HOTCHKISS BRAIN INSTITUTE

XXI WORLD CONGRESS OF NEUROLOGY
Vienna, Austria 2013

Teaching Course	
25.09.2013, 2:30PM - 4:00PM	Hall J
Neurotrauma (CNS and PNS)	

CNS Trauma P.E. Vos, The Netherlands
PNS Trauma Douglas W. Zochodne Calgary, Canada and Ahmet Hoke Baltimore,USA

PNS
PERIPHERAL NERVE SOCIETY

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DISCLOSURES FOR D.W. ZOCHODNE

None related to this work

Neurobiology of Peripheral Nerve Regeneration, Cambridge, 2008

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Regenerative steps and barriers to regrowth

Douglas W. Zochodne
Department of Clinical Neurosciences and the Hotchkiss Brain Institute
University of Calgary

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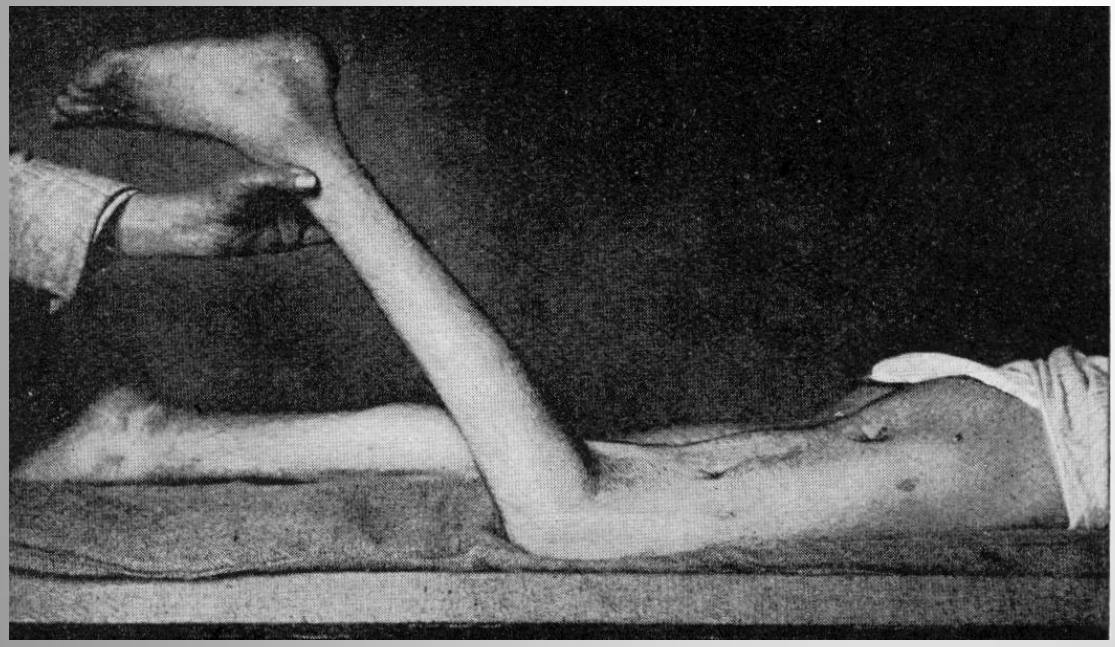
Neurological deficits from peripheral nerve damage are severe, intractable

sciatic missile injury



GBS-axonal damage

From:
Neurobiology
of peripheral
nerve regeneration,
Cambridge, 2008

The regenerative process involves a series of complex and highly interesting neurobiological events. These events require a coordination to translate into regenerative success. After transection for example, these include:

(i) Active Wallerian degeneration
(ii) Reprogramming of the cell body and a change in its architecture
(iii) Responses of perineuronal glial cells
(iv) Invasion of macrophages
(v) Changes in Schwann cell phenotype- reactive, myelinolytic and proliferative
(vi) The emergence of new sprouts from axons
(vii) Growth, reconnection, maturation and remyelination of axons

From:
Neurobiology of peripheral nerve regeneration, Cambridge, 2008

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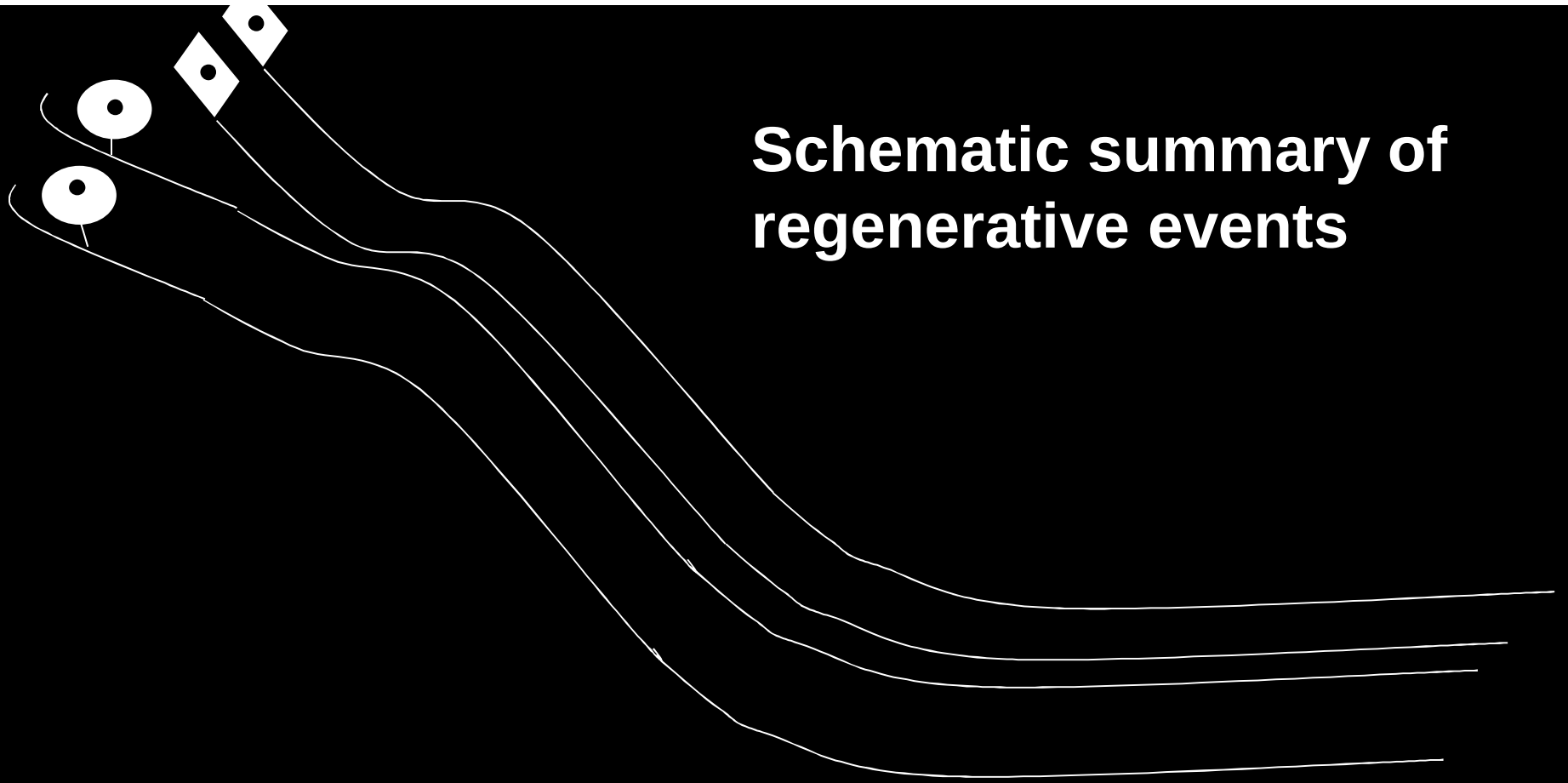
Schematic summary of regenerative events



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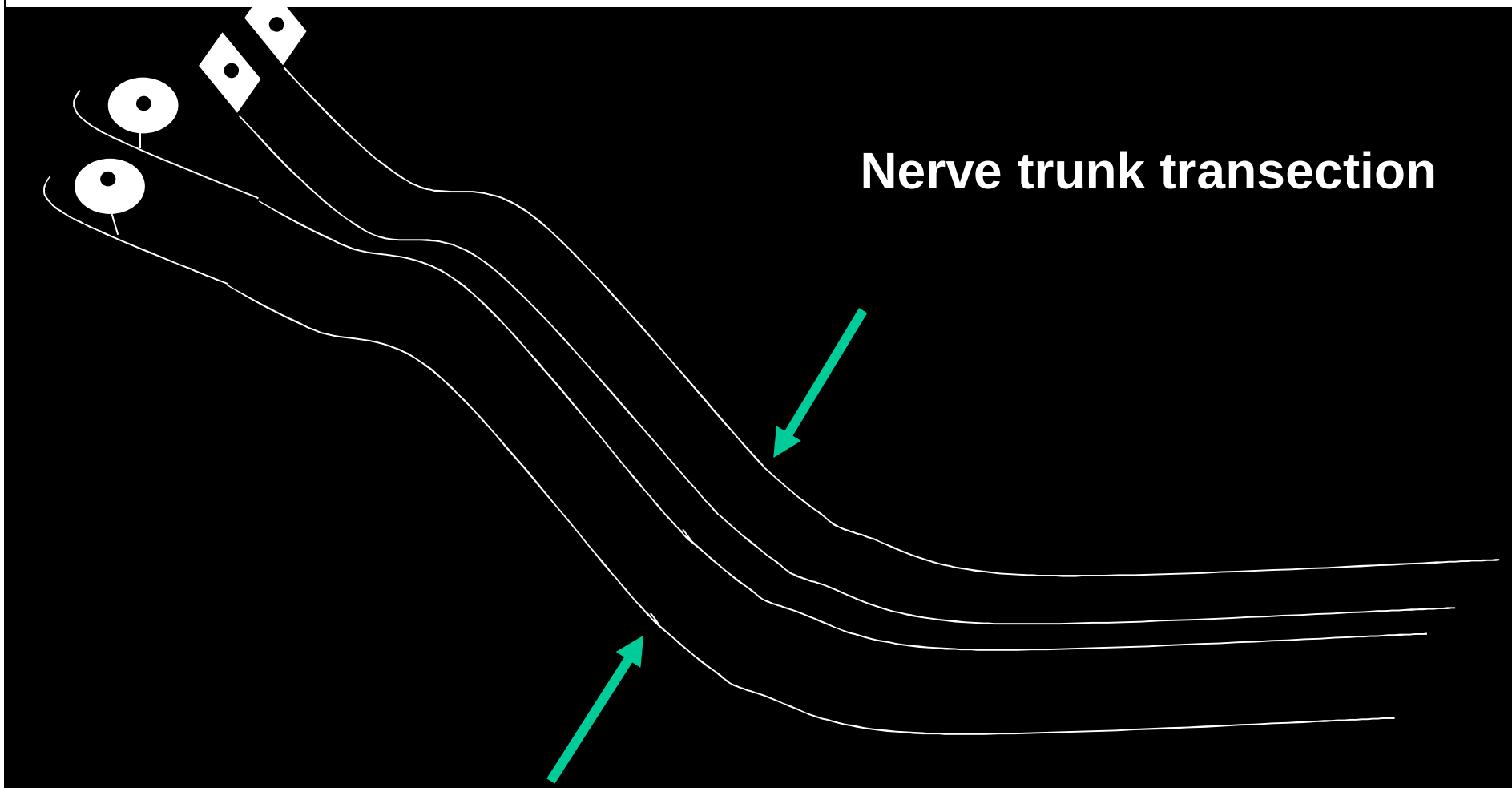
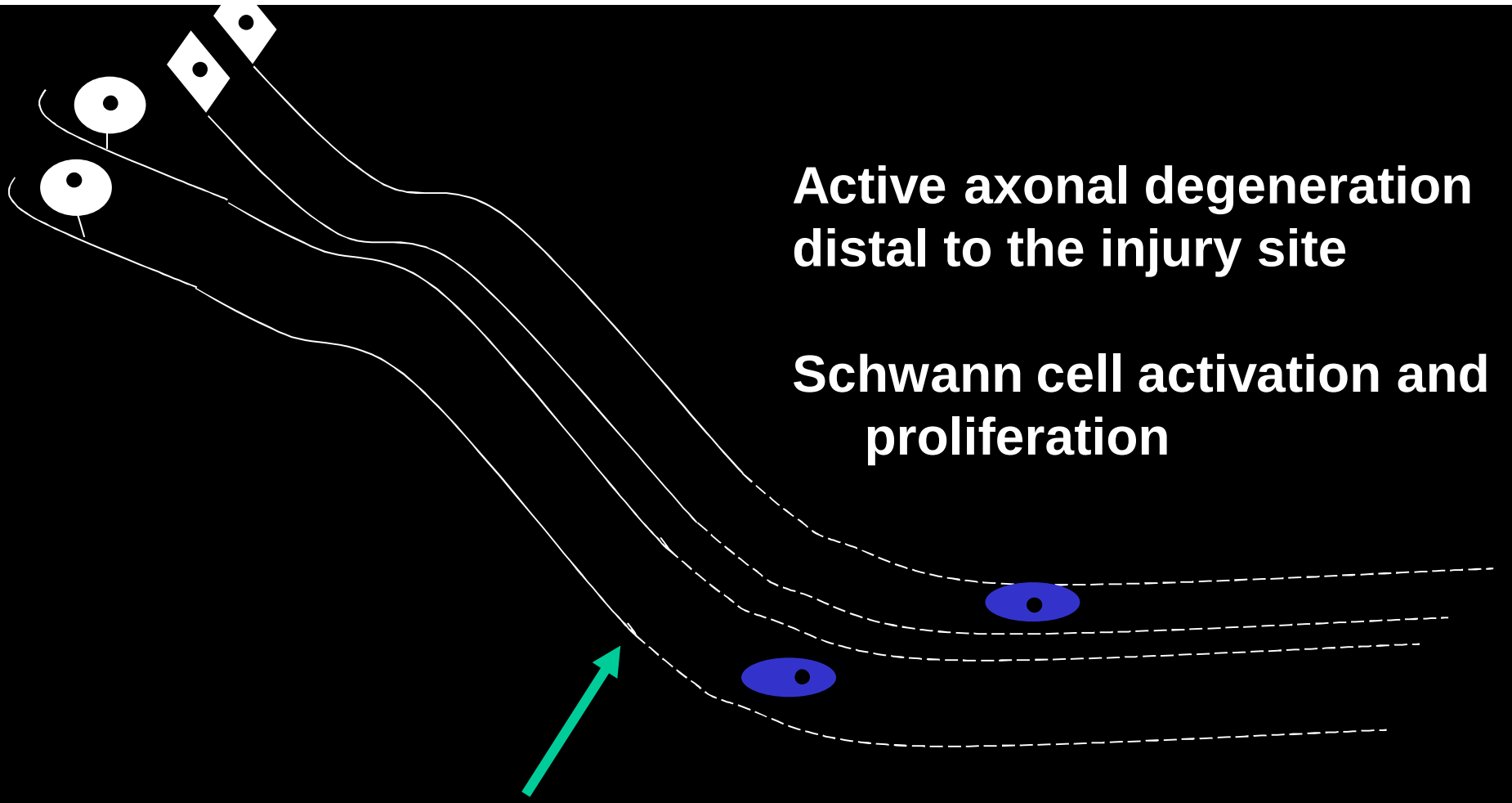
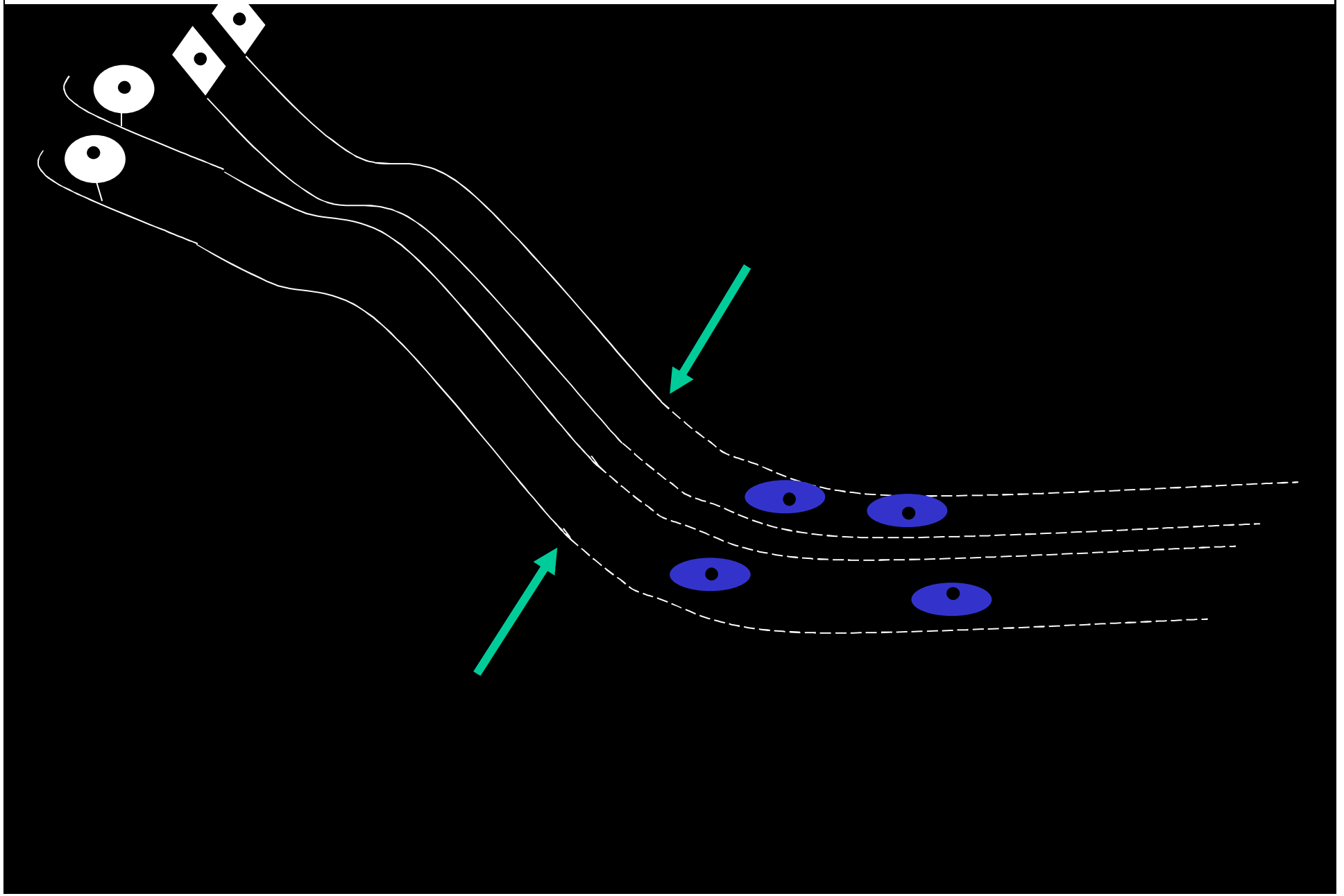
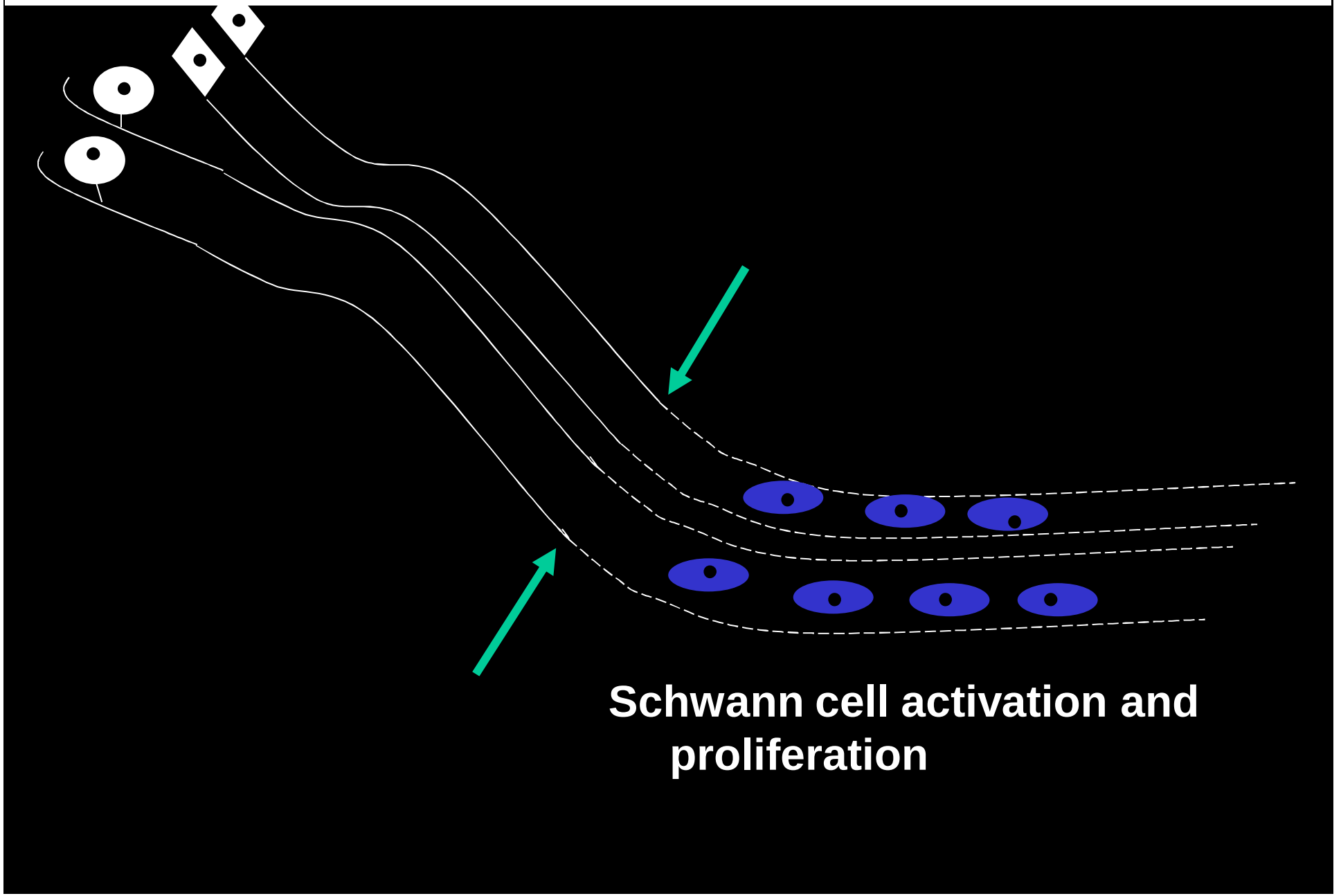




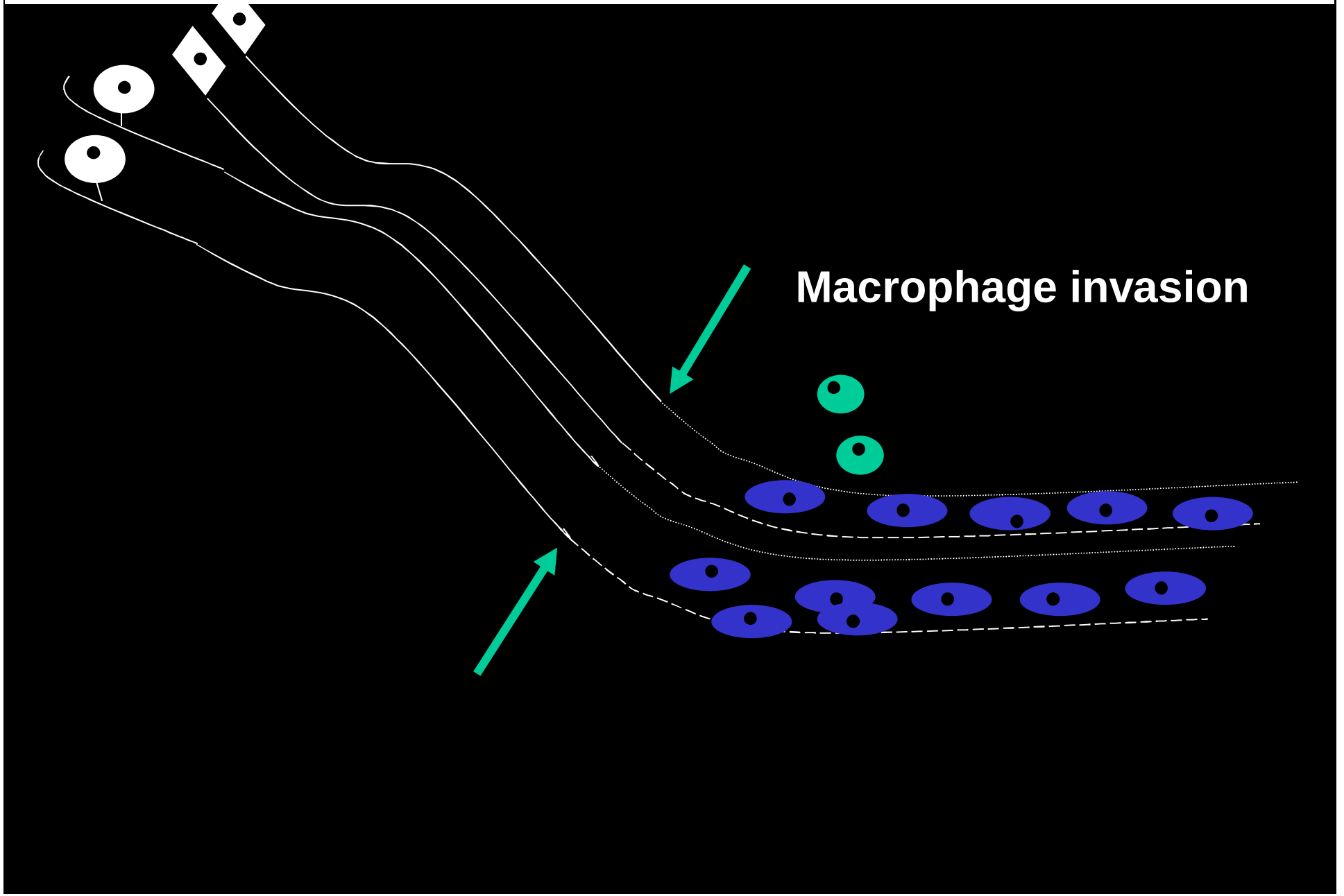
Active axonal degeneration distal to the injury site

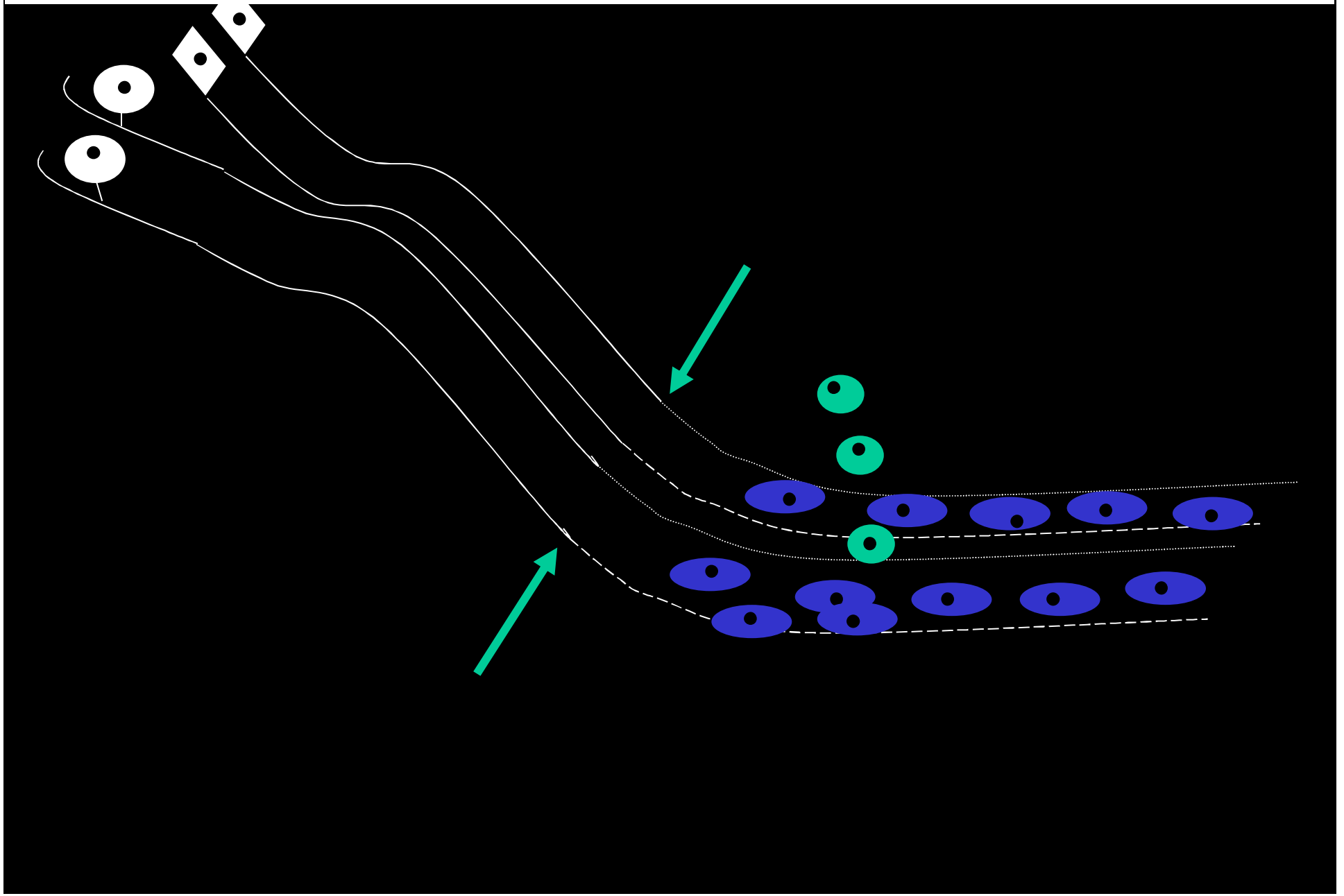
Schwann cell activation and proliferation

