stump.



The regeneration process is complete **(d)**, once the axons reinnervate their original targets and the SCs recognising the axons, redifferentiate and the inflammatory response resolves.

Neurotmesis without substance loss
direct epineurial/perineural suture
“termino-terminal neurorrhaphy” or
“end-to-end nerve repair”



(Siemionow and Brzezicki 2009

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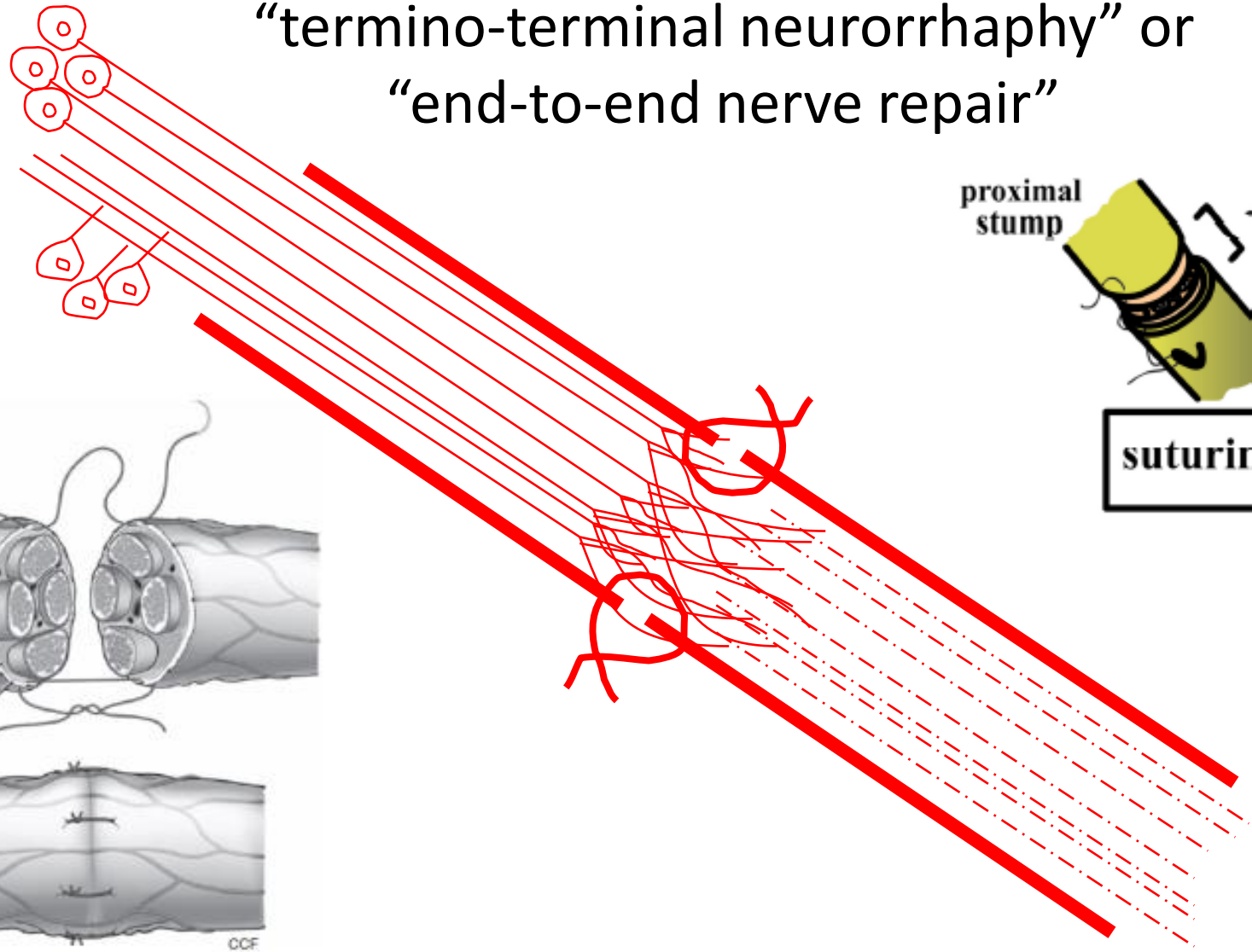
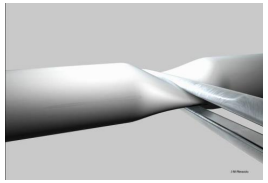


(Siemionow and Brzezicki 2009

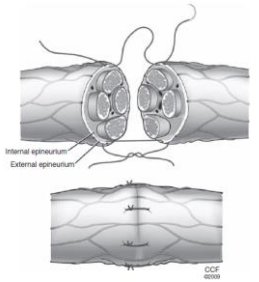
Axonotmesis (crush)
- mild nerve lesion -



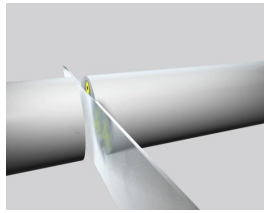
Nerve injury

— bridge formation

gap < 2 mm — end-to-end repair



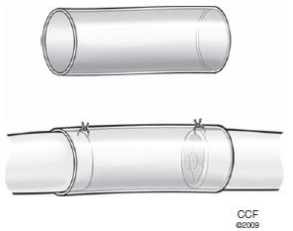
Neurotmesis (cut)
- severe nerve lesion -



