The role of the immune system in the generation of neuropathic pain

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The role of the immune system in the generation of neuropathic pain –

Agenda:

I Definition and clinical presentation

II Animal models of neuropathic pain

III Inflammation in peripheral nerve injury

IV Neuroimmunology in the CNS

V Translational considerations

VI Q&A and References

I Definition and clinical presentation

Definition and clinical presentation

from: Ropper, A. H., Adams, R. D., Victor, M., Brown, R. H., & Victor, M. (2014). *Adams and Victor's principles of neurology*. New York: McGraw-Hill Medical Pub. Division.

"Pain that **arises from** direct stimulation of **nervous tissue itself**, central or (far more often) peripheral, exclusive of pain as a consequence of stimulation of C fibers by lesions of other bodily structures"

Key clinical features:

- hyperesthesia, hyperalgesia, allodynia, and hyperpathia.
- Often coexisting sensory deficit and local autonomic dysfunction.
- The pain generally responds poorly to treatment, including the administration of opioid medications.

Neurogenic, or Neuropathic Pain includes a variety of diffrent entities:

- Any trauma or lesion involving single and multiple nerves
- Trigeminal neuralgia
- Herpes zoster and other mostly viral infections
- Diabetic neuropathia
- Neuromas and Neurofibromas,
- A number of polyneuropathies of diverse type; root irritation, e.g., from a prolapsed disc; spinal arachnoiditis and spinal cord injuries;
- Guillain-Barré syndrome
- Complex regional pain syndrome (CRPS)
- Toxic injury (e.g. Chemotherapeutic agents, such as Vinca Alcaloids, Taxols, Oxaliplatin, Antiretroviral drugs)

- Multiple Sclerosis
- Thalamic pain syndrome of DejerineRoussy;
- Rarely, parietal lobe infarction
- As a rule, lesions of the cerebral cortex and white matter are associated not with pain but with hypalgesia.

Please note that this overview is far from extensive!

Common clinical scenarios with a common thread

- Trauma (e.g. spinal cord injuries)
- Infection (e.g. Herpes zoster)
- Toxins (e.g. palitaxel)
- Metabolic agents (Diabethic polyneuropathia)

All of these Ethiologies are associated with a **robust immune response**

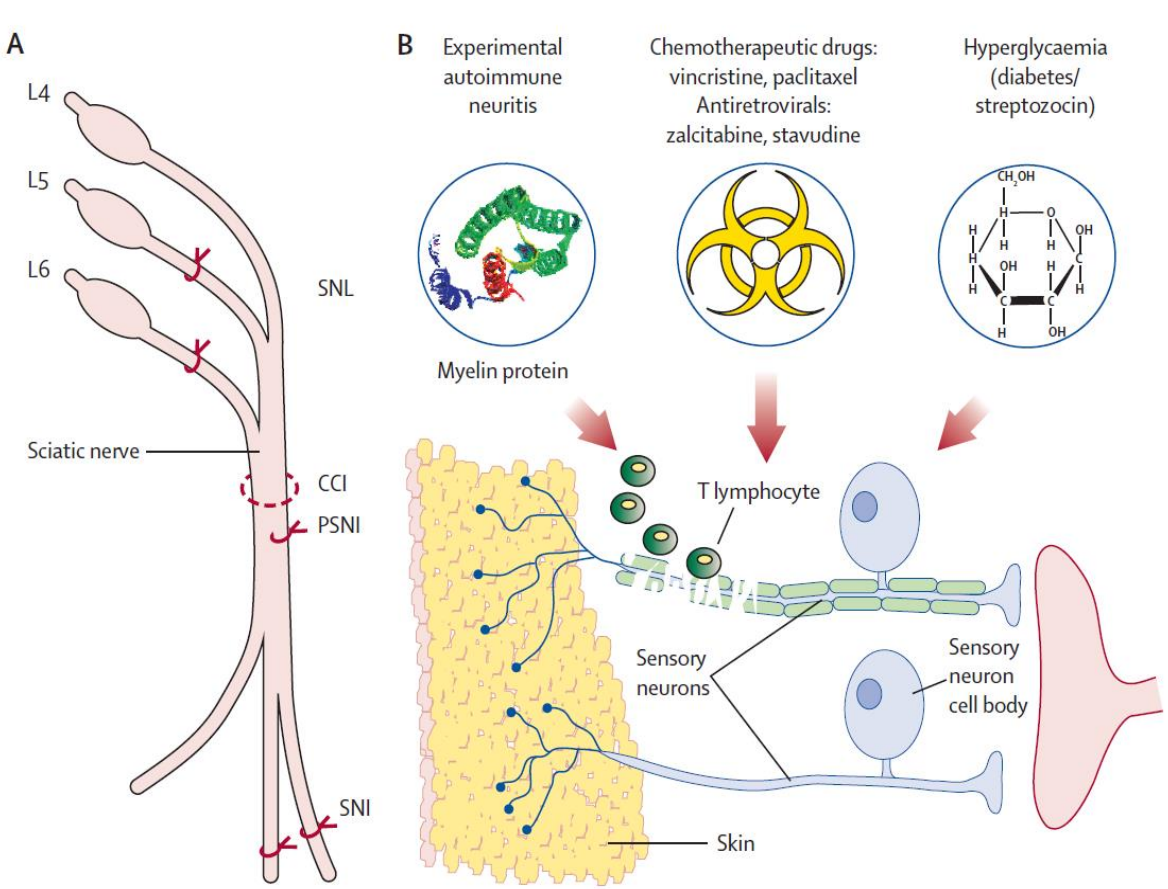
Injured neurons and their associated glial cells release factors that activate resident immune cells and recruit more immune cells from the circulation.