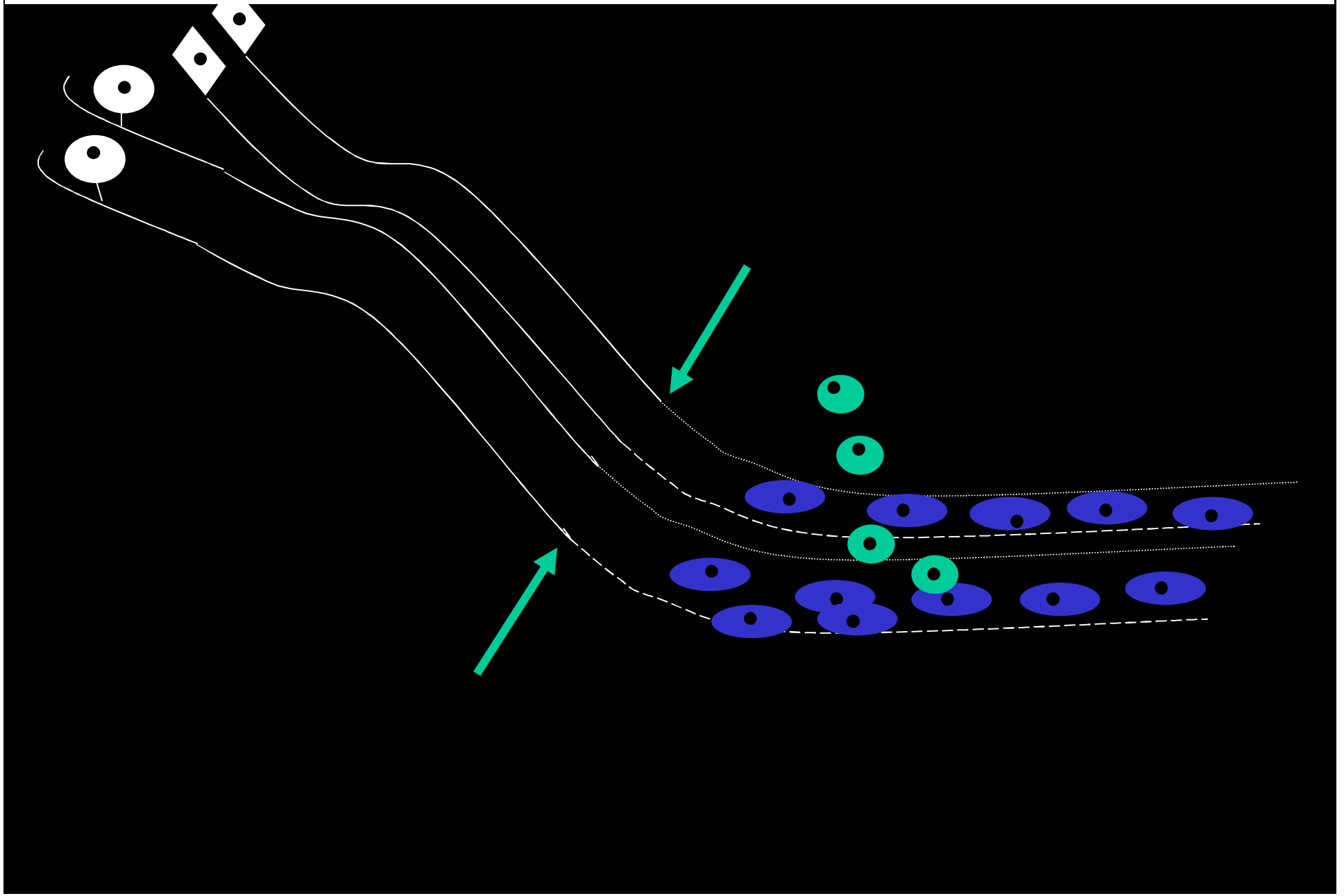


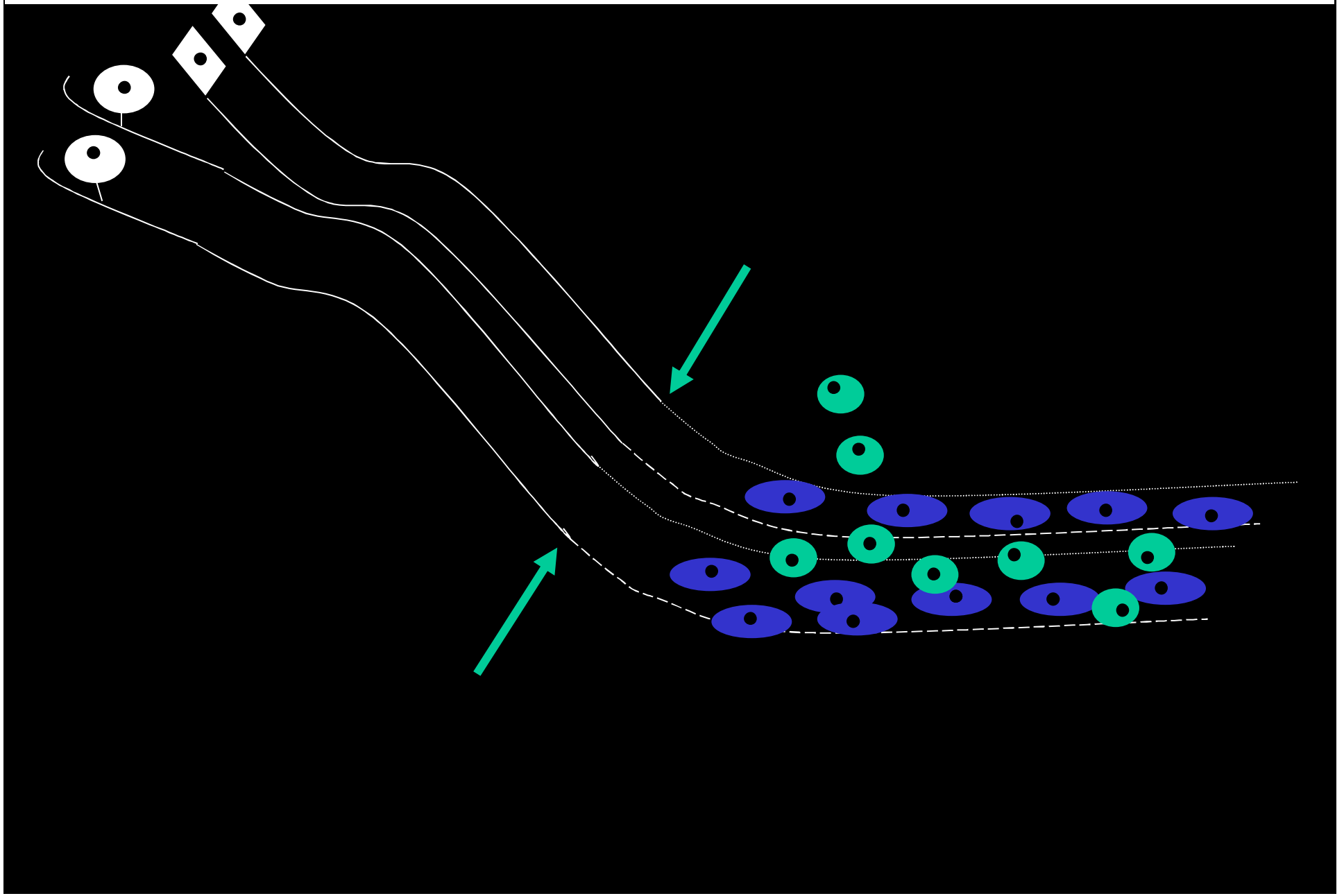


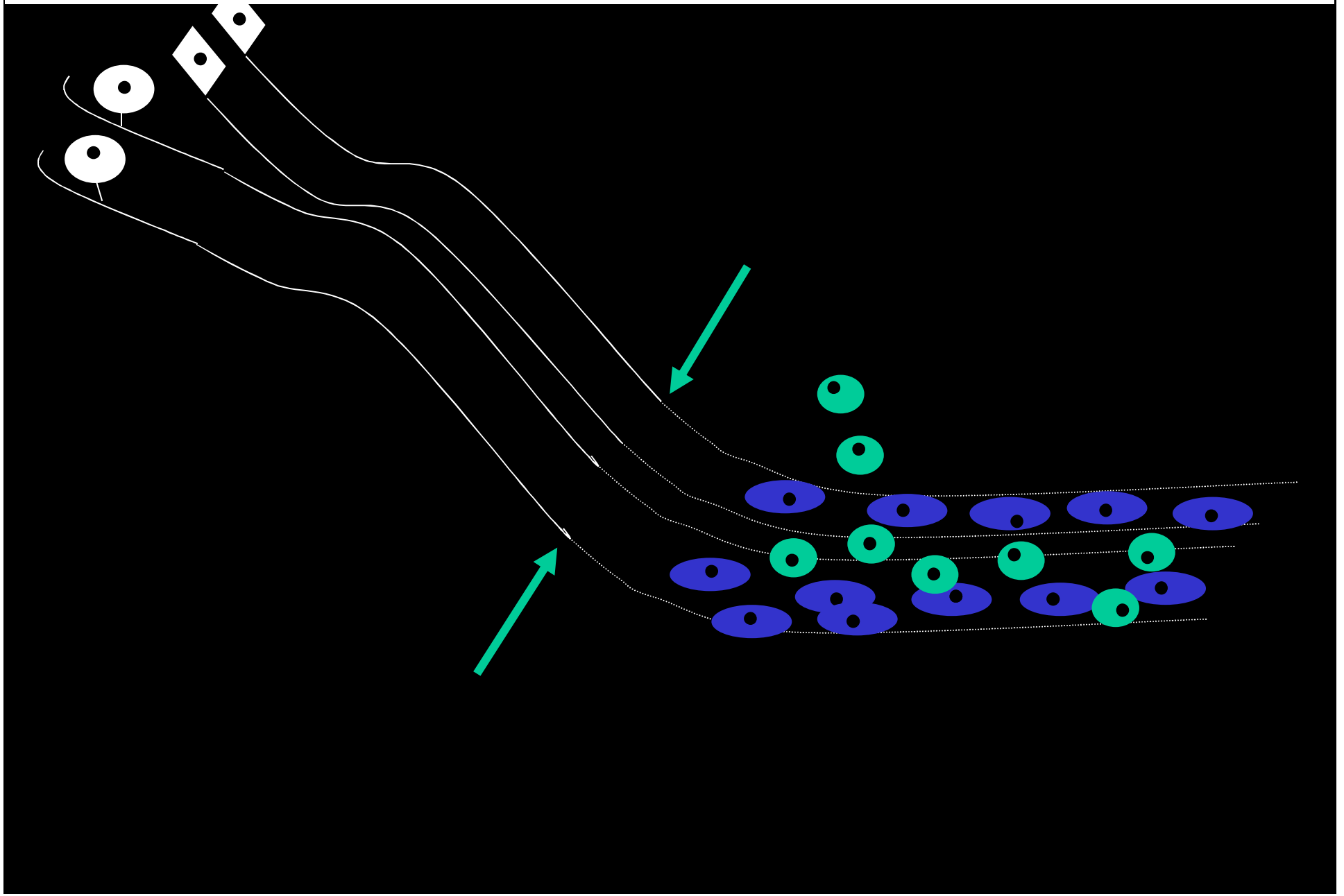
Schwann cell activation and proliferation