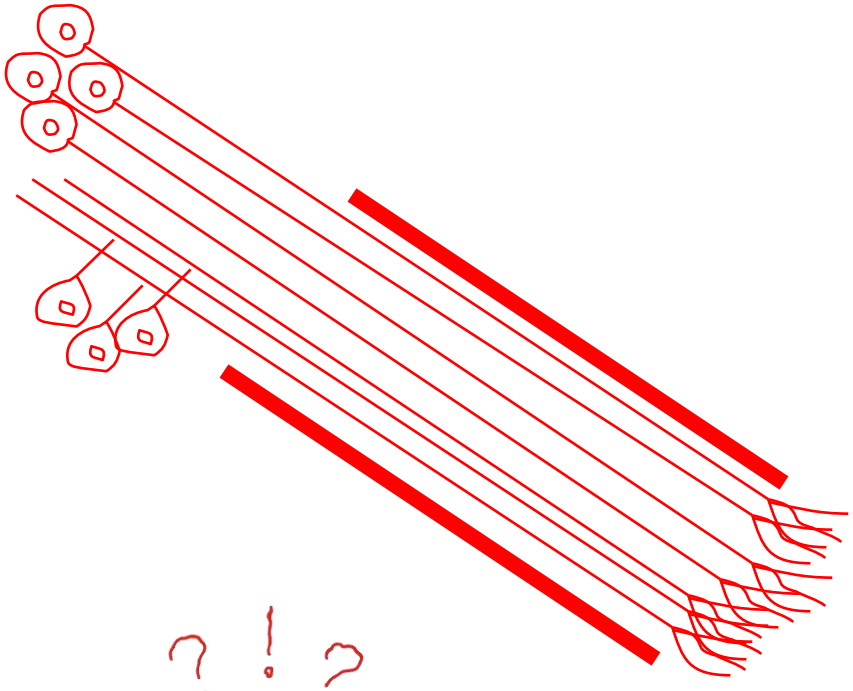
gap > 2 mm

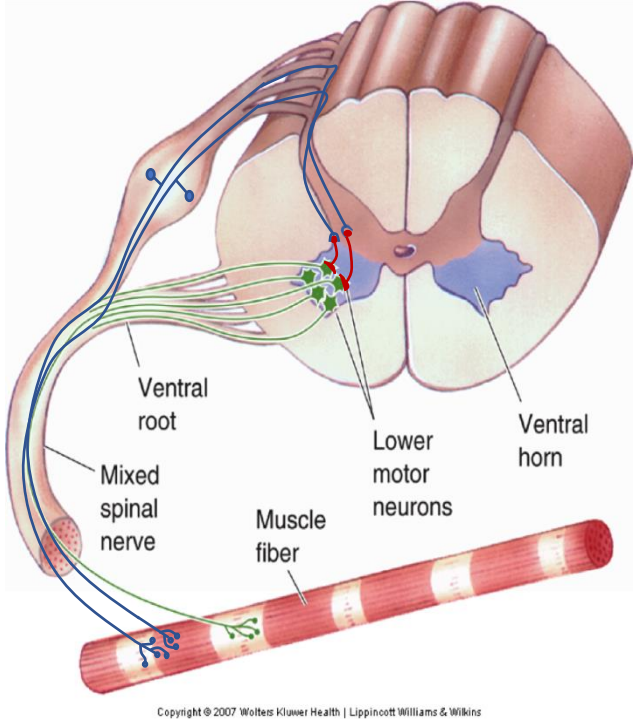
autologous graft

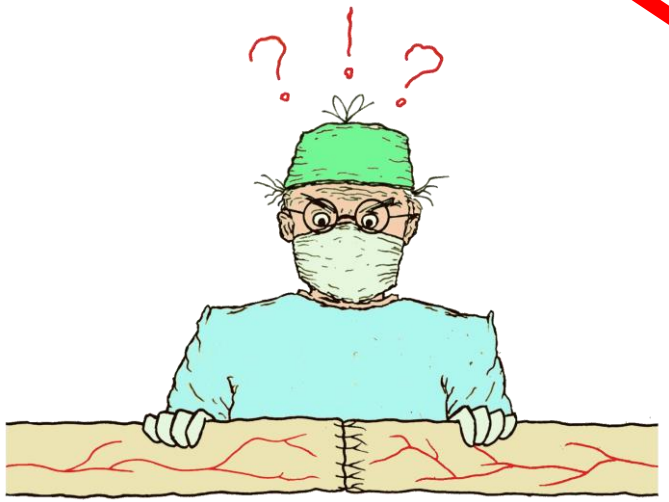
artificial graft

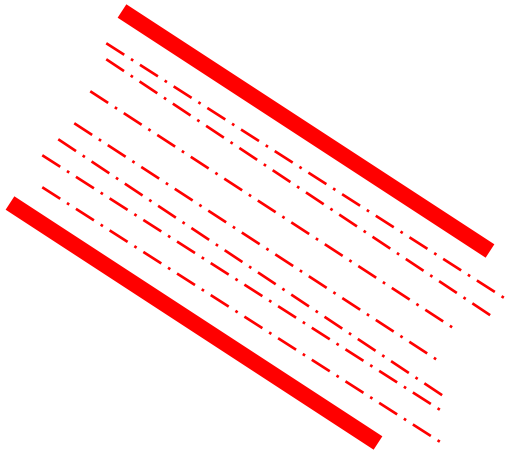


Neurotmesis WITH substance loss

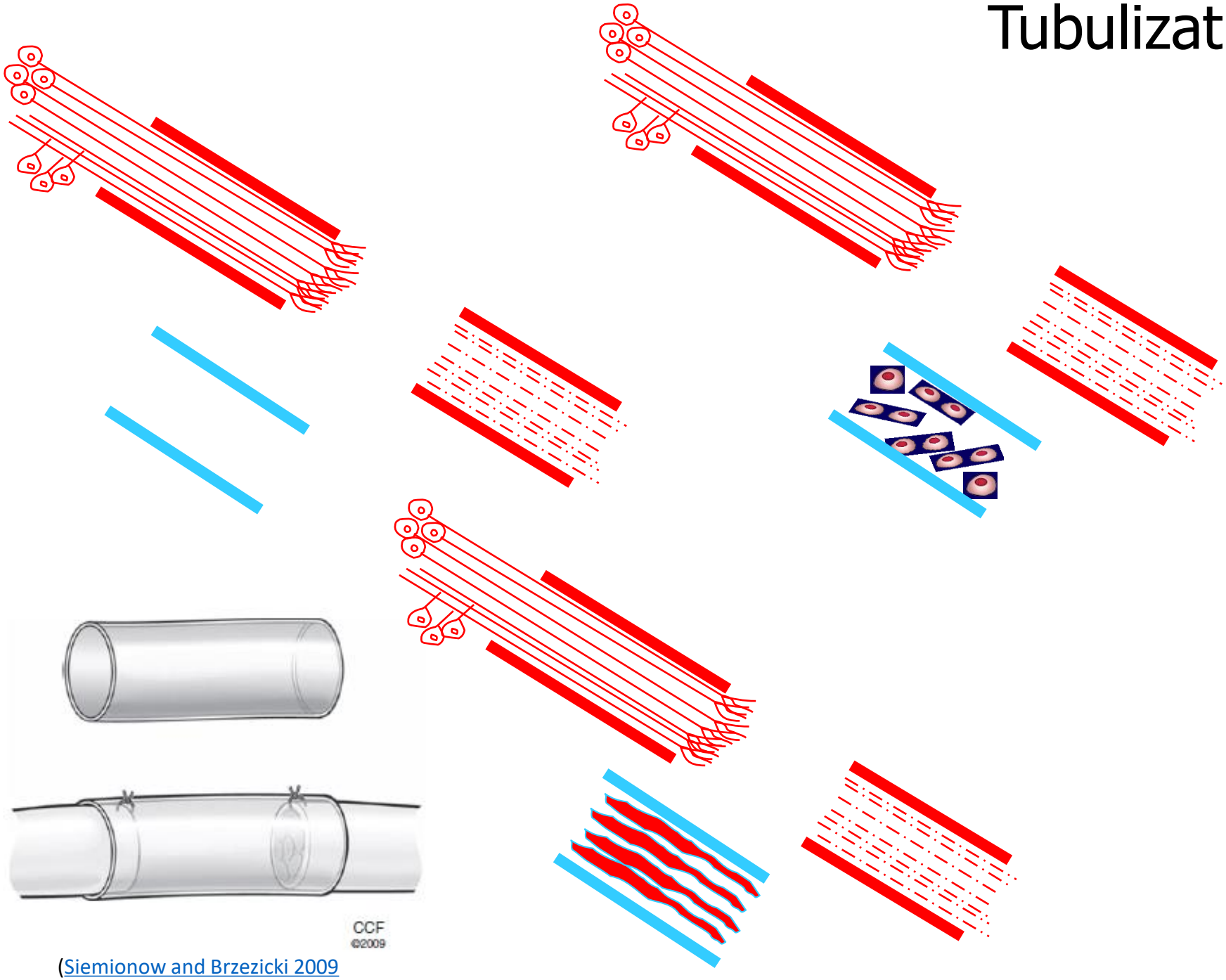




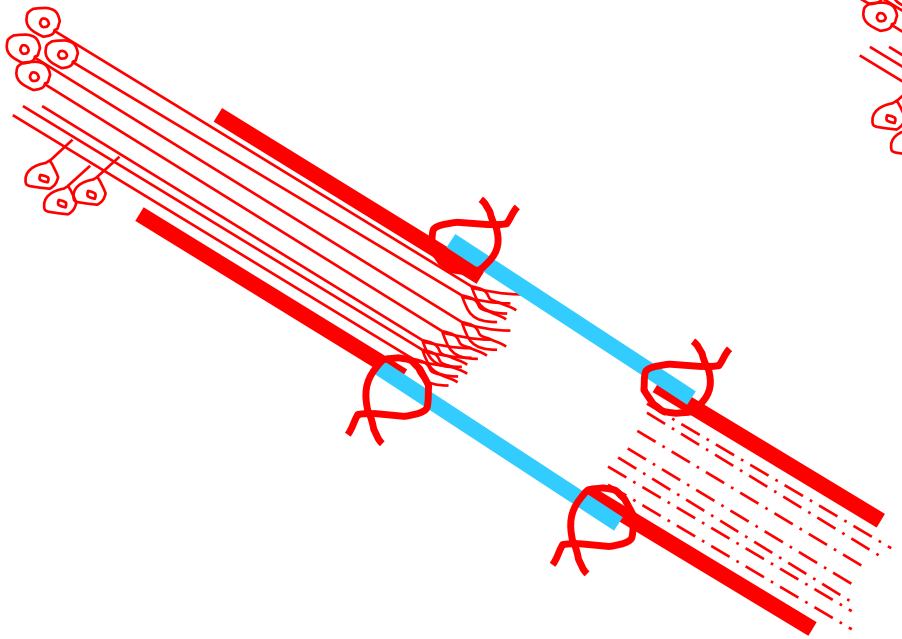


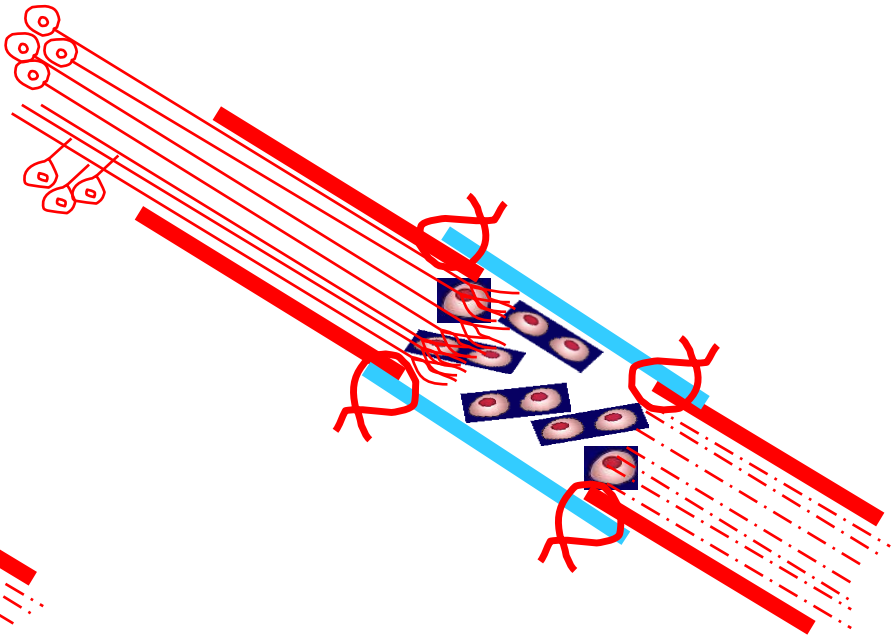


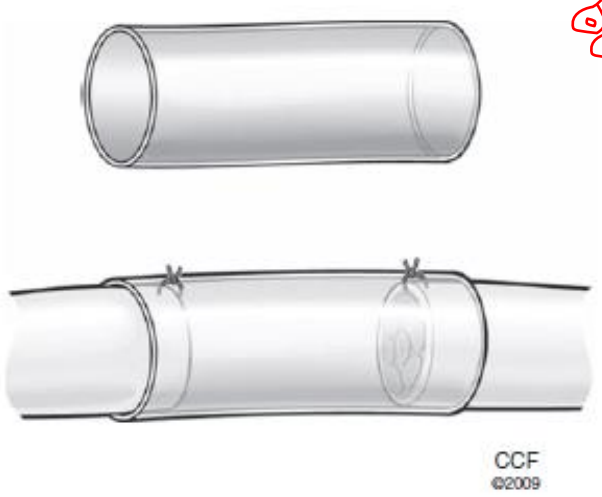
Tubulization

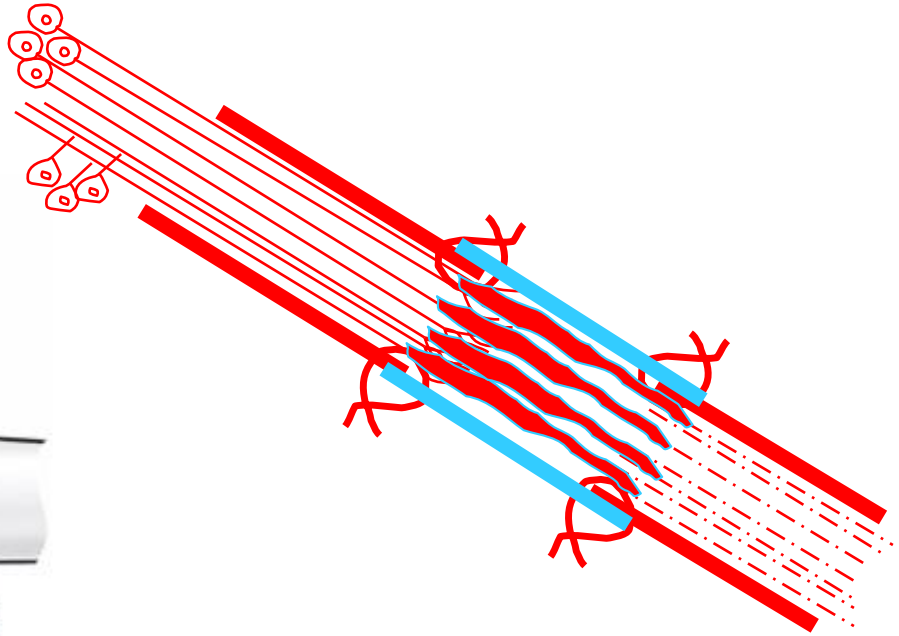


(Siemionow and Brzezicki 2009



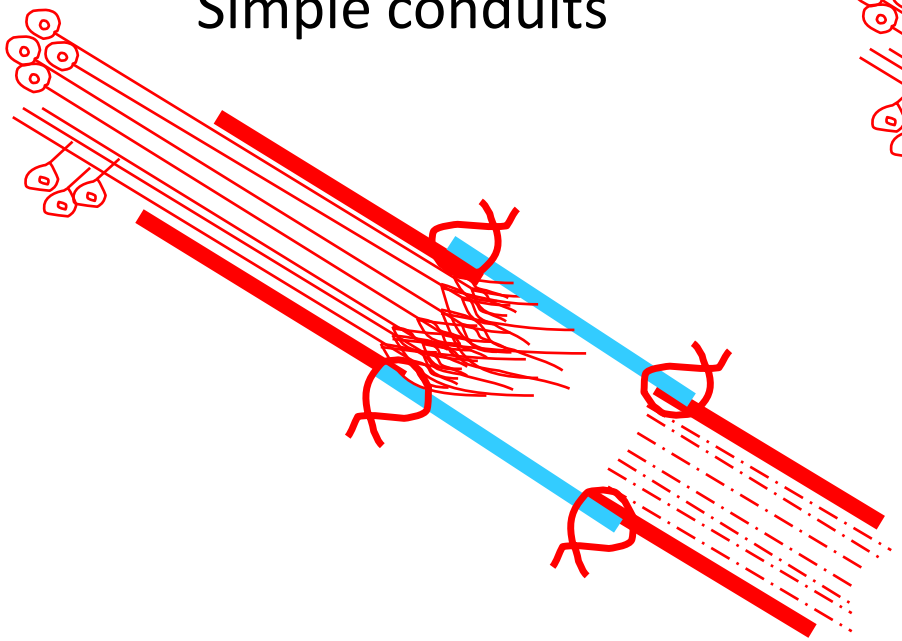




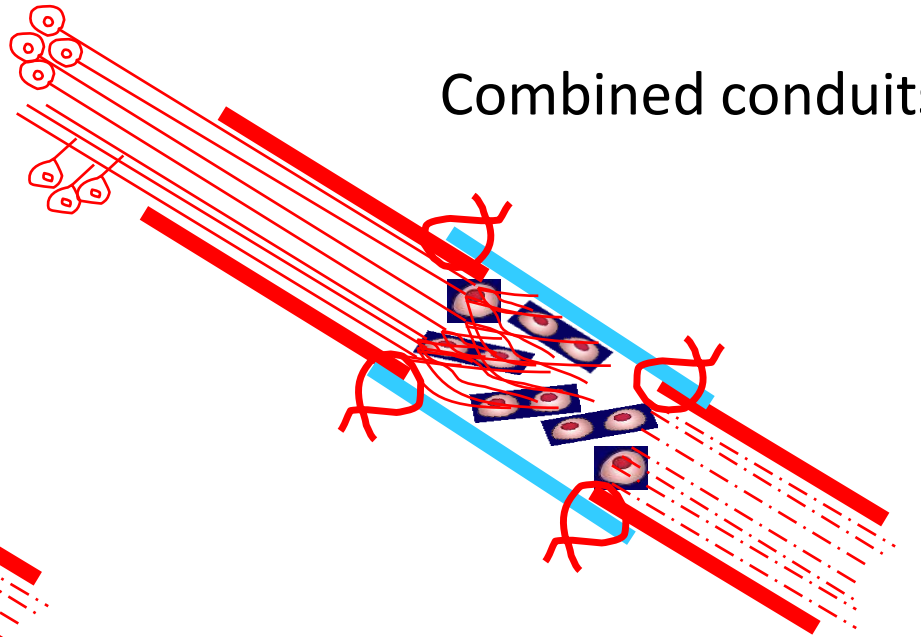


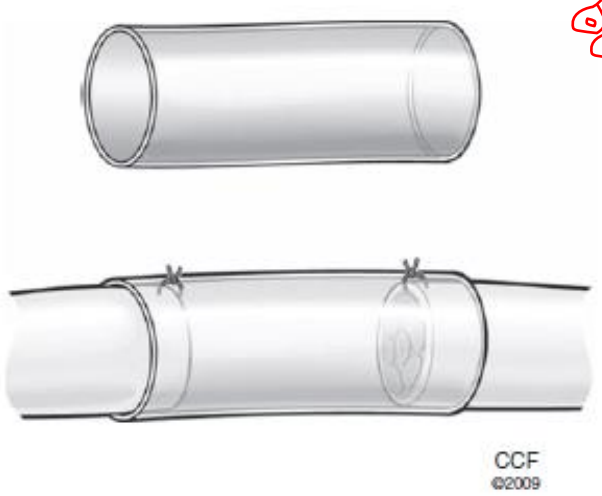
(Siemionow and Brzezicki 2009

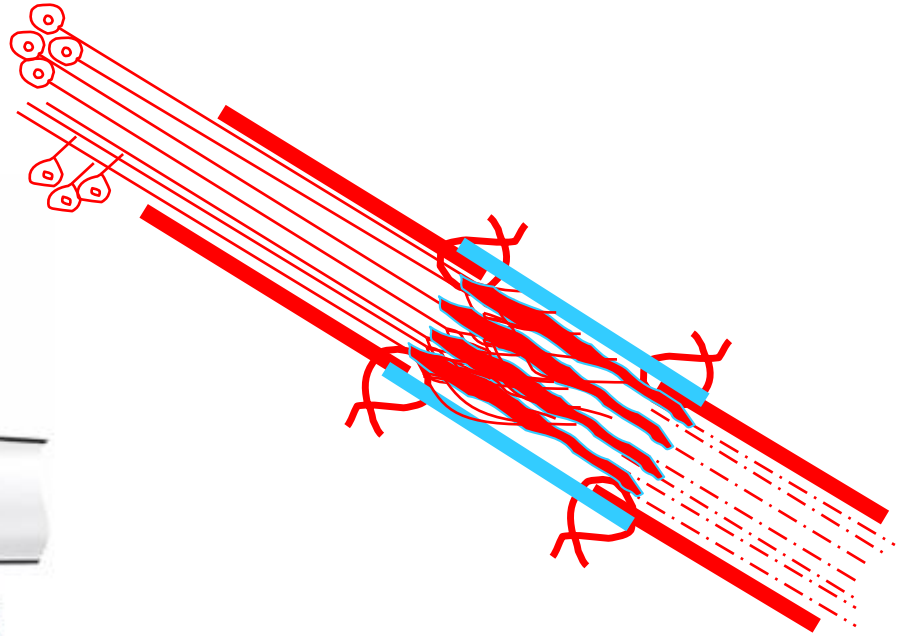
Simple conduits



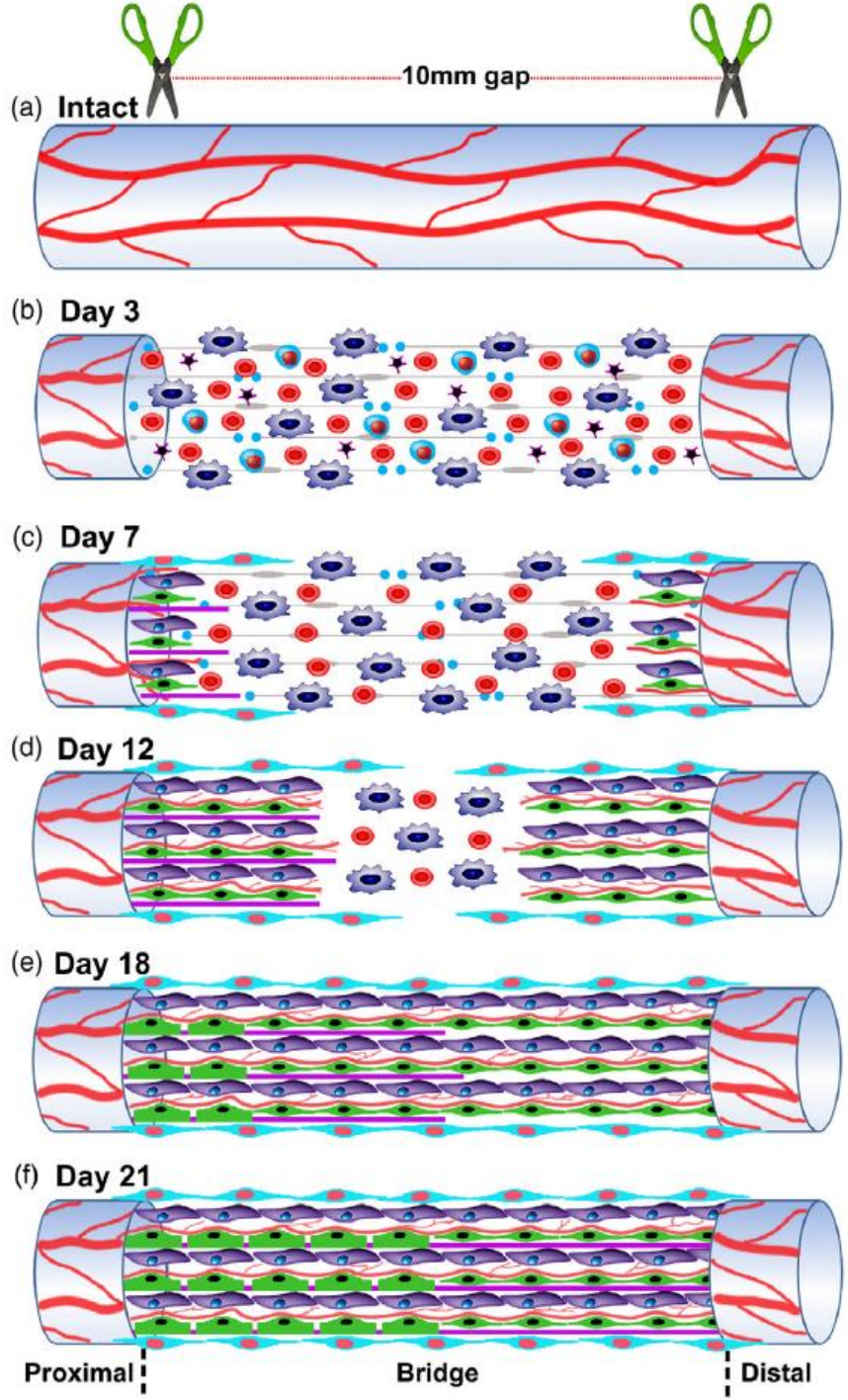
Combined conduits

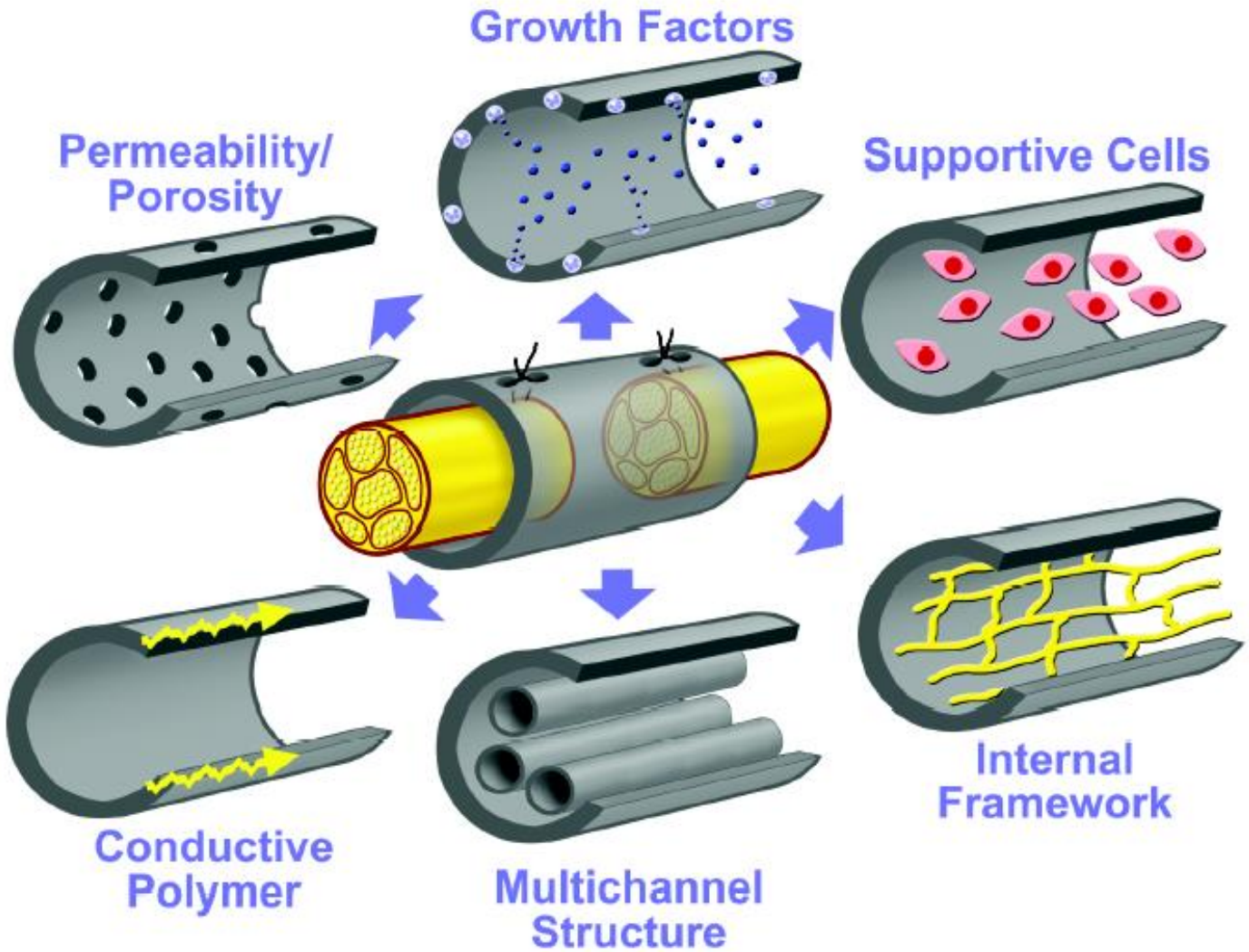




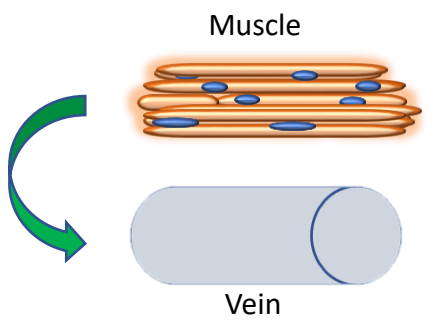


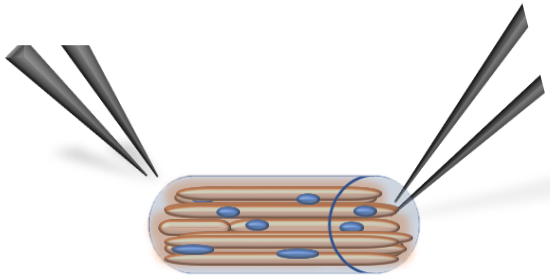
(Siemionow and Brzezicki 2009

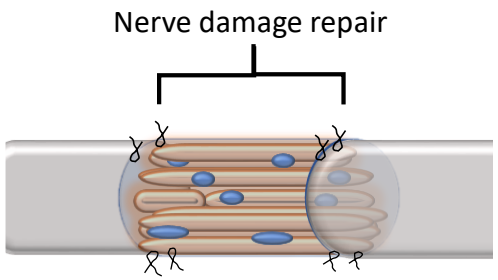

10mm gap
(a) Intact
(b) Day 3
(c) Day 7
(d) Day 12
(e) Day 18
(f) Day 21
Proximal
Bridge
Distal

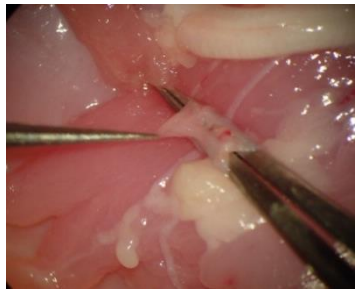

Growth Factors
Permeability/
Porosity
Supportive Cells
Internal
Framework
Conductive
Polymer
Multichannel
Structure

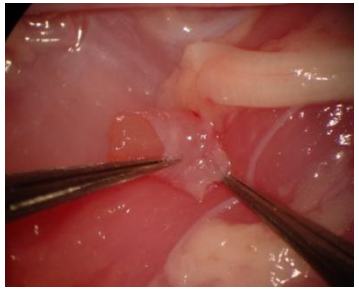
The **M**uscle **I**n **V**ein combined technique

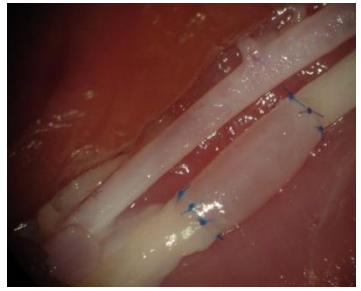












MIV avoids withdrawal of autologous nerve

The vein prevents axon dispersion

The muscle avoids vein collapse and guide axon regrowth and Schwann cell migration

This technique was applied in repair of 40 cases of sensory and mixed nerve defects (0.5-6 cm) with good results achieved in 85% of patients" (Battiston et al.,2000).

?

Which genes are regulated during peripheral nerve regeneration?

Which proteins are expressed during peripheral nerve regeneration?

Which factors can promote peripheral nerve regeneration?

Which biomaterials can be used to promote peripheral nerve regeneration?

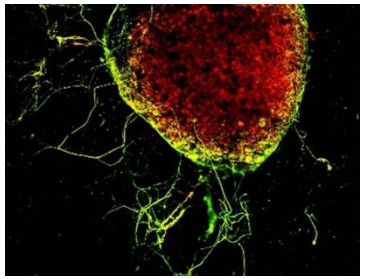
Which cells are involved in peripheral nerve regeneration after different injury types?

Animal models

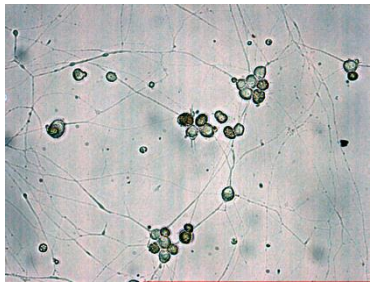
RAT

MOUSE

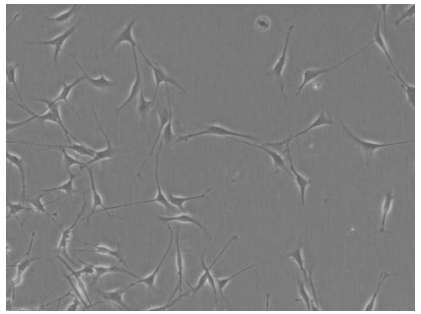
in vitro models



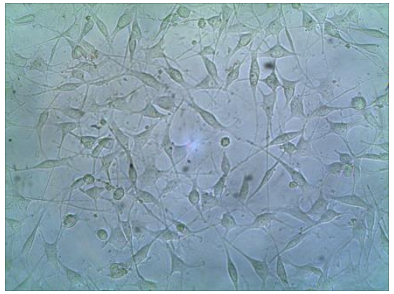
DRG EXPLANTS



PRIMARY CULTURES OF DRG DISSOCIATED SENSITIVE NEURONS

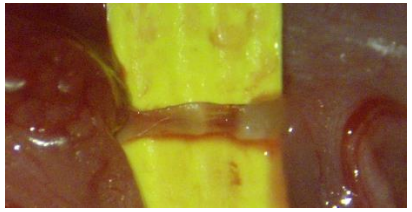


PRIMARY CULTURES OF SCHWANN CELLS



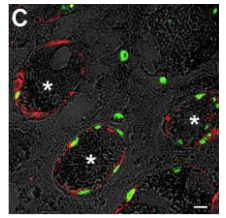
IMMORTALIZED CELL LINES OF SCHWANN CELLS OR SENSITIVE NEURONS

Methods

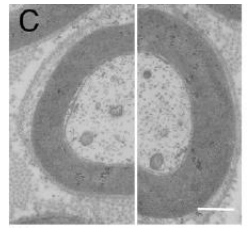


Morphological analysis

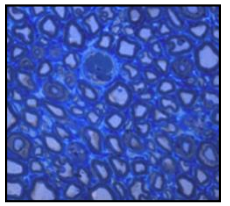
- immunohistochemistry



- electron microscopy

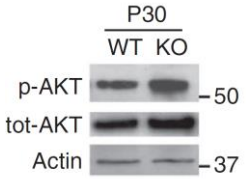


- high resolution light microscopy

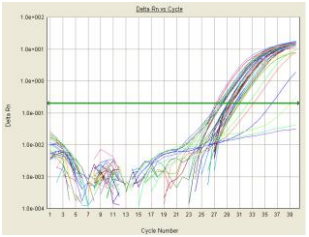


Biomolecolar analysis

- proteins — western blot



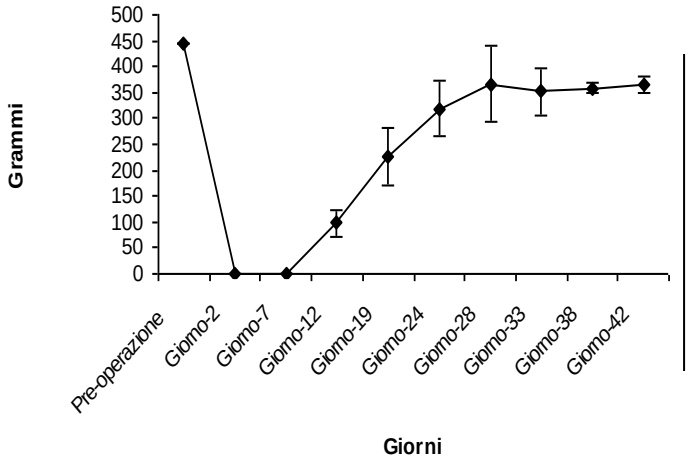
- mRNA
  - quantitative real time PCR

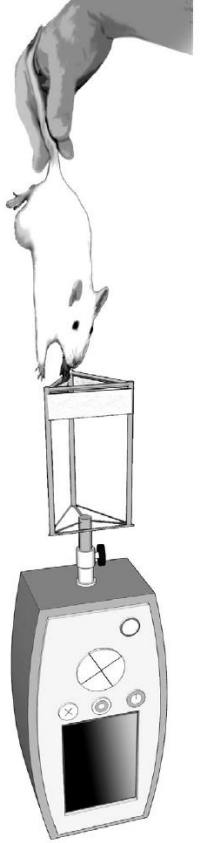


Methods

Functional analysis

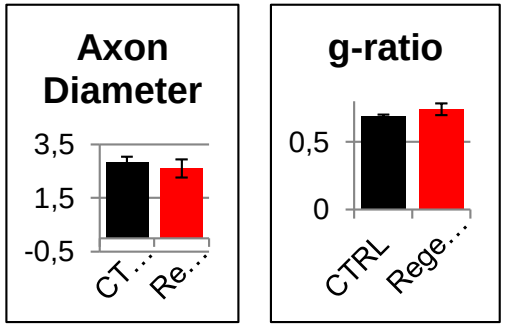
Evaluation of functional recovery of medial nerve through grasping test

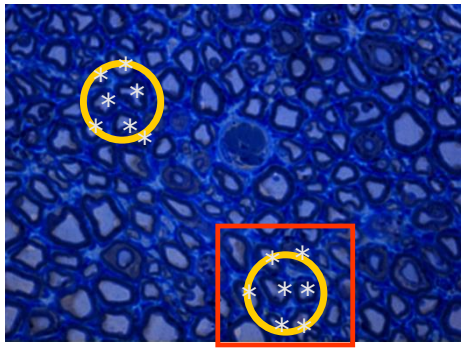




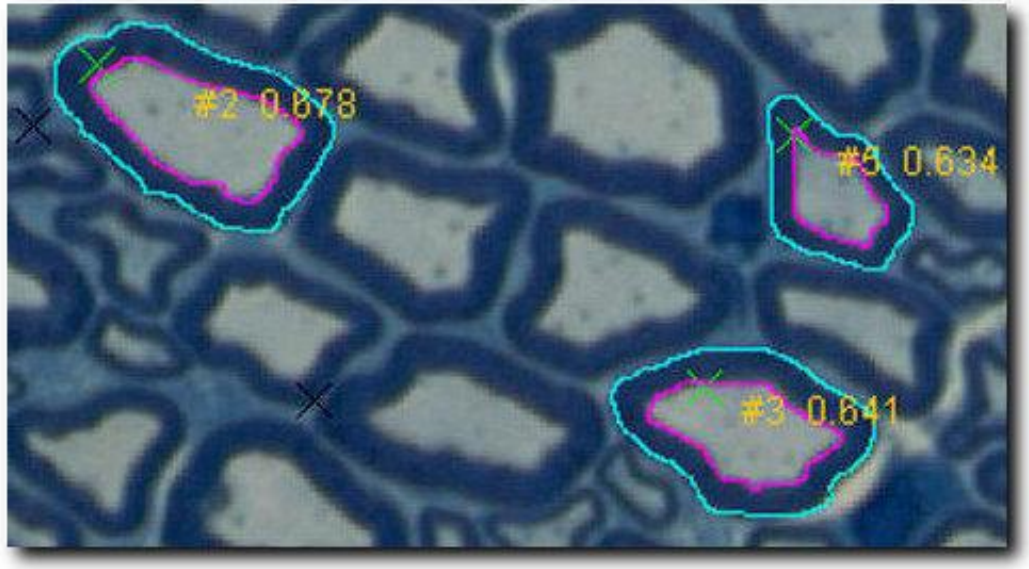
Morphometric analysis

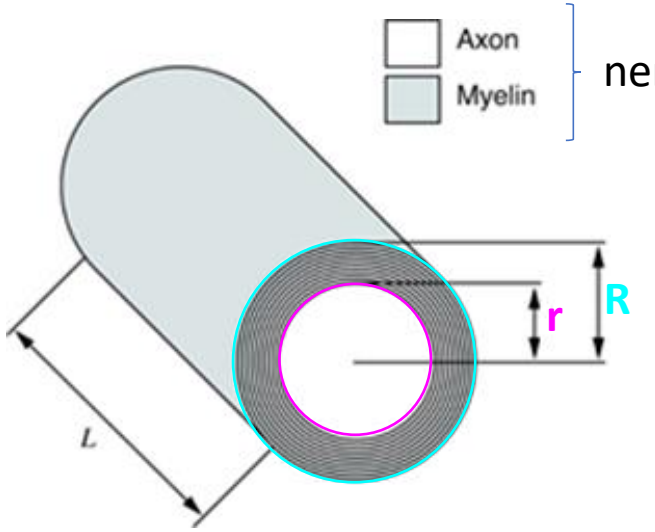
Evaluation of number and dimension of regenerating fibers





g-*ratio* = d/D = r/R

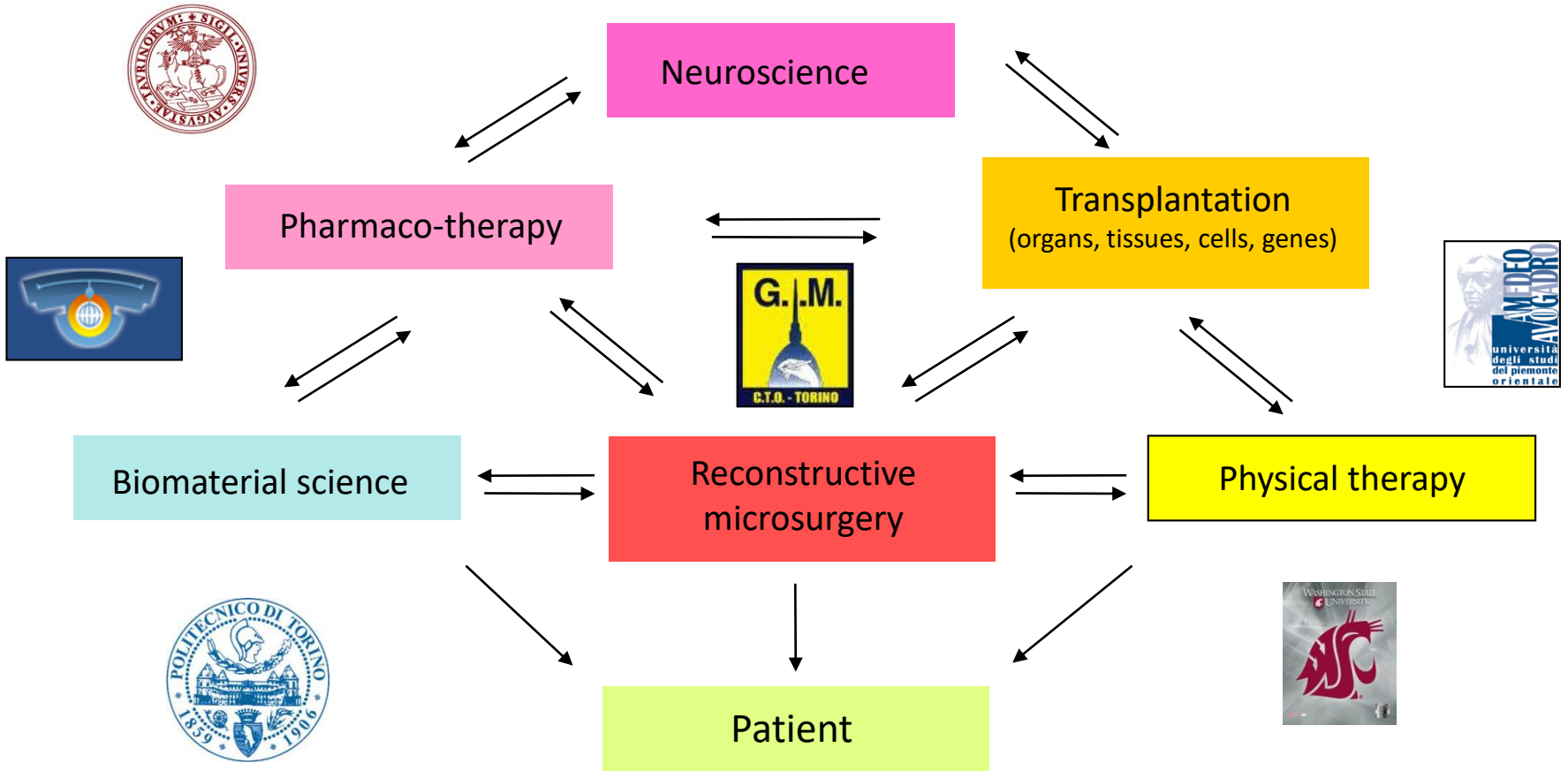




$$g = \frac{\text{axon circumference}}{\text{nerve fiber circumference}}$$

$$g = \frac{2\pi r}{2\pi R} = g = \frac{r}{R} = g = \frac{d}{D}$$

To answer these questions
an interdisciplinary approach is necessary



?