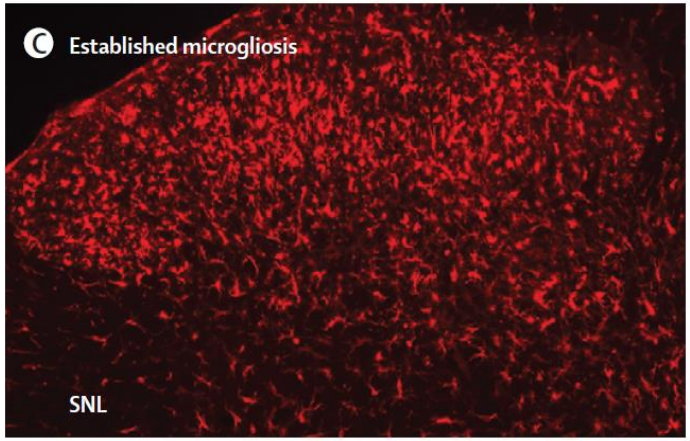
II Animal models of neuropathic pain

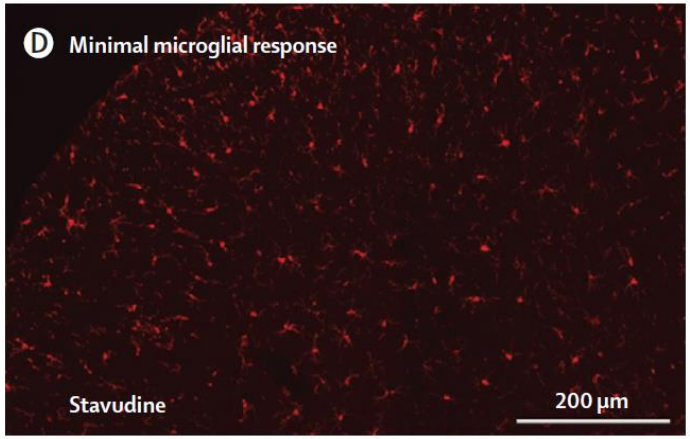
Animal models of neuropathic pain

A Traumatic injury: SNL: Spinal nerve ligation, CCI: Chronic constriction injury, PSNI: Partial scitaic nerv injury, SNI: Spared nerve injury