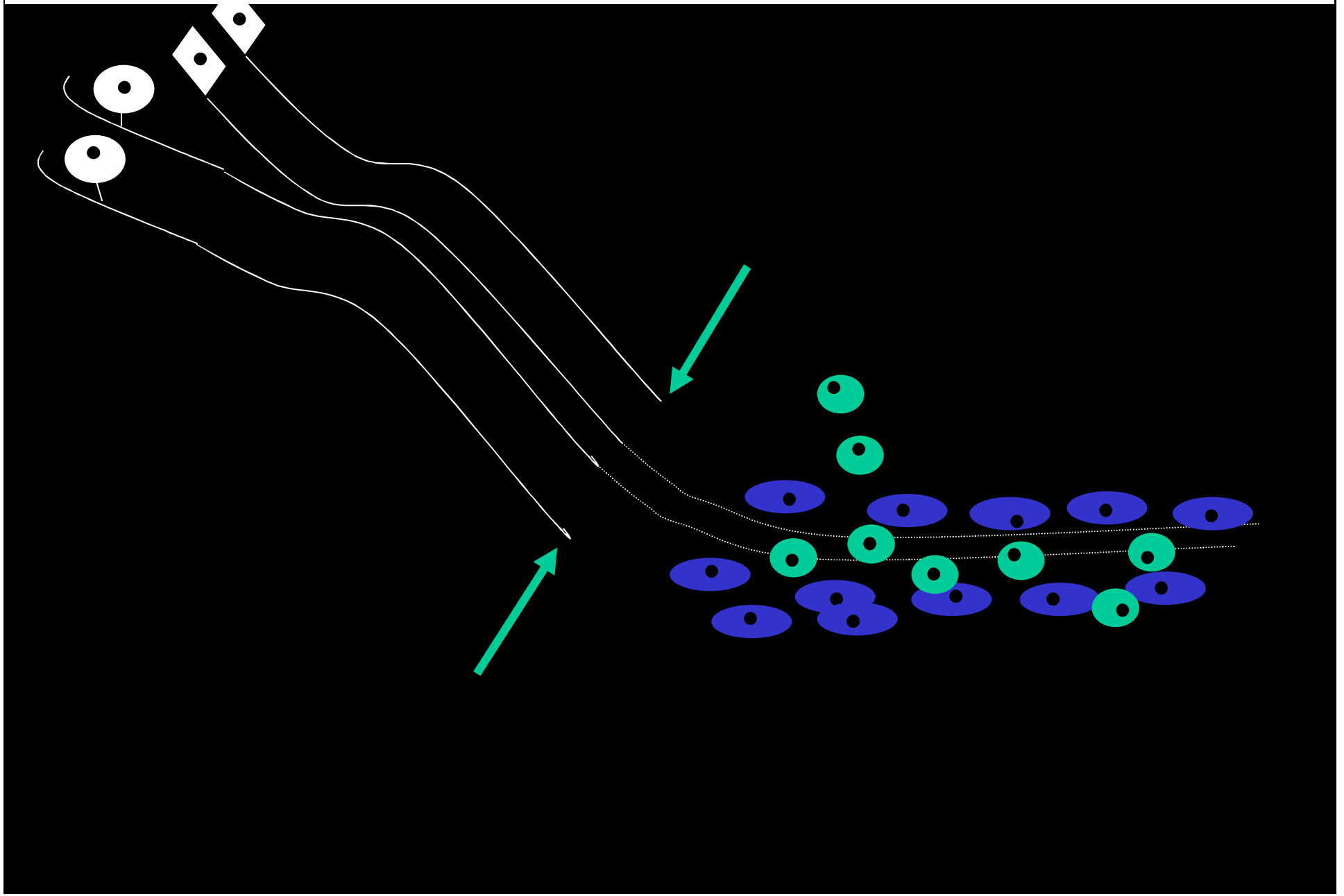

Macrophage invasion

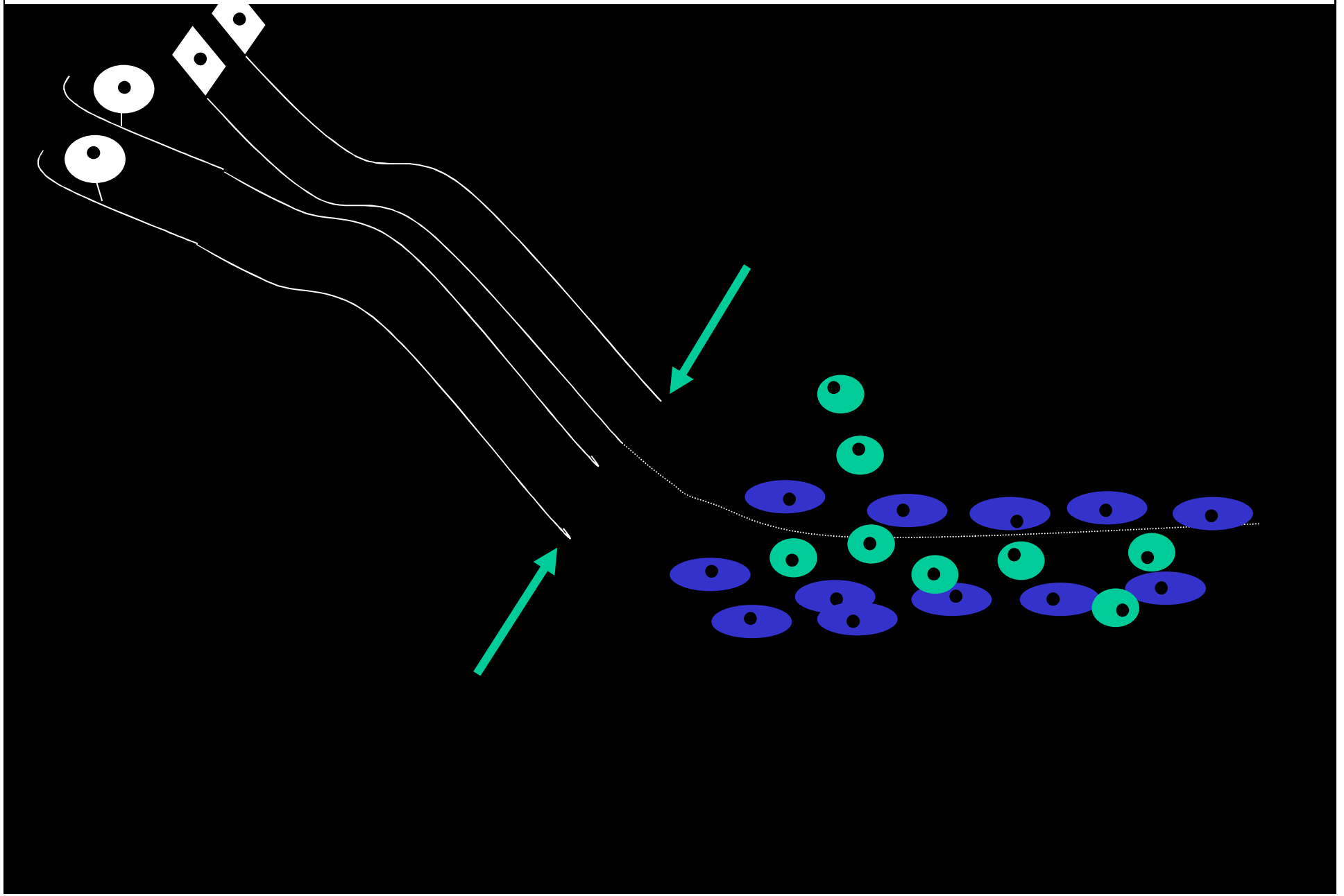


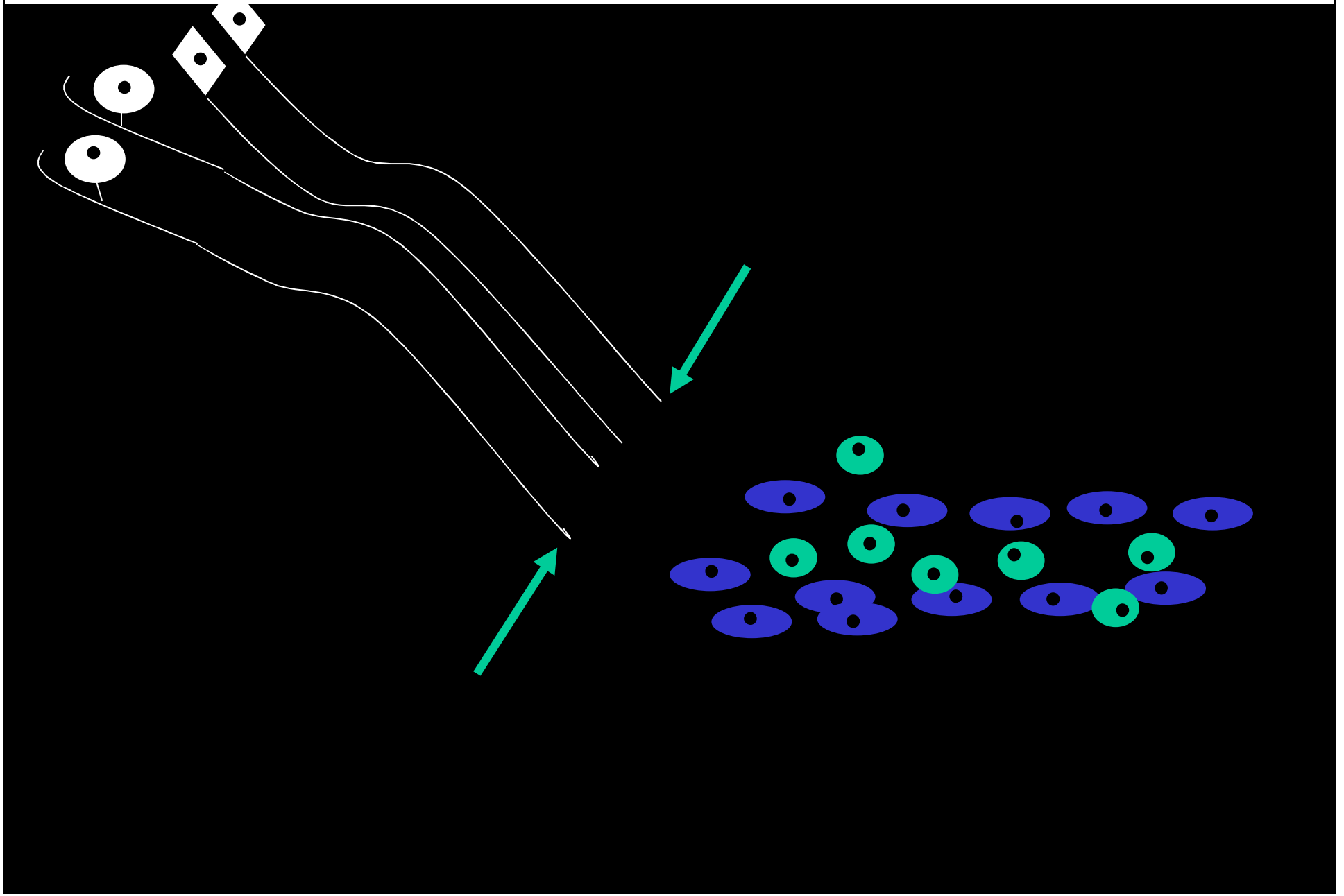


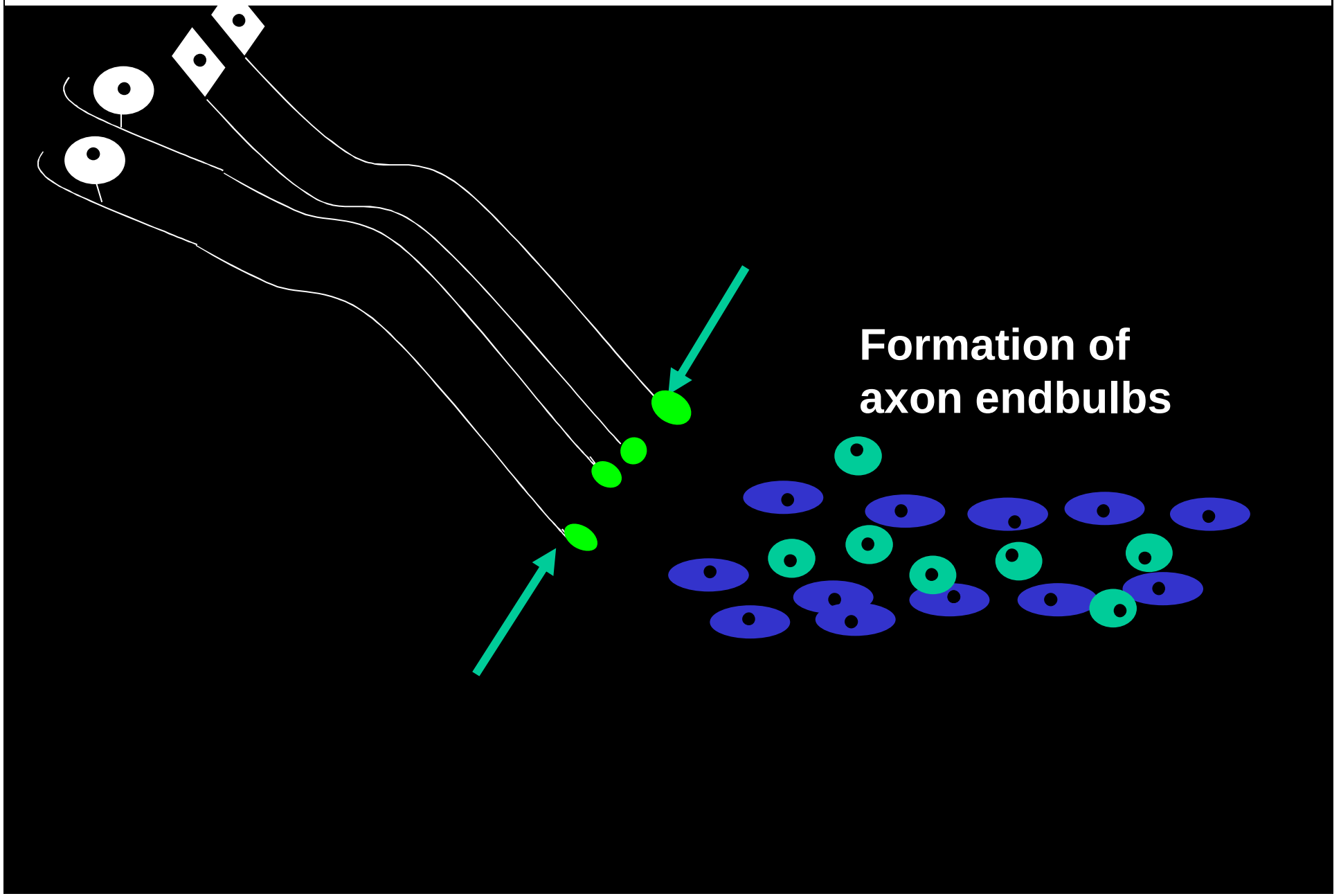


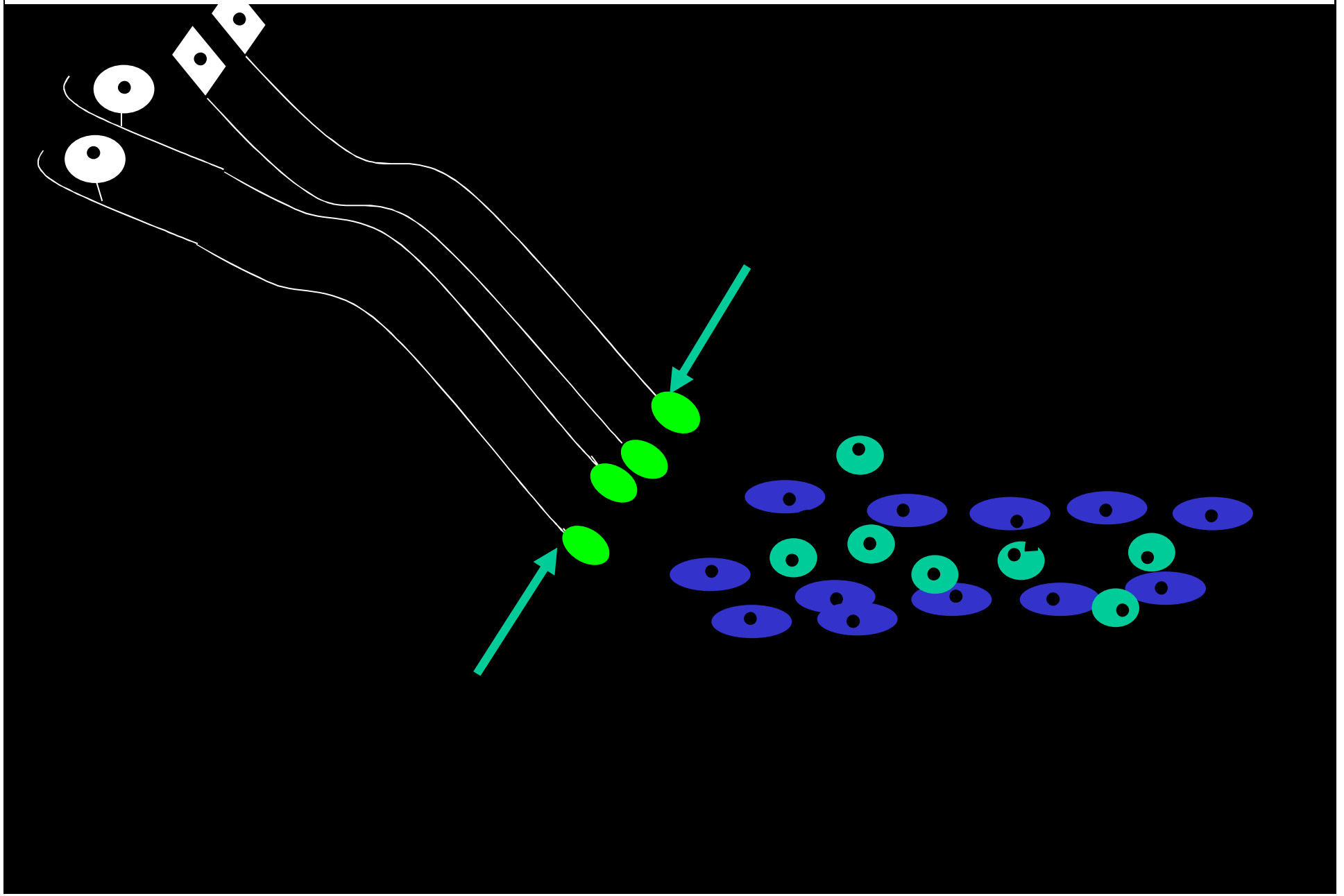









Formation of
axon endbulbs



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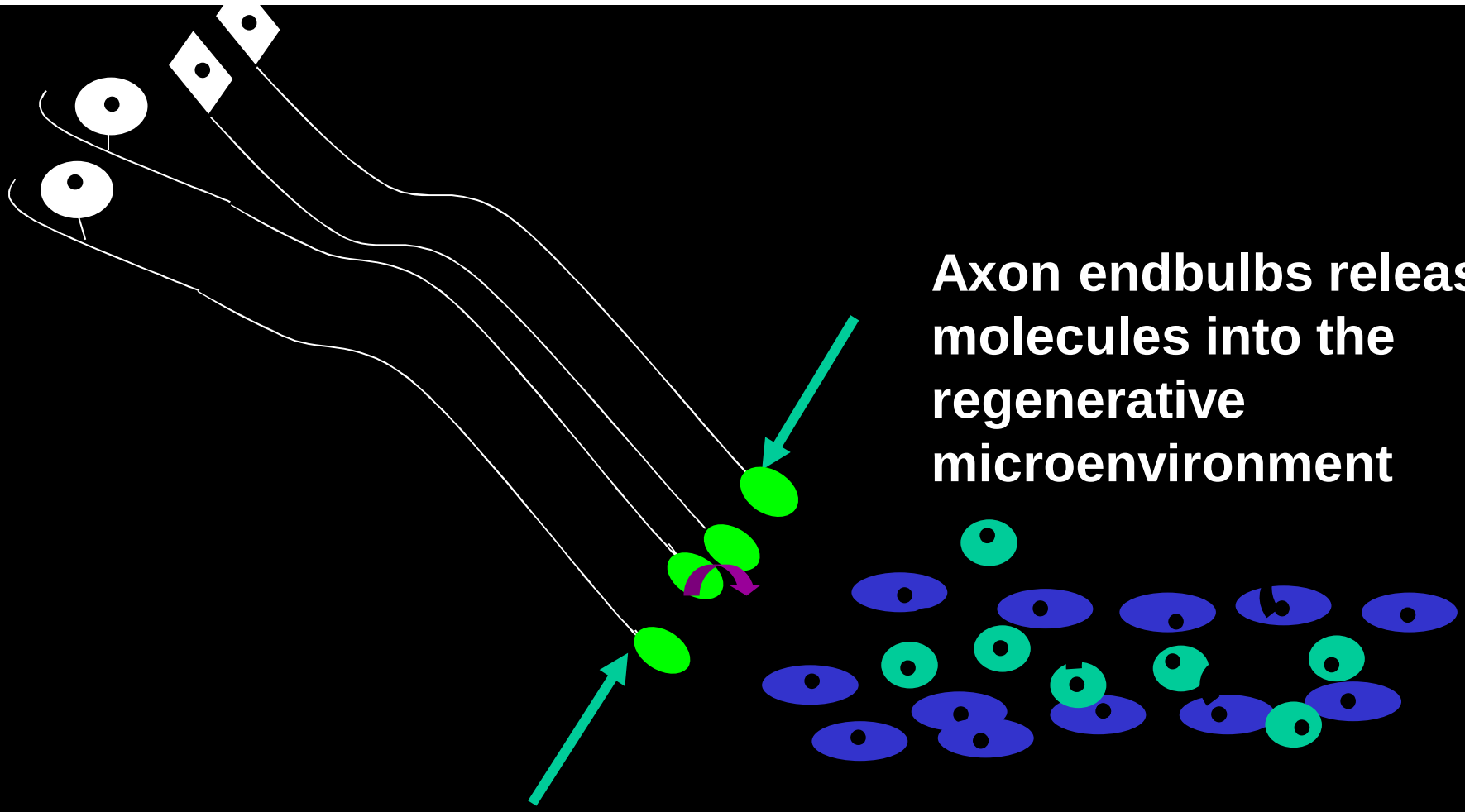
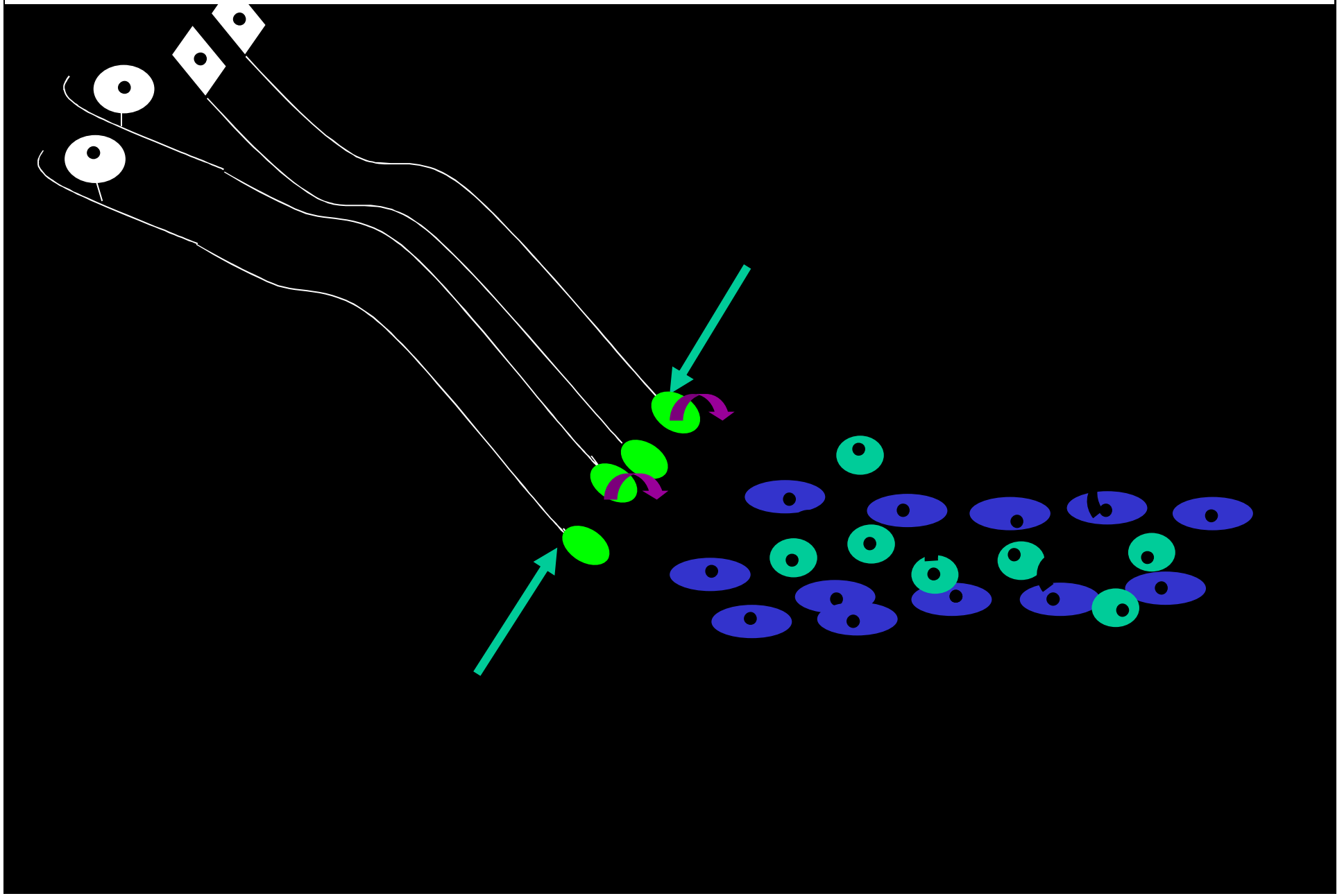
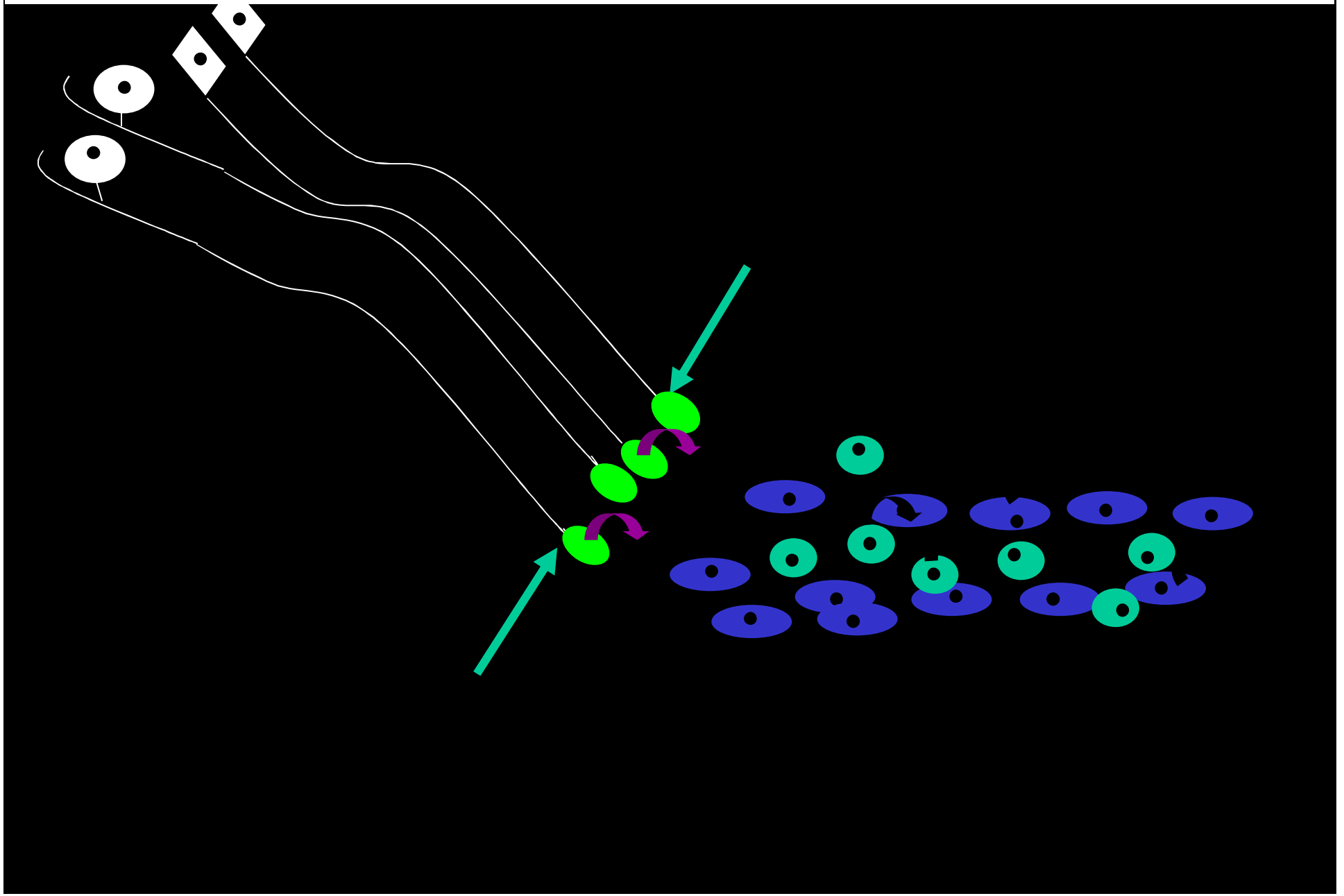
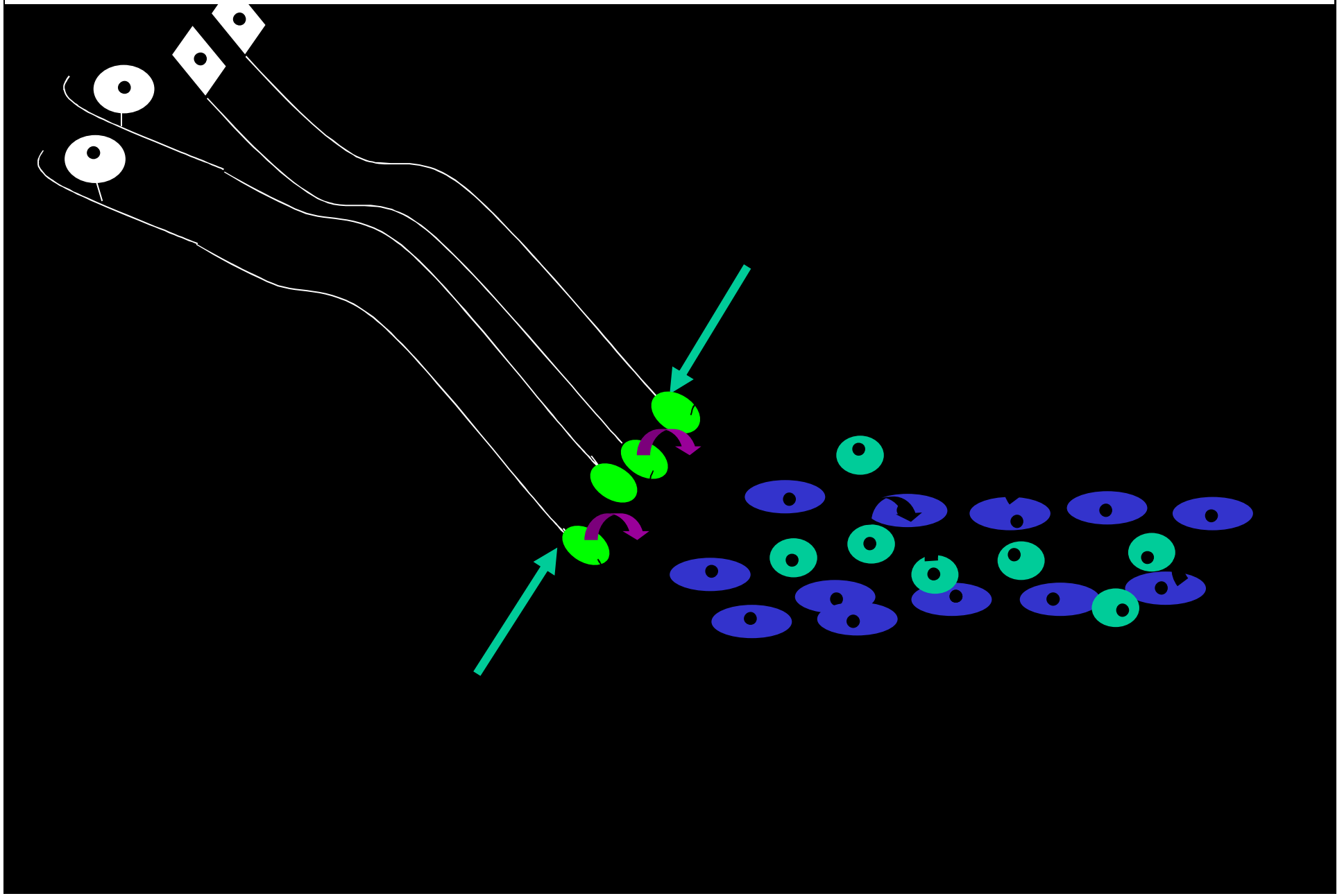
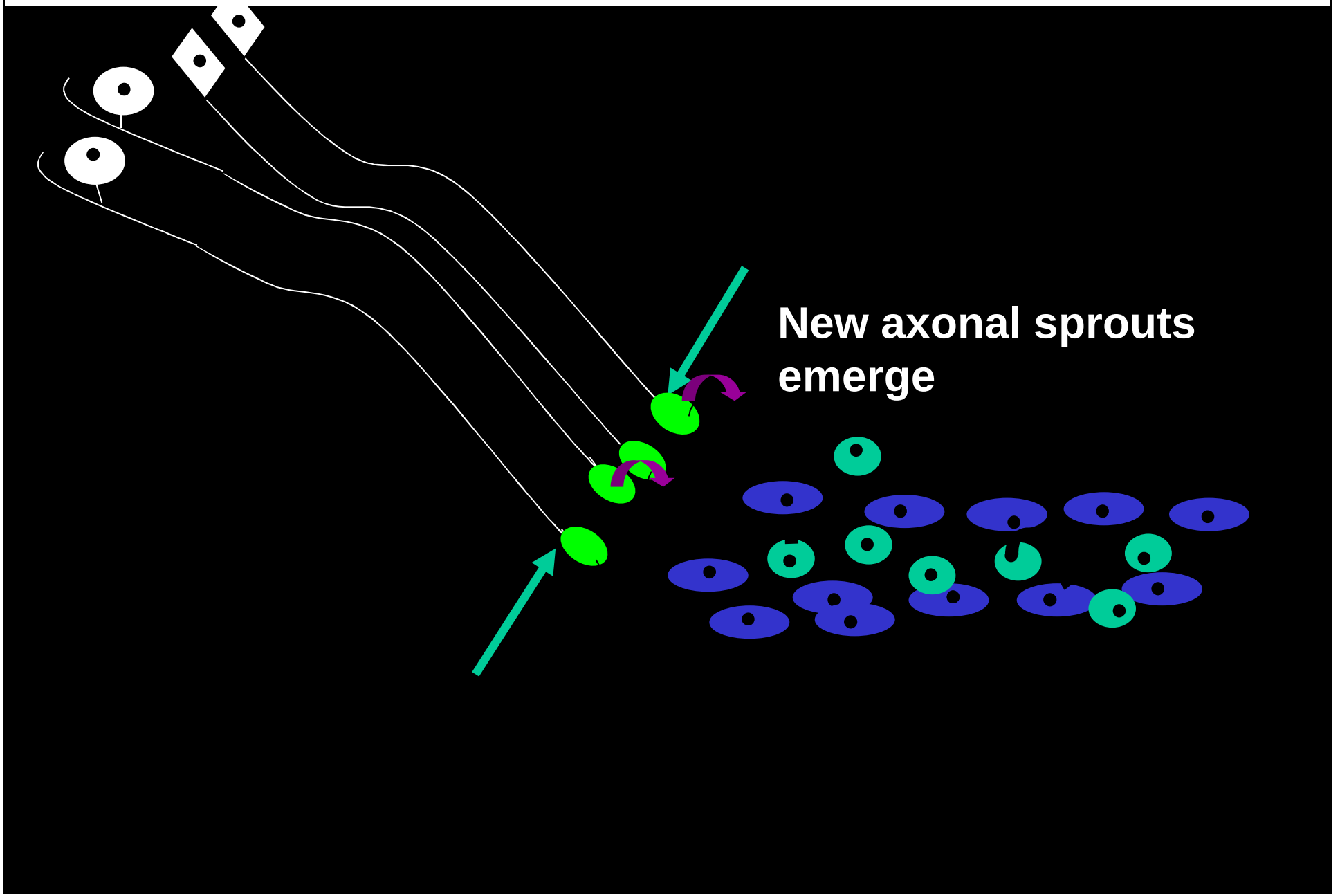


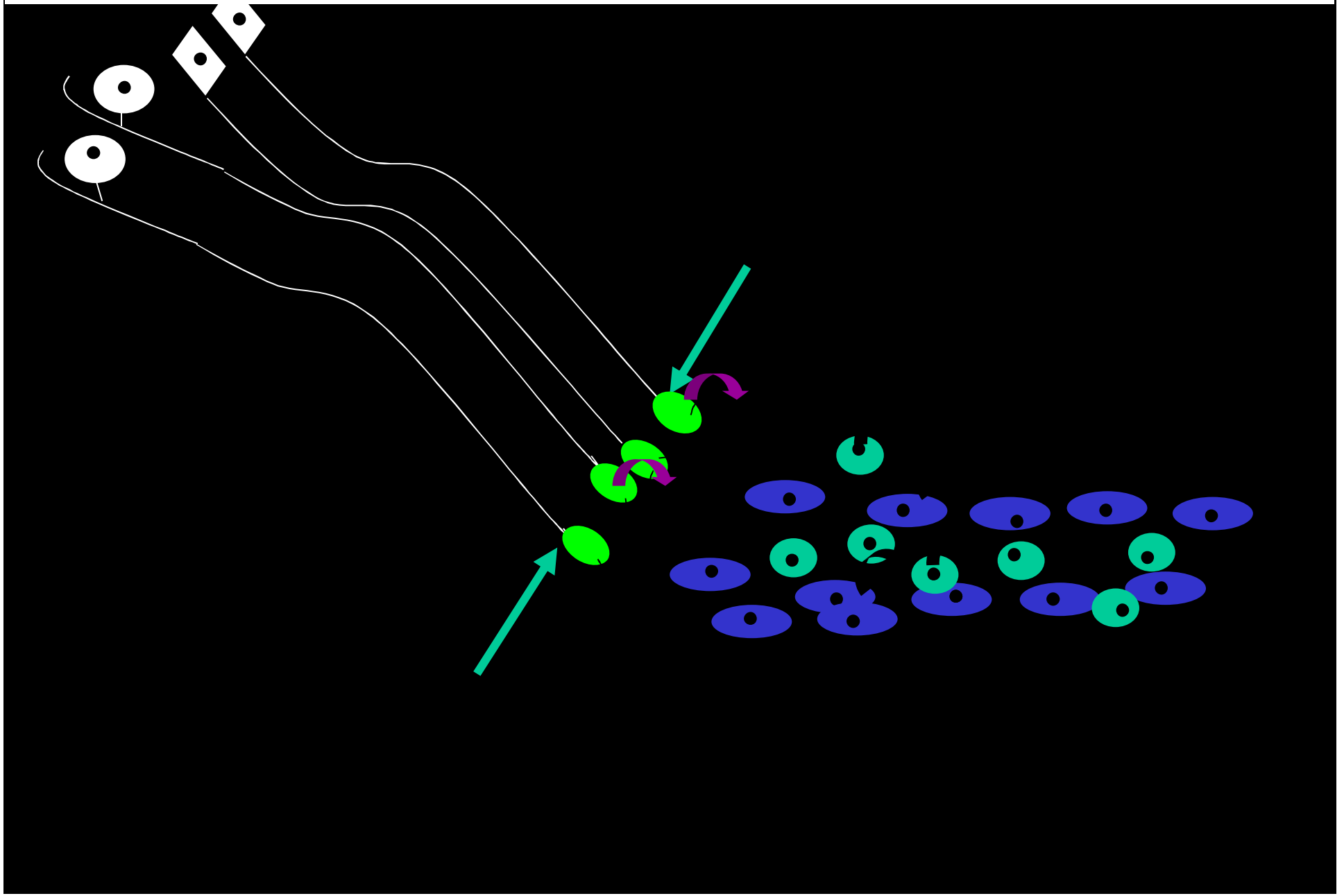
Axon endbulbs release molecules into the regenerative microenvironment