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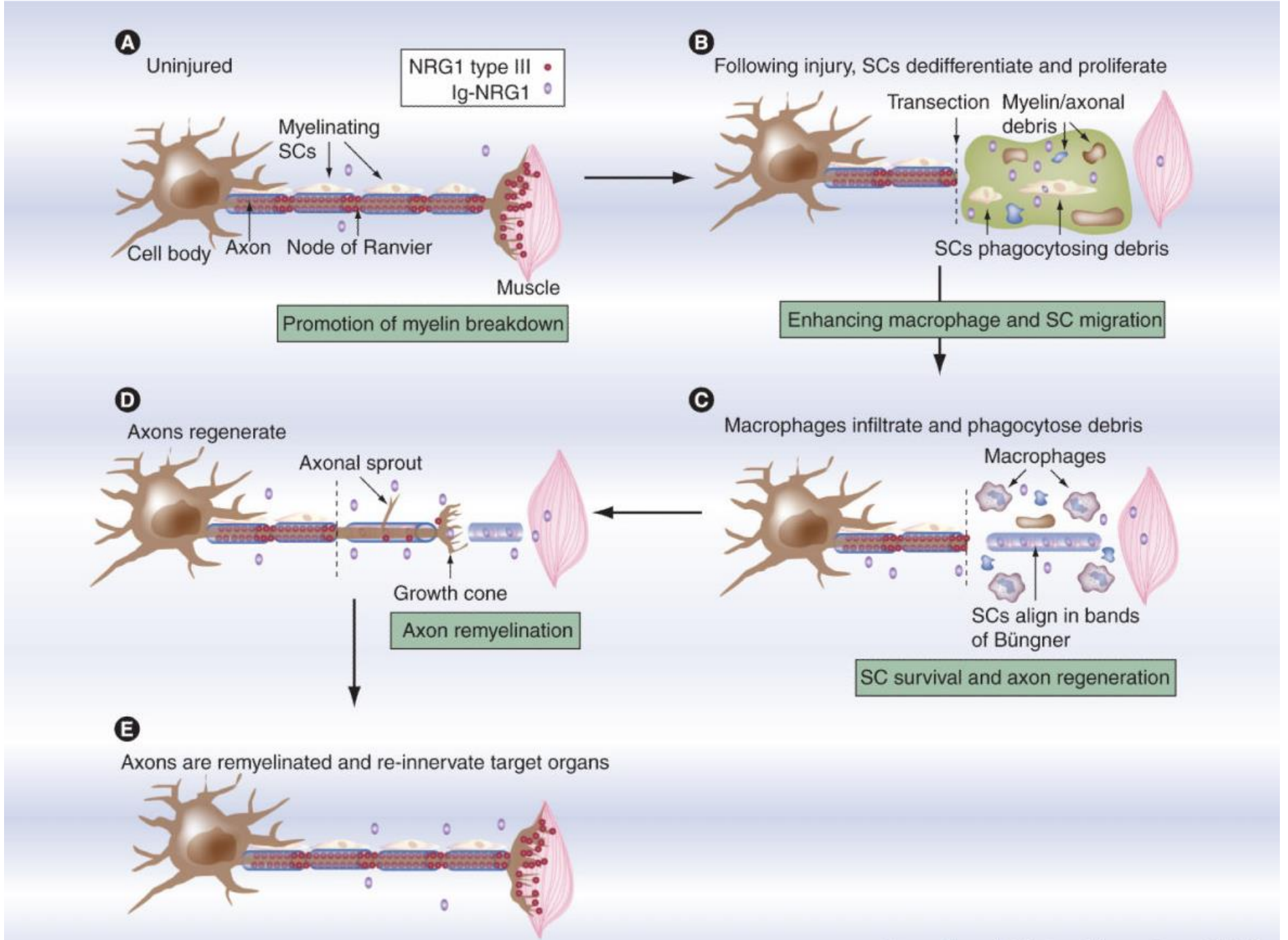
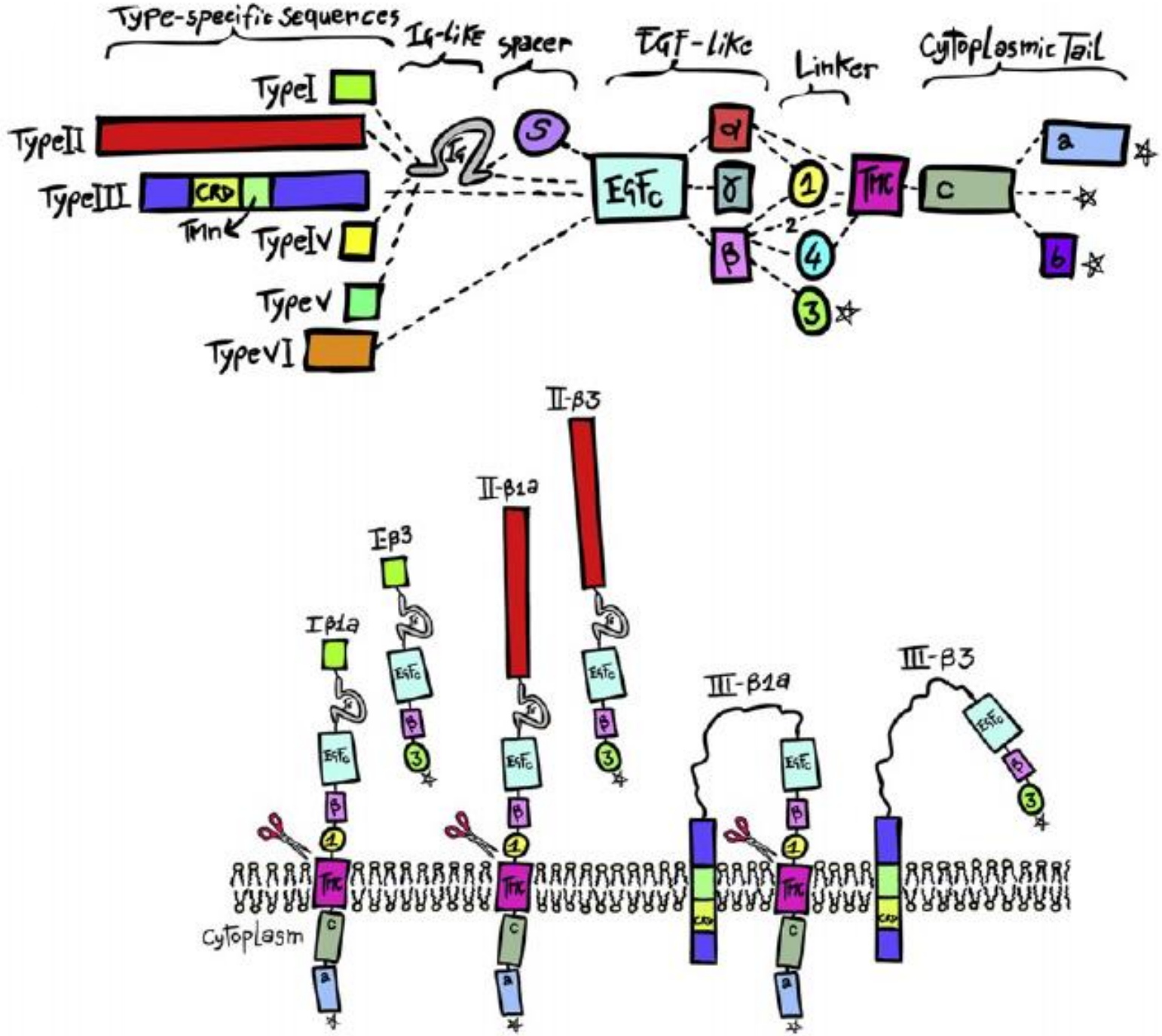
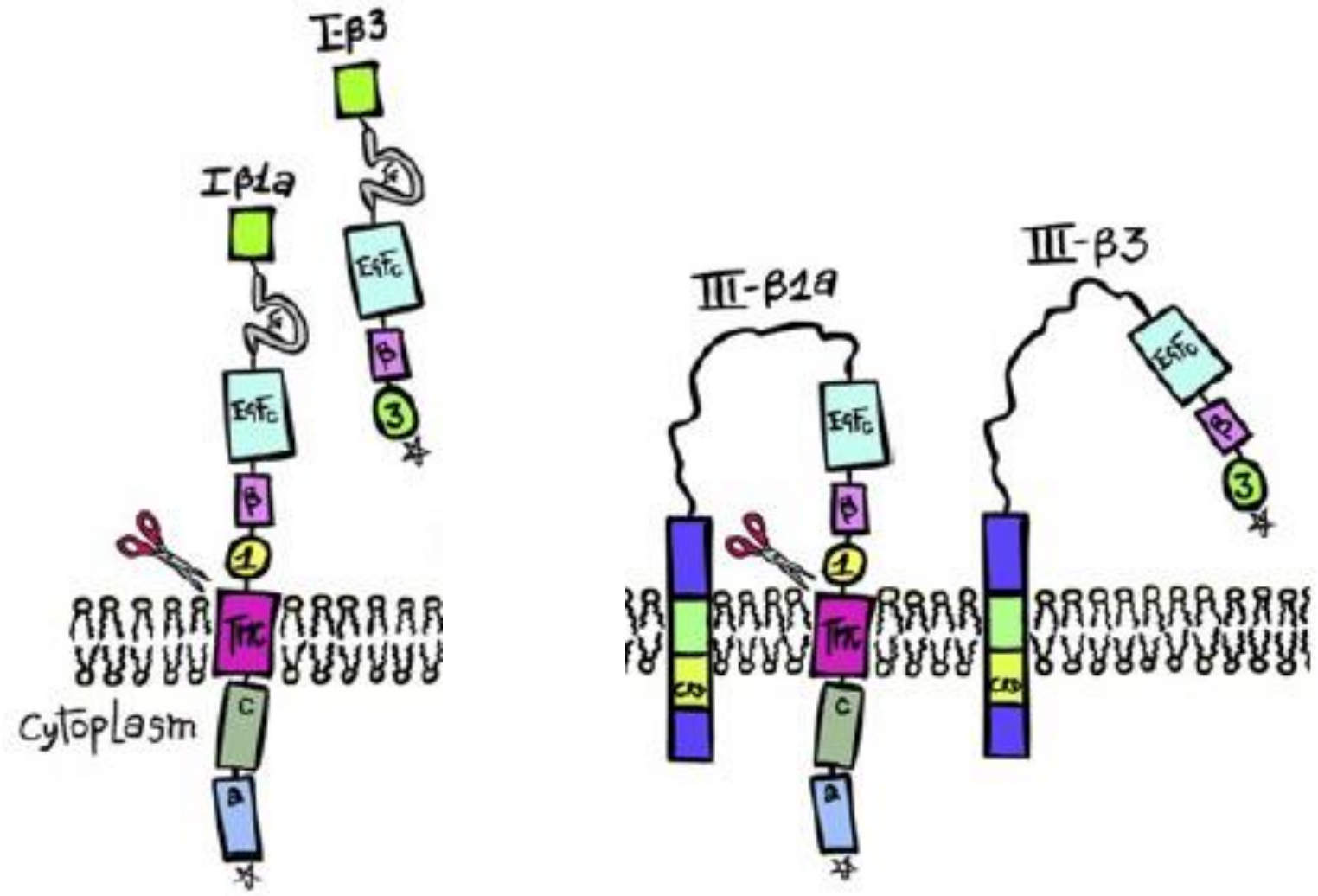
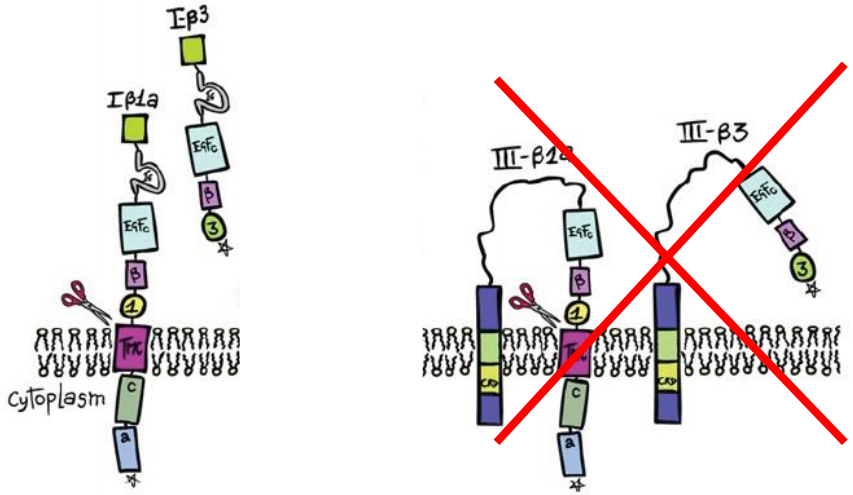



Type-specific sequences
Ig-like
spacer
EGF-like
Linker
Cytoplasmic Tail
TypeI
TypeII
TypeIII
CRD
TypeIV
TypeV
TypeVI
Ig
S
EGFc
TMc
c
a
b
II-β3
II-β1a
I-β3
I-β1a
III-β1a
III-β3
Cytoplasm

Soluble and transmembrane NRG1 isoforms
play different roles during myelination and re-myelination



Soluble and transmembrane NRG1 isoforms play different roles during myelination and re-myelination

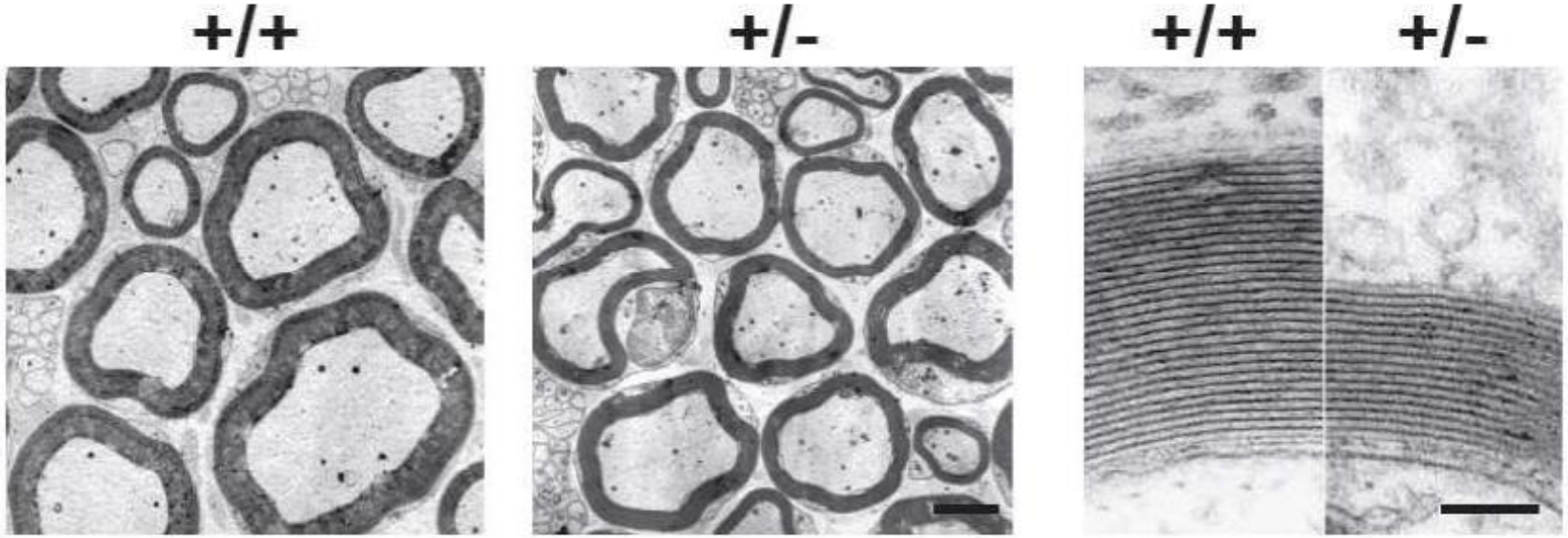


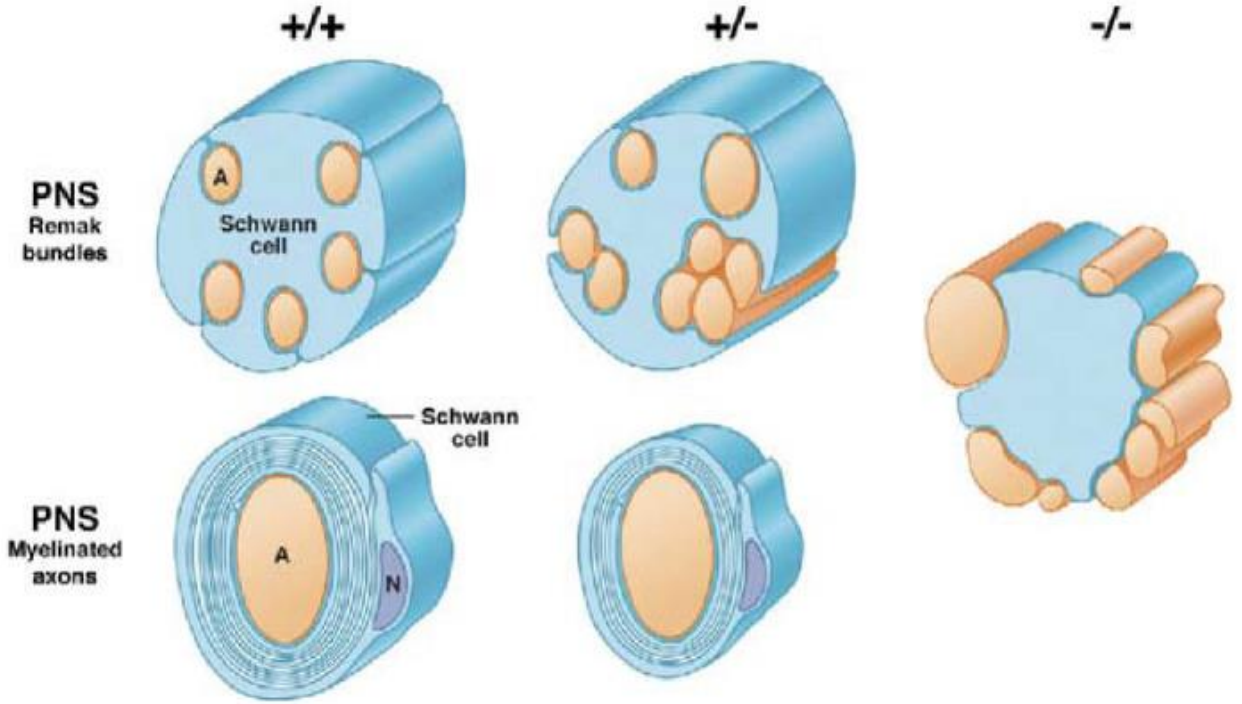
Ablation of axonal transmembrane NRG1 (type III)

→ **hypo-myelination**

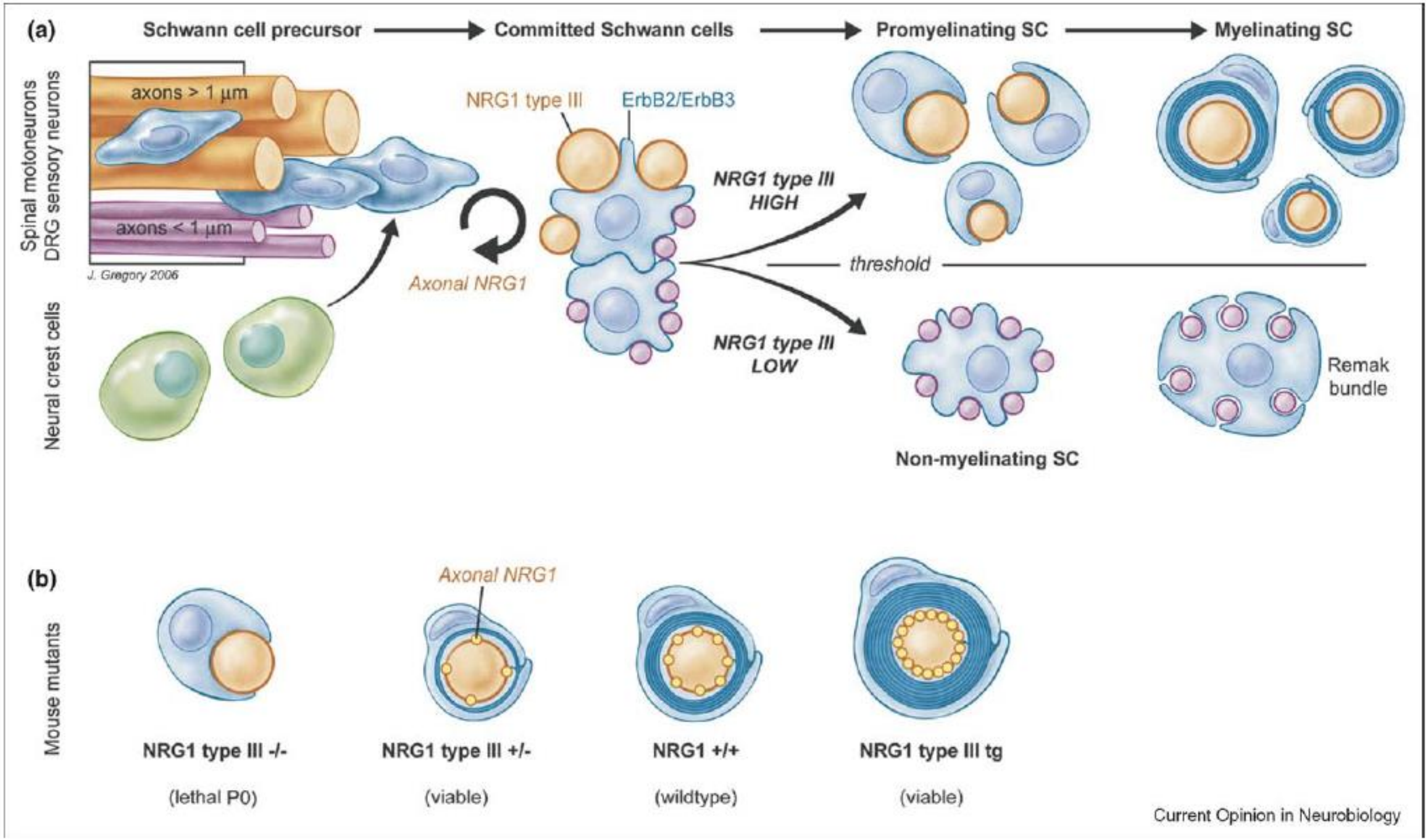
→ **impairs re-myelination**

(Michailov et al, 2004; Taveggia et al., 2005; Stassart et al., 2013, Fricker et al., 2013)