B Myeline sensitisation, neurotoxic drugs, streptozocin -> toxic to β-Islets

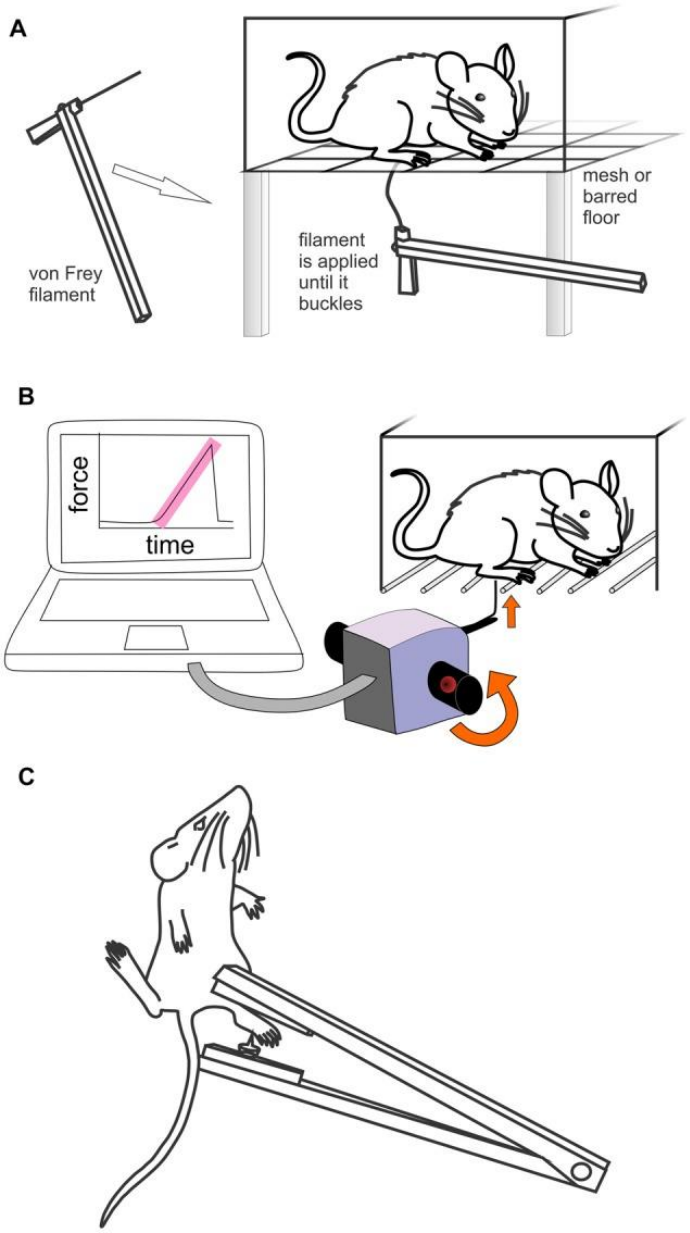






The following figures are from: Calvo, M., et al. (2012). "The role of the immune system in the generation of neuropathic pain." Lancet Neurol 11(7): 629-642.

Does it hurt?



Von Frey or Randall Selitto test

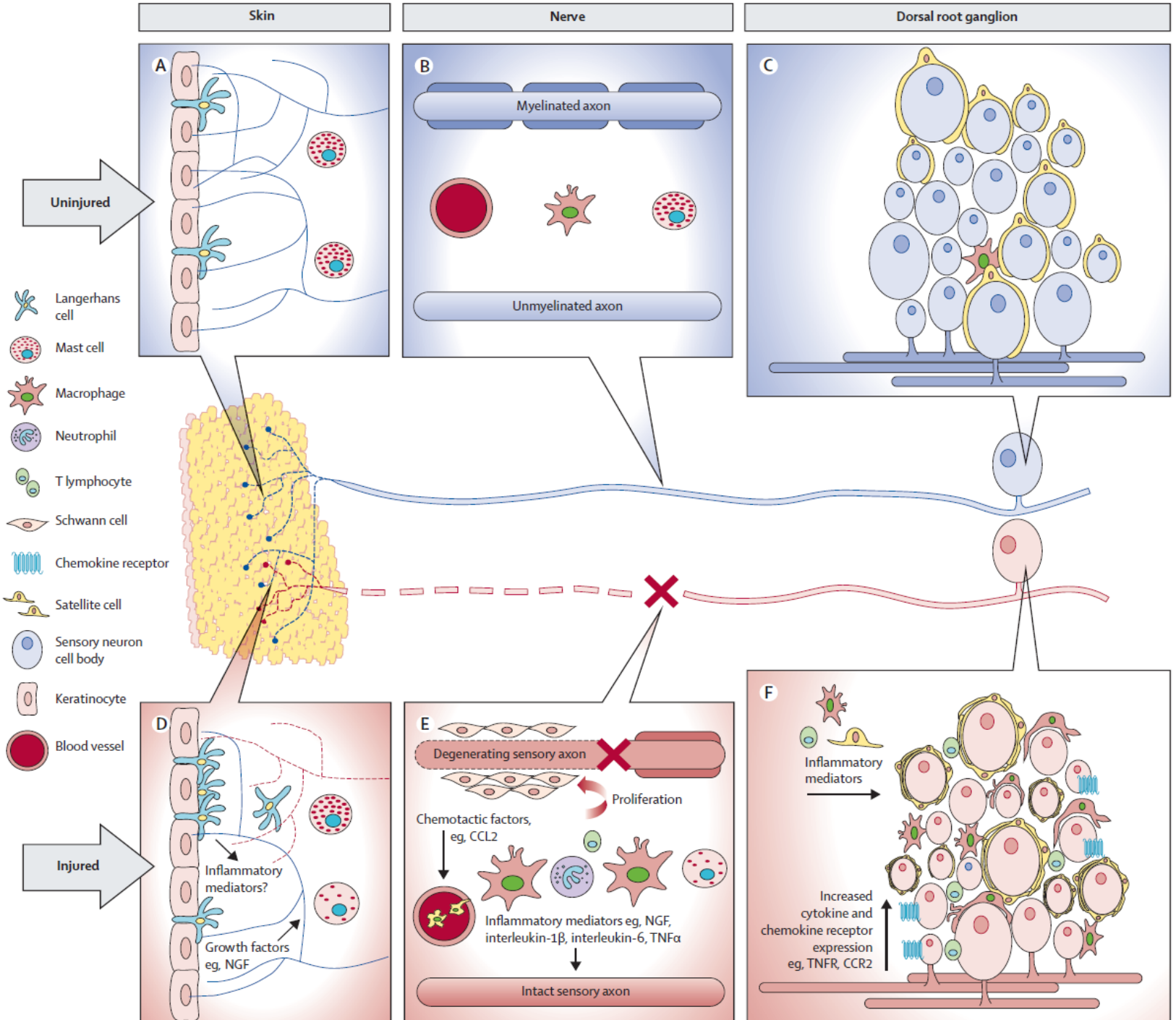
-> In a nutshell:
“Pain like behavior” is measured.