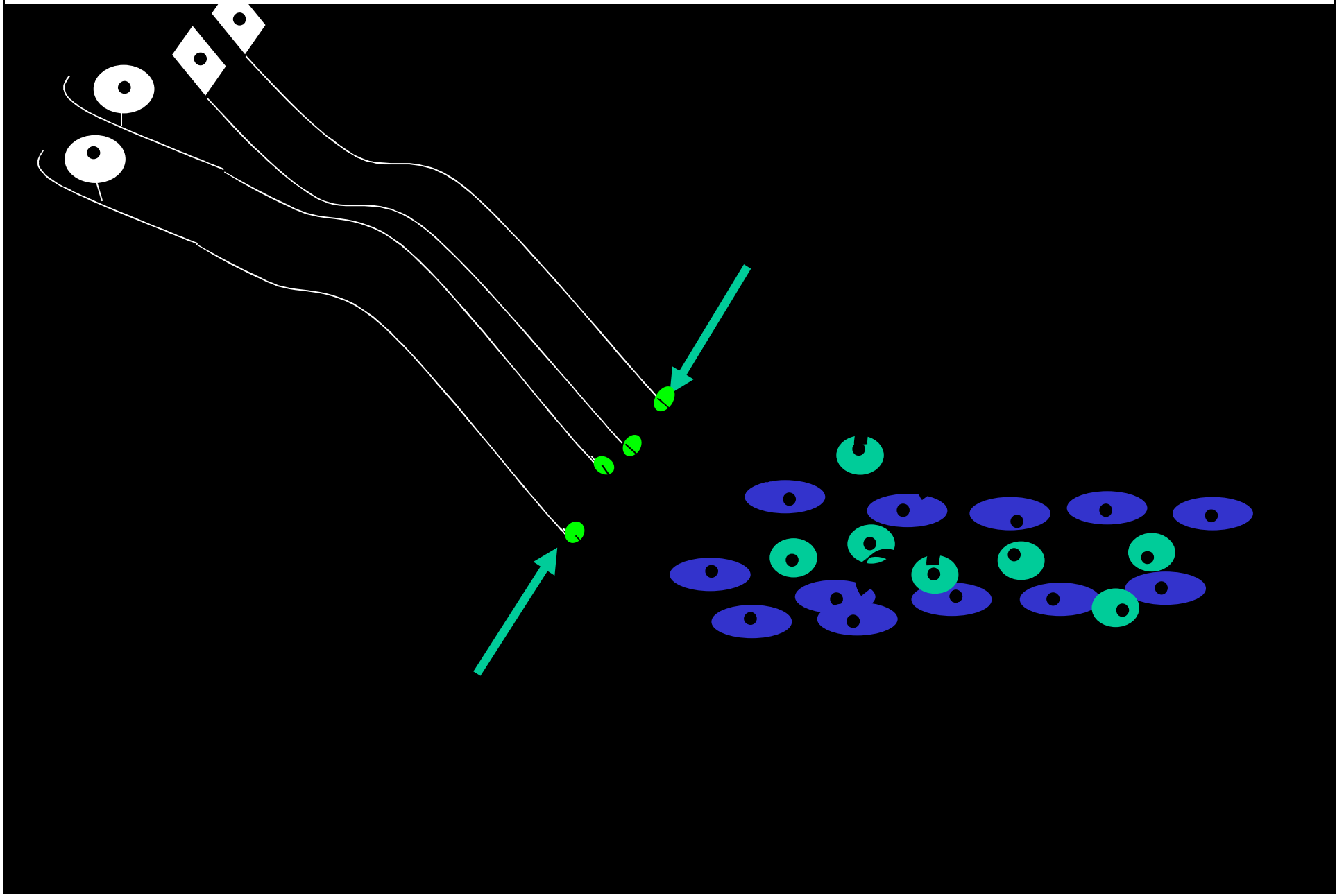


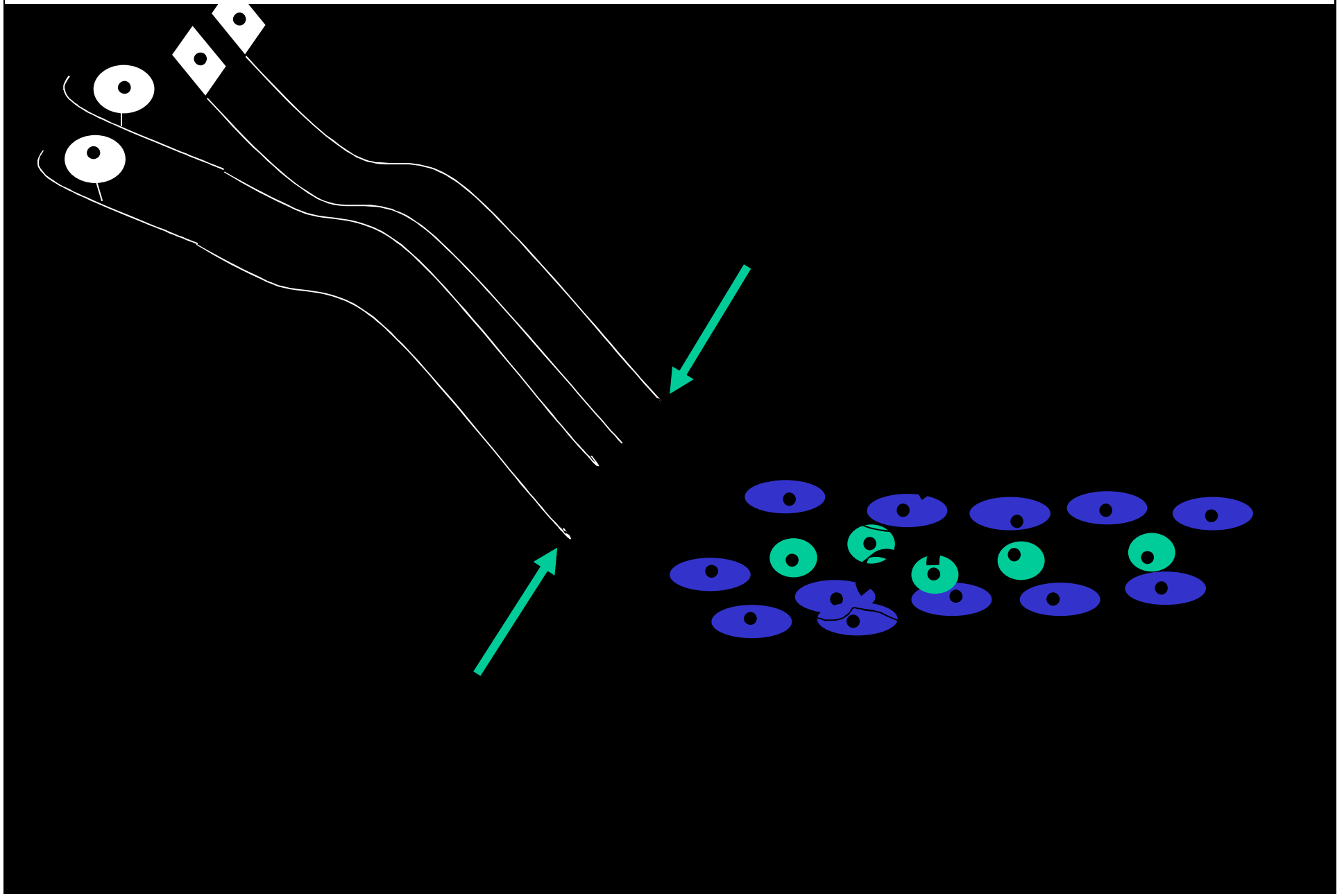


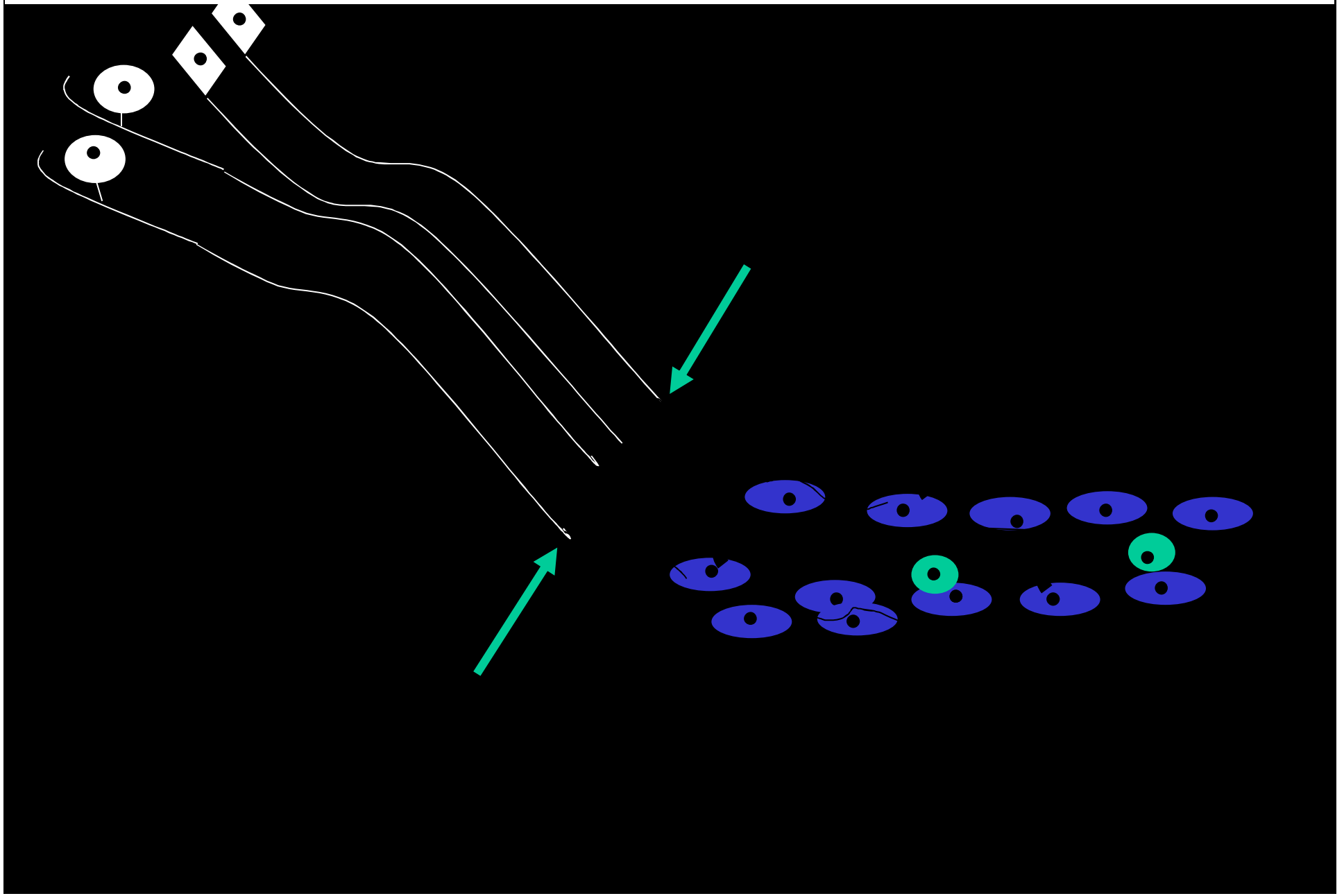


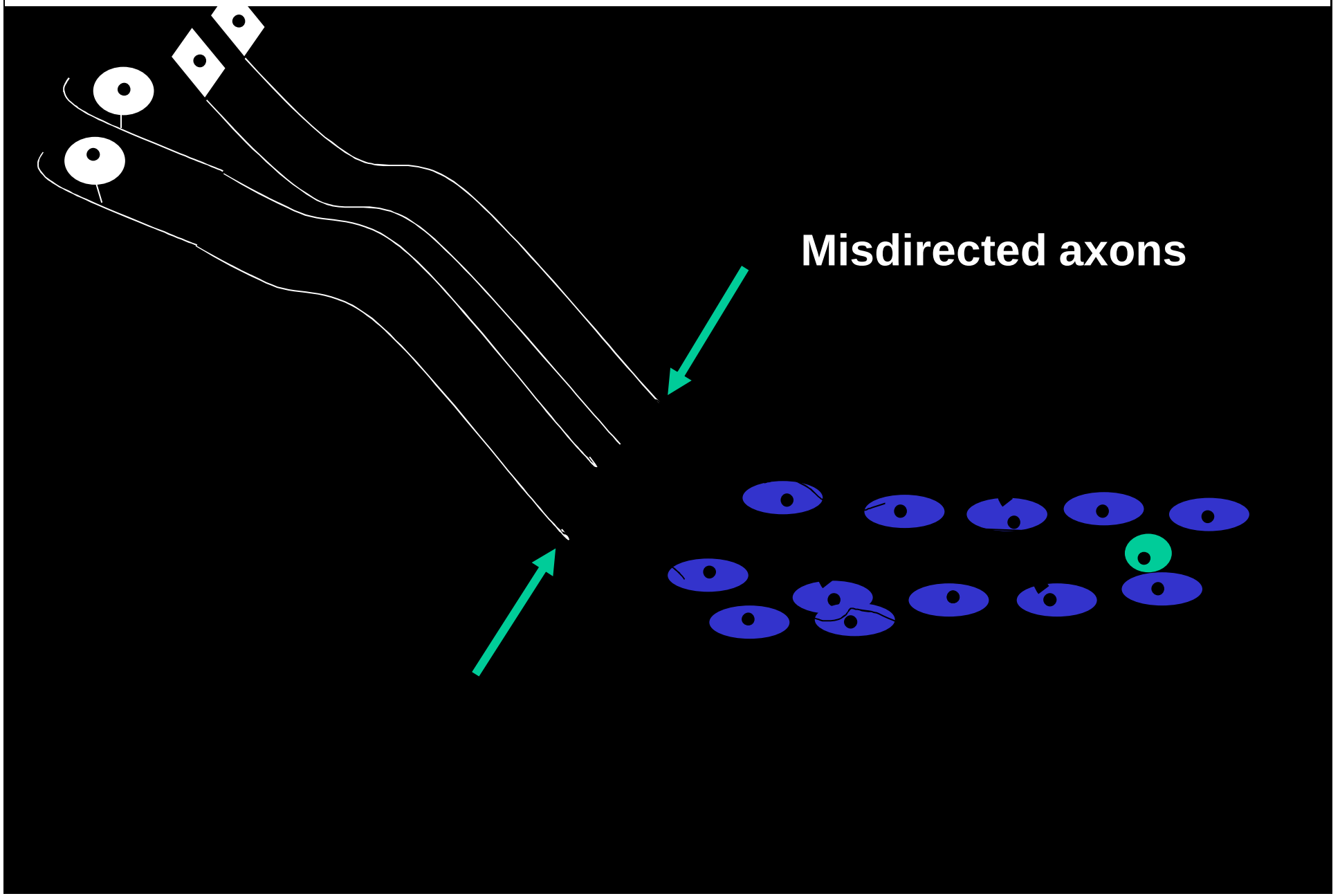

New axonal sprouts emerge

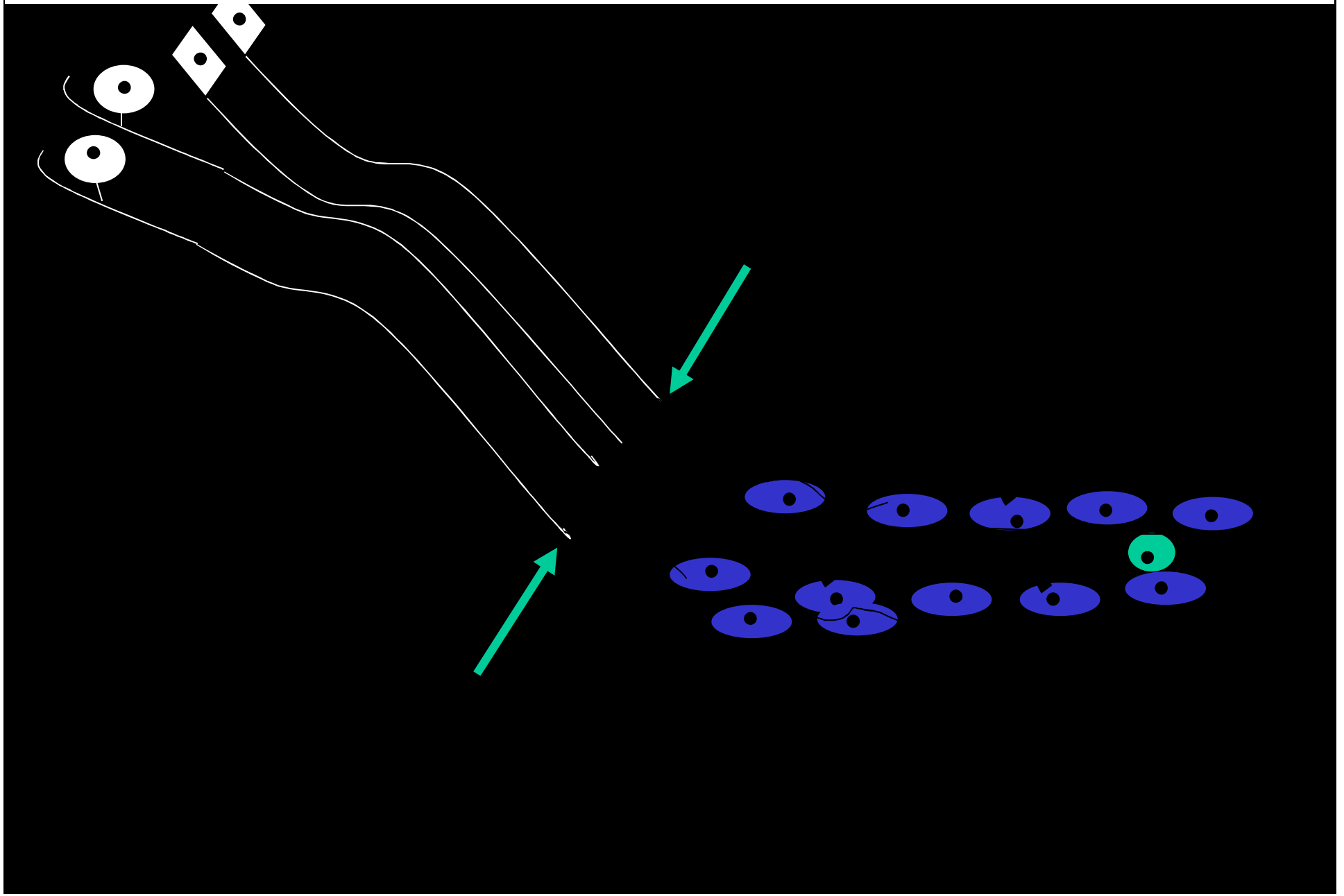









Misdirected axons



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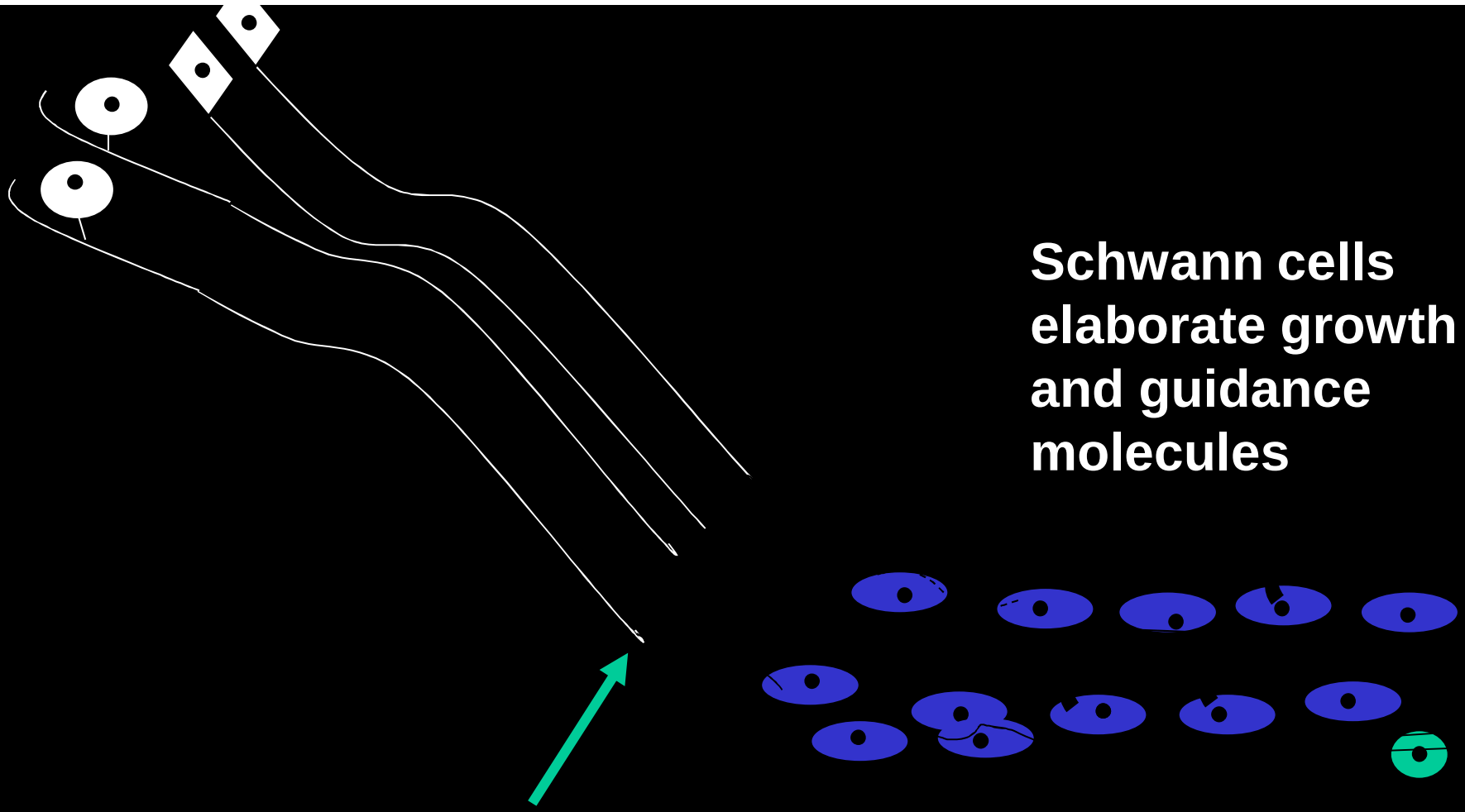
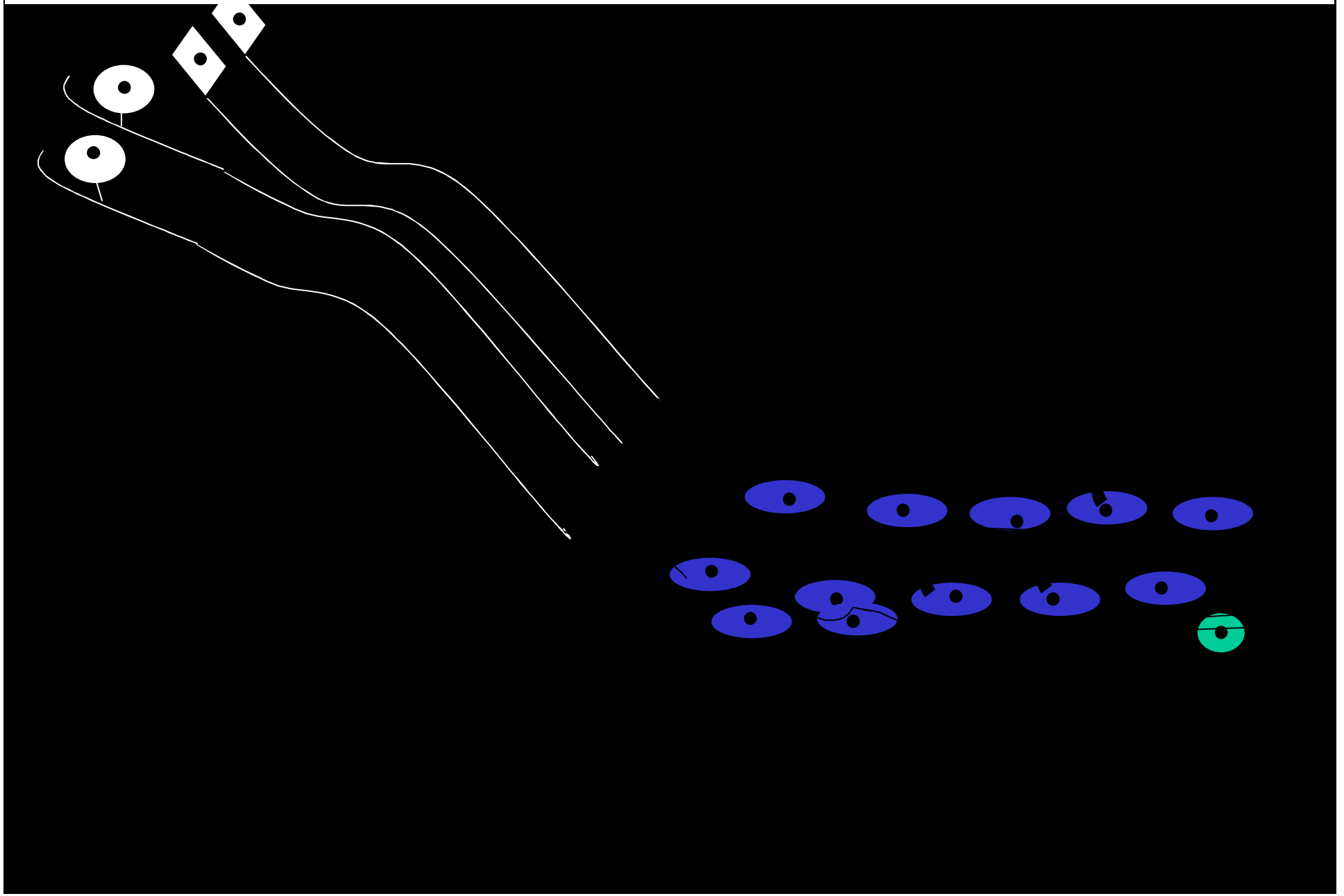
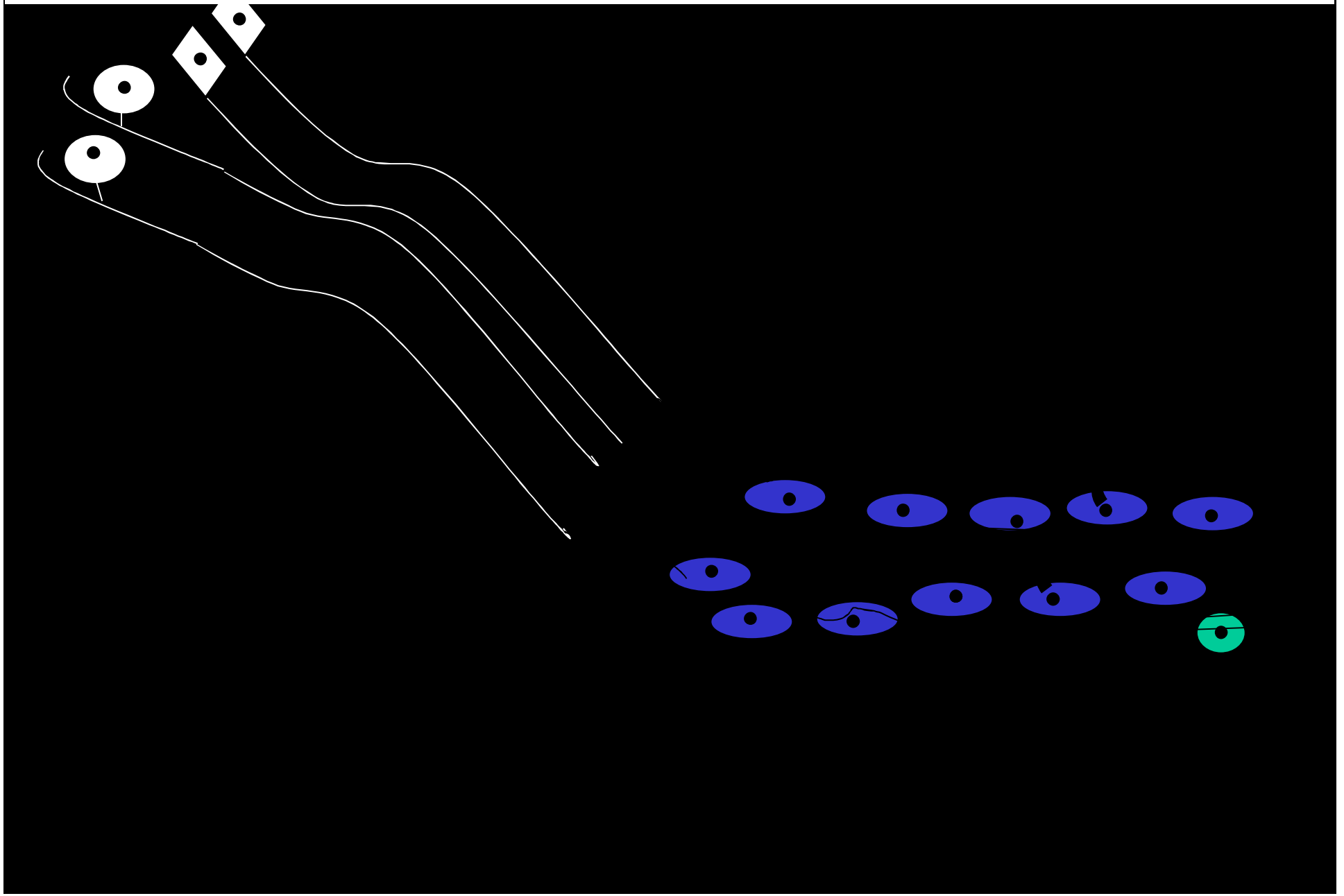
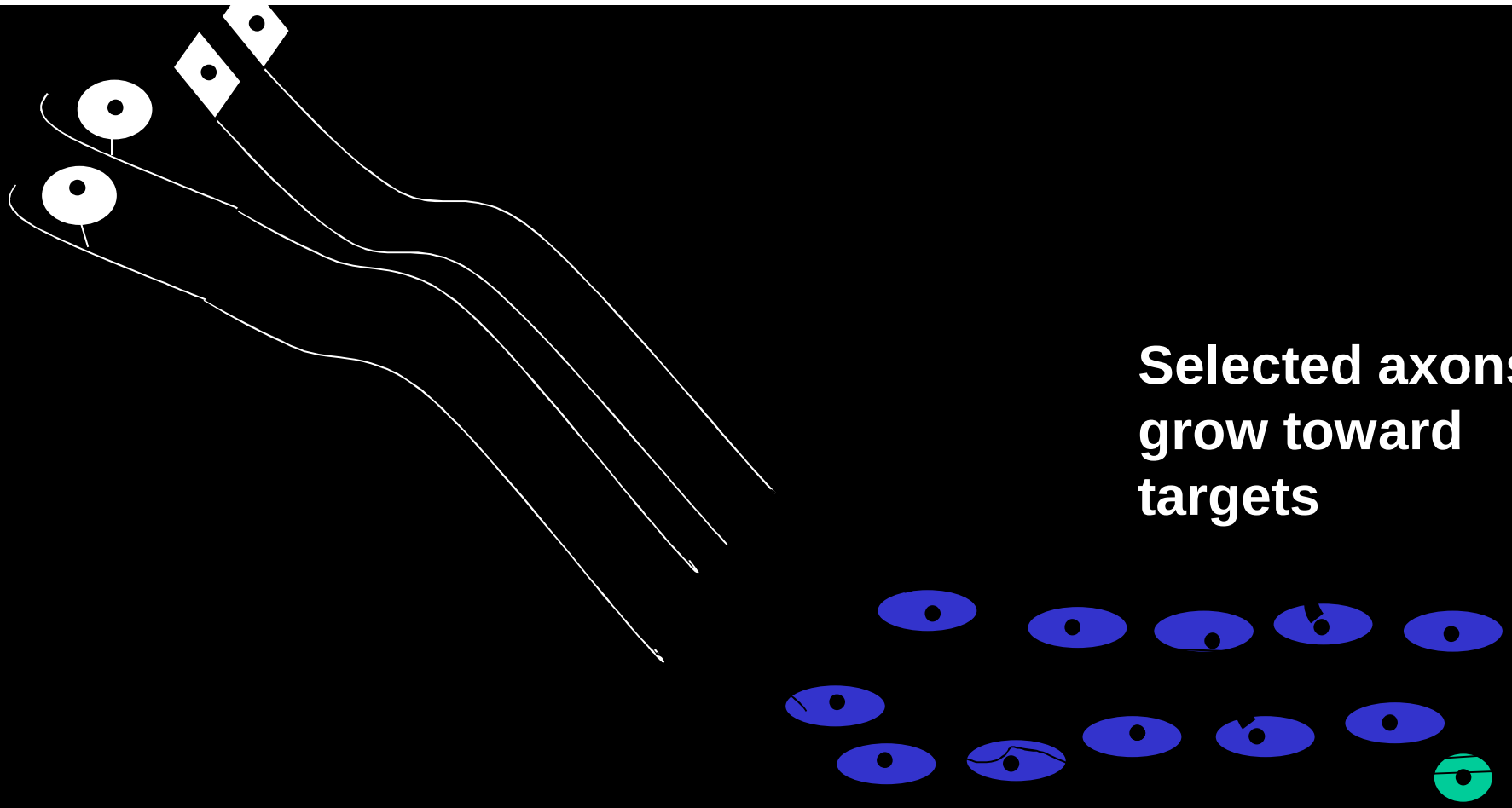


Schwann cells elaborate growth and guidance molecules







Selected axons grow toward targets

Regeneration and neurobiological themes

- The pace of Wallerian/Wallerian-like degeneration influences subsequent regenerative success

Delayed axon breakdown is associated with delayed axon regrowth

Examples: (i) Wld^s mouse

(ii) Macrophage depletion

(iii) iNOS knockout mouse

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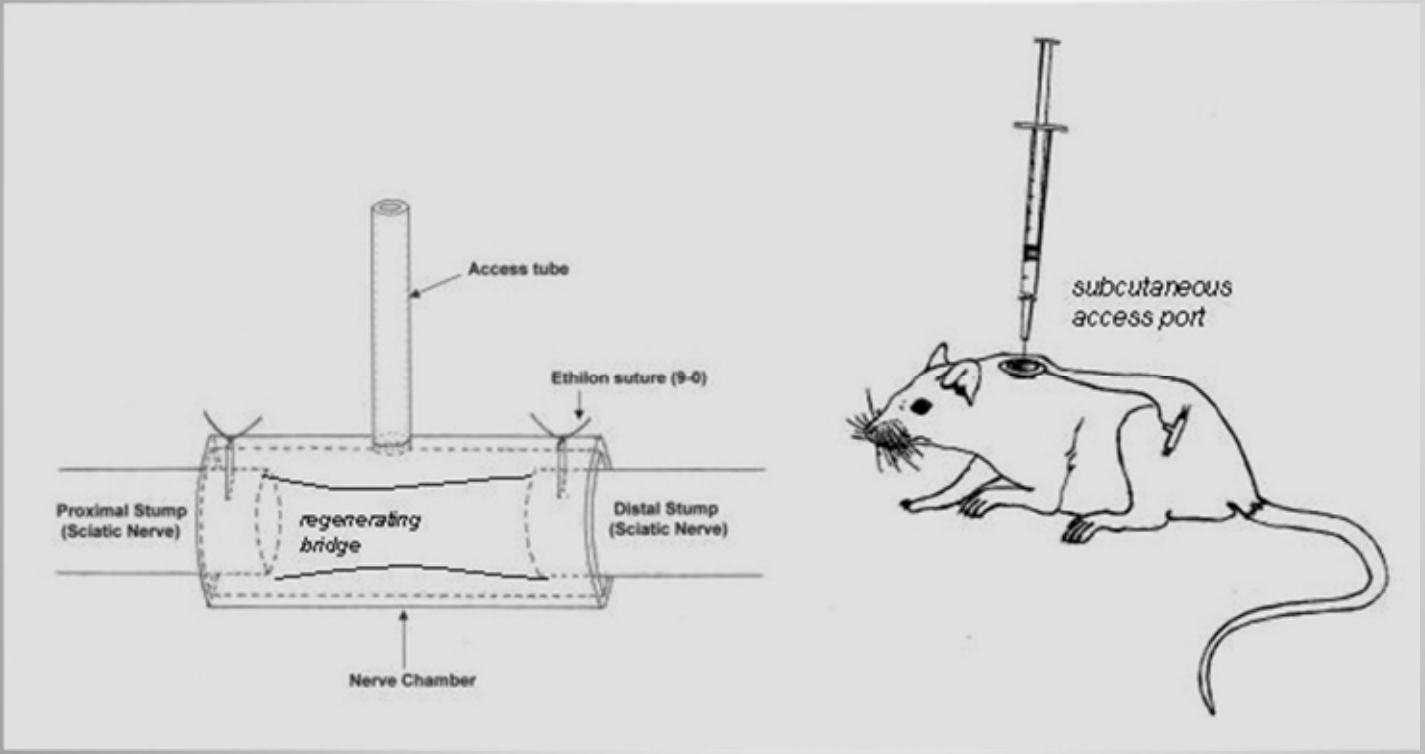
Regeneration and neurobiological themes

- The pace of Wallerian/Wallerian-like degeneration influences subsequent regenerative success
- The Schwann cell is the essential player and partner guiding axon outgrowth

Physical and molecular partnership between new axons and Schwann cells (SCs)

- **Schwann cell outgrowth almost always precedes and closely accompanies axon growth [Chen *et al.* 2005;McDonald *et al.* 2006]**
- **Inhibition of SC proliferation results in severe regenerative failure[Chen, McDonald, Cheng, Magnowski, Durand, and Zochodne2005]**
- **Synthesis of laminin by SCs may help direct axon trajectories**
- **Molecular interchange: Neuregulin by axons act on erbB2/3-SCs elaborate growth factors in response**
- **CGRP is a neuropeptide that acts as a SC mitogen [Toth *et al.* 2009]**
- **DCC and Unc5H netrin receptors are expressed on SCs and facilitate both SC and follow along axon growth [Webber *et al.* 2011]**

Examining and manipulating the regenerative microenvironment: McDonald regeneration chamber: a tool for examining early axon outgrowth



From:
Neurobiology
of peripheral
nerve regeneration,
Cambridge, 2008

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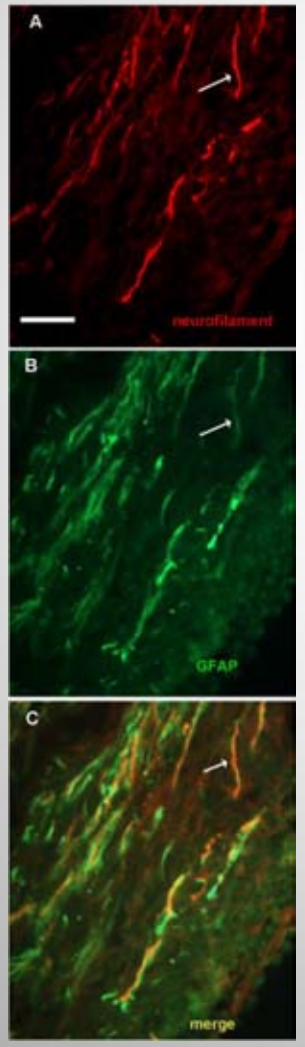
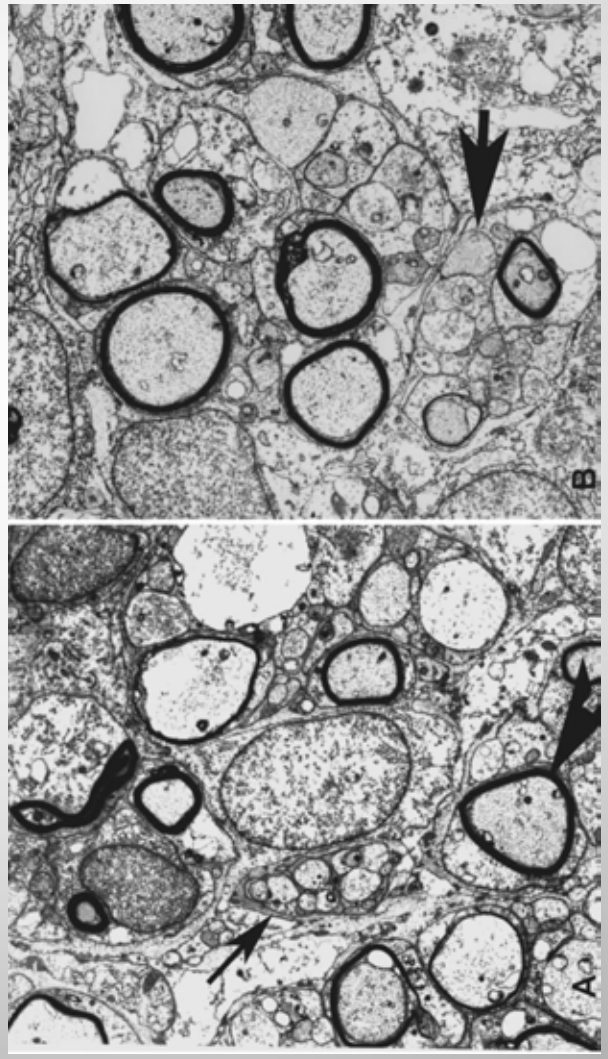
Axon regrowth is accompanied by Schwann cells (immunohistochemistry [middle; neurofilament stains axons, GFAP reactive SCs], EM showing clusters of axons associated with SCS