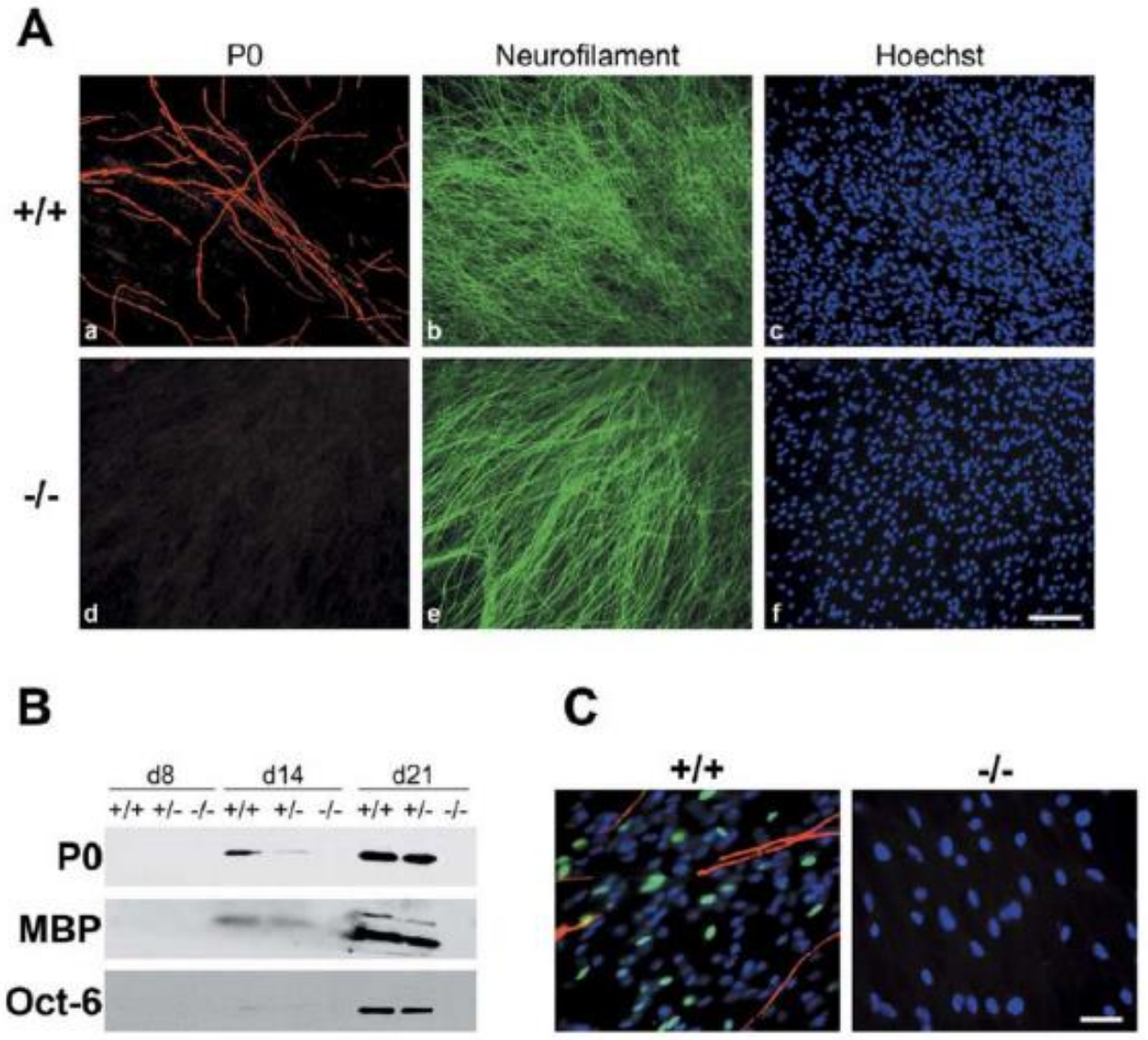




Carla Taveggia

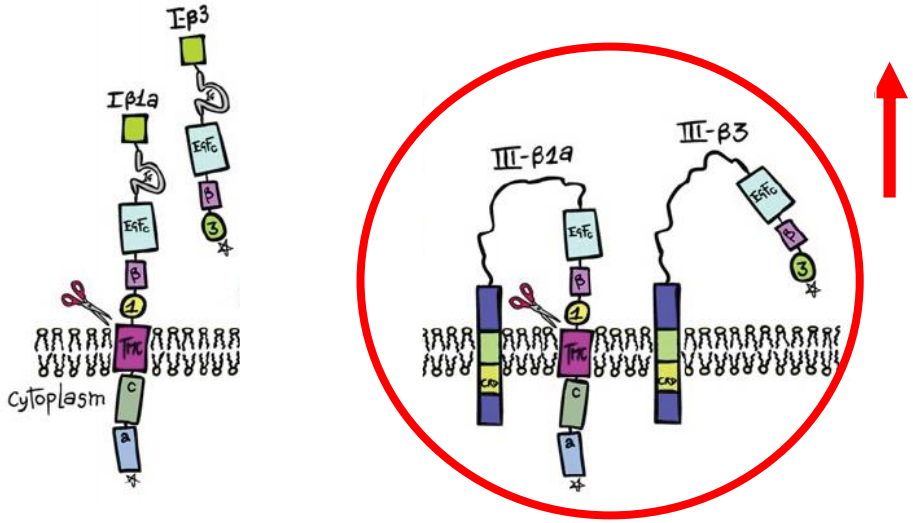

(a)
Schwann cell precursor
Committed Schwann cells
Promyelinating SC
Myelinating SC
Spinal motoneurons
DRG sensory neurons
axons > 1 µm
axons < 1 µm
J. Gregory 2006
NRG1 type III
ErbB2/ErbB3
Axonal NRG1
NRG1 type III HIGH
threshold
NRG1 type III LOW
Neural crest cells
Remak bundle
Non-myelinating SC
(b)
Mouse mutants
Axonal NRG1
NRG1 type III -/-
(lethal P0)
NRG1 type III +/-
(viable)
NRG1 +/+
(wildtype)
NRG1 type III tg
(viable)
Current Opinion in Neurobiology

NRG1 Type III Is Essential for Schwann Cell Ensheathment and Myelination



(Carla Taveggia et al., Neuron, 2005)

Soluble and transmembrane NRG1 isoforms play different roles during myelination and re-myelination

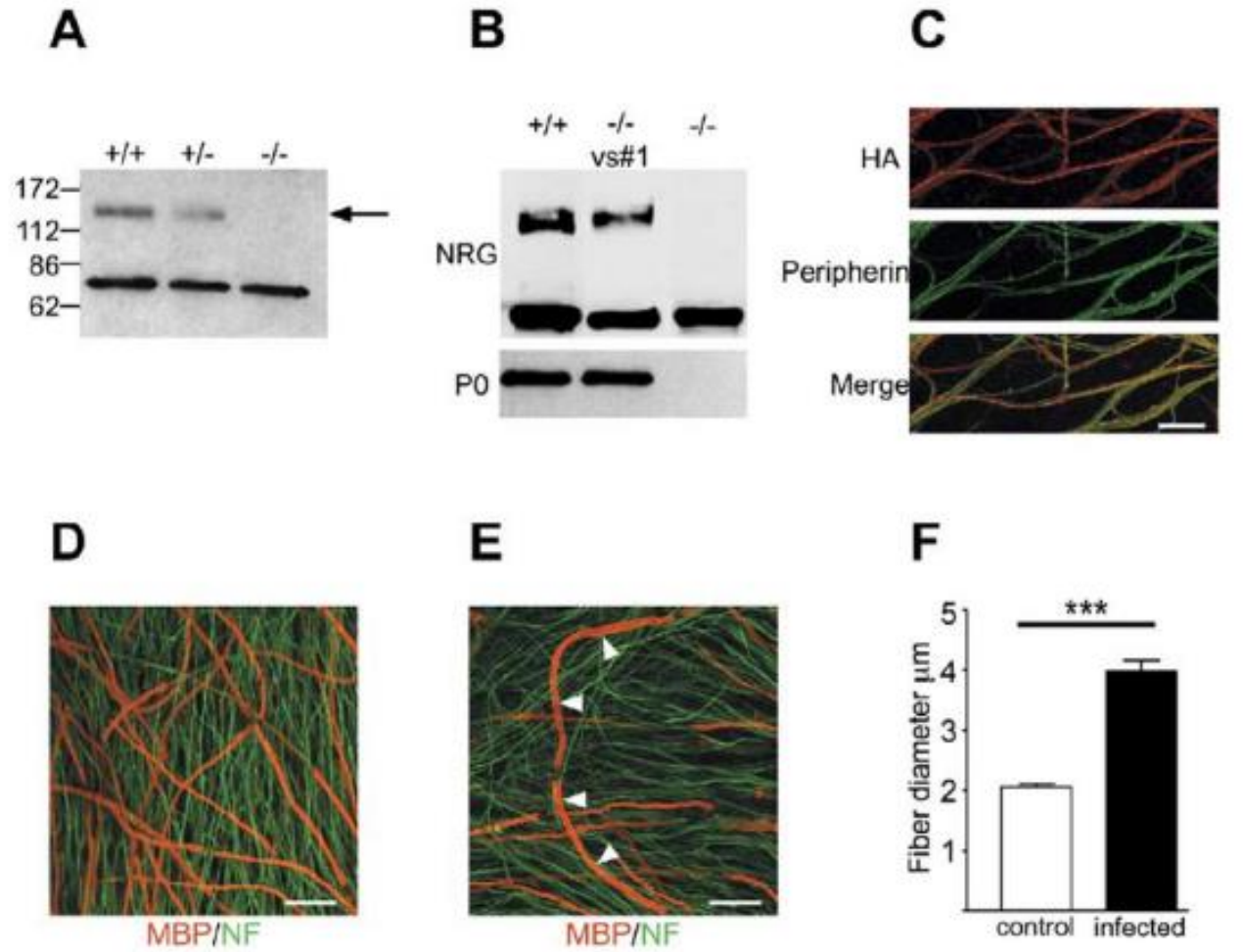


Ablation of axonal transmembrane NRG1 (type III)
→ hypo-myelination
→ impairs re-myelination

Overexpression of transmembrane NRG1
→ **hyper-myelination**
→ **improves re-myelination**

(Michailov et al, 2004; Taveggia et al., 2005)

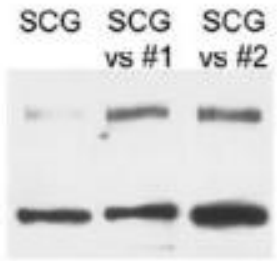
Forced Expression of NRG1 Type III Rescues the Myelination Defect of NRG1 Type III−/− Neurons and Results in Hypermyelination

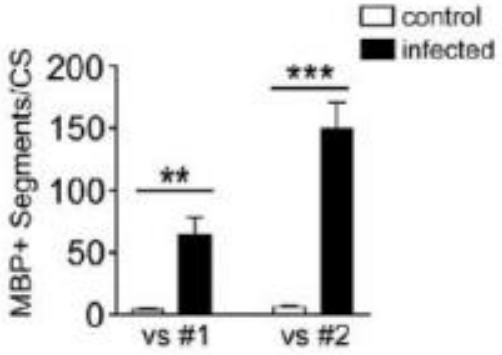


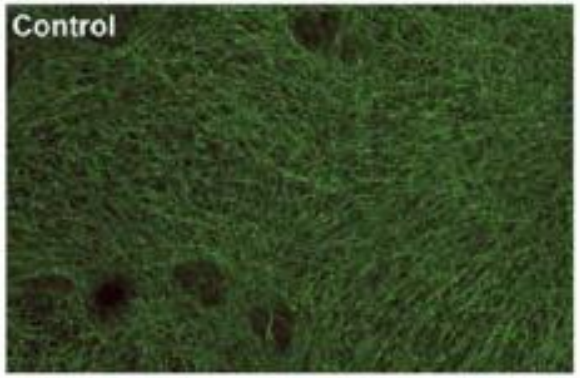
D-Rescue of myelination of NRG1 type III−/− neurons by expression of NRG1 type III is shown in cocultures double stained for MBP (rhodamine) and neurofilament (fluorescein). Scale bar, 40 µm.

(Carla Taveggia et al., Neuron, 2005)

NRG1 Type III Expression Induces Myelination



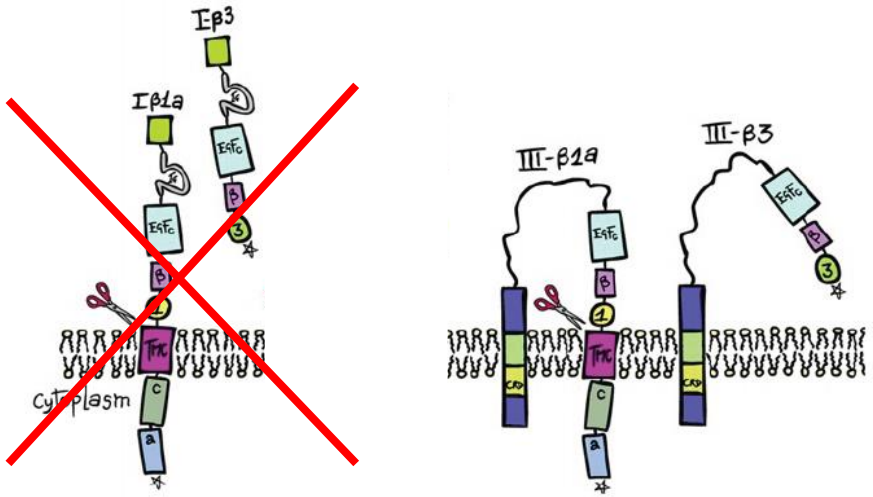




superior cervical ganglia (SCG) are ensheathed but unmyelinated

(Carla Taveggia et al., Neuron, 2005)

Soluble and transmembrane NRG1 isoforms play different roles during myelination and re-myelination



Ablation of soluble NRG1 (type I/II) expressed by Schwann cells

→ **no defects in myelination;**

→ **impairs re-myelination** *(Stassart et al., 2013)*

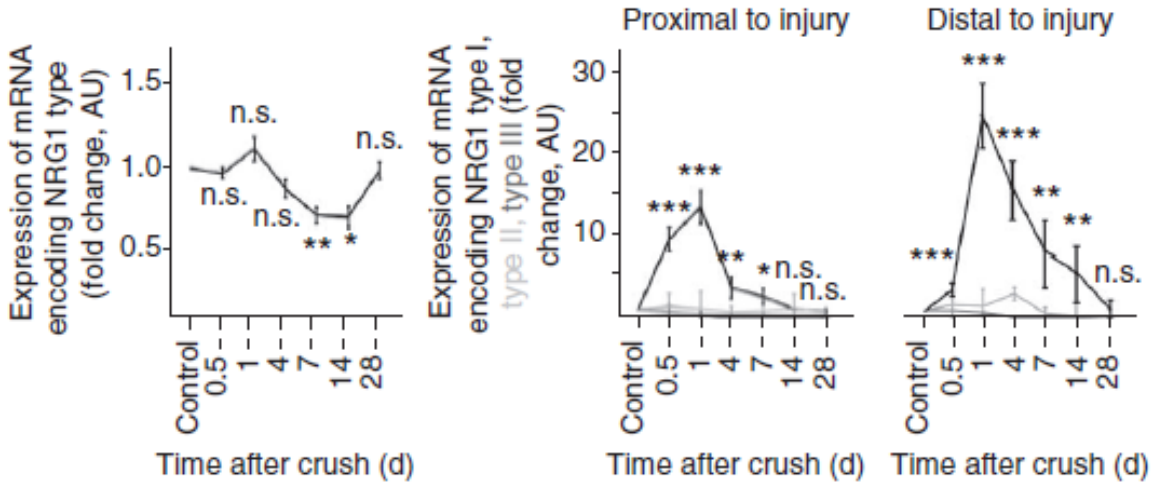
nature neuroscience

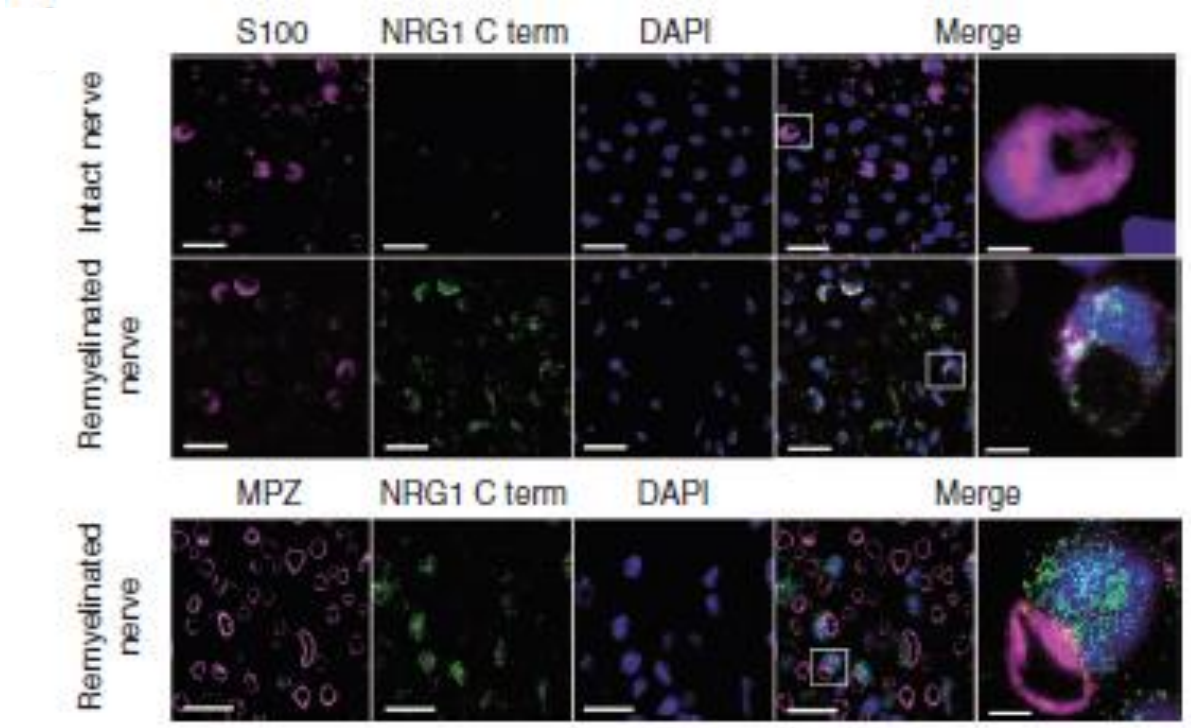
A role for Schwann cell–derived neuregulin-1 in remyelination

Ruth M Stassart[1,2,7], Robert Fledrich[1,7], Viktorija Velanac[1,3], Bastian G Brinkmann[1,4], Markus H Schwab[1], Dies Meijer[5], Michael W Sereda[1,6] & Klaus-Armin Nave[1]

After peripheral nerve injury, axons regenerate and become remyelinated by resident Schwann cells. However, myelin repair never results in the original myelin thickness, suggesting insufficient stimulation by neuronal growth factors. Upon testing this hypothesis, we found that axonal neuregulin-1 (NRG1) type III and, unexpectedly, also NRG1 type I restored normal myelination when overexpressed in transgenic mice. This led to the observation that Wallerian degeneration induced *de novo* NRG1 type I expression in Schwann cells themselves. Mutant mice lacking a functional *Nrg1* gene in Schwann cells are fully myelinated but exhibit impaired remyelination in adult life. We suggest a model in which loss of axonal contact triggers denervated Schwann cells to transiently express NRG1 as an autocrine/paracrine signal that promotes Schwann cell differentiation and remyelination.

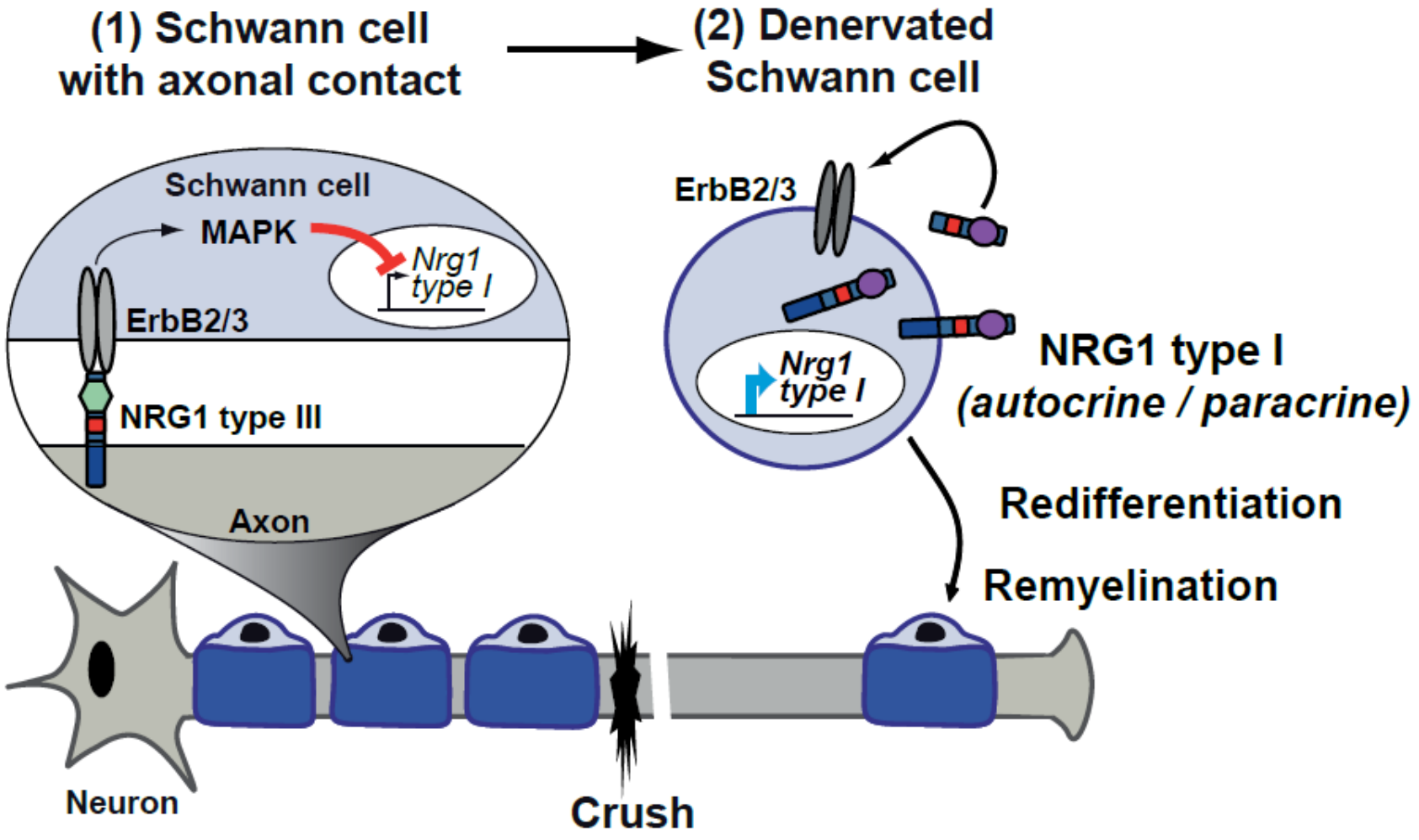
Schwann cell–derived NRG1 is induced after nerve injury





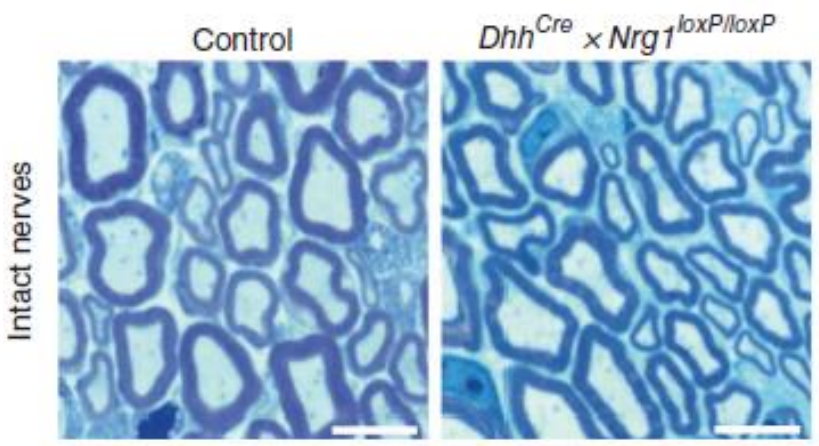
Ruth Stassart

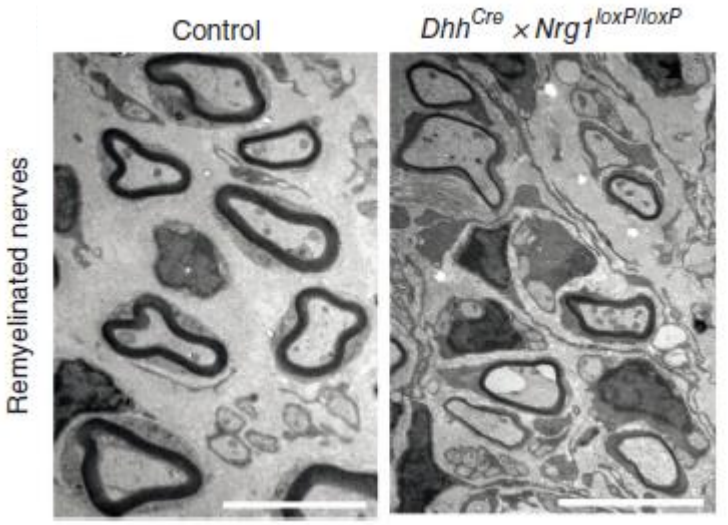
Schwann cell NRG1 expression is controlled by axonal NRG1

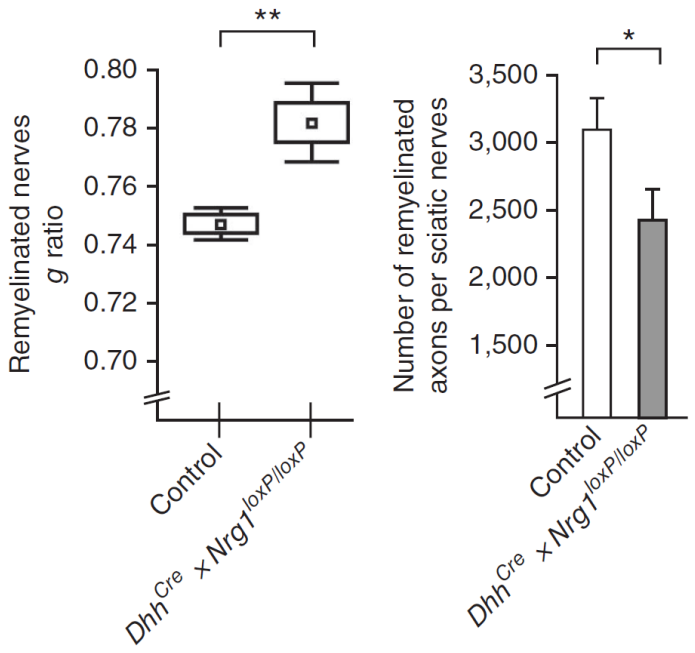


(Ruth Stassart, et al. Nature Neuroscience, 2013)