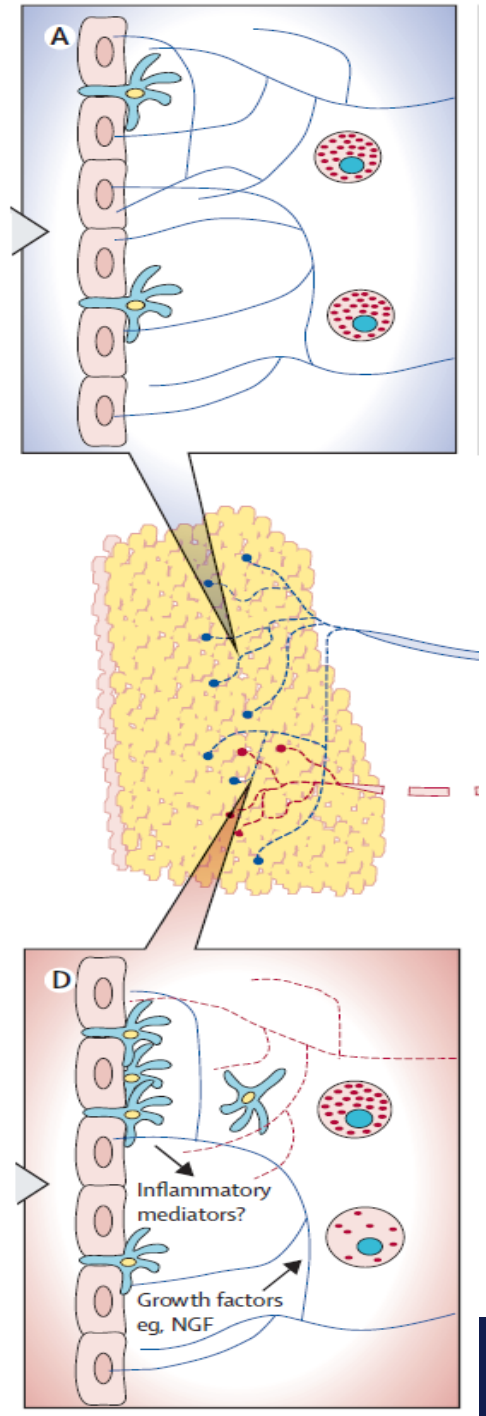
Evaluation of withdrawal reflex:

If a stimulus is applied that physiologically does not evoke a response, but the animal withdraws, the animal is considered to have allodynia.

Figure taken from: Deuis, Jennifer R et al. “Methods Used to Evaluate Pain Behaviors in Rodents” *Frontiers in molecular neuroscience* vol. 10 284. 6 Sep. 2017, doi:10.3389/fnmol.2017.00284

III Inflammation processes in peripheral nerve injury