From:
Neurobiology
of peripheral
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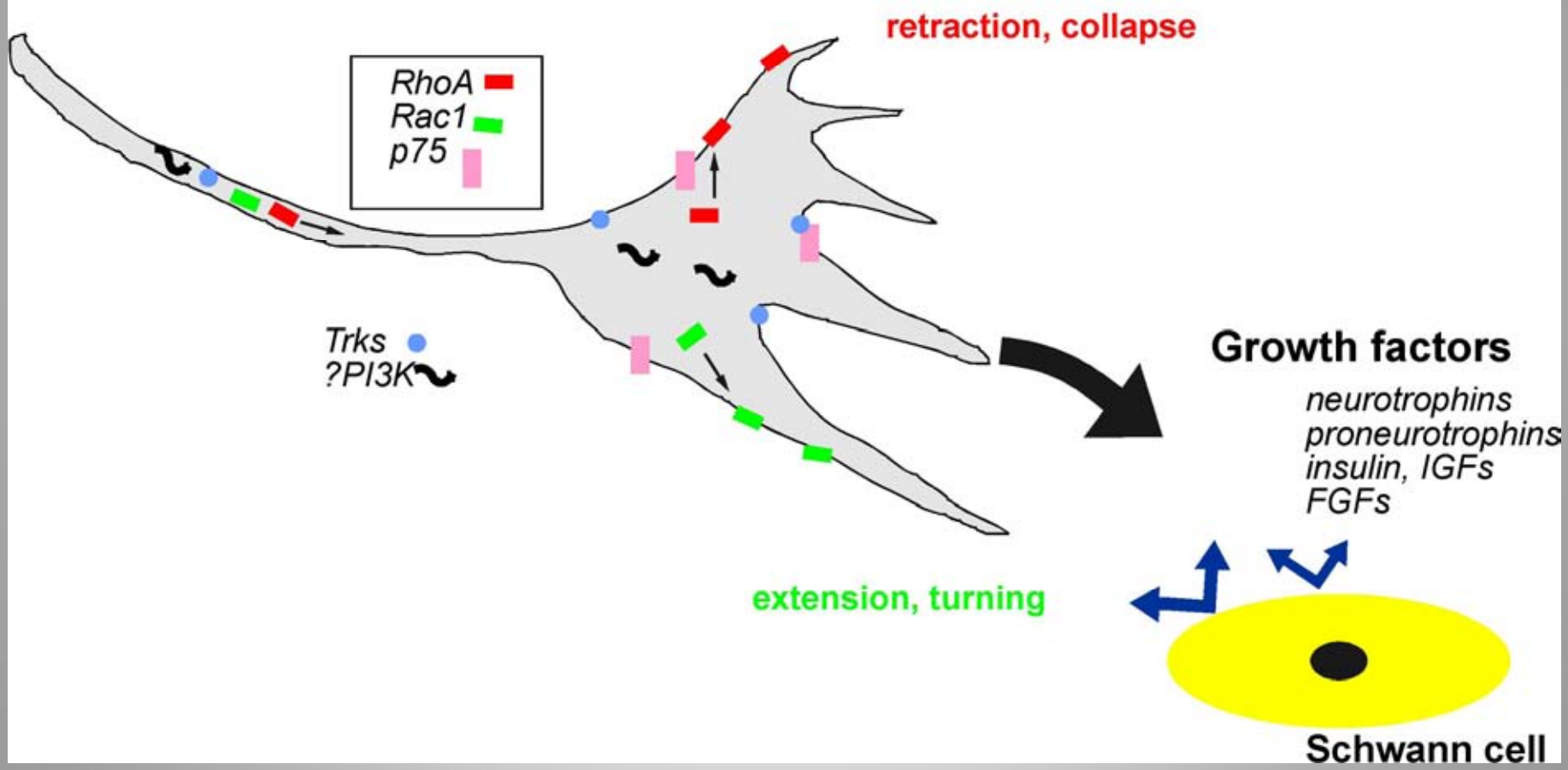
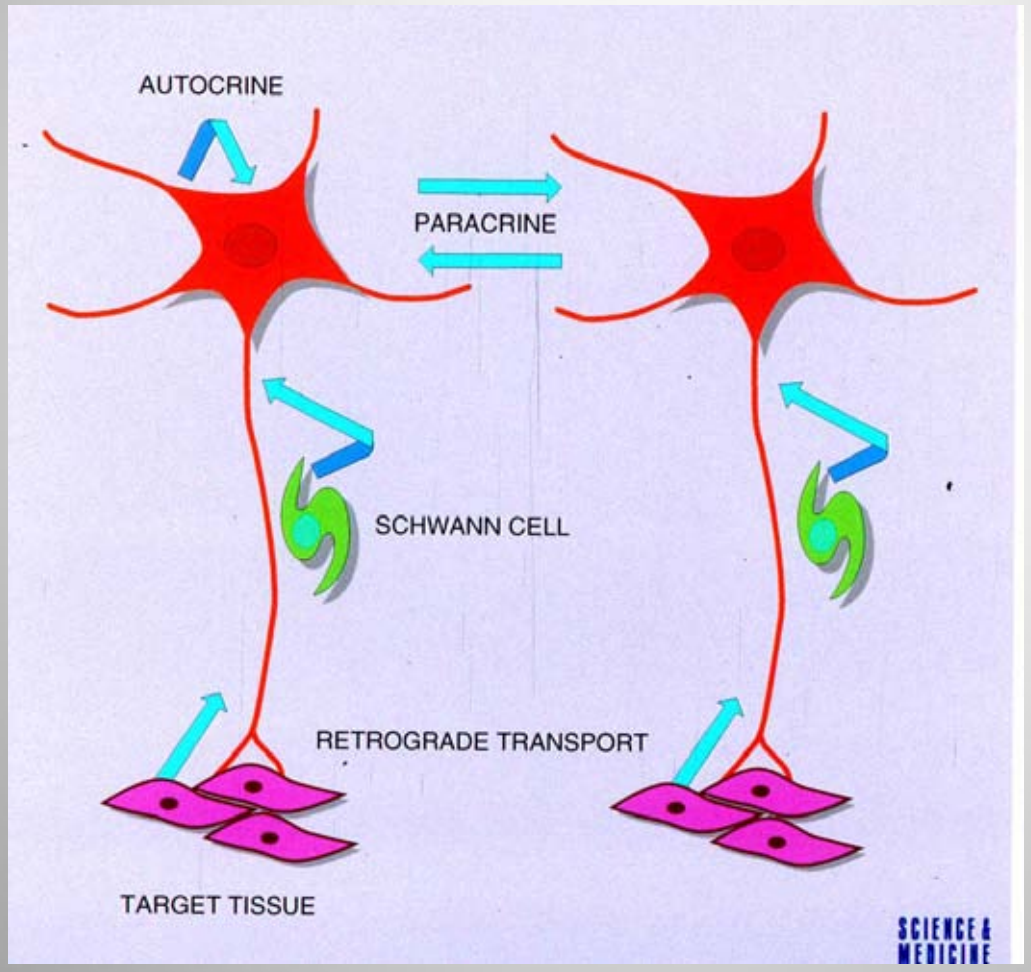
Growth cones in vivo extend and turn with guidance from Schwann cells and their repetoire of growth factors

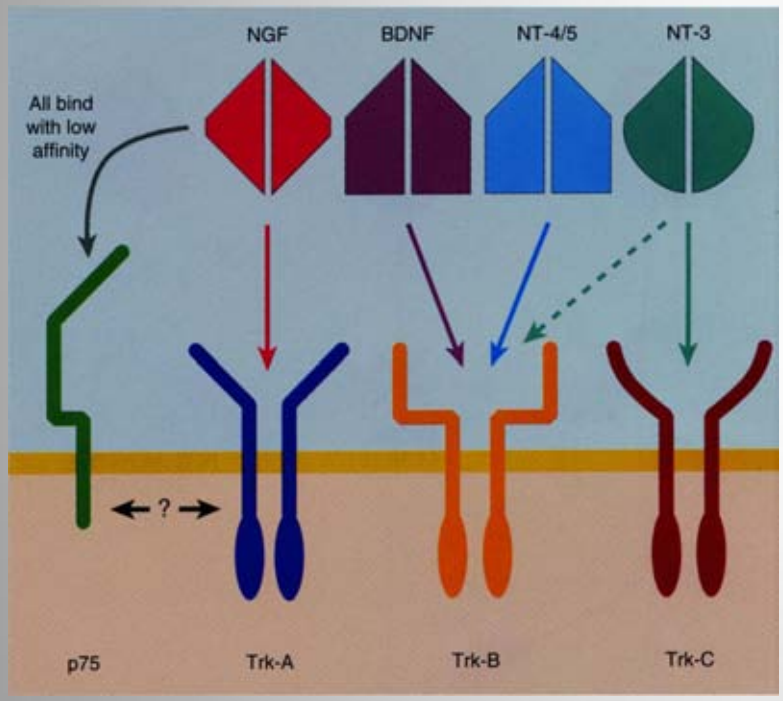


Growth factors are synthesized by neurons, Schwann cells and target tissues

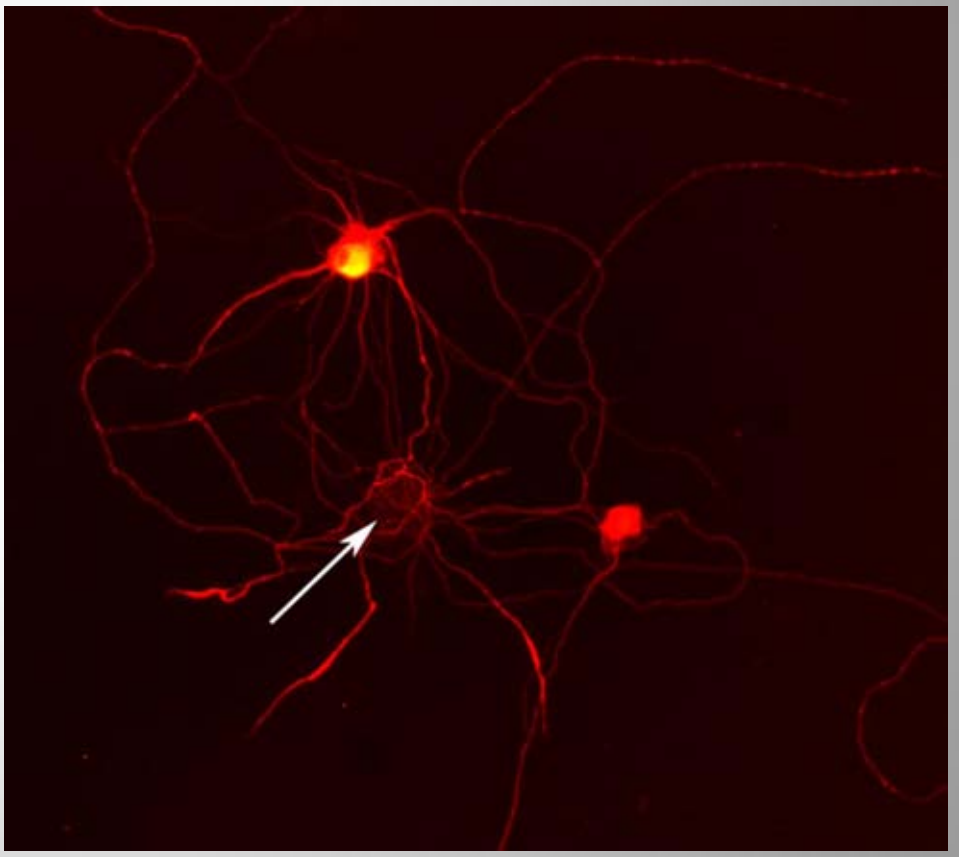


From:
Neurobiology of peripheral nerve regeneration, Cambridge, 2008 and Science and Medicine,1999

Neurotrophin growth factors (NGF family) act on specific receptors to attract axons

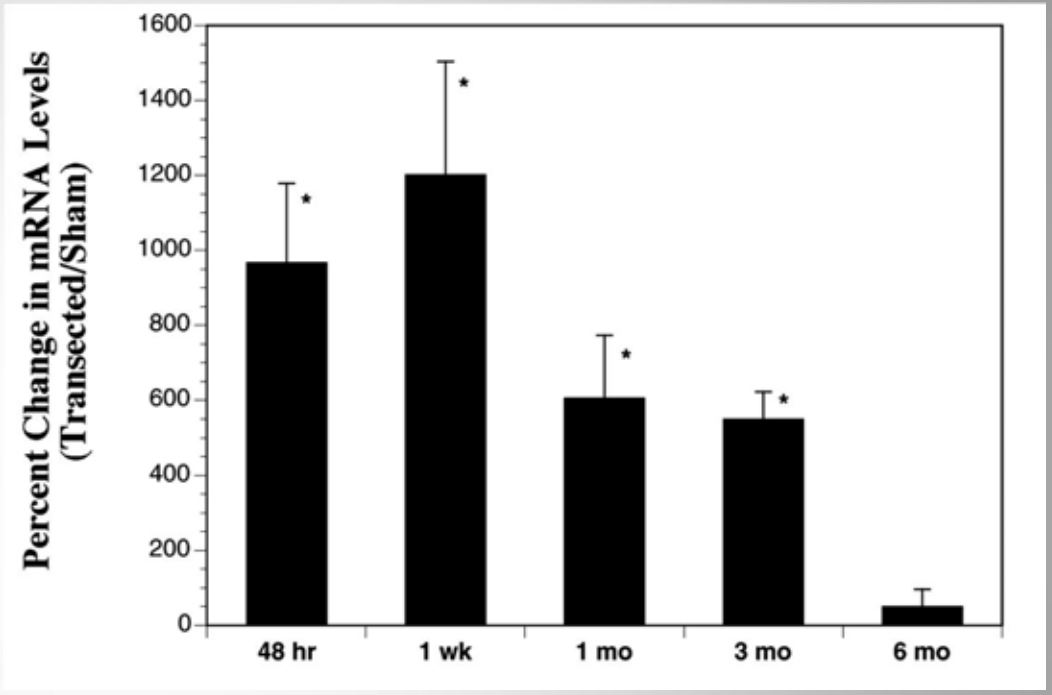


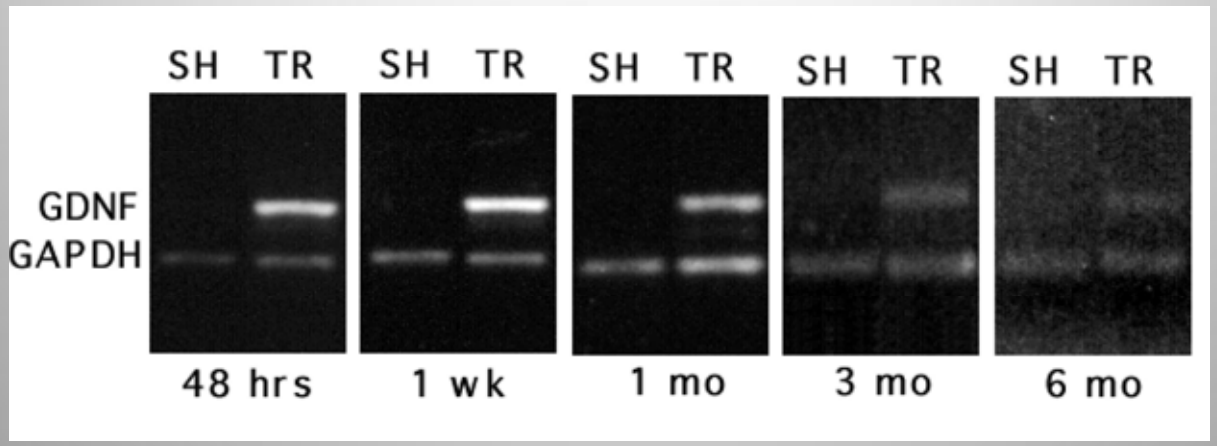
From: Neurobiology of peripheral nerve regeneration, Cambridge, 2008 and Science and Medicine, 1999



Adult sensory neurons in vitro send multiple axon projections into a cluster of Schwann cells (unstained; arrow)

Glial-derived neurotrophic factor (GDNF) in long term denervated nerve Trunks (Hoke et al, 2000)





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Regeneration and neurobiological themes

- The pace of Wallerian/Wallerian-like degeneration influences subsequent regenerative success
- The Schwann cell is the essential player and partner guiding axon outgrowth
- Trajectory is as important as outgrowth during axon regeneration

Misdirected axons contribute to regenerative failure

- Neuromas in continuity are connected, injured nerves that fail to fully reconnect from proximal to distal stumps;

The severity of the lesion depends on how many axons traverse the lesion (variable);
Surgeons may resect and resuture if few axons have traversed (intra-operative electrophysiology)

- Axons may circle backwards up the proximal stump, into and around the epineurium
- 'Wrong way' axons can be detected experimentally
- Laminin and SC guidance reduce numbers of 'wrong way' axons
- Some axons may be directed and turned by growth factors: NGF, HGF (adult neuron growth cone turning assay)

[Webber *et al.* 2008;Cheng *et al.* 2010]

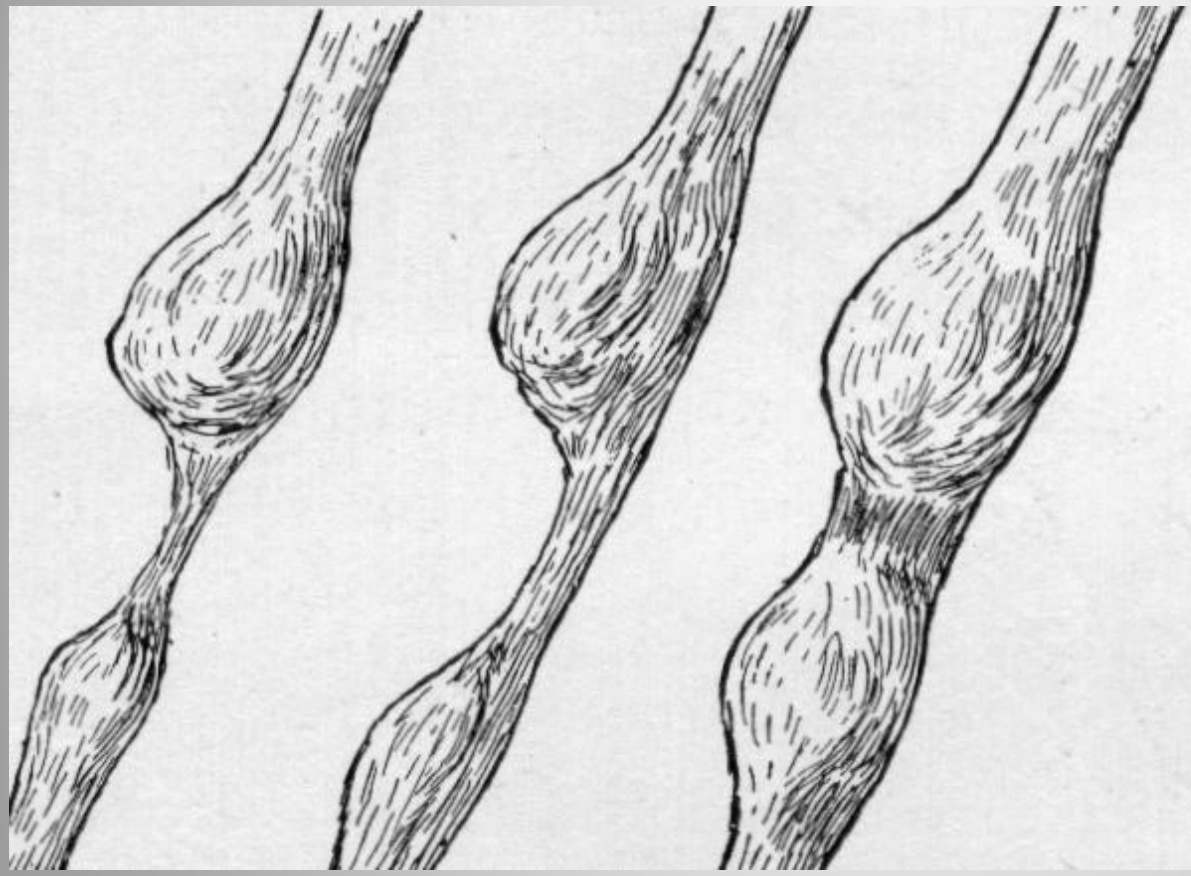
References

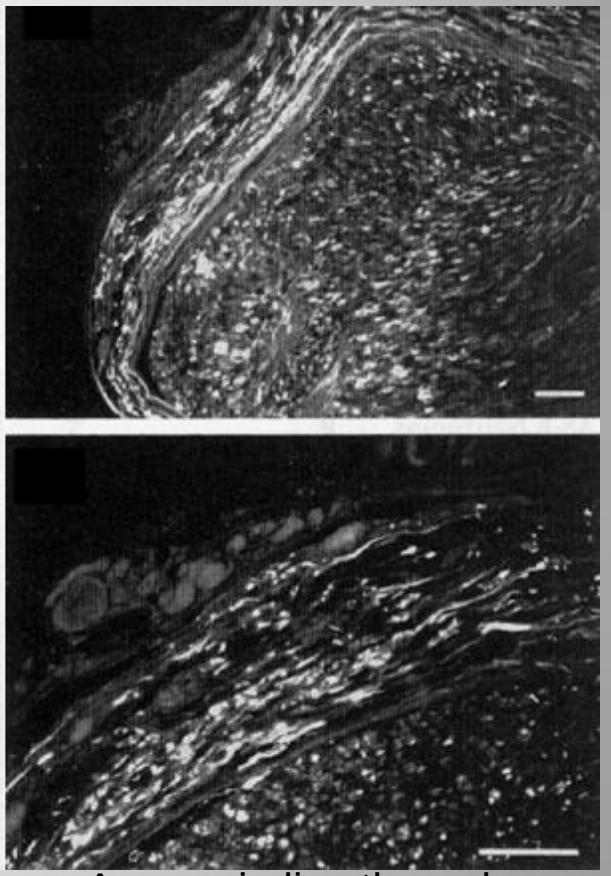
Cheng C, Guo GF, Martinez JA, Singh.V., Zochodne DW. Dynamic plasticity of axons within a cutaneous milieu. J Neurosci 2010; 30: 14735-44.

Webber CA, Xu Y, Vanneste KJ, Martinez JA, Verge VMK, Zochodne DW. Guiding adult mammalian sensory axons during regeneration. J Neuropathol Exp Neurol 2008; 67: 212-222.

The problem of misdirected peripheral nerve axons

Neuromas in continuity





Axons circling through the epineurium

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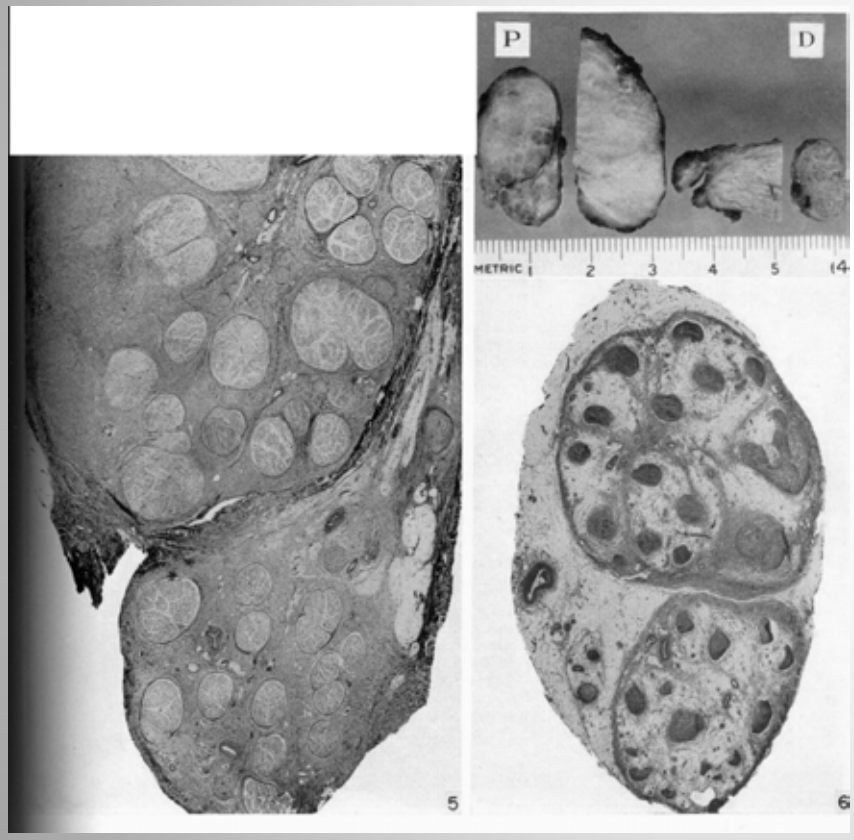
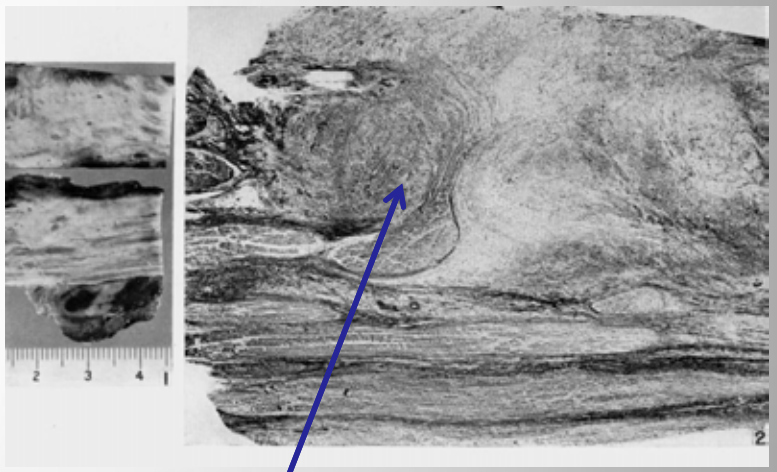
Misdirection in missile injuries to peripheral nerves





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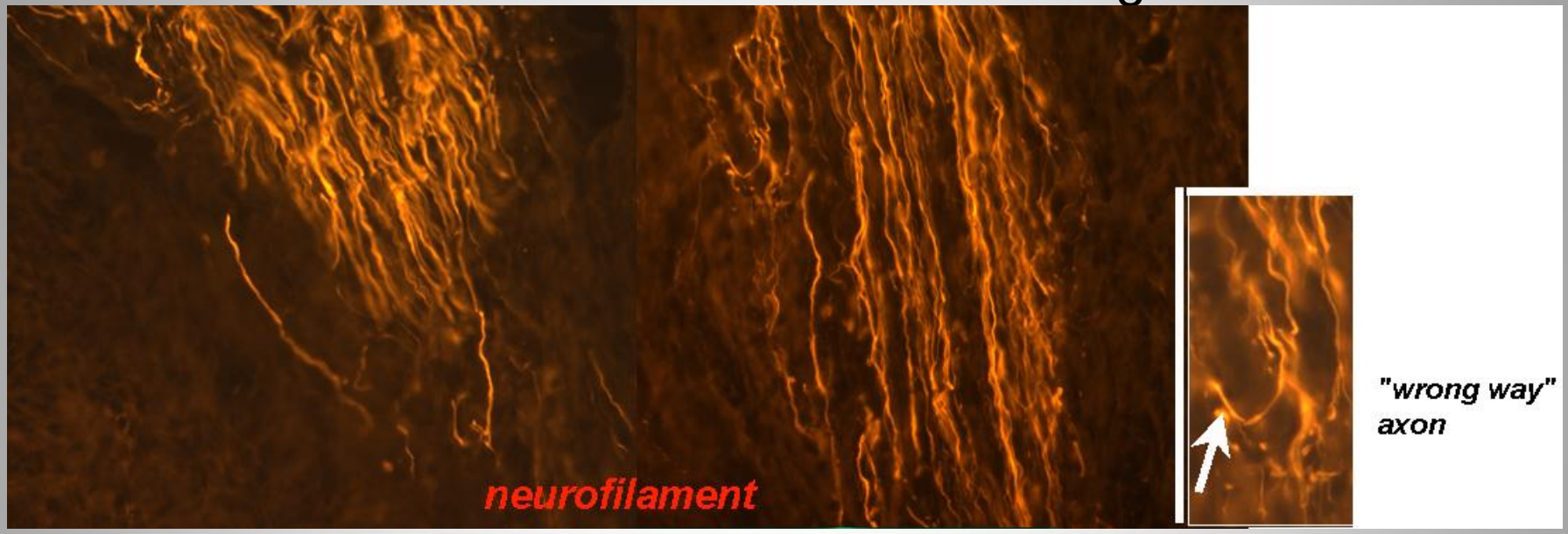
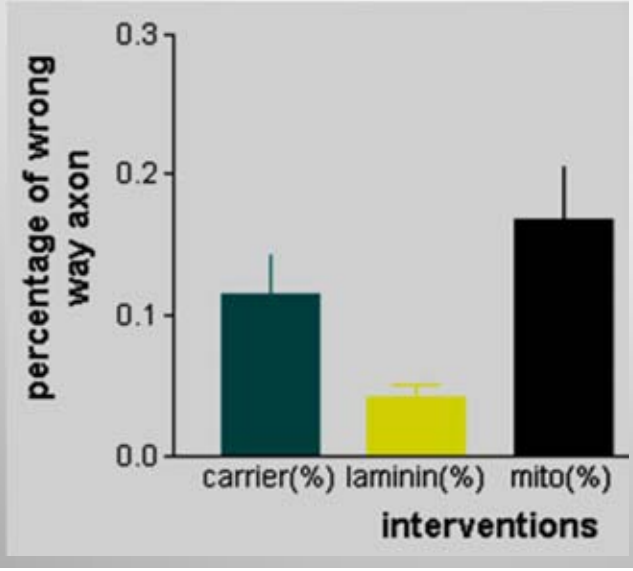
In vivo axon direction finding





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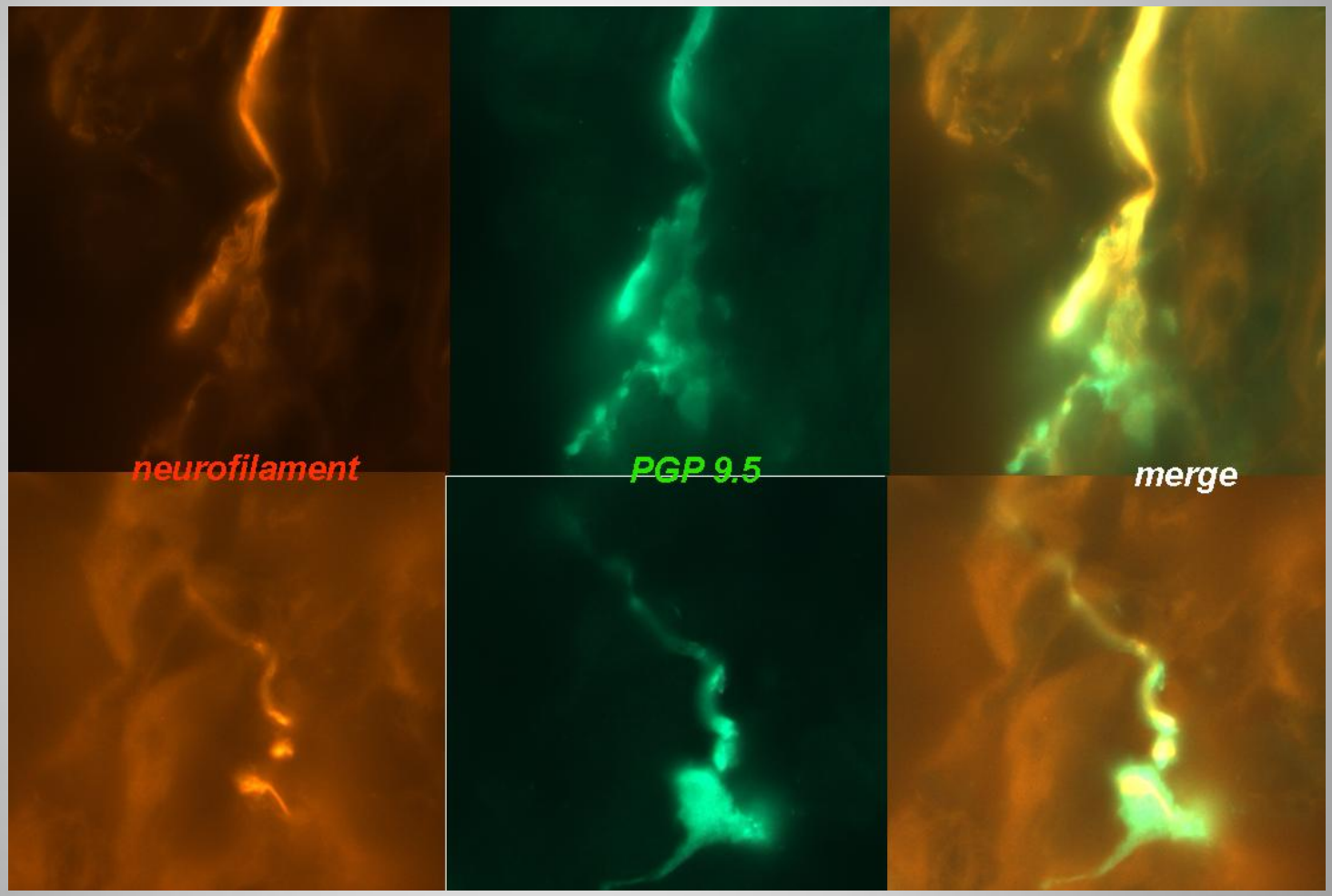
Complexities of in vivo growth cones