Schwann cell–derived NRG1 is required for efficient remyelination, but does not affect myelination during development

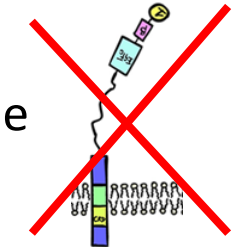
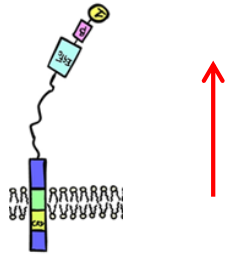
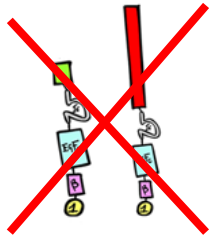




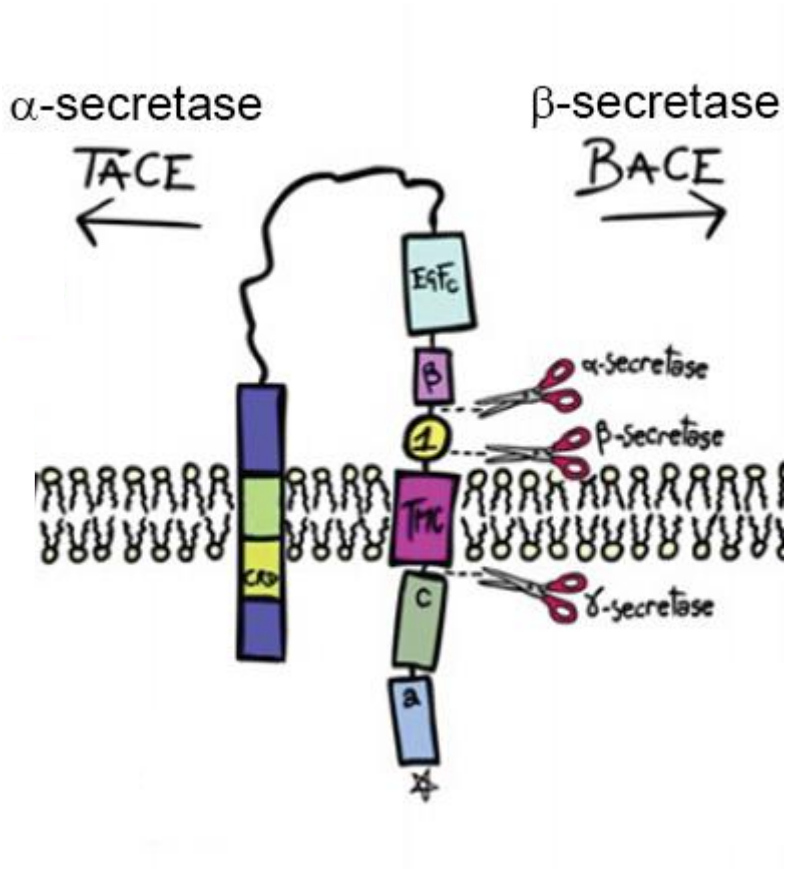


(Ruth Stassart, et al. Nature Neuroscience, 2013)

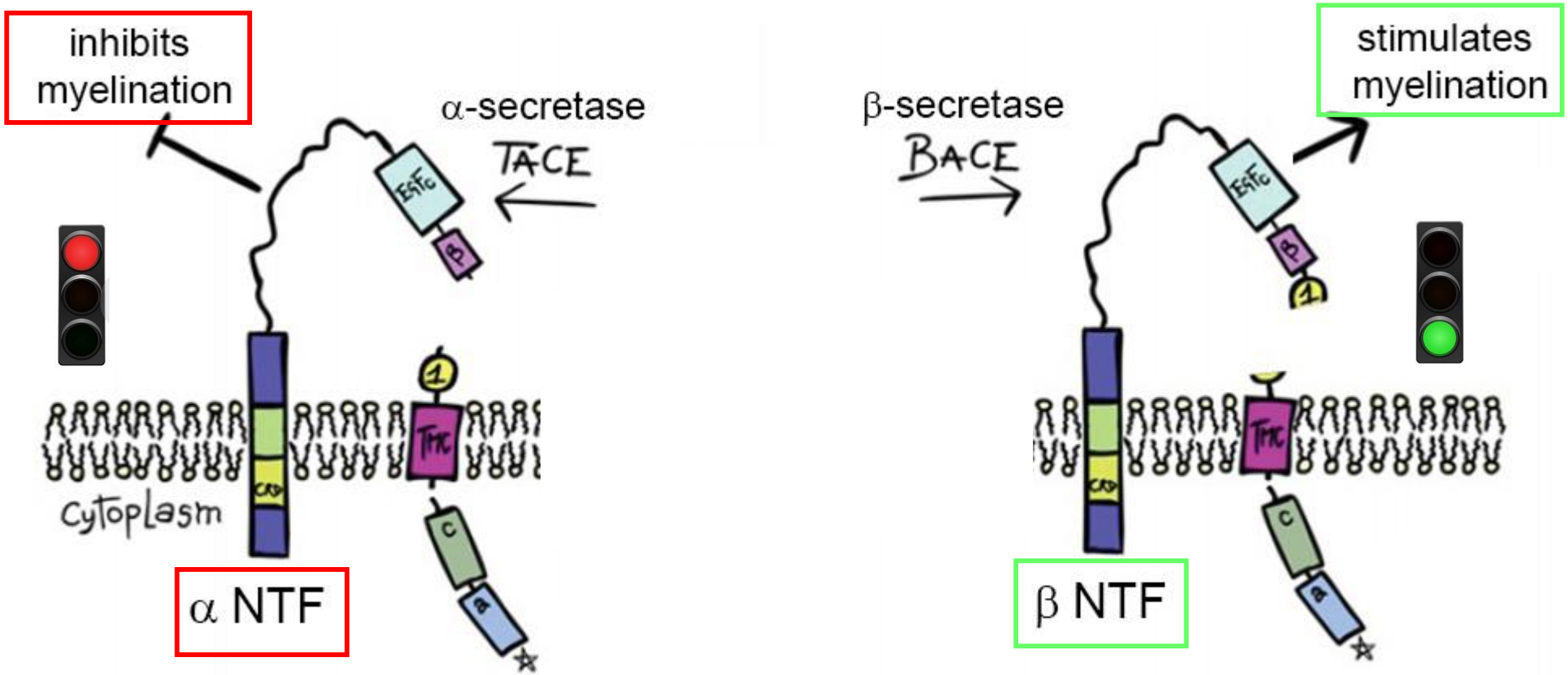
TRANSGENIC MICE MODELS

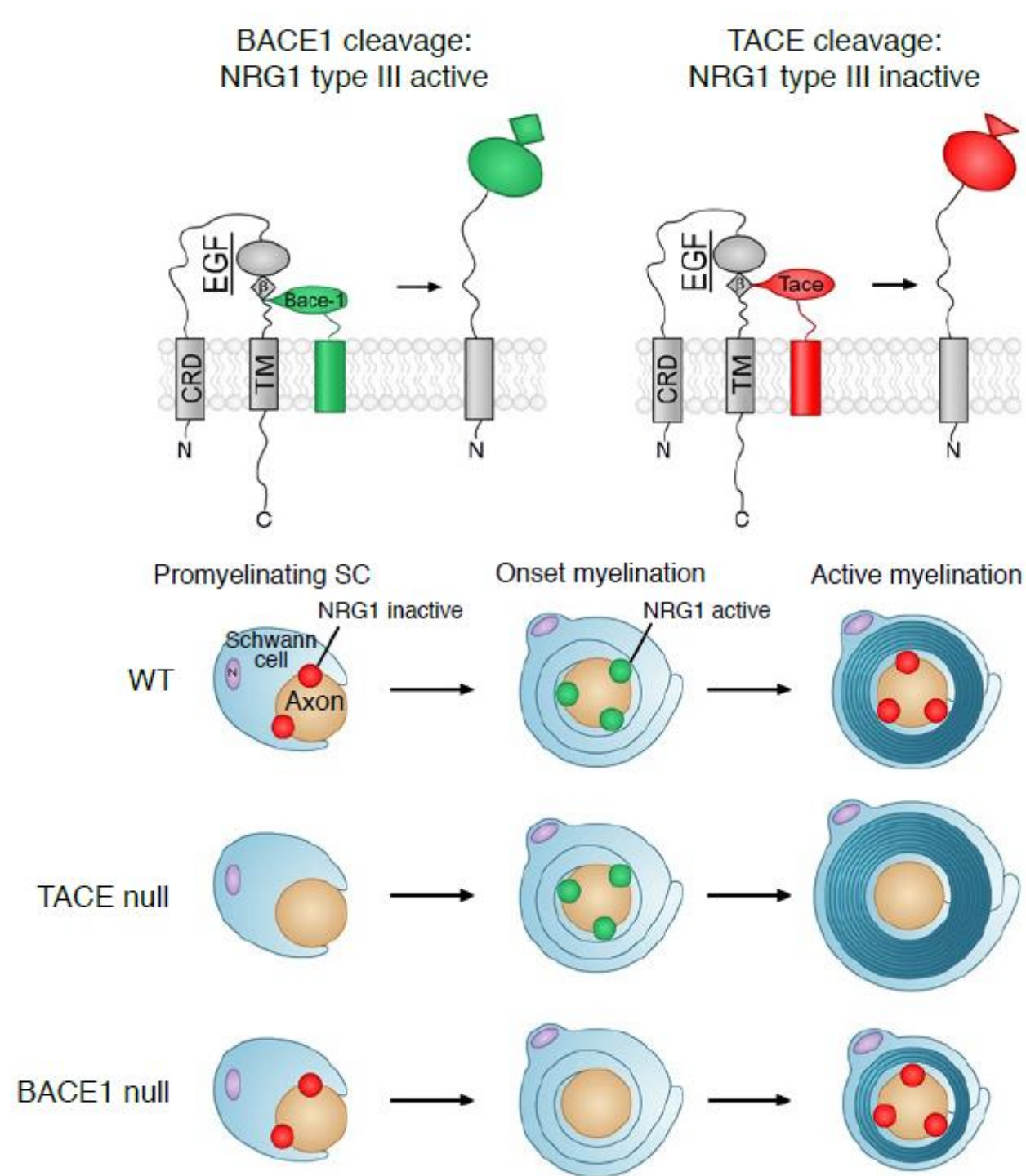
	myelination	remyelination	
transmembrane 	-	-	
transmembrane 	+	+	
soluble 	/	-	

Axonal transmembrane NRG1 processing affects myelination



Axonal transmembrane NRG1 processing affects myelination



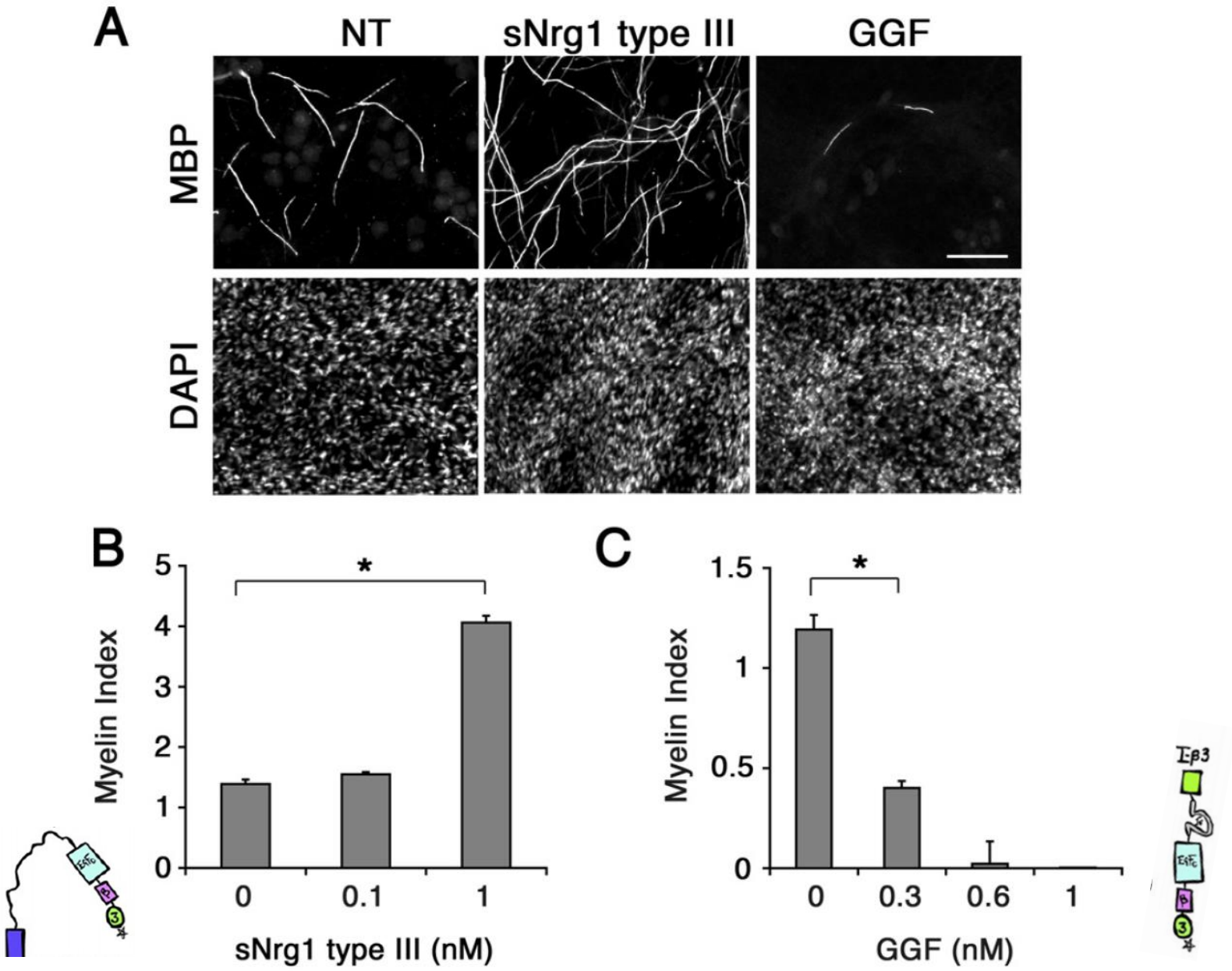

BACE1 cleavage:
NRG1 type III active
TACE cleavage:
NRG1 type III inactive
EGF
Bace-1
Tace
CRD
TM
N
C
Promyelinating SC
Onset myelination
Active myelination
NRG1 inactive
NRG1 active
Schwann cell
Axon
WT
TACE null
BACE1 null

Soluble Neuregulin-1 Has Bifunctional, Concentration-Dependent Effects on Schwann Cell Myelination

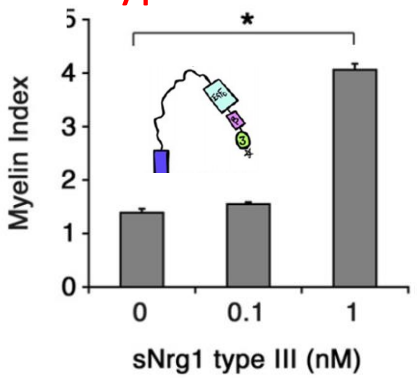
Neeraja Syed,[1]* Kavya Reddy,[1]* David P. Yang,[1] Carla Taveggia,[2] James L. Salzer,[3] Patrice Maurel,[1] and Haesun A. Kim[1]

Members of the neuregulin-1 (Nrg1) growth factor family play important roles during Schwann cell development. Recently, it has been shown that the membrane-bound type III isoform is required for Schwann cell myelination. Interestingly, however, Nrg1 type II, a soluble isoform, inhibits the process. The mechanisms underlying these isoform-specific effects are unknown. It is possible that myelination requires juxtacrine Nrg1 signaling provided by the membrane-bound isoform, whereas paracrine stimulation by soluble Nrg1 inhibits the process. To investigate this, we asked whether Nrg1 type III provided in a paracrine manner would promote or inhibit myelination. We found that soluble Nrg1 type III enhanced myelination in Schwann cell-neuron cocultures. It improved myelination of Nrg1 type III $^{+/-}$ neurons and induced myelination on normally nonmyelinated sympathetic neurons. However, soluble Nrg1 type III failed to induce myelination on Nrg1 type III $^{-/-}$ neurons. To our surprise, low concentrations of Nrg1 type II also elicited a similar promyelinating effect. At high doses, however, both type II and III isoforms inhibited myelination and increased c-Jun expression in a manner dependent on Mek/Erk (mitogen-activated protein kinase kinase/extracellular signal-regulated kinase) activation. These results indicate that paracrine Nrg1 signaling provides concentration-dependent bifunctional effects on Schwann cell myelination. Furthermore, our studies suggest that there may be two distinct steps in Schwann cell myelination: an initial phase dependent on juxtacrine Nrg1 signaling and a later phase that can be promoted by paracrine stimulation.

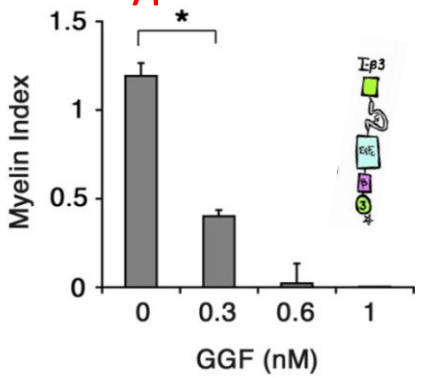
« Soluble » Nrg1 type III promotes Schwann cell myelination

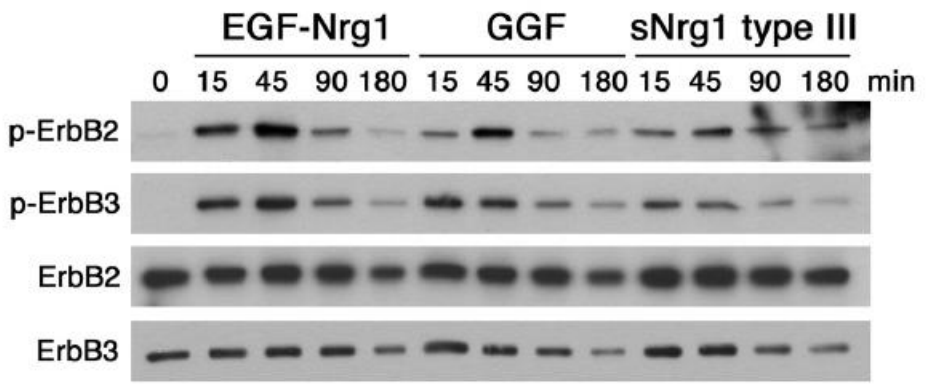


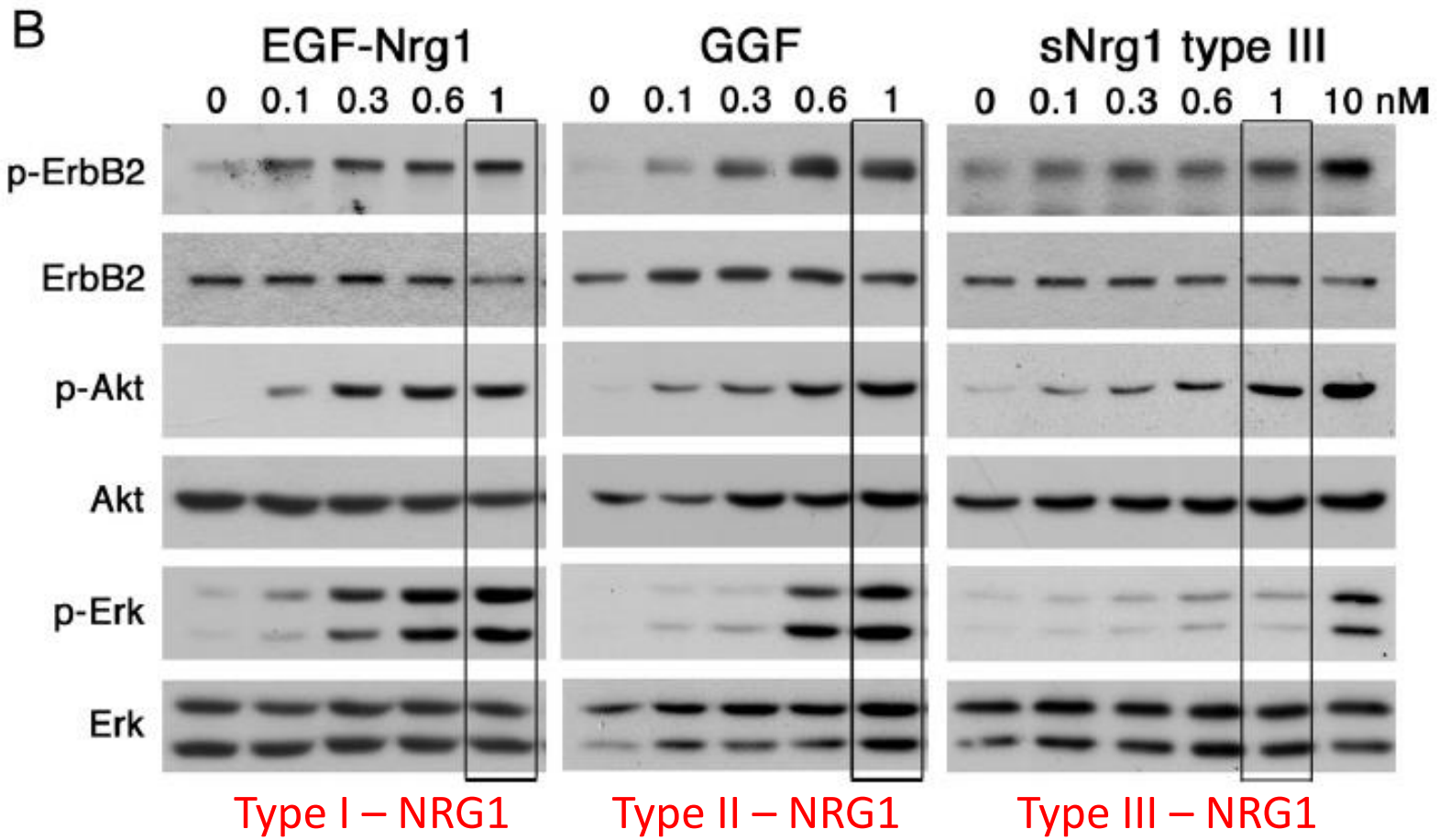
Type III – NRG1



Type II – NRG1





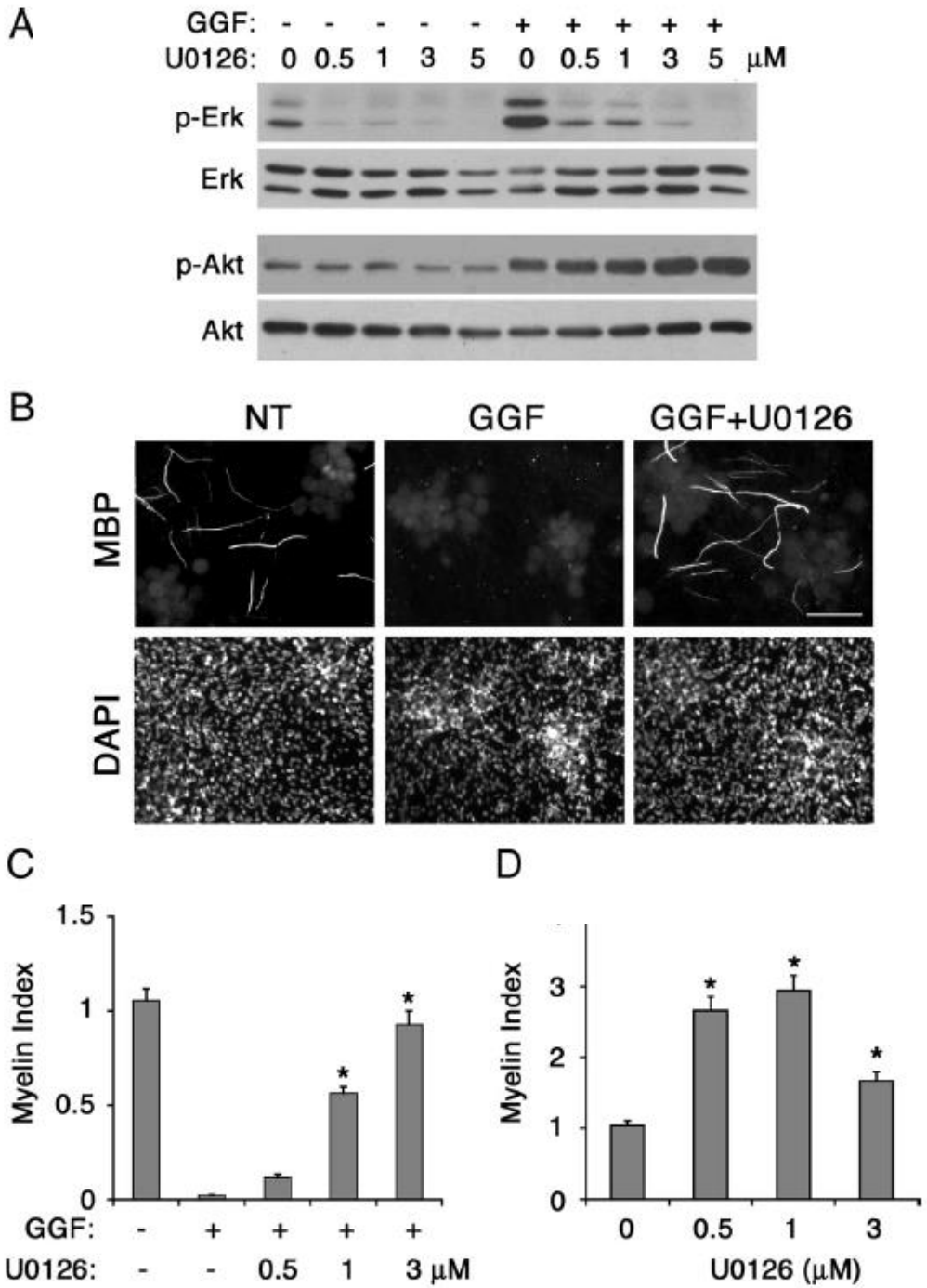


Type I – NRG1

Type II – NRG1

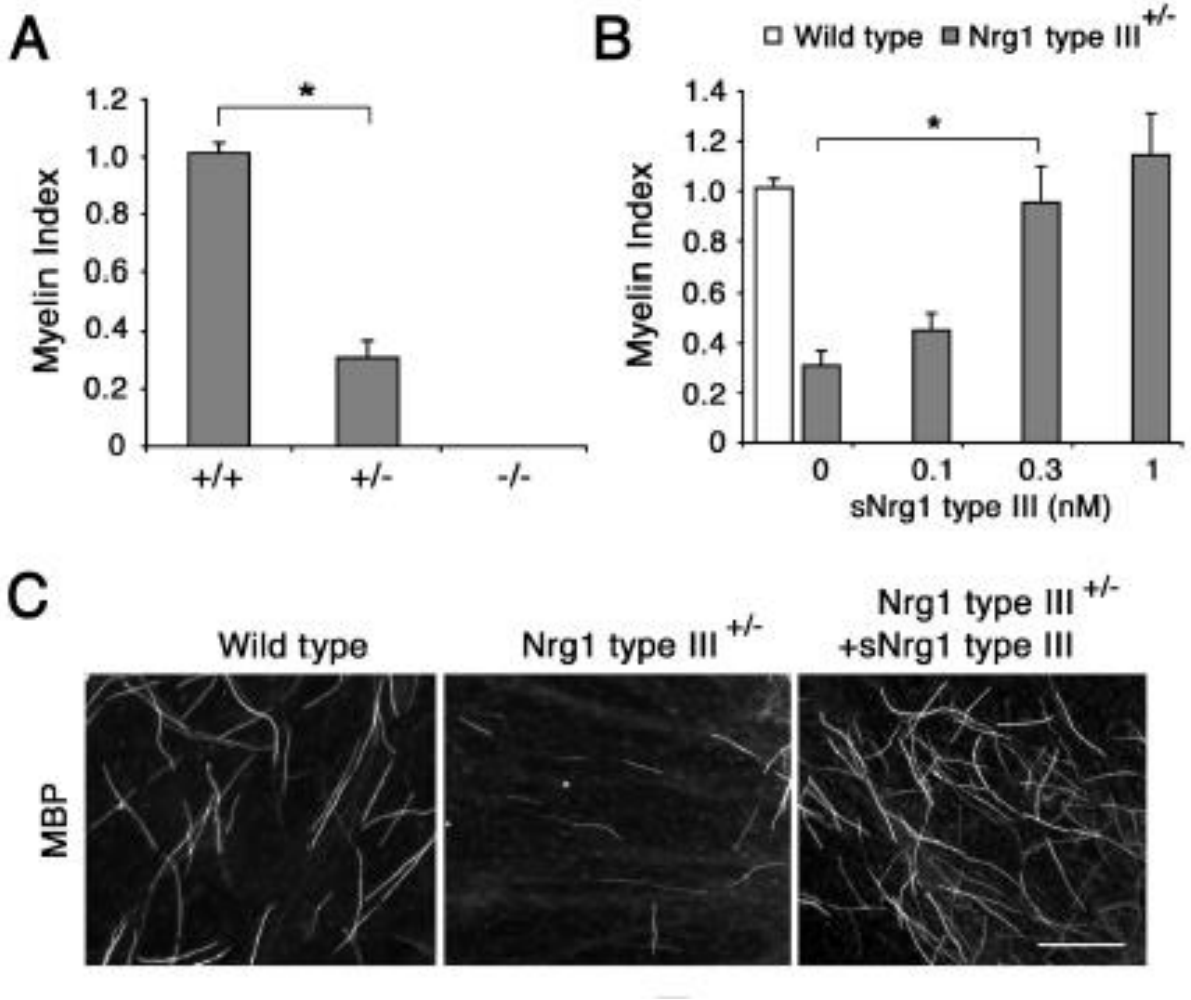
Type III – NRG1

(Syed et al, JNeuroscience 2010)



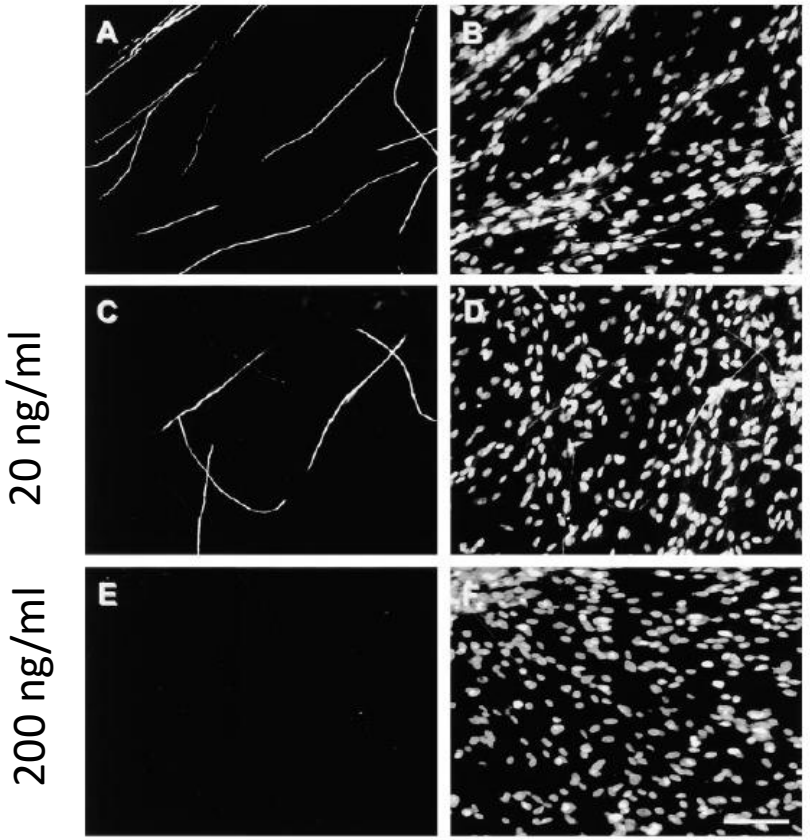
(Syed et al, JNeuroscience 2010)

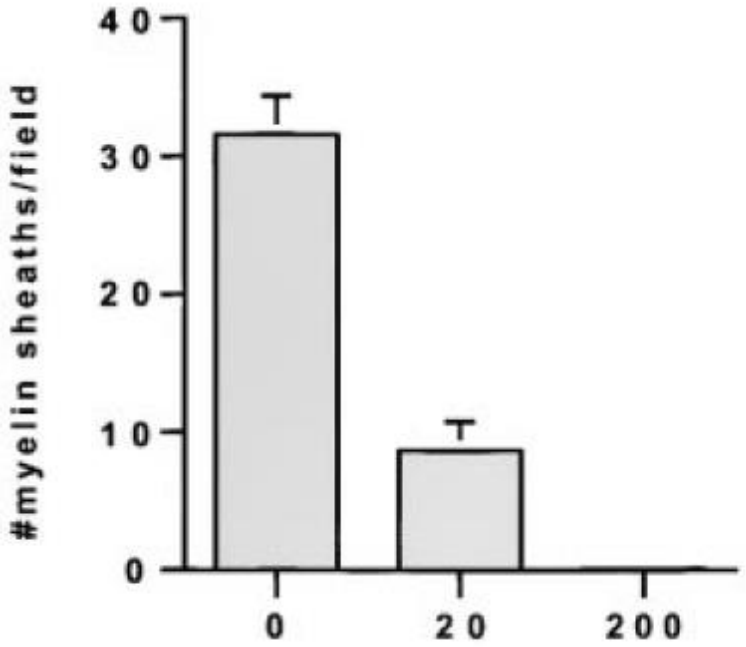
Soluble Nrg1 type III rescues the myelination defect on transmembrane Nrg1 KO neurons



Glial Growth Factor/Neuregulin Inhibits Schwann Cell Myelination and Induces Demyelination

George Zanazzi,* Steven Einheber,* Richard Westreich,* Melanie-Jane Hannocks,* Debra Bedell-Hogan,‡ Mark A. Marchionni,‡ and James L. Salzer*§‖





GGF and FGF-2 inhibit myelination

(A–F) Schwann cell-neuron cocultures were grown in myelin-promoting media without (A and B) or with 20 (C and D) or 200 (E and F) ng/ml GGF and maintained for 7 days. The cocultures were fixed and stained for MBP (A, C, and E) and Hoechst dye to visualize Schwann cell nuclei (B, D, and F). GGF strikingly inhibited myelination in a dose-dependent manner. Bar, 100 mm.

Glial Growth Factor/Neuregulin Inhibits Schwann Cell Myelination and Induces Demyelination

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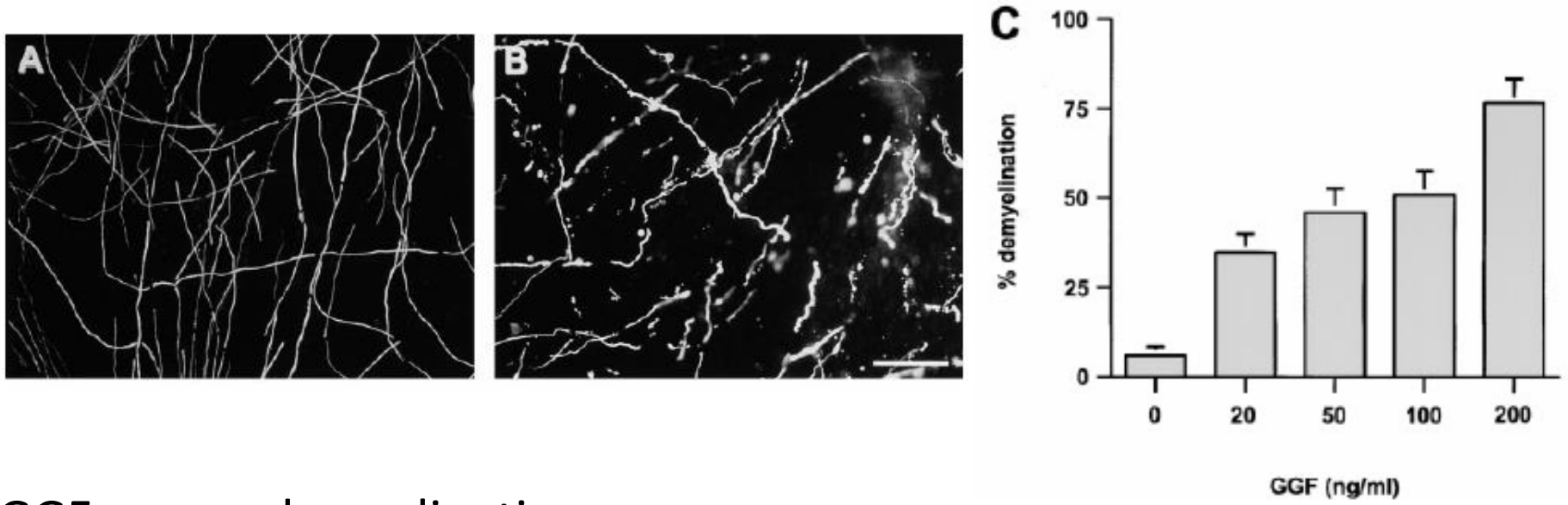


GGF causes demyelination

Immunofluorescence micrographs of cocultures that had myelinated for 3 weeks demonstrating normal myelination in control cultures (A) or substantial demyelination in companion cultures after 3 days of treatment with 200 ng/ml GGF (B). The mean percentage of damaged myelin sheaths in the cultures treated with GGF for 2 days was significantly higher than that in control cultures (*P* , 0.0001) (C).

European Journal of Neuroscience, Vol. 43, pp. 351–364, 2016 doi:10.1111/ejn.12974

The Neuregulin1/ErbB system is selectively regulated during peripheral nerve degeneration and regeneration

Giulia Ronchi,[1,2] Kirsten Haastert-Talini,[3,4] Benedetta Elena Fornasari,[1] Isabelle Perroteau,[1,5] Stefano Geuna[1,2,5] and Giovanna Gambarotta[1,5]

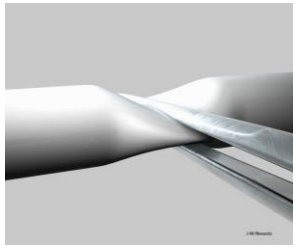
Abstract

The peripheral nervous system has an intrinsic capability to regenerate, crucially related to the ability of Schwann cells (SC) to create a permissive environment, for example, through production of regeneration-promoting neurotrophic factors. Survival, proliferation, migration and differentiation of SC into a myelinating phenotype during development and after injury is regulated by different Neuregulin1 (NRG1) isoforms. This study investigates the expression of different NRG1 isoforms and of their ErbB receptors in distal rat median nerve samples under regenerating conditions after a mild (crush) and more severe (end-to-end repair) injury and under degenerating condition. The expression of the NRG1/ErbB system was evaluated at mRNA and protein level, and demonstrated to be specific for distinct and consecutive phases following nerve injury and regeneration or the progress in degeneration. For the first time a detailed analysis of expression profiles not only of soluble and transmembrane NRG1 isoforms, but also of alpha and beta as well as type a, b and c isoforms is presented. The results of mRNA and protein expression pattern analyses were related to nerve ultrastructure changes evaluated by electron microscopy. In particular, transmembrane NRG1 isoforms are differentially regulated and proteolytically processed under regeneration and degeneration conditions. Soluble NRG1 isoforms alpha and beta, as well as type a and b, are strongly upregulated during axonal regrowth, while type c NRG1 isoform is downregulated. This is accompanied by an upregulation of ErbB receptors. This accurate regulation suggests that each molecule plays a specific role that could be clinically exploited to improve nerve regeneration.

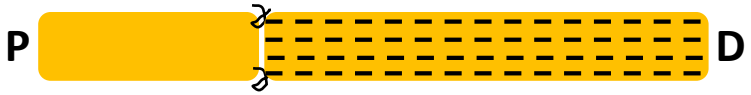
Giulia Ronchi

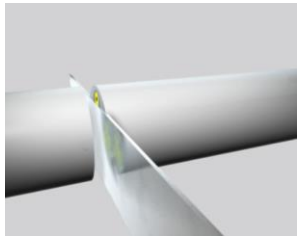
Three injury models of rat median nerve characterized by different severity degrees

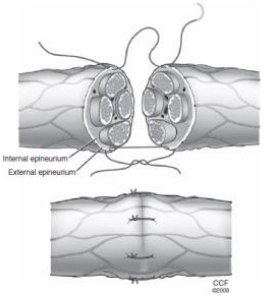
1 -*axonotmesis*
"CRUSH INJURY"



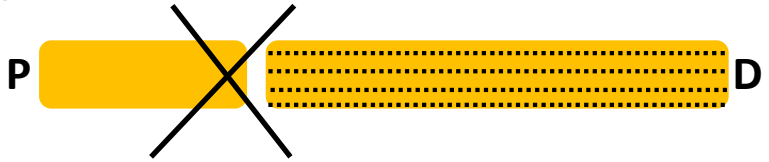
2 -*neurotmesis*
"END-TO-END REPAIR"

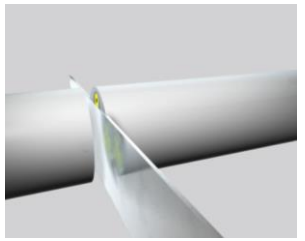






3 -*neurotmesis* (not repaired)
"DEGENERATING NERVE"

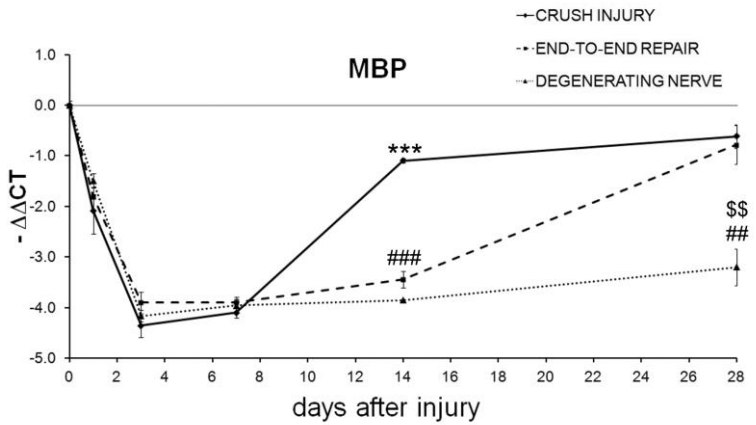


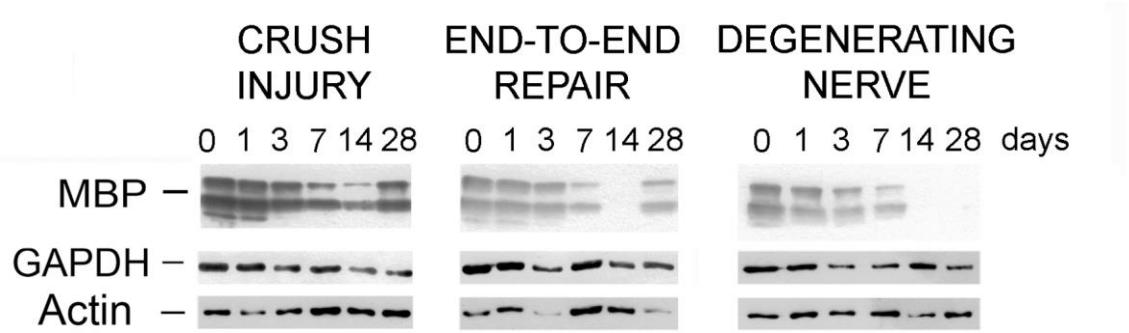


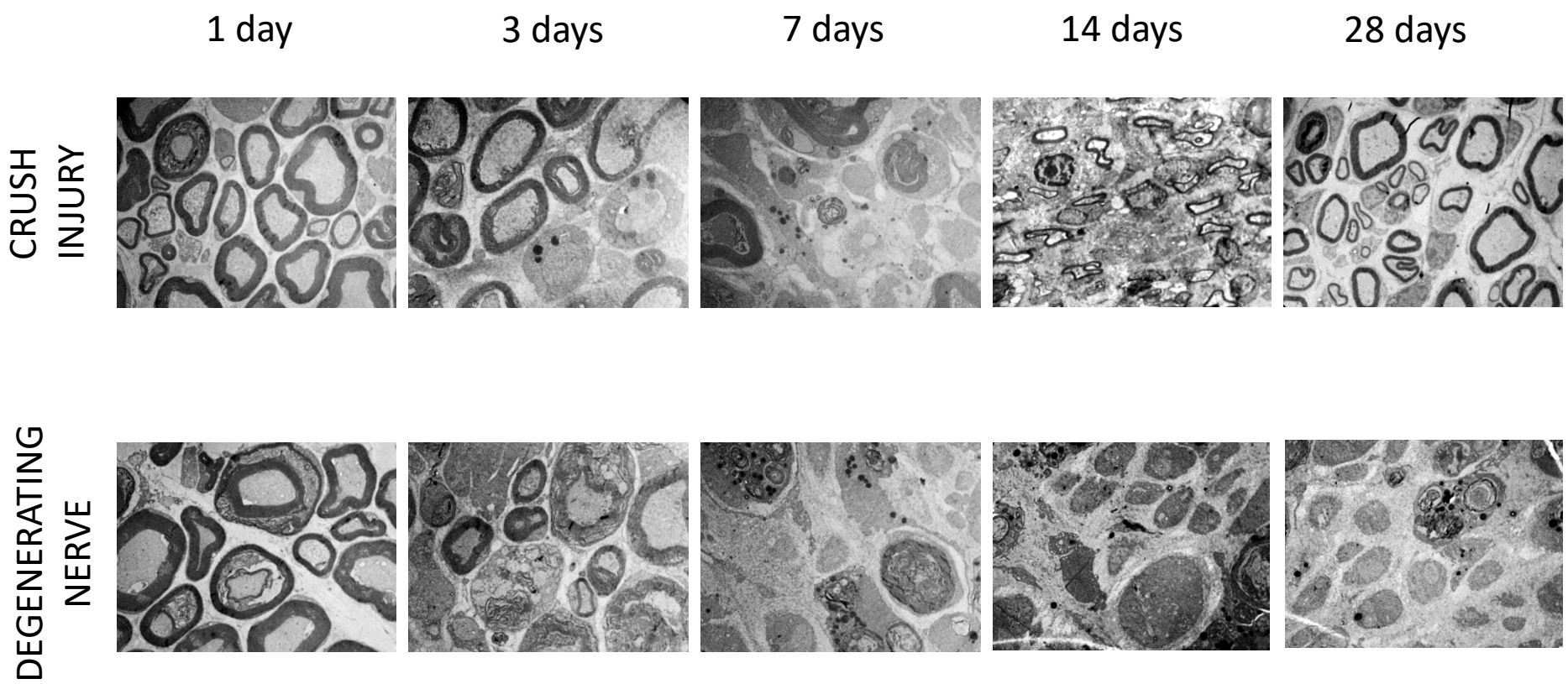
Time course: 1, 3, 7, 14, 28 days

biomolecular &
morphological analysis

(Ronchi et al., 2016)