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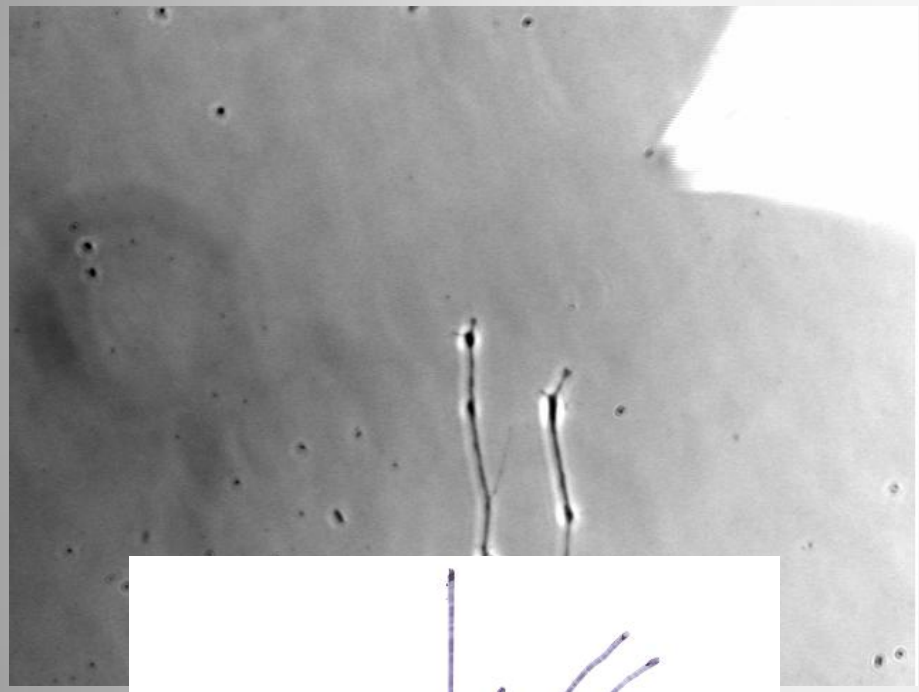
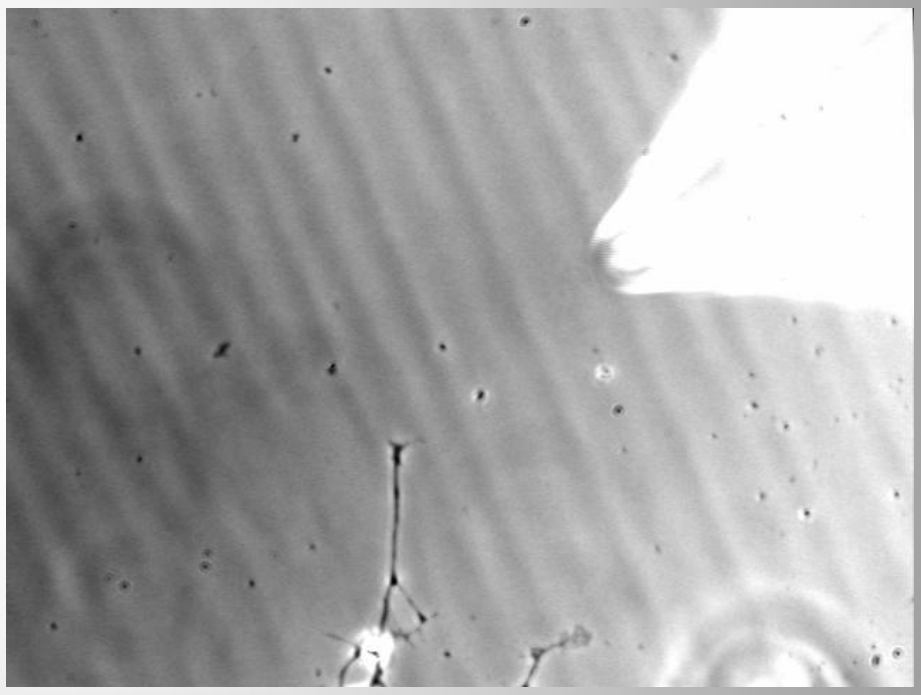
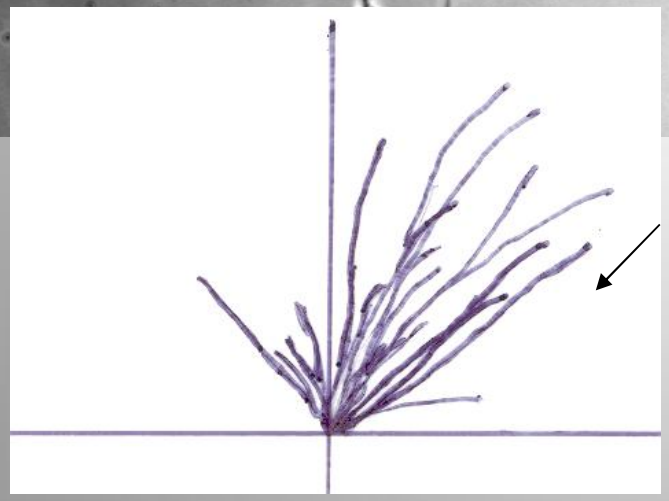
Growth cone turning in adult primary sensory neurons in response to NGF







Webber et al,
J NP Exp Neurol 2008

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- The Schwann cell is the essential player and partner guiding axon outgrowth
- Trajectory is as important as outgrowth during axon regeneration
- There are intrinsic roadblocks to regenerative success

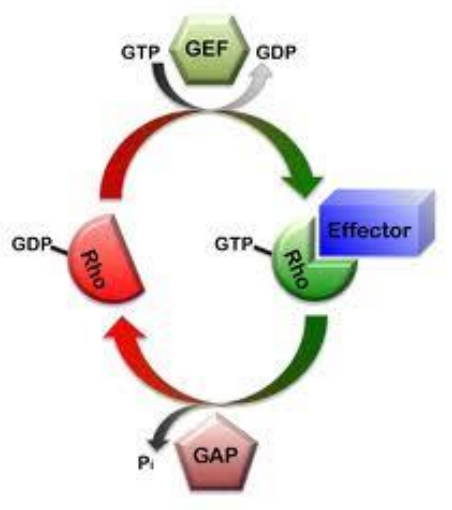
Examples of regenerative roadblocks

(i) RhoA acting through Rho kinase (ROK)

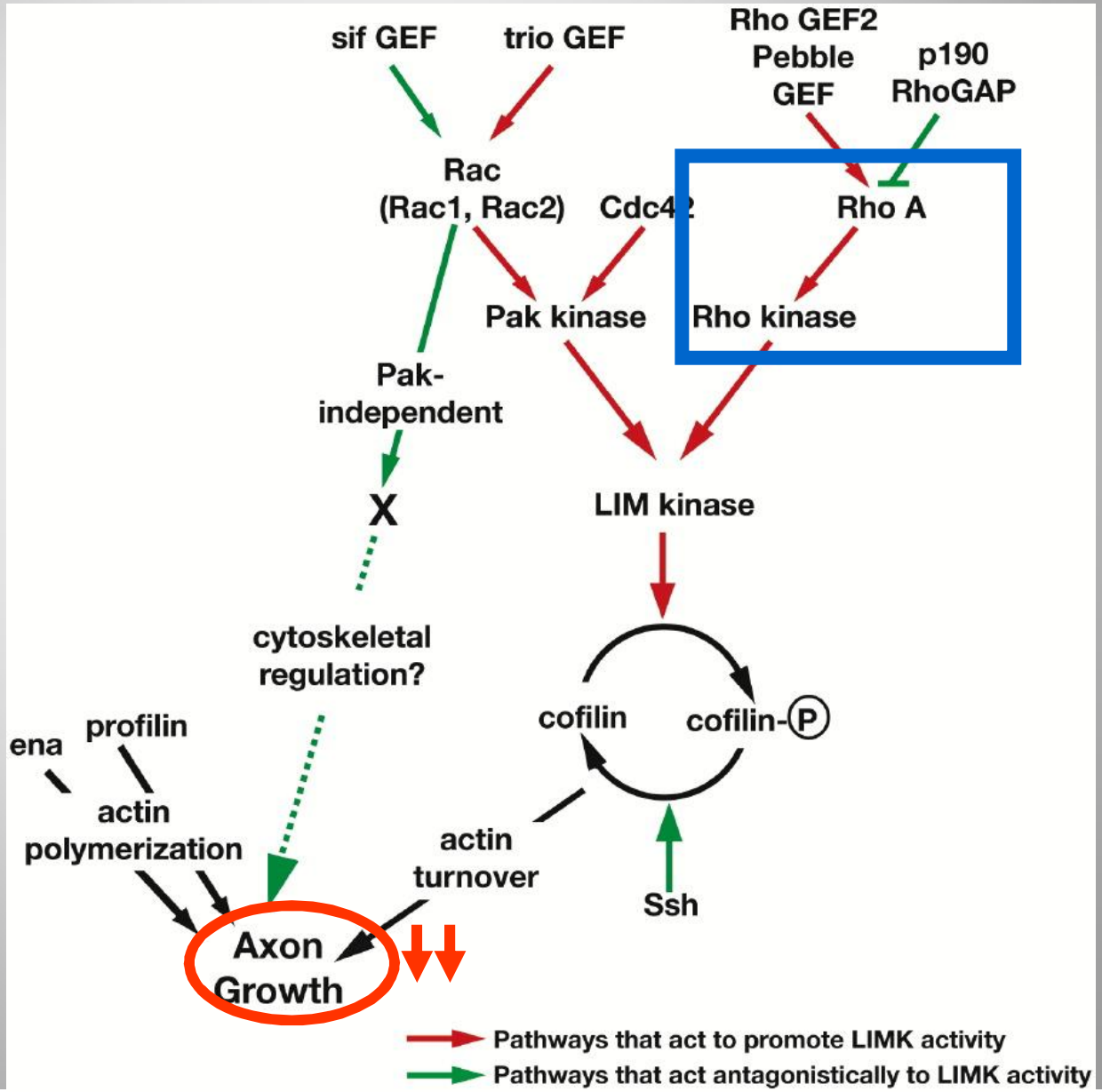
(ii) PTEN (a tumour suppressor)

(iii) Extracellular myelin, CSPGs

Central regulators of growth cones: RhoA (collapse)



Nance and Zallen
Development 2011



Ng and Luo,
Neuron 2004

Rho (Ras) family GTPases: molecular switches on growth cone membranes

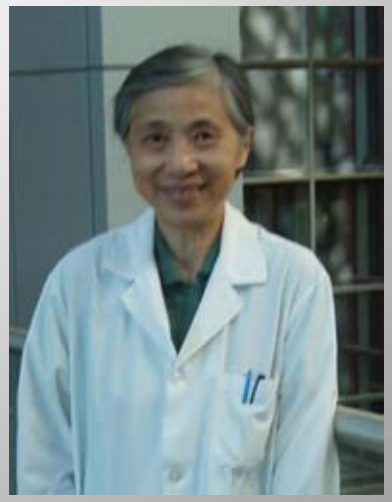
Family members:

RhoA: activates ROCK (ROK; Rho kinase), may phosphorylate actin-associated growth cone myosin to cause retraction; interacts with p75 to mediate NOGO 66, MAG inhibition

Rac 1: activates PAK, facilates lamellopodia extension

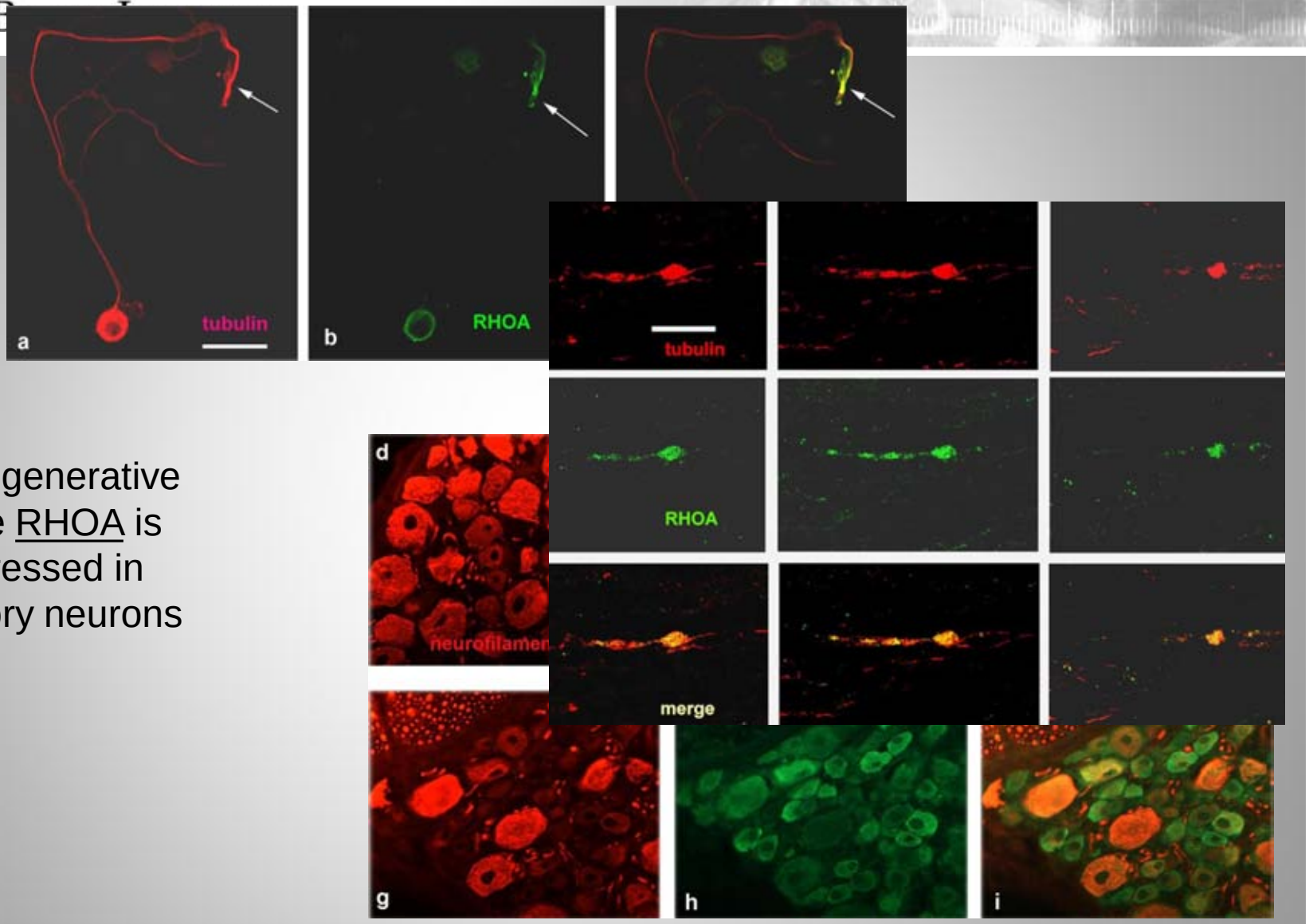
CDC42: actions similar to Rac 1

Others: Other Rhos, Rac2, Mtl



Cheng C, et al Exp Neurol. 2008

The regenerative brake RHOA is expressed in sensory neurons



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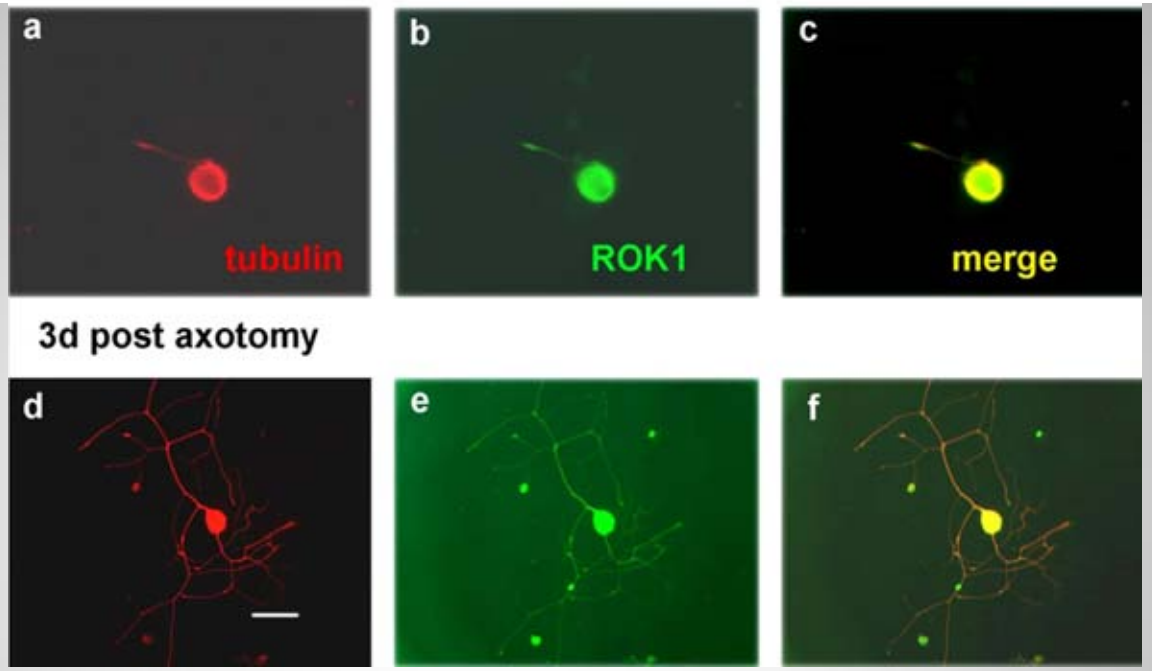
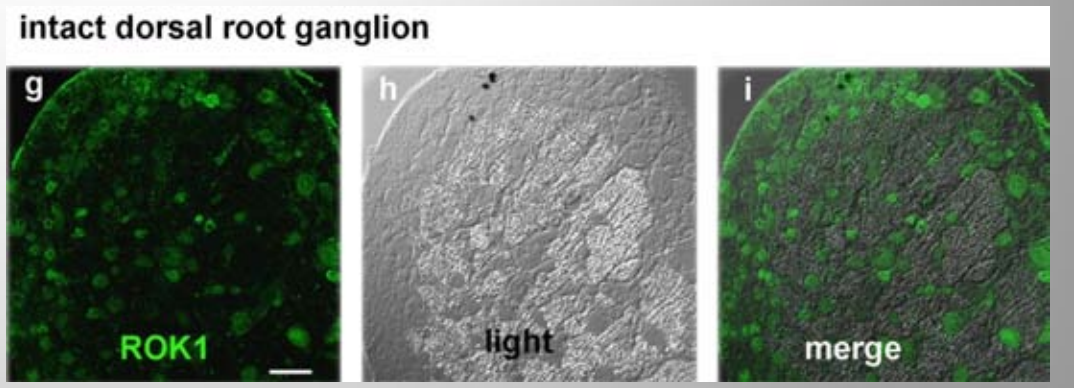
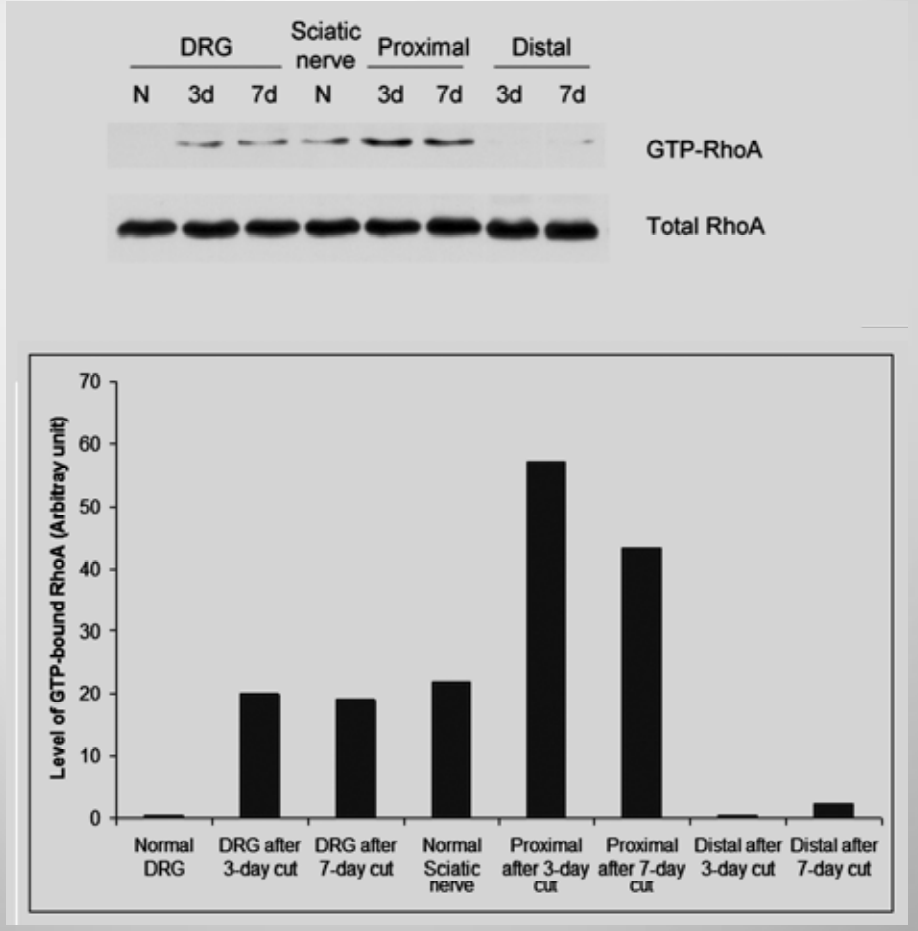


Expression of RHO kinase (ROK1)



Activate (GTP bound) RHOA
is absent in intact nerve,
but upregulated after injury



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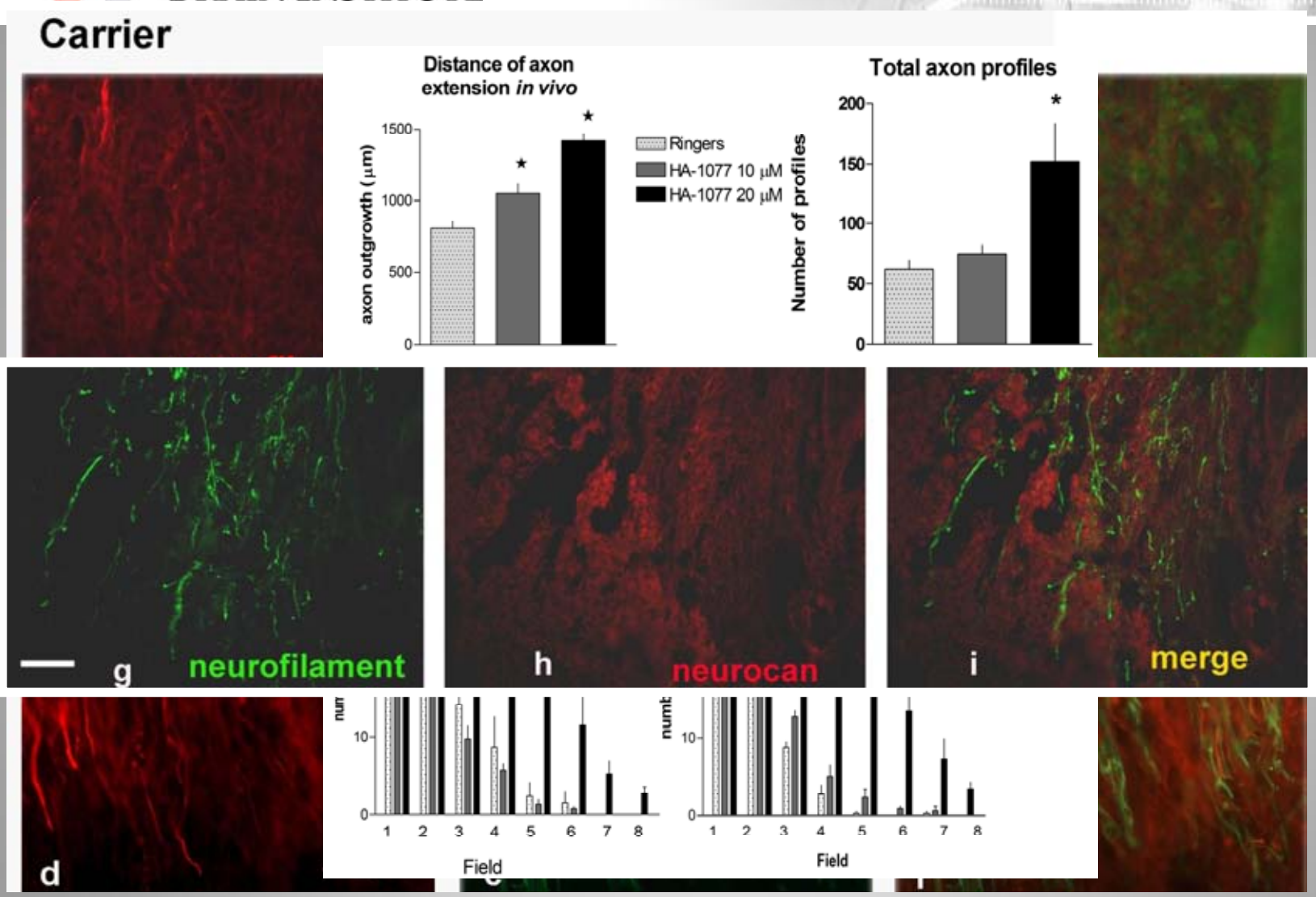

RHOA_ROK inhibition facilitates outgrowth of axons in vivo

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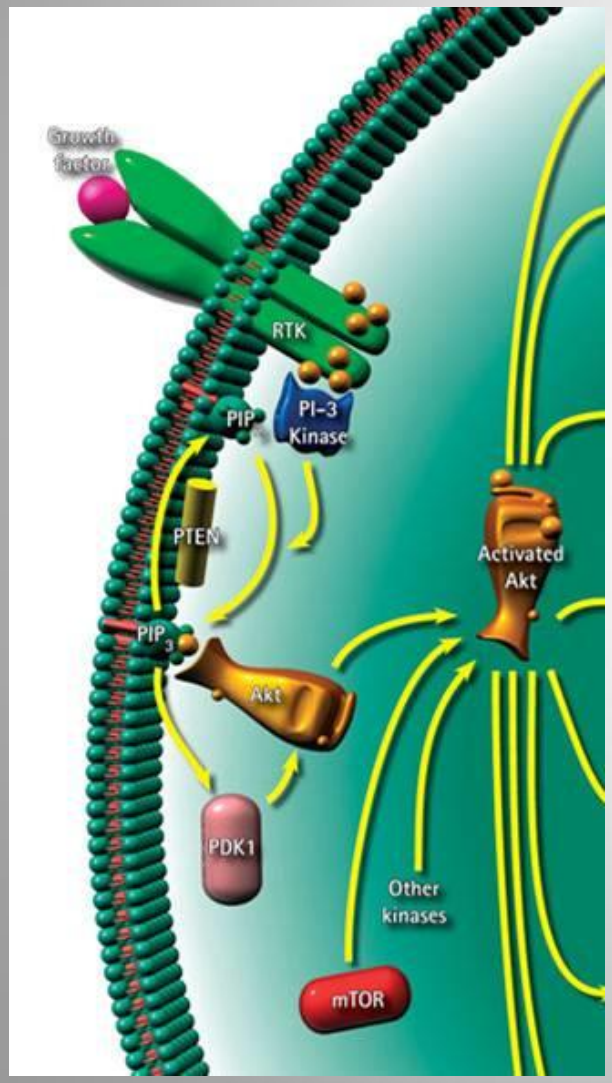
PTEN



- **Phosphatase and tensin homolog deleted on chromosome ten**
- **Tumor suppressor**
- **Inhibits motility and cell cycle progression**
- **Induces apoptosis**
- **Hydrolyze PIP3**
- **Phosporylation inactivation**

Christie et al,
J Neurosc. 2010

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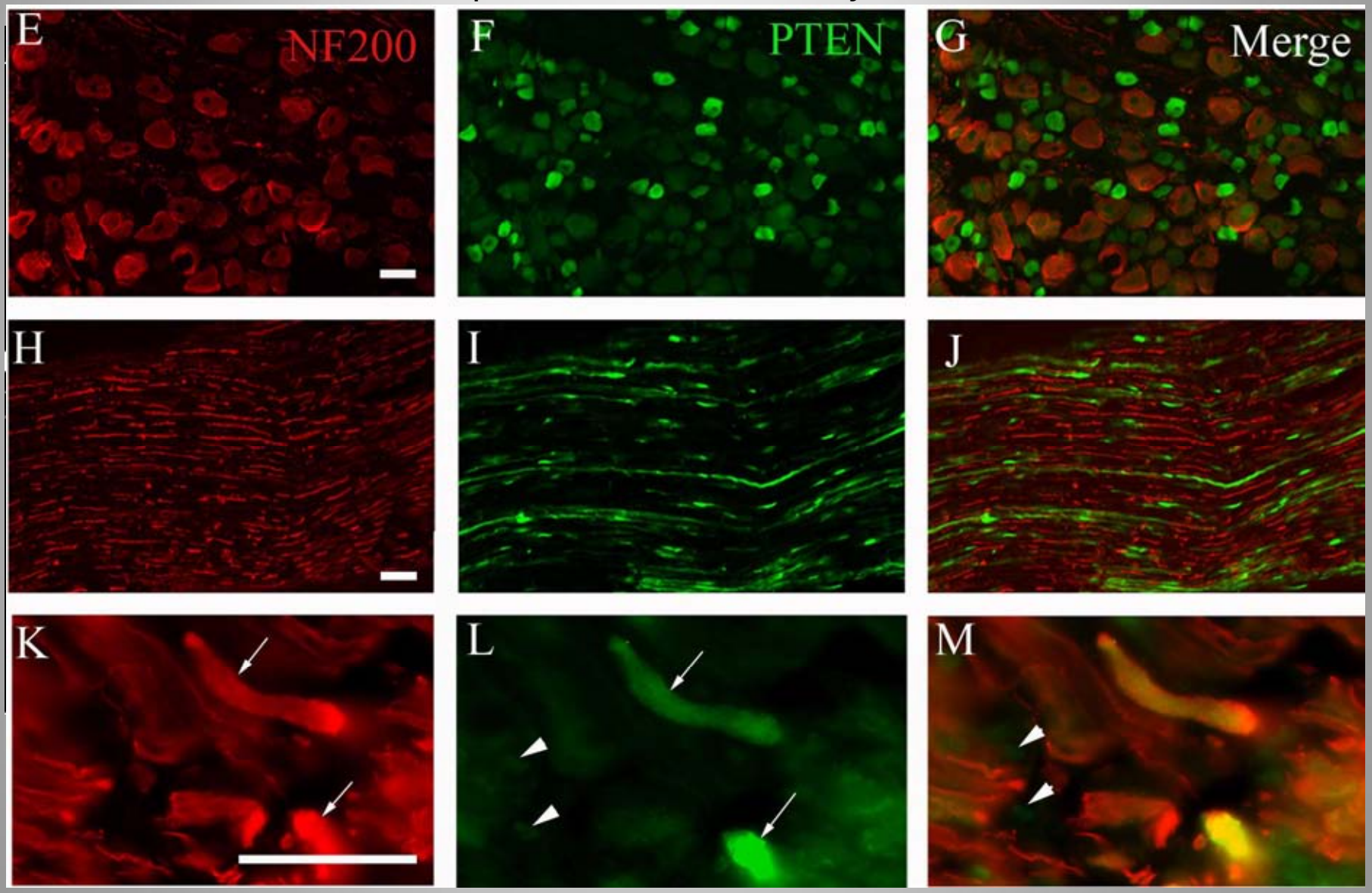
Is PTEN expressed in adult sensory neurons?