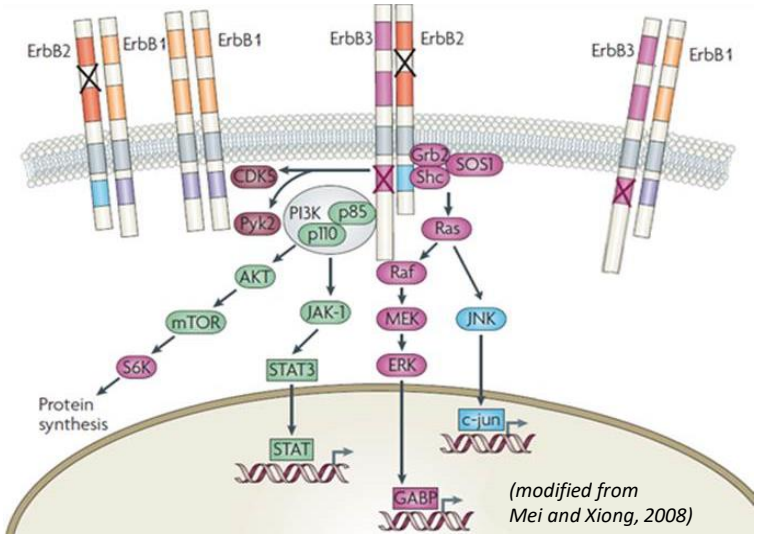


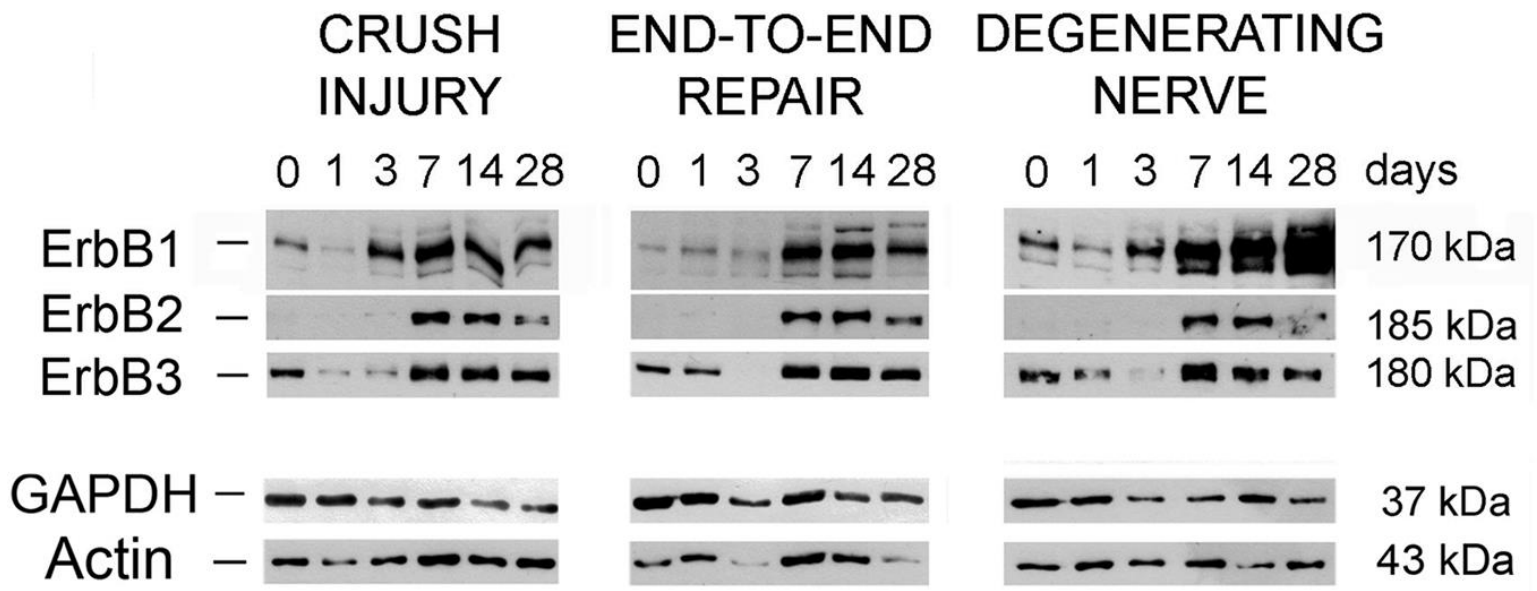




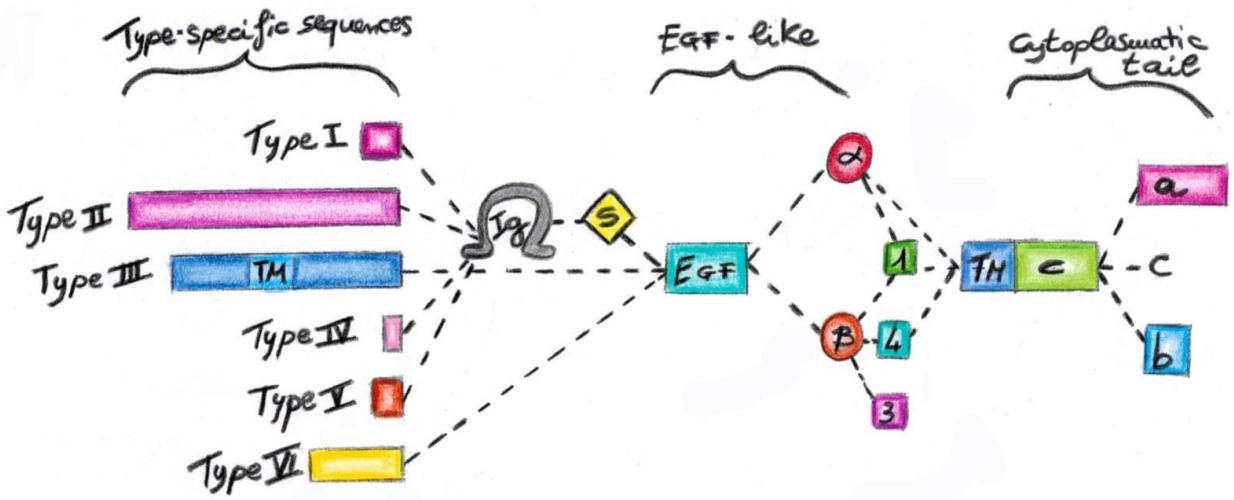
(Ronchi et al., EJNeuroscience 2016)

ErbB receptors are regulated after injury





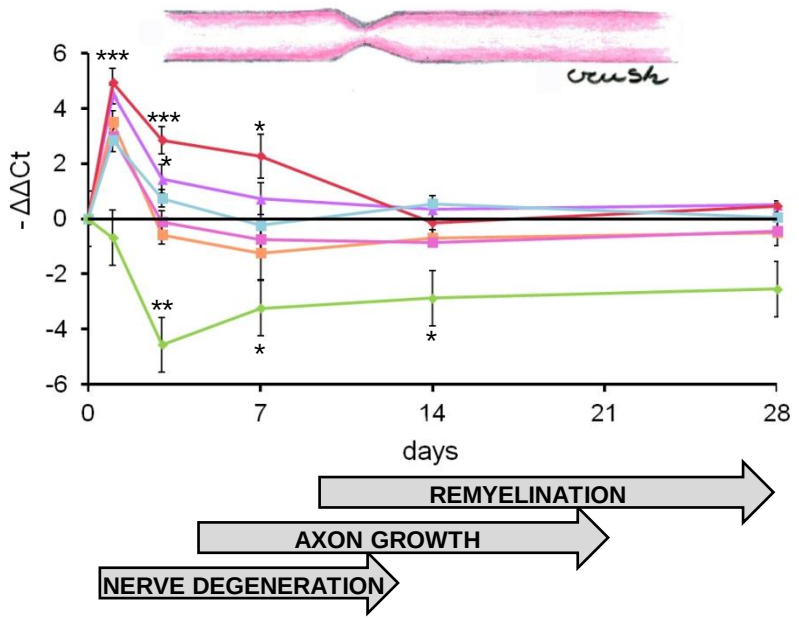
(Ronchi et al., EJNeuroscience 2016)

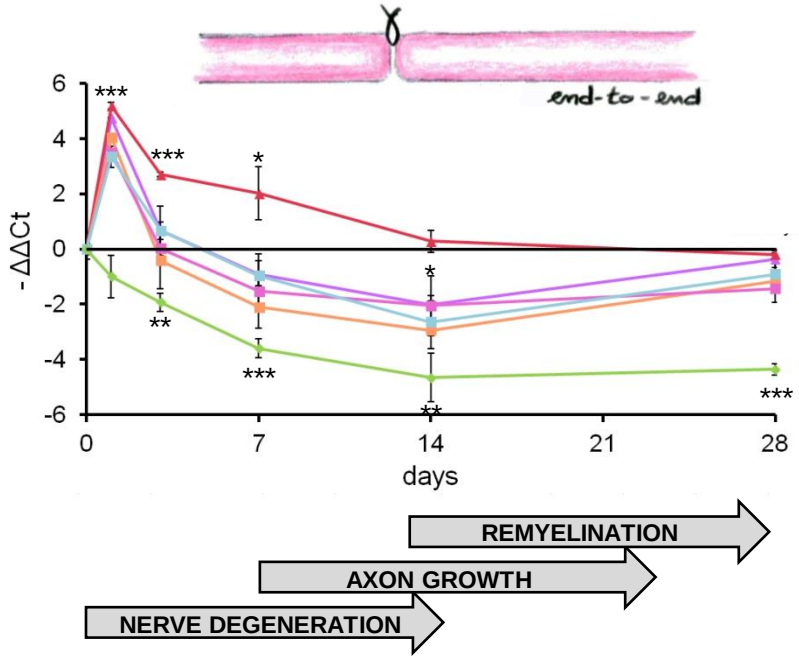


transmembrane NRG1
soluble NRG1
NRG1α
NRG1β
NRG1a
NRG1b
NRG1c

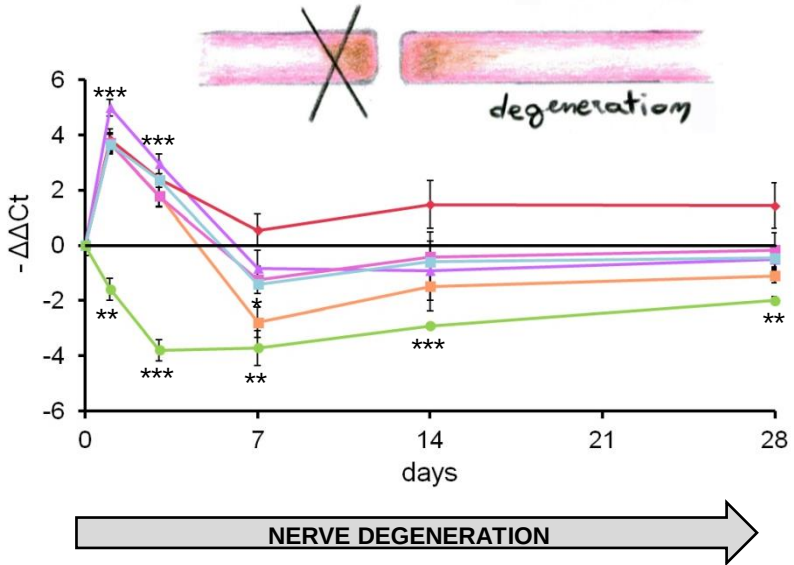
Type-specific sequences
EGF-like
Cytoplasmatic tail

Soluble NRG1, both α and β, both type a and type b, are highly expressed immediately after nerve injury





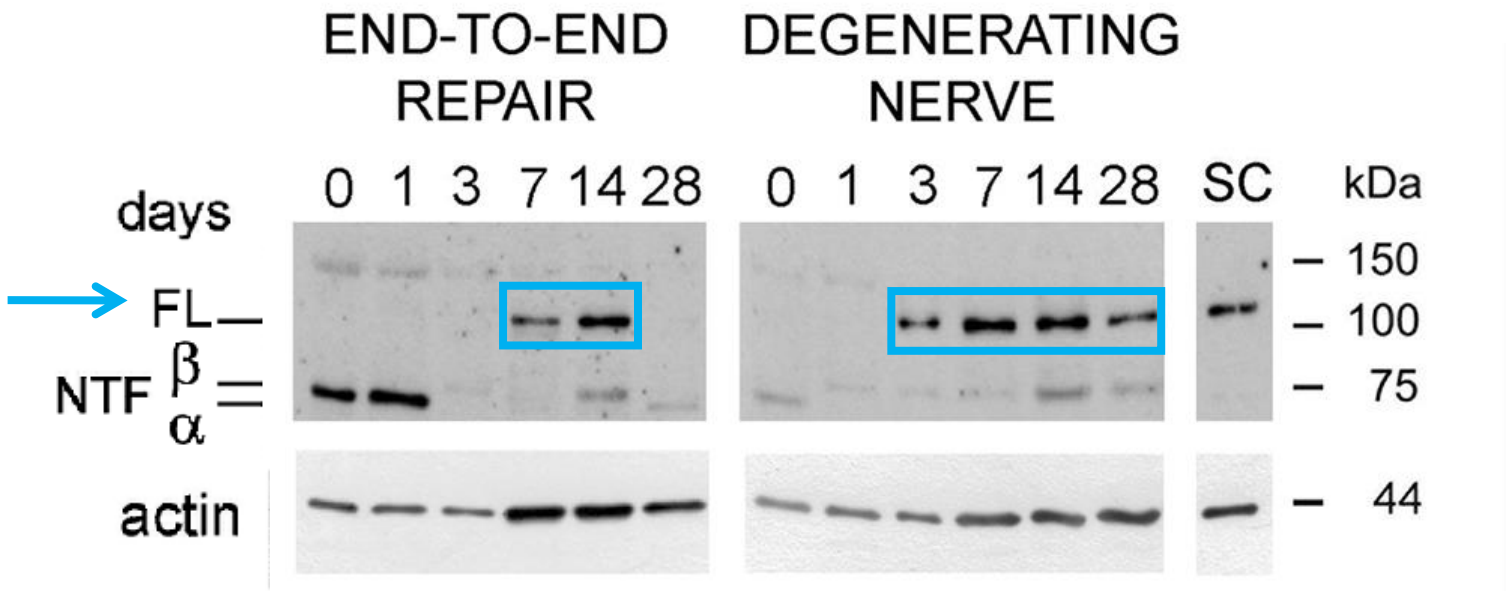
soluble NRG1
NRG1α
NRG1β
NRG1a
NRG1b
NRG1c
Healthy nerve



(Ronchi et al., EJNeuroscience 2016)

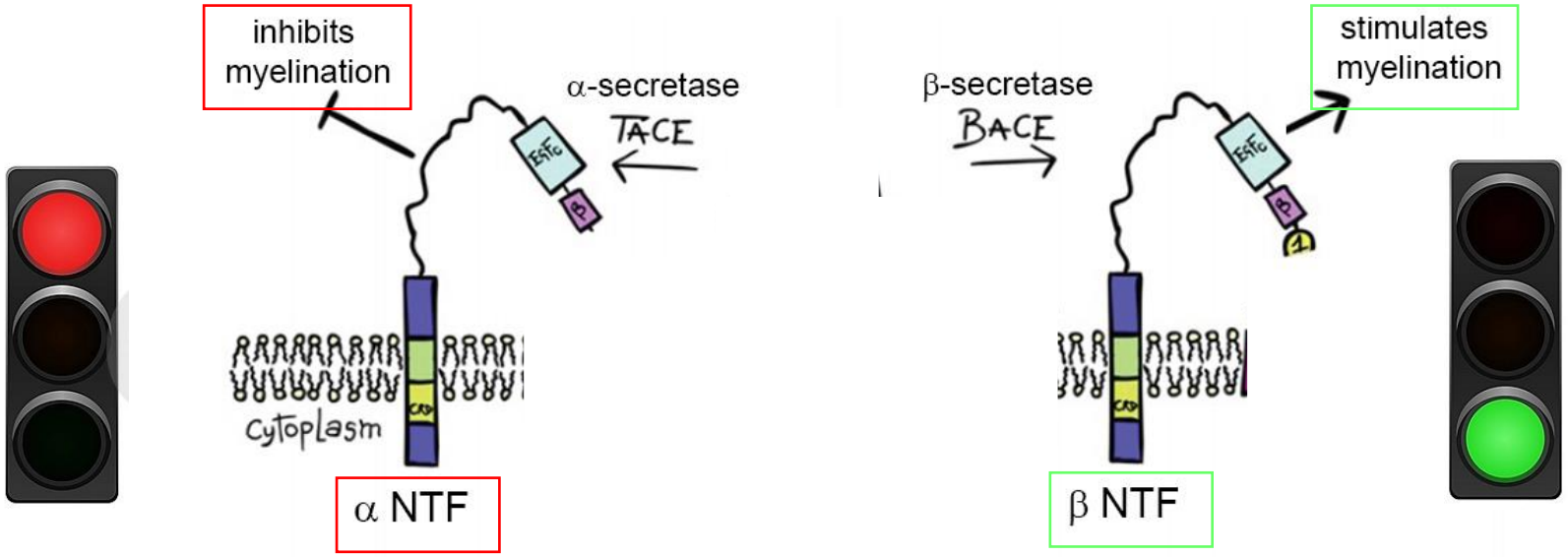
Full length 100-kDa transmembrane NRG1 isoform

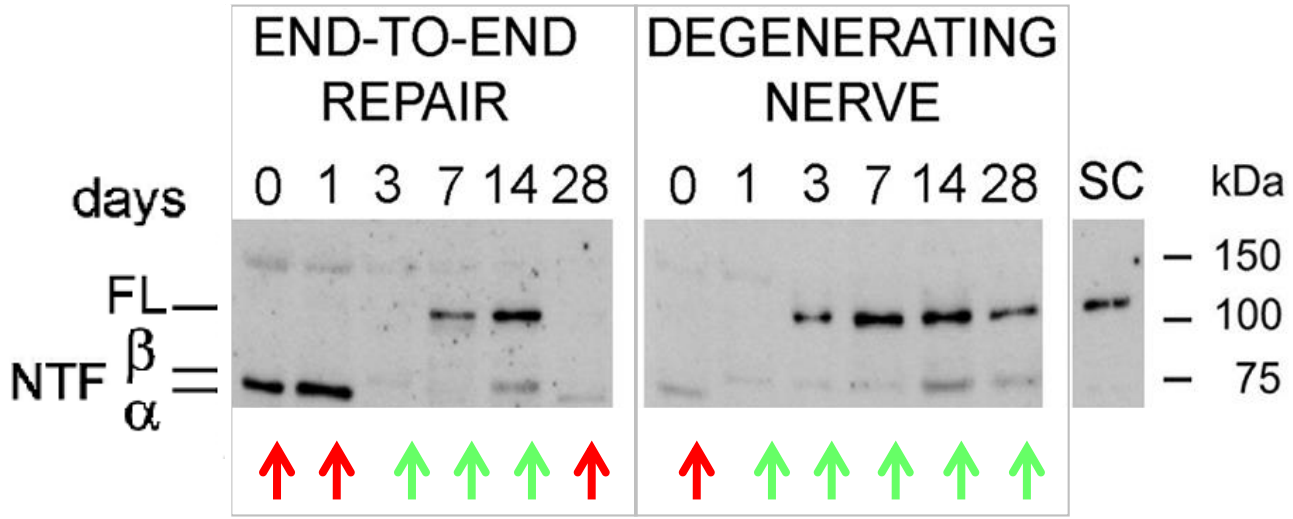
- is switched on after nerve injury and in degenerating conditions,
- is switched off under regenerating conditions



(Ronchi et al., EJNeuroscience 2016)

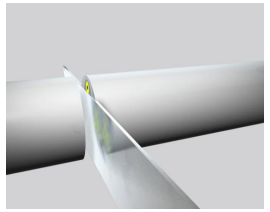
NRG1 proteolytic cleavage is selectively regulated after injury

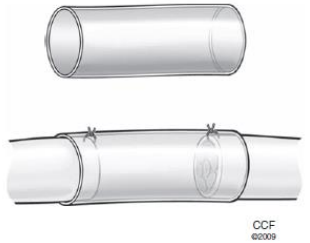




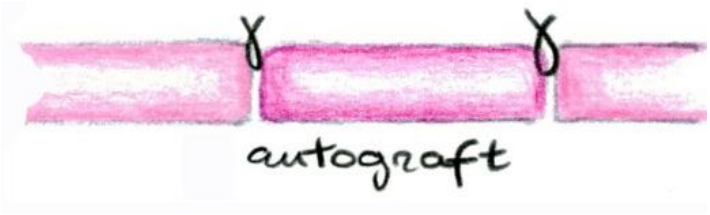
(Ronchi et al., EJNeuroscience 2016)