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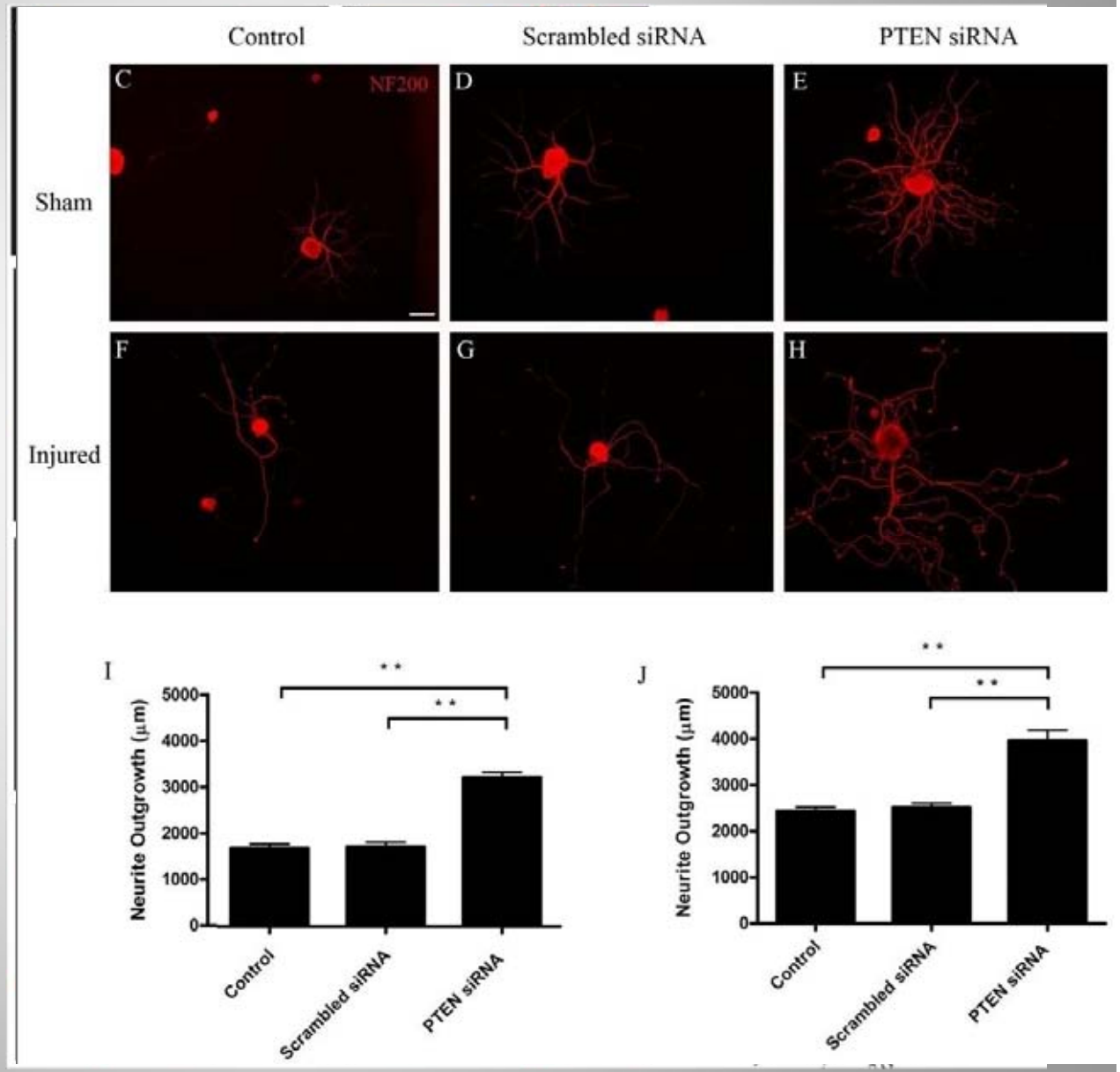
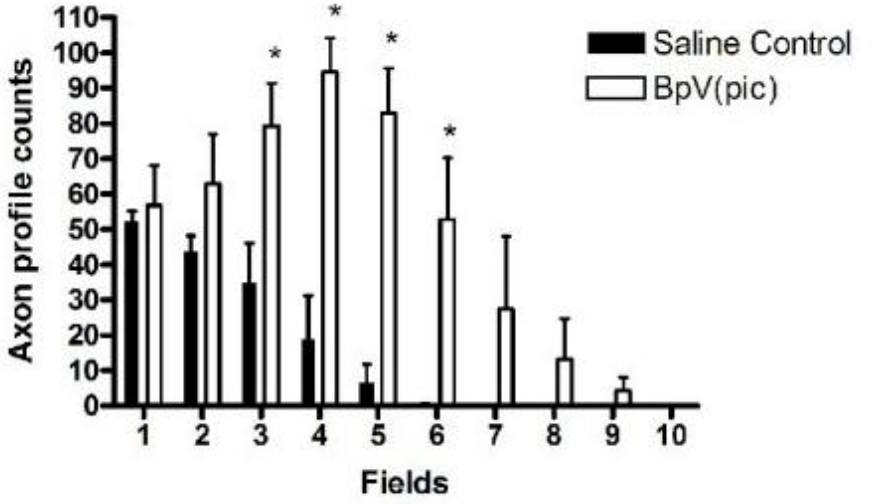
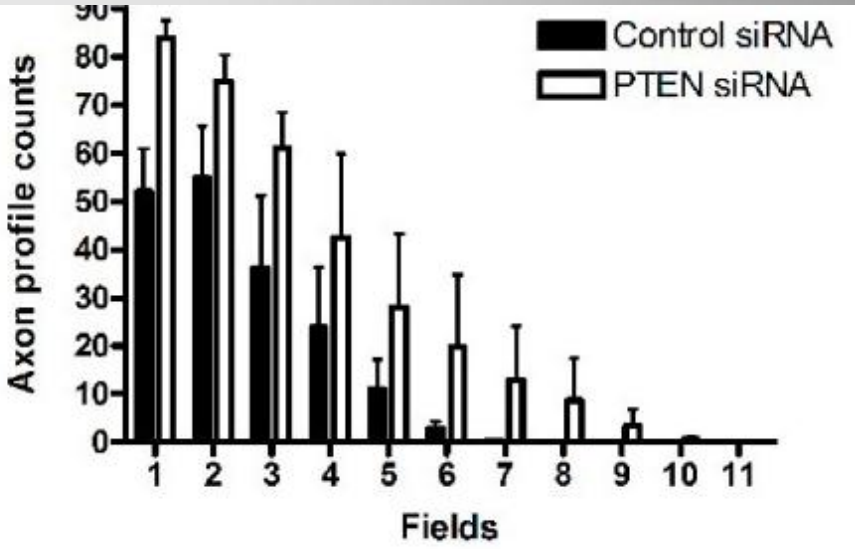
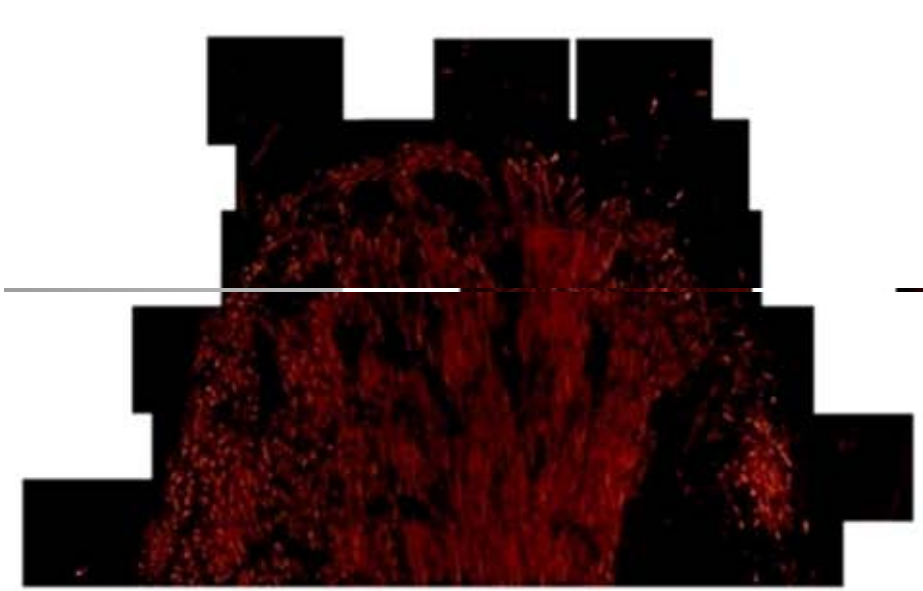
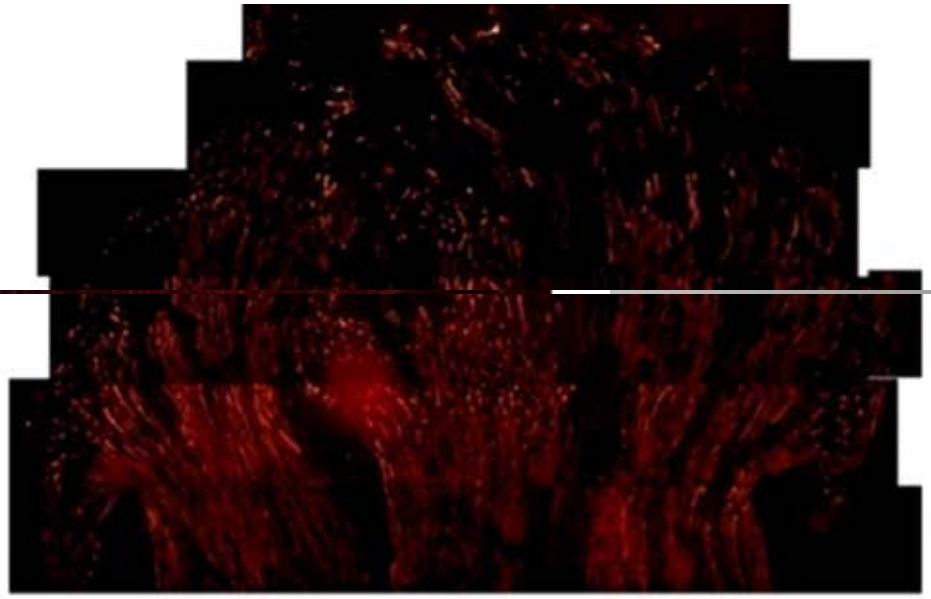
PTEN inhibition enhances sensory neuron outgrowth in vitro




Axon profile counts
Fields
Saline Control
BpV(pic)
*
*
*


Axon profile counts
Fields
Control siRNA
PTEN siRNA





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Regeneration and neurobiological themes

- The pace of Wallerian/Wallerian-like degeneration influences subsequent regenerative success
- The Schwann cell is the essential player and partner guiding axon outgrowth
- Trajectory is as important as outgrowth during axon regeneration
- There are intrinsic roadblocks to regenerative success
- Regeneration can recapitulate neurodevelopmental events
- Reinnervation can be accomplished by collateral sprouting in target tissues

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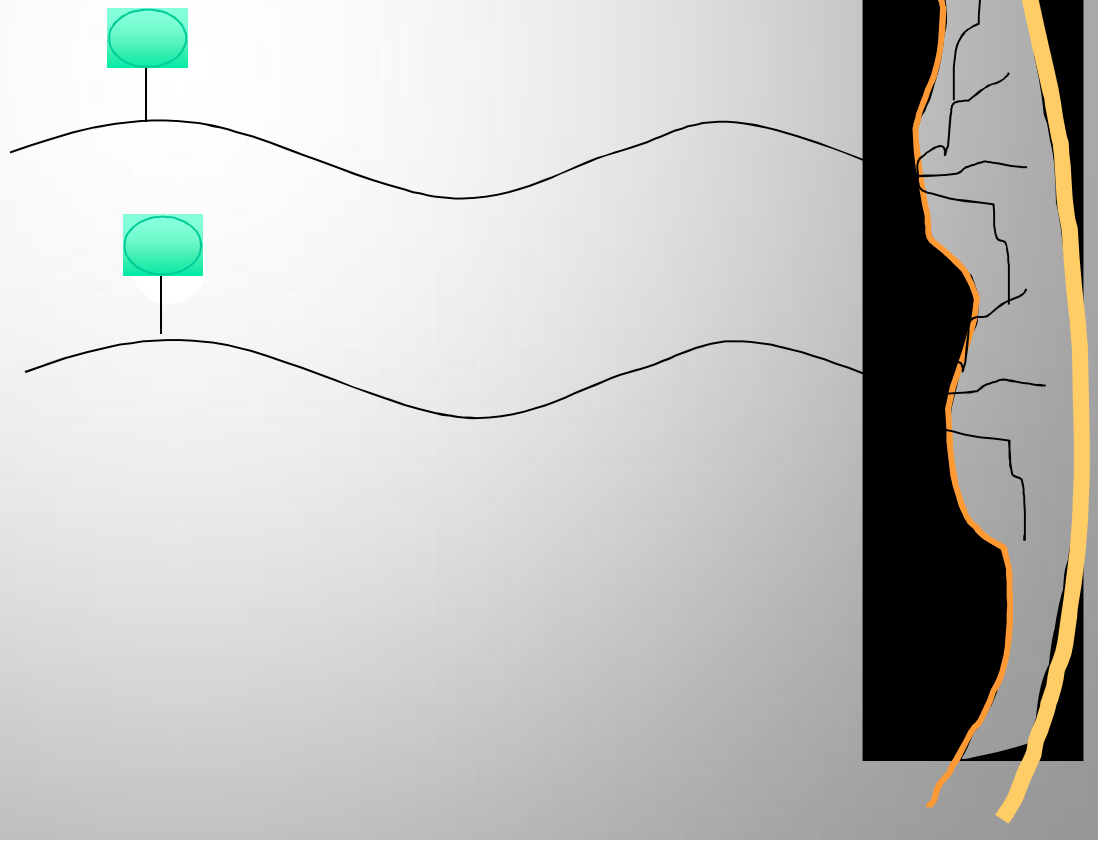
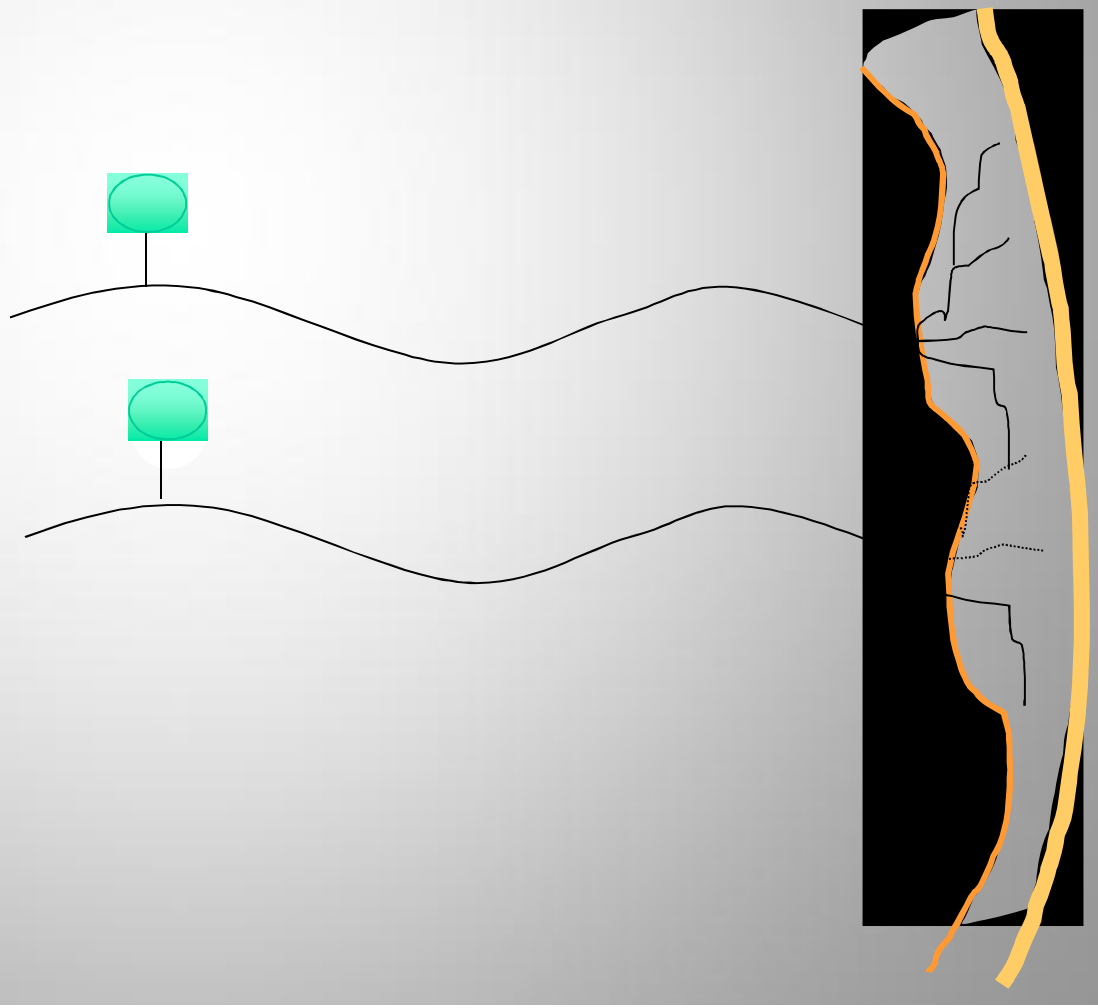
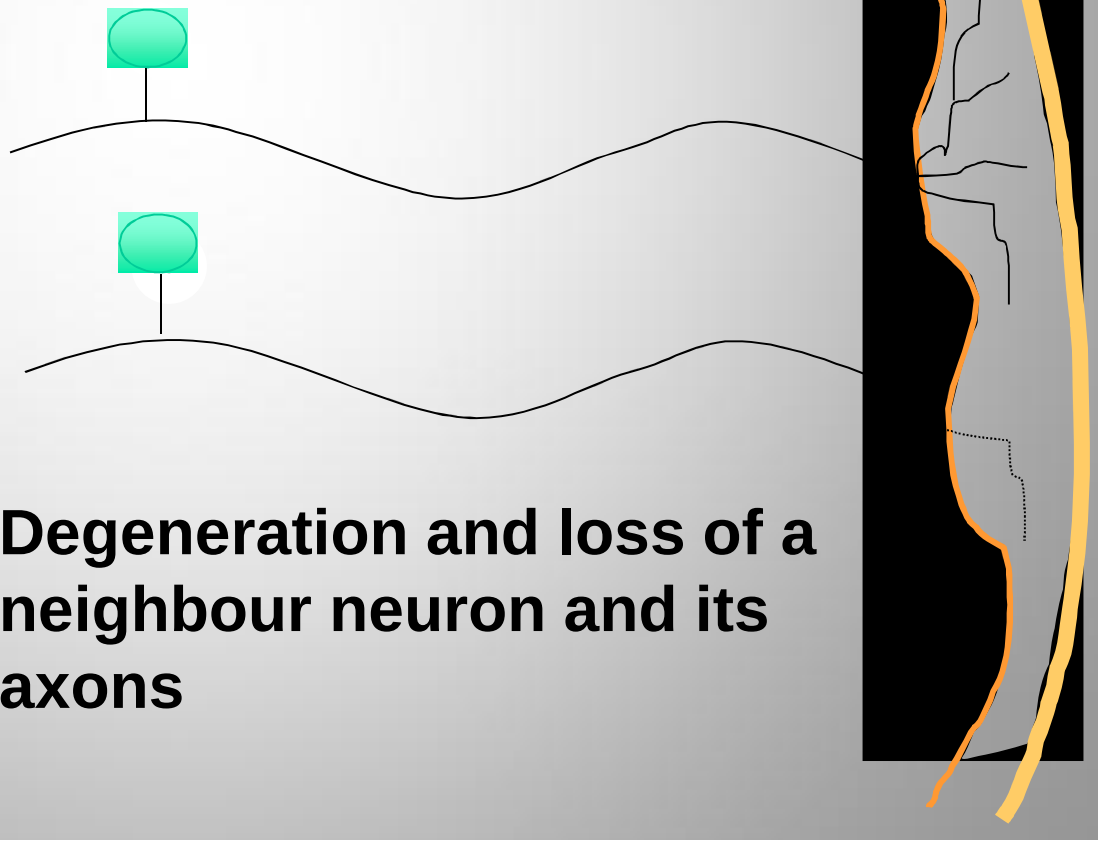
Collateral reinnervation:

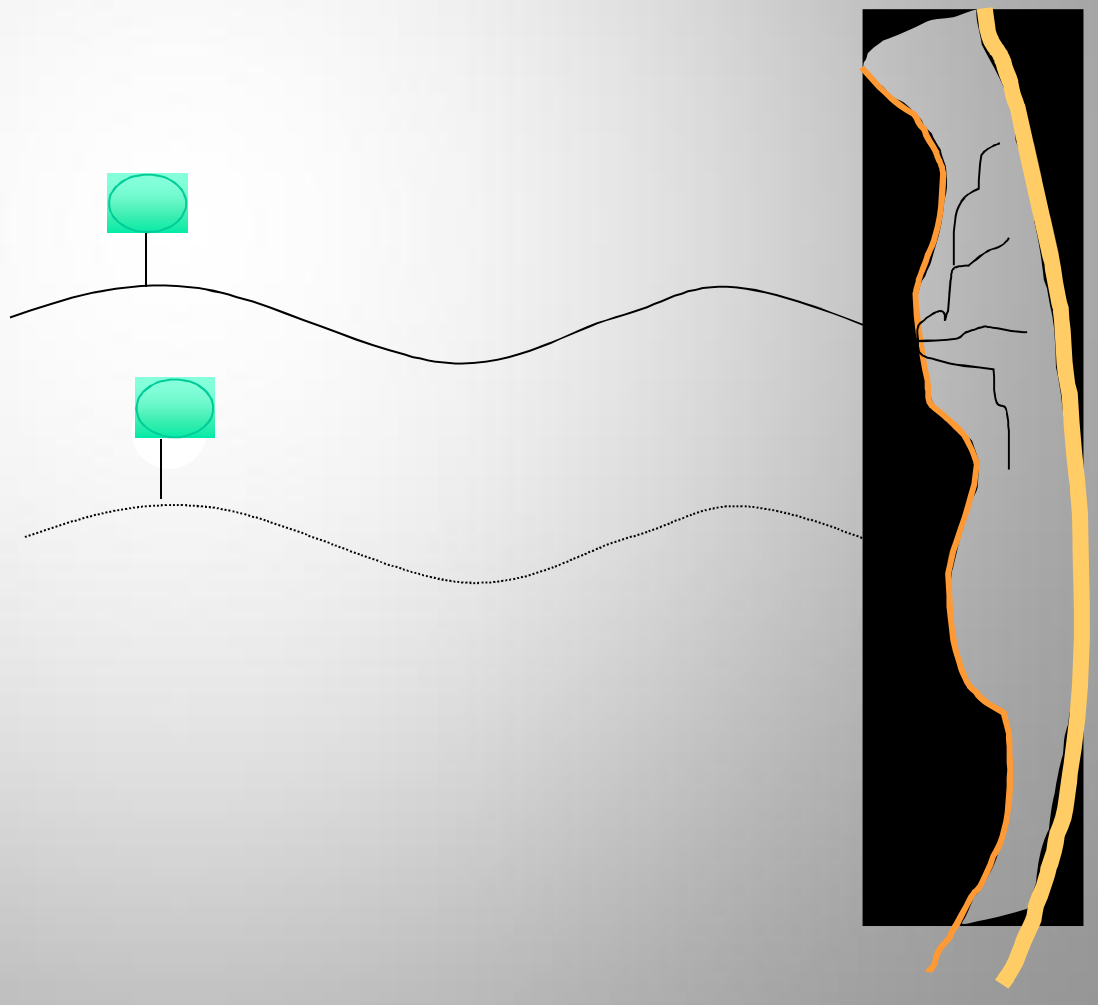
An alternative form of peripheral axon repair

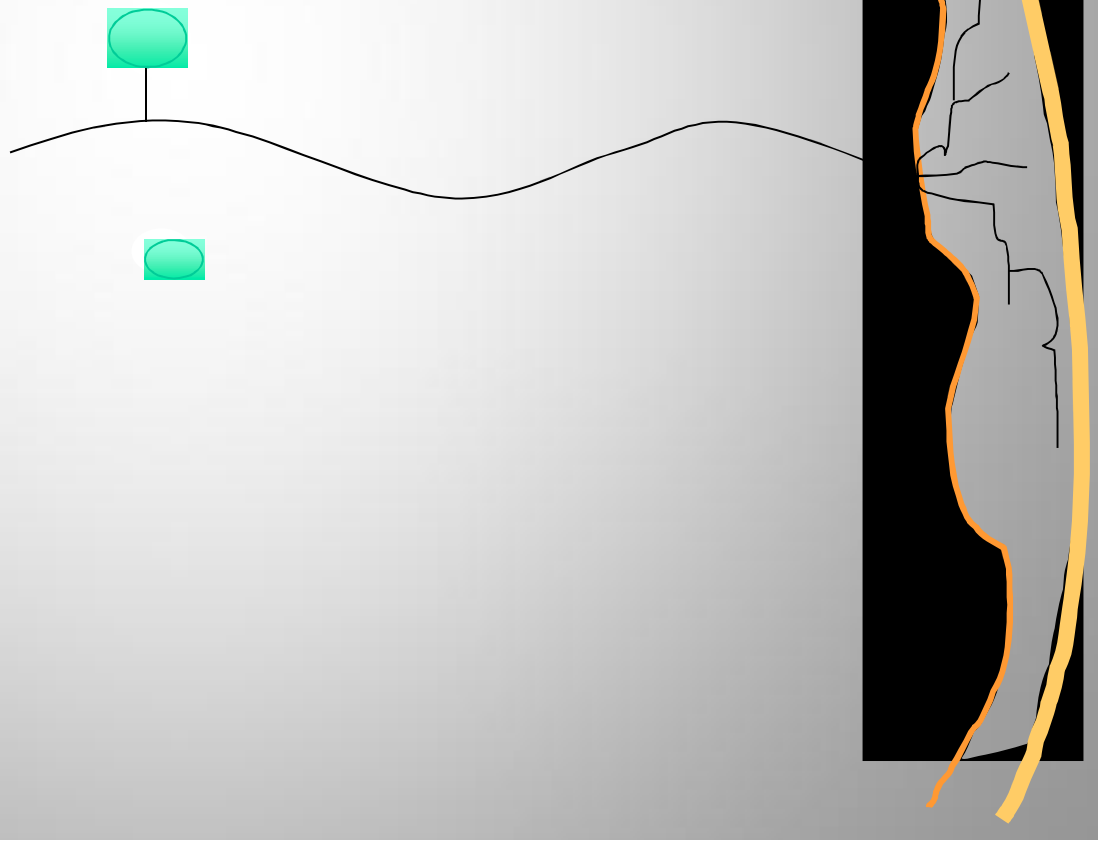


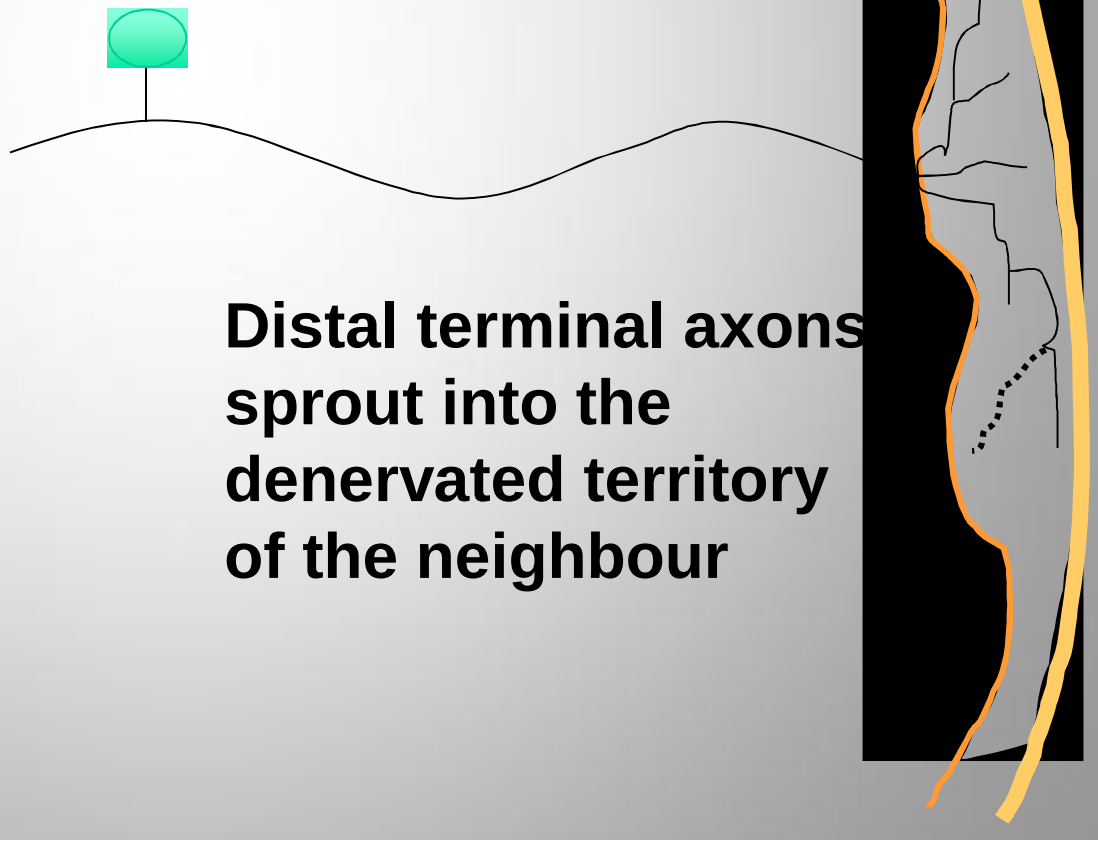




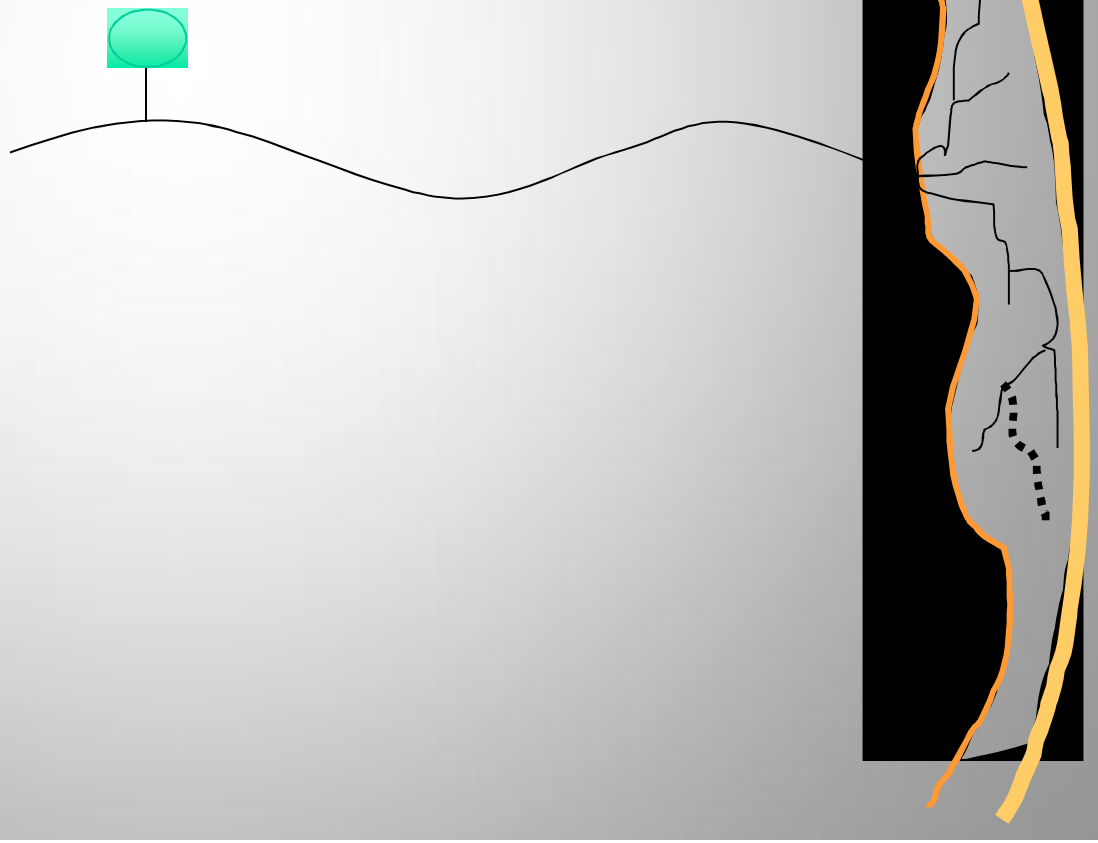
Degeneration and loss of a neighbour neuron and its axons

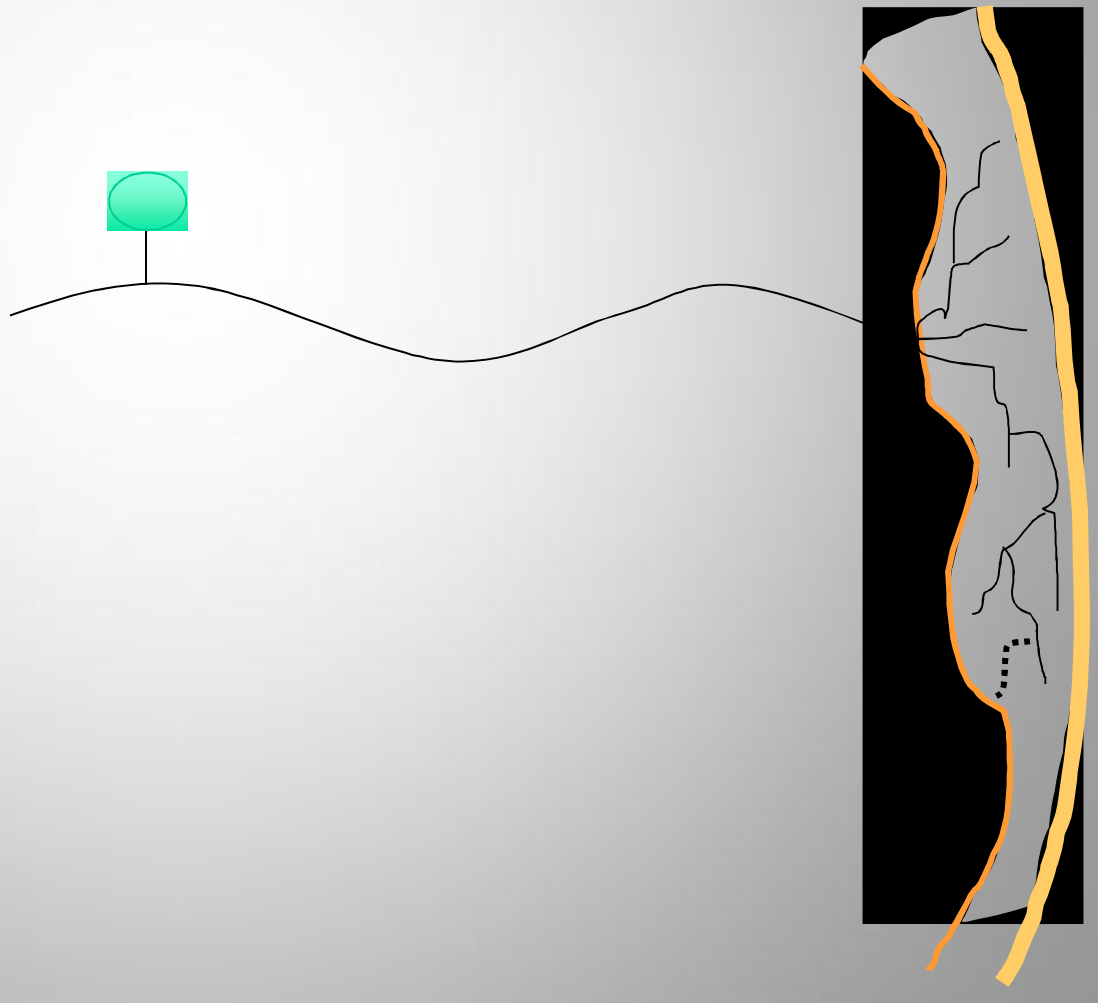


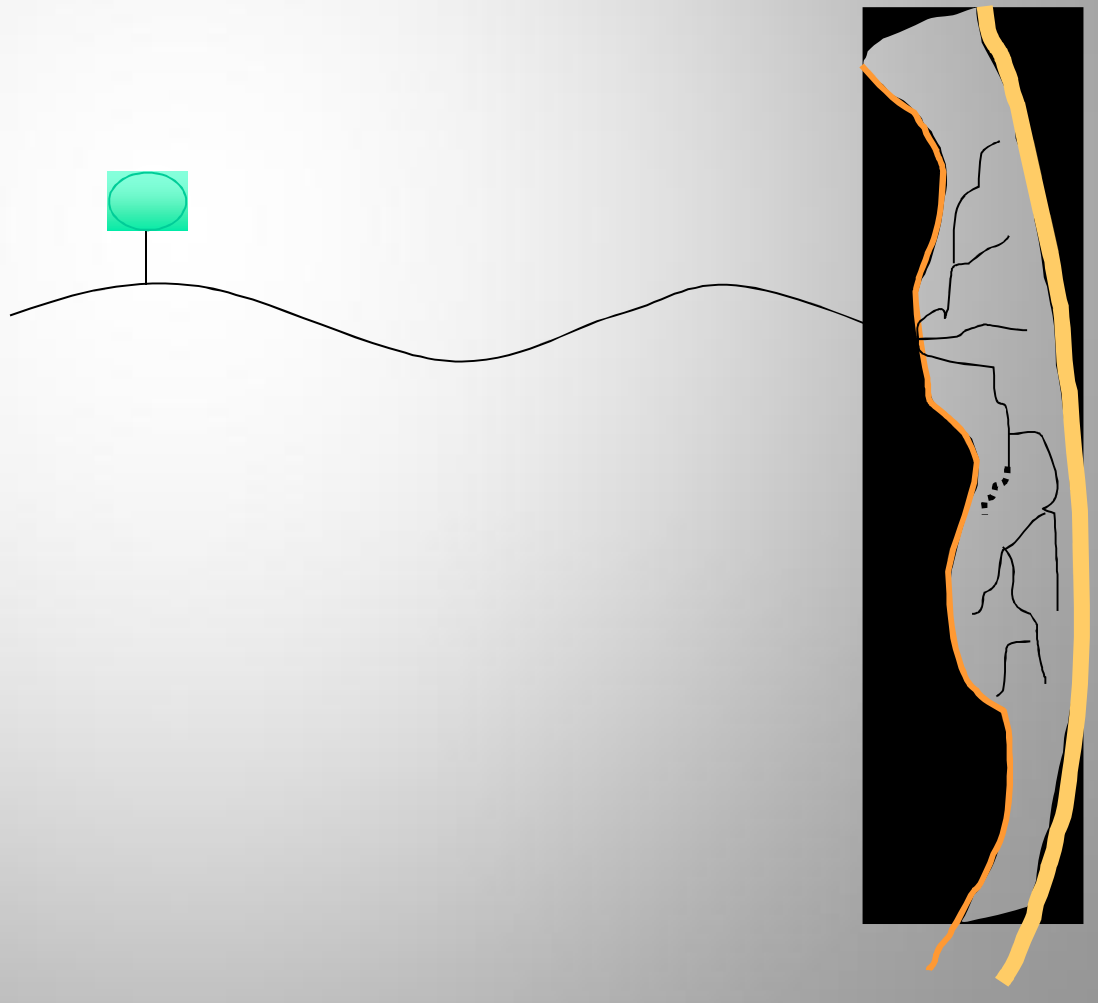


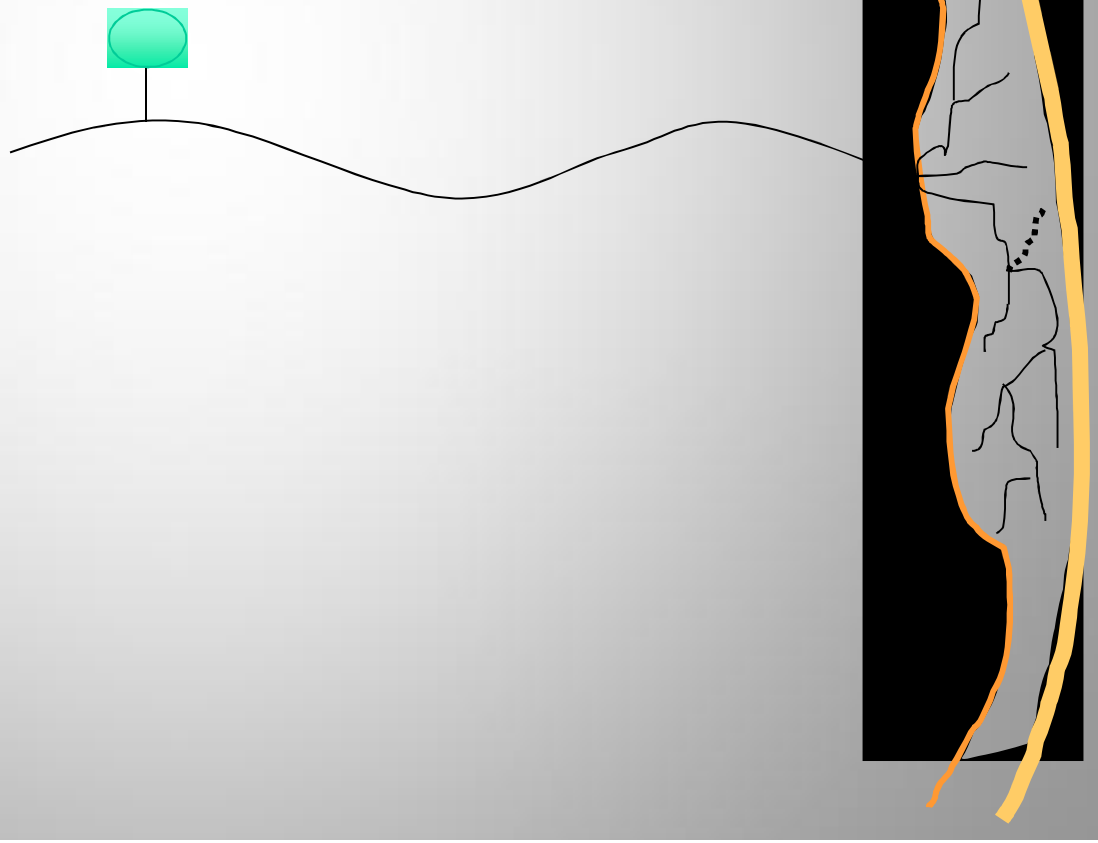


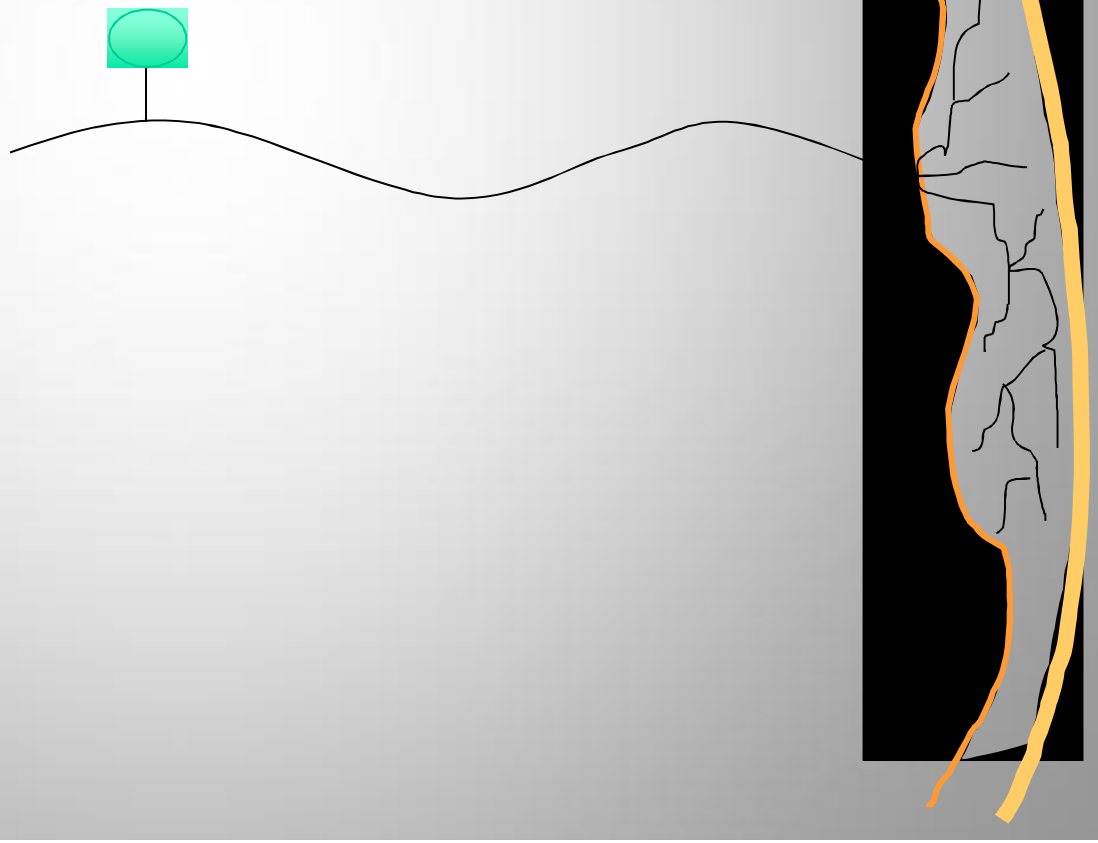
Distal terminal axons sprout into the denervated territory of the neighbour











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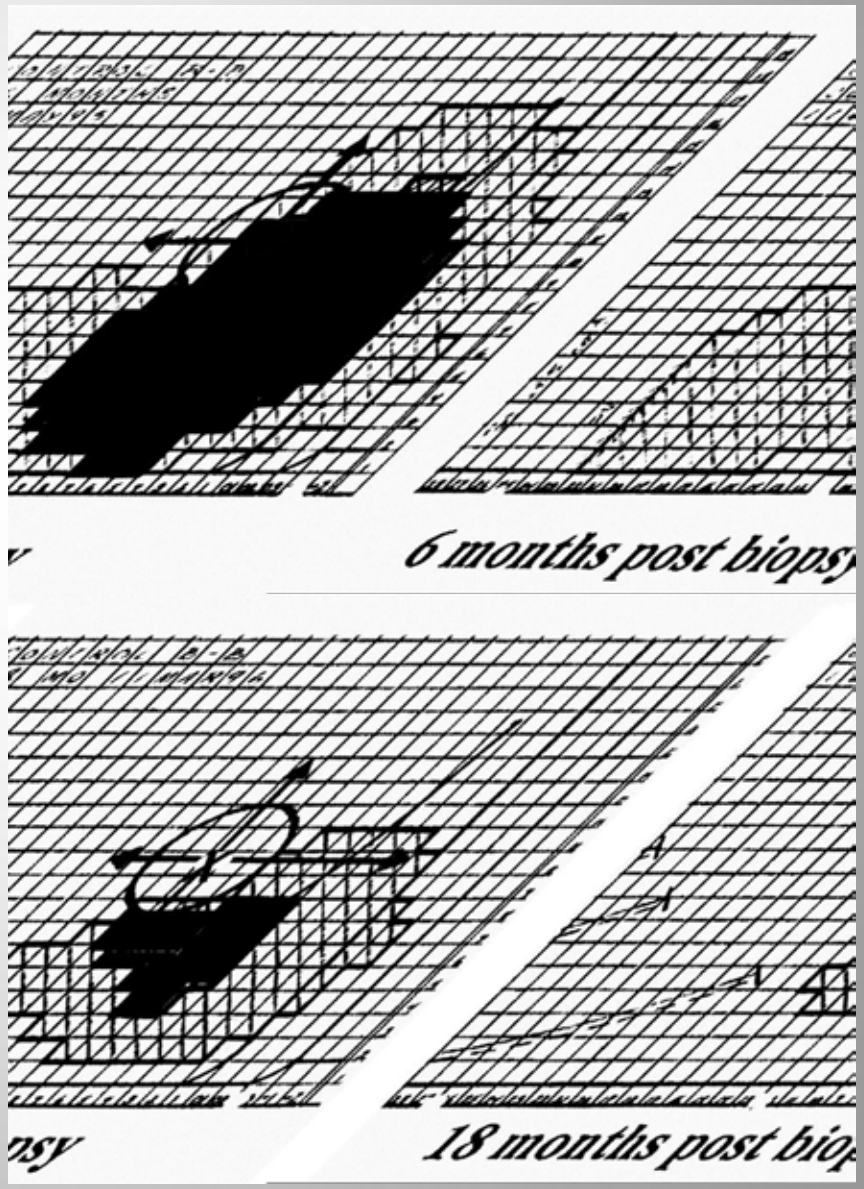
Collateral sprouting and repair of neurological deficits: recovery from a sural nerve biopsy

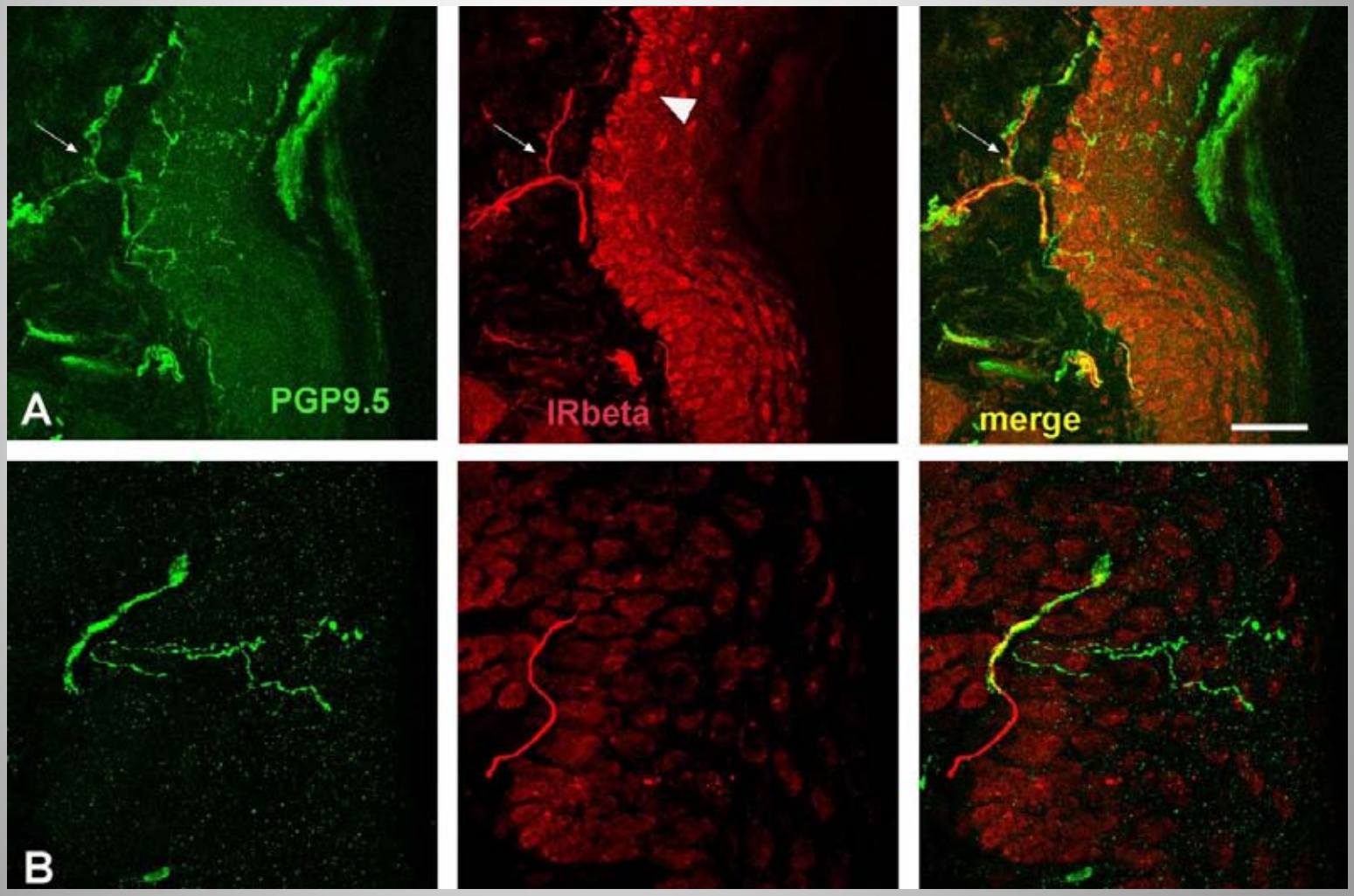


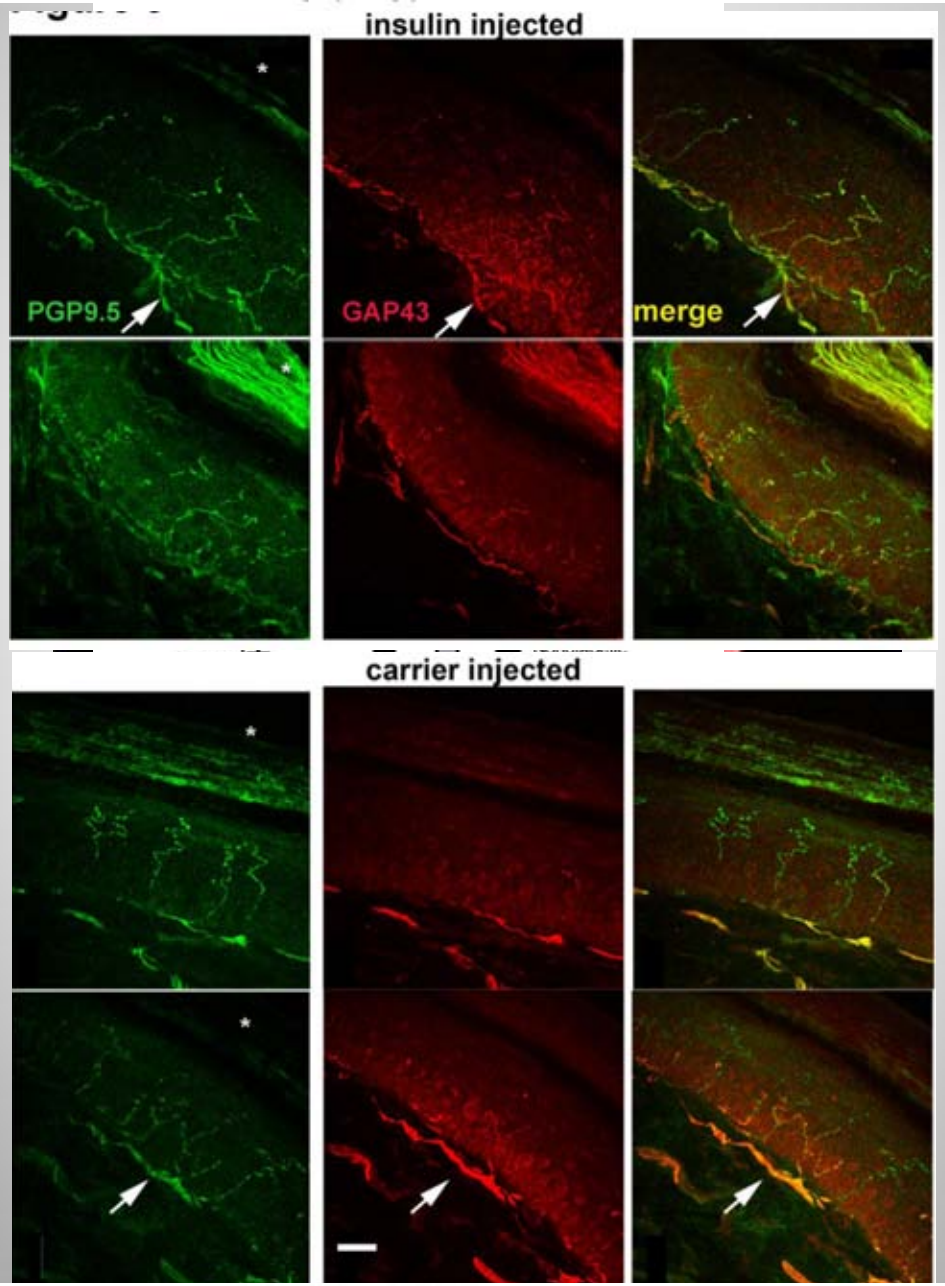
From:
Neurobiology
of peripheral
nerve regeneration,
Cambridge, 2008

Cutaneous axon plasticity

- Epidermal axons are highly plastic and express growth molecules
- There is generation of new axon branches by noninvasive stimuli (e.g. shaving skin)
- HGF and its receptor c-met are expressed in the skin and in axons
- Insulin receptors are found on dermal and some epidermal axons
- Loss of epidermal axons in experimental diabetes can be reversed by local nonsystemic injections of low dose insulin (without altering systemic glucose levels)

Insulin receptors are expressed on terminal sensory axons




insulin injected
PGP9.5
GAP43
merge
carrier injected

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The compelling case for better regeneration of peripheral neurons

"If you can find any other view of the world which agrees over the entire range where things have already been observed, but disagrees somewhere else, you have made a great discovery. It is very nearly impossible, but not quite, to find any theory which agrees with experiments over the entire range in which all theories have been checked....A new idea is extremely difficult to think of. It takes a fantastic imagination."

Richard Feynmann
The Character of Physical Law, 1965

"No class of cases with which we have been called to deal seemed to us, at one time, so sadly hopeless as injuries of nerves; none has better rewarded enduring and steady efforts to afford relief" Silas Weir Mitchell

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Acknowledgements

Zochodne lab

Regeneration studies

Chu Cheng (RHOA)

Kim Christie (PTEN)

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Jose Martinez

Gui Fang Guo

Bhagat Singh

Vandana Singh

Yuanyuan Chen

YQ Xu

David McDonald

Cory Toth

Alberta Heritage Foundation for Medical Research (AHFMR)

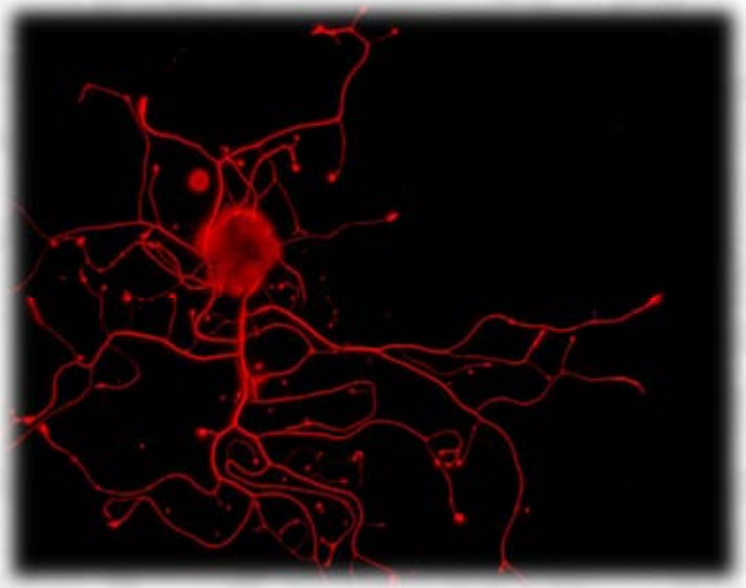
Canadian Institutes of Health Research (CIHR)

Canadian Diabetes Association (CDA)

Department of Clinical Neurosciences

R.U.N.

THE REGENERATION UNIT IN NEUROBIOLOGY



Dedicated to reversing neurological deficits in patients

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UNIVERSITY OF CALGARY

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•Hoke A. Mechanisms of Disease: what factors limit the success of peripheral nerve regeneration in humans? Nat Clin Pract Neurol 2006; 2: 448-454.

•Webber C, Zochodne D. The nerve regenerative microenvironment: early behavior and partnership of axons and Schwann cells. Exp Neurol 2010; 223: 51-59.

•Zochodne DW. Neurobiology of peripheral nerve regeneration. Cambridge: Cambridge; 2008.

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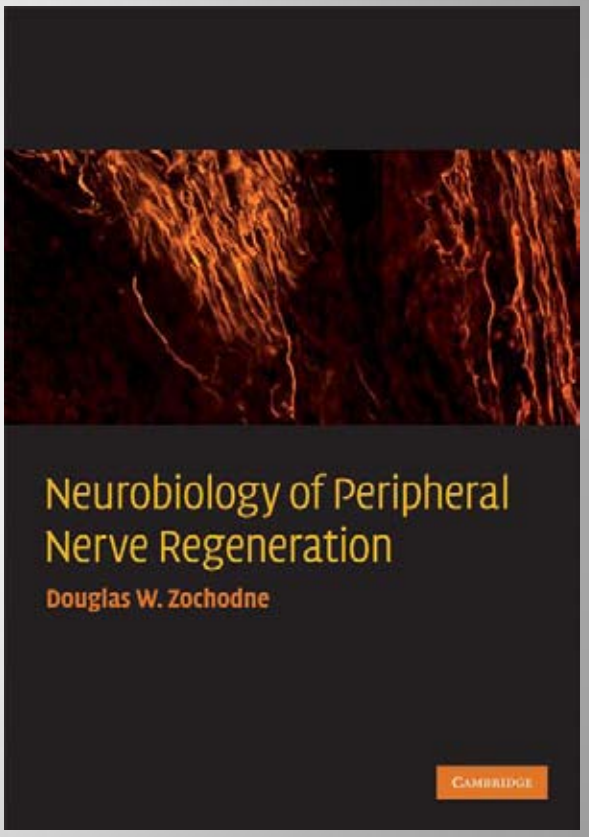

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