Peripheral nerve regeneration in more severe injuries





1 - *neurotmesis*, with substance loss, followed by AUTOGRAFT

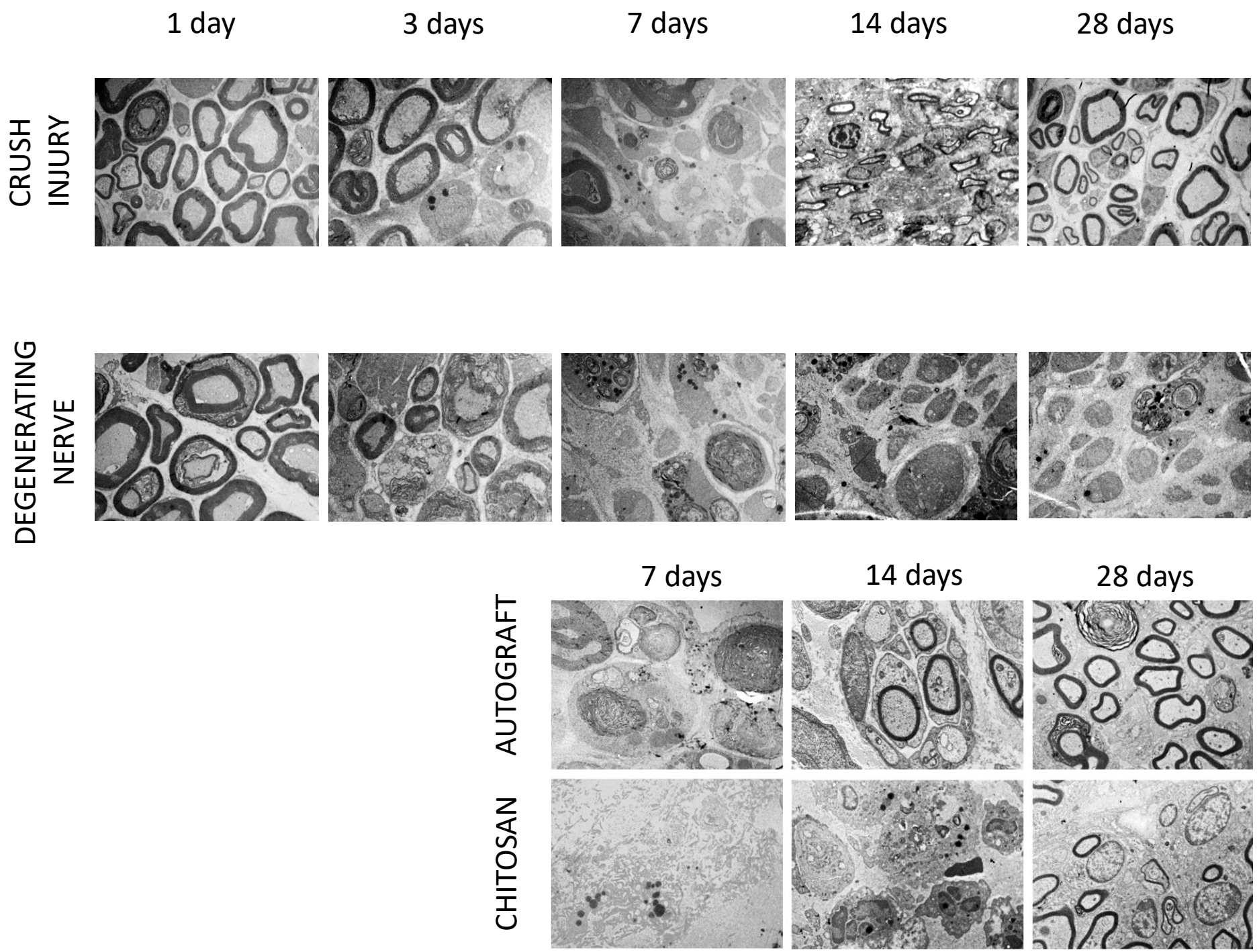


2 - *neurotmesis*, with substance loss, followed by CHITOSAN tubulization

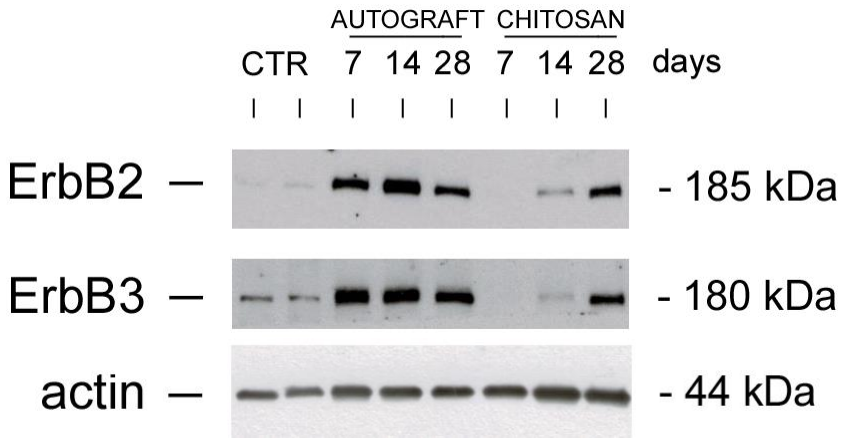
↓

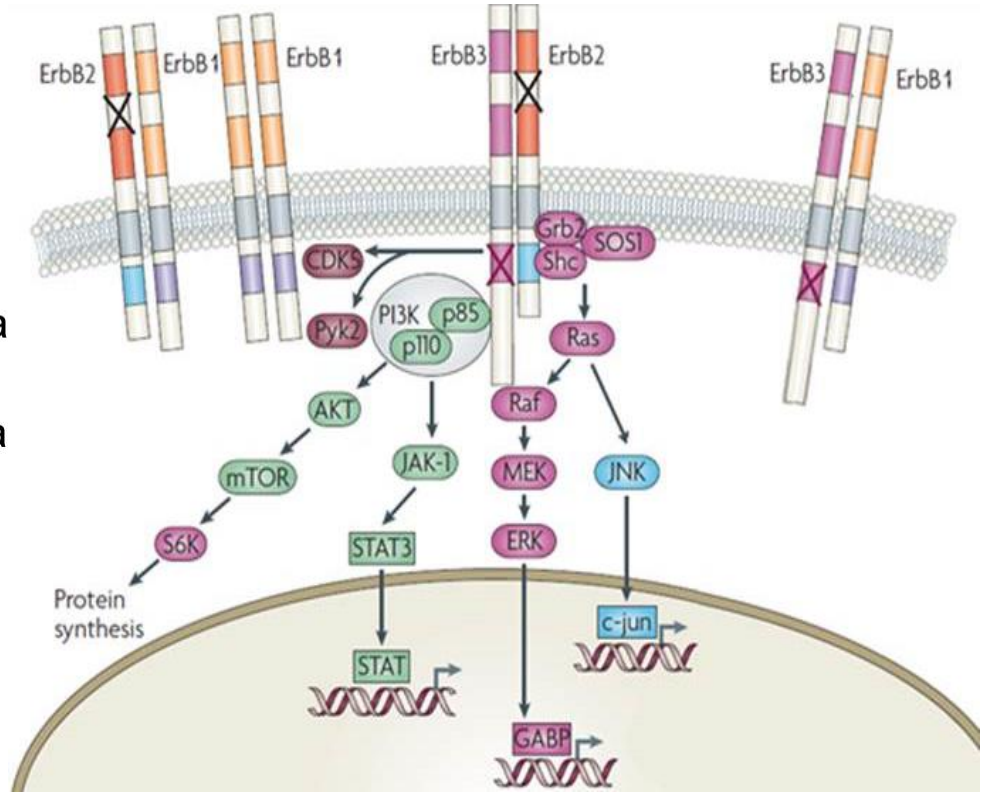
biomolecular &
morphological analysis

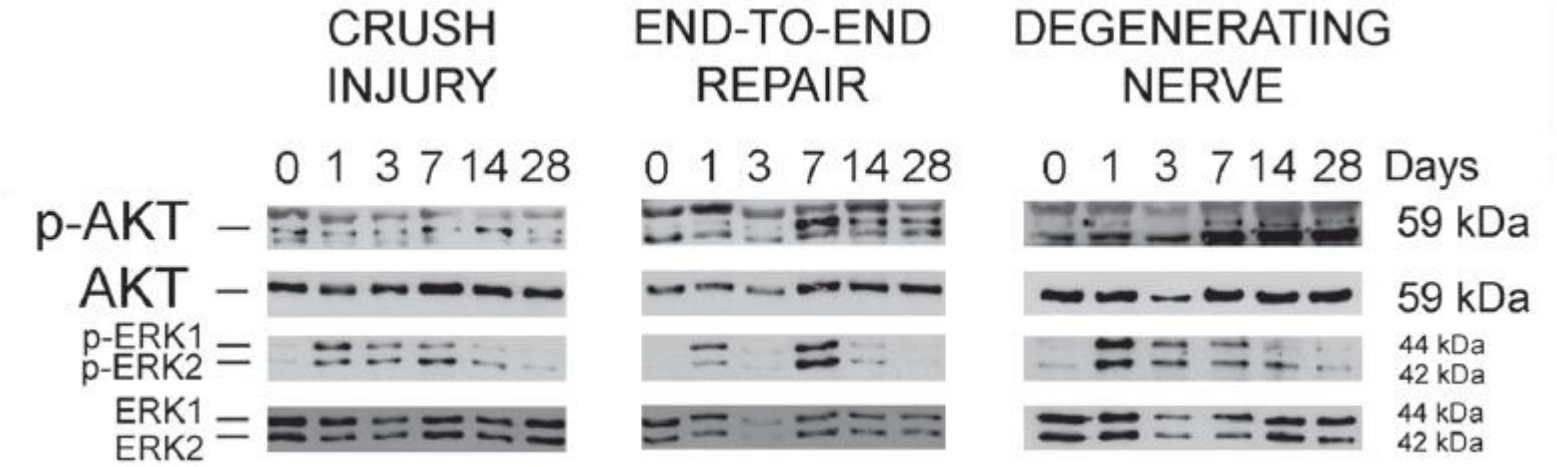
Time course: 7, 14, 28 days

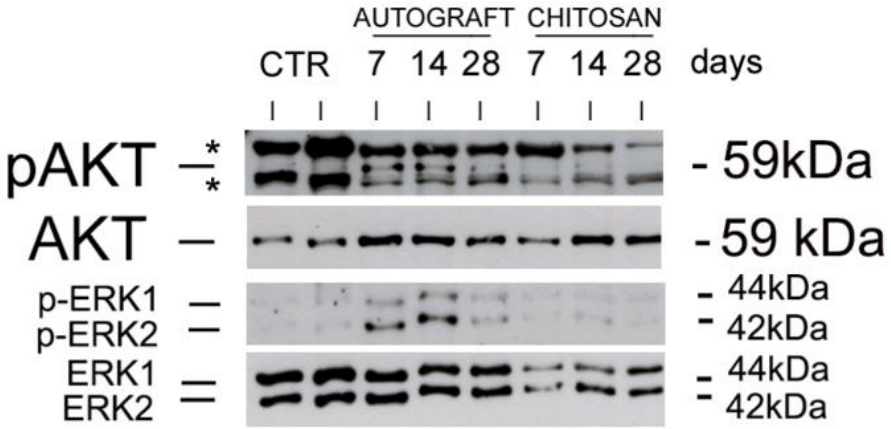

1 day
3 days
7 days
14 days
28 days
CRUSH INJURY
DEGENERATING NERVE
7 days
14 days
28 days
AUTOGRAFT
CHITOSAN

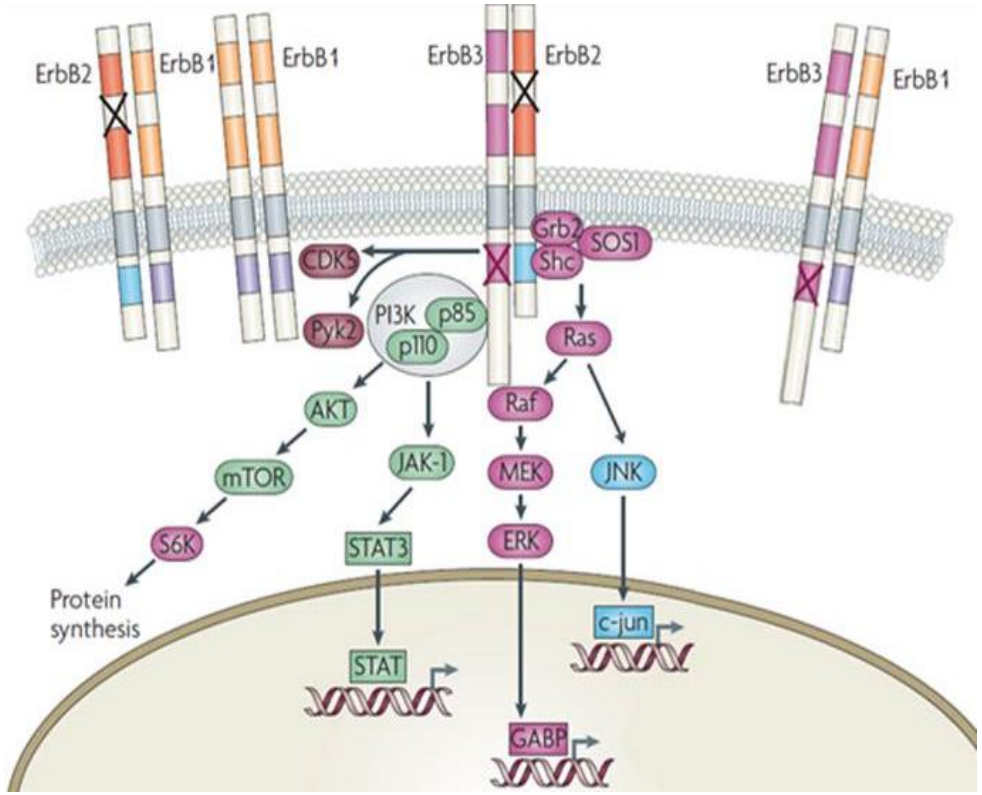

CRUSH INJURY
END-TO-END REPAIR
DEGENERATING NERVE
0 1 3 7 14 28
0 1 3 7 14 28
0 1 3 7 14 28 Days
ErbB2
ErbB3
Actin
185 kDa
180 kDa
44 kDa


AUTOGRAFT
CHITOSAN
CTR 7 14 28 7 14 28 days
ErbB2
ErbB3
actin
- 185 kDa
- 180 kDa
- 44 kDa

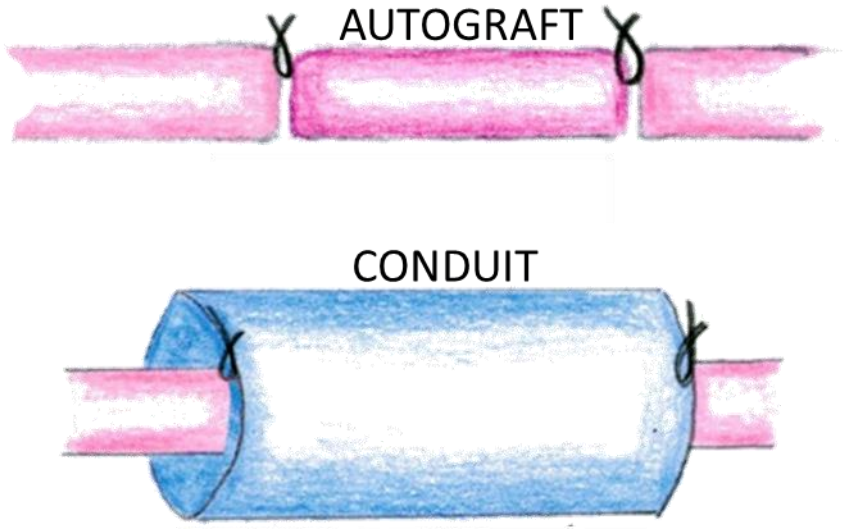

ErbB2
ErbB1
ErbB1
ErbB3
ErbB2
ErbB3
ErbB1
Grb2
SOS1
Shc
CDK5
PI3K
p85
p110
Pyk2
Ras
AKT
Raf
mTOR
JAK-1
MEK
JNK
S6K
STAT3
ERK
Protein synthesis
c-jun
STAT
GABP

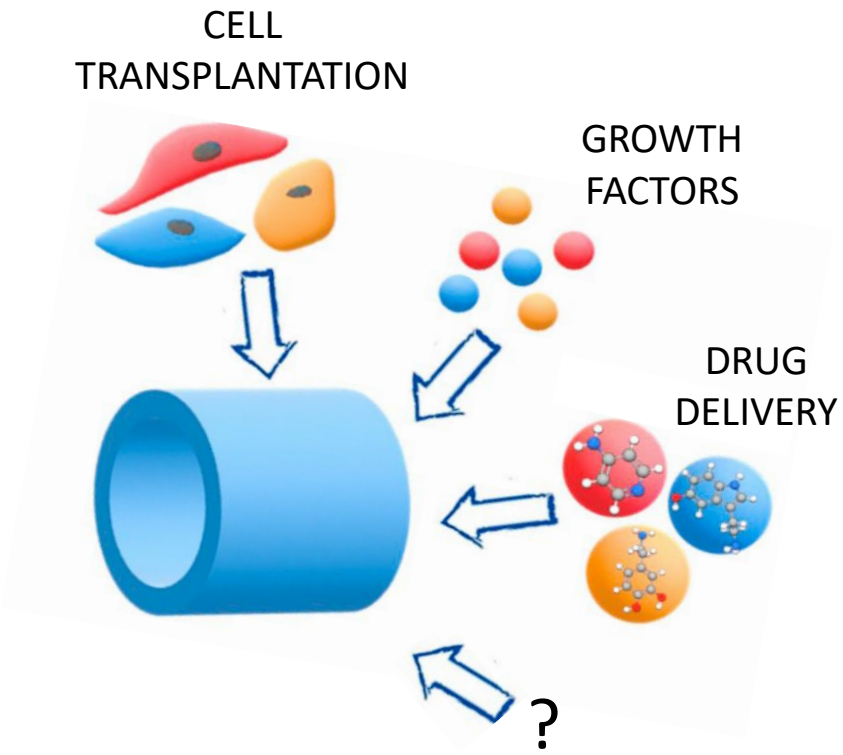

CRUSH INJURY
END-TO-END REPAIR
DEGENERATING NERVE
0 1 3 7 14 28
0 1 3 7 14 28
0 1 3 7 14 28 Days
p-AKT
AKT
p-ERK1
p-ERK2
ERK1
ERK2
59 kDa
59 kDa
44 kDa
42 kDa
44 kDa
42 kDa


AUTOGRAFT
CHITOSAN
CTR 7 14 28 7 14 28 days
pAKT
AKT
p-ERK1
p-ERK2
ERK1
ERK2
- 59kDa
- 59 kDa
- 44kDa
- 42kDa
- 44kDa
- 42kDa


ErbB2
ErbB1
ErbB1
ErbB3
ErbB2
ErbB3
ErbB1
Grb2
SOS1
Shc
CDK5
Pyk2
PI3K
p85
p110
Ras
AKT
Raf
mTOR
JAK-1
MEK
JNK
S6K
STAT3
ERK
Protein synthesis
c-jun
STAT
GABP

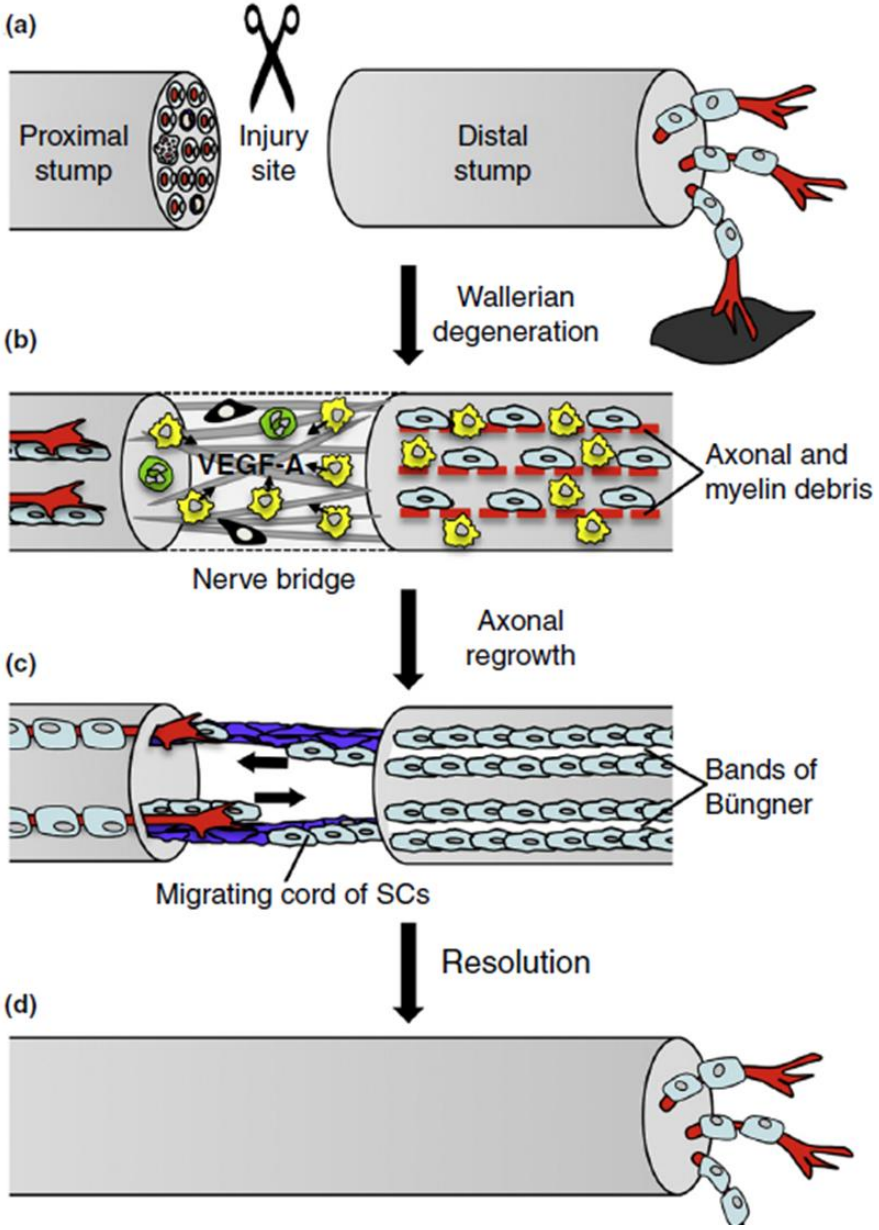
Nerve conduit characterization for efficient enrichment

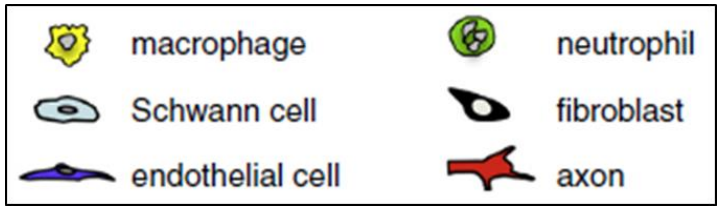


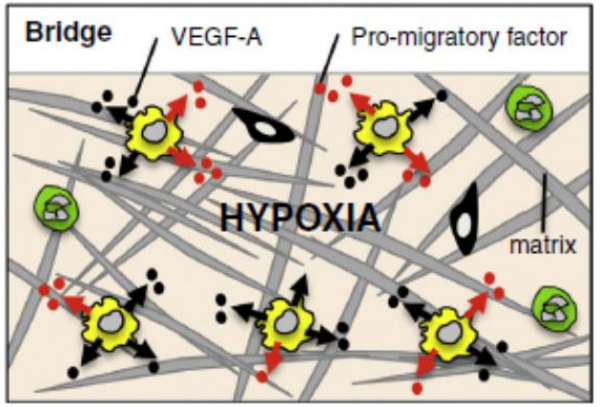


(from Manoukian et al, 2020)

In the nerve bridge blood vessels provide a track for Schwann cell migration

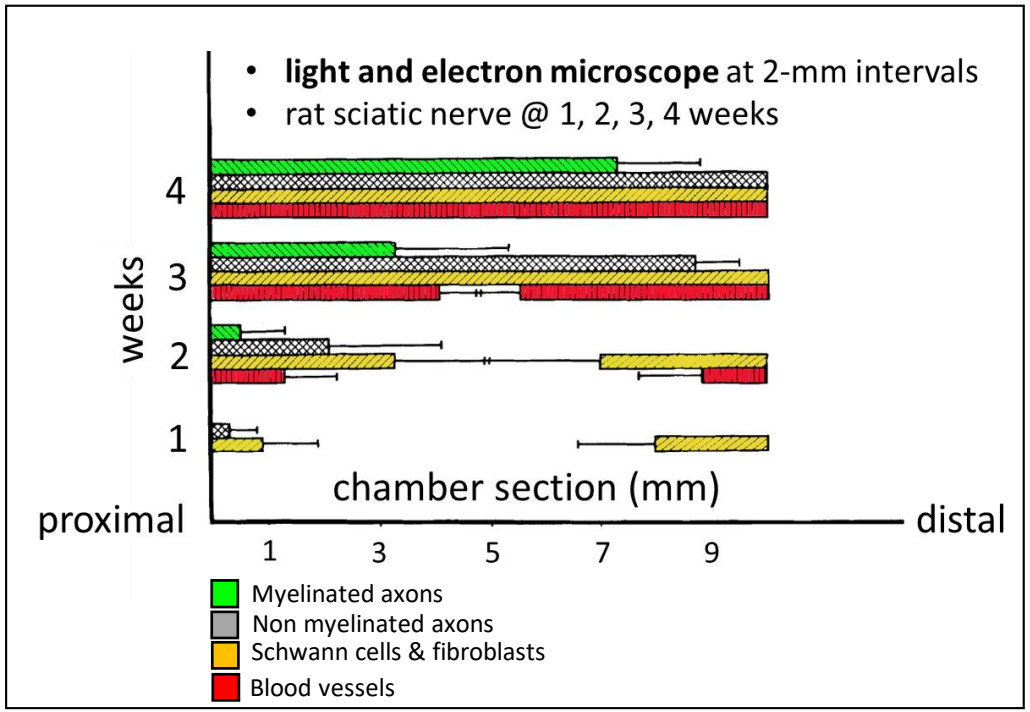




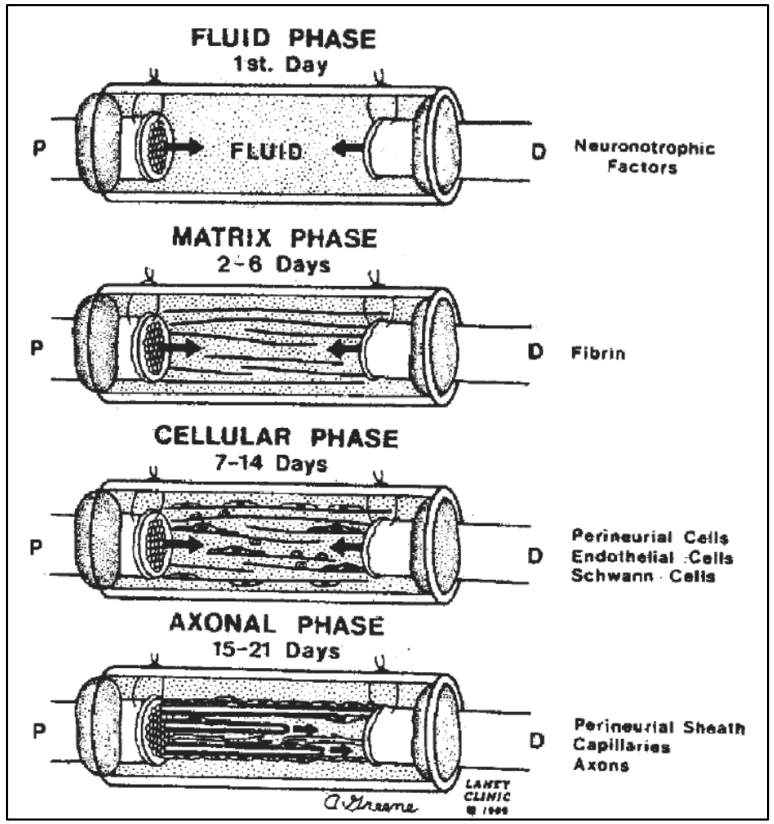


(A-L Cattin and AC Lloyd, 2016)

Do blood vessels guide Schwann cell migration also within nerve conduits?



(Williams et al., 1983)



(Belkas et al., 2004)

-Williams, Longo, Powell, Lundborg, Varon, 1983
-Schroder, May, Weiss, 1993, 1994
-Li, Yan, Ai, Hu, Gu, Matloub, Sanger, 2004

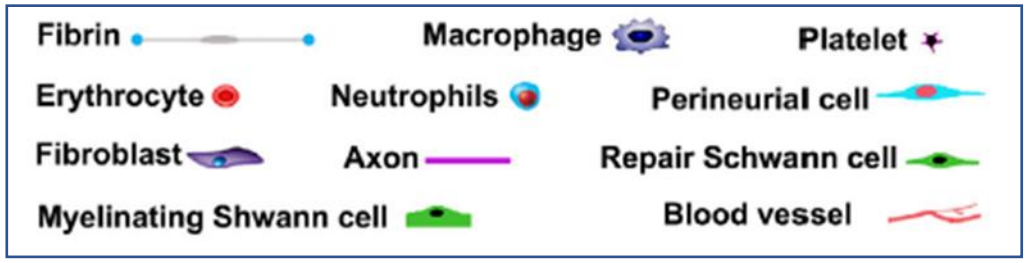
Do blood vessels guide Schwann cell migration also within nerve conduits?

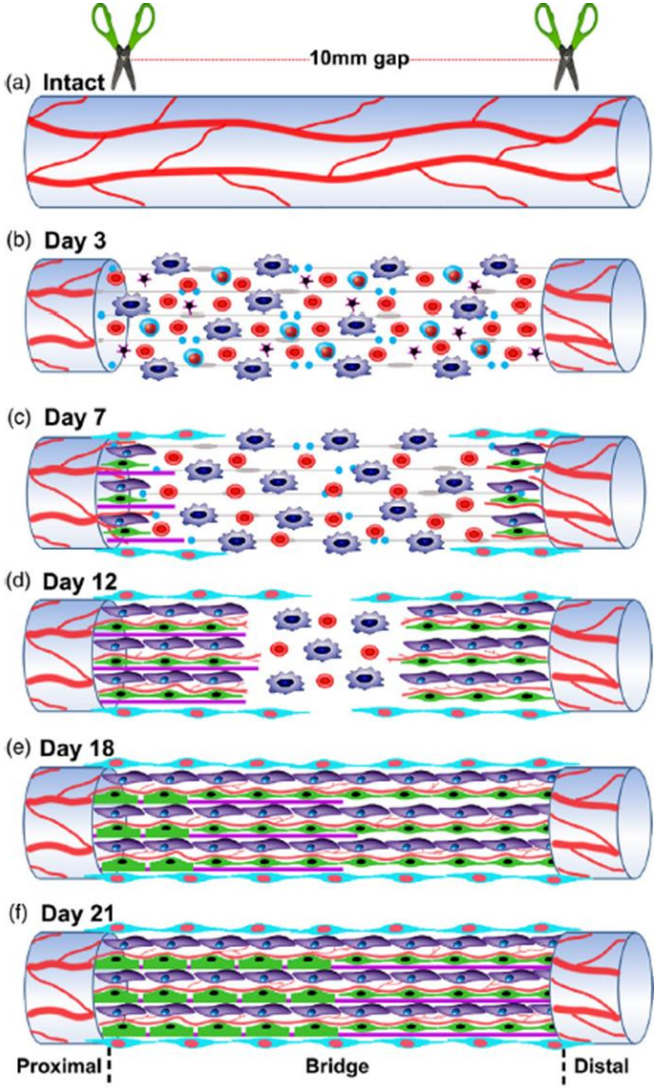
Table 1 Percentage of cells in the nerve conduit

Postoperative days	7	14	21	28	35	42
Monocytes	10.5	9.0	0	0	0	0
Macrophages	32.5	17.5	21.5	6.0	0	0
Fibroblasts[a]	31.0	28.5	0	0	0	0
Capillaries	10.5	6.0	16.0	0	0	0
Erythrocytes	13.0	15.5	10.5	0	0	0
Schwann's cells[b]	2.5	9.0	21.5	31.0	18.5	13.5
Nerve fibers[c]	0[d]	14.5	30.5	63.0	81.5	86.5
Fibroprotein[e]	+++	++	+	–	–	–

- 5-mm nerve gap, sciatic nerve rat
- silicon conduit
- TEM analysis

(Li et al., 2004)





(Min et al., 2020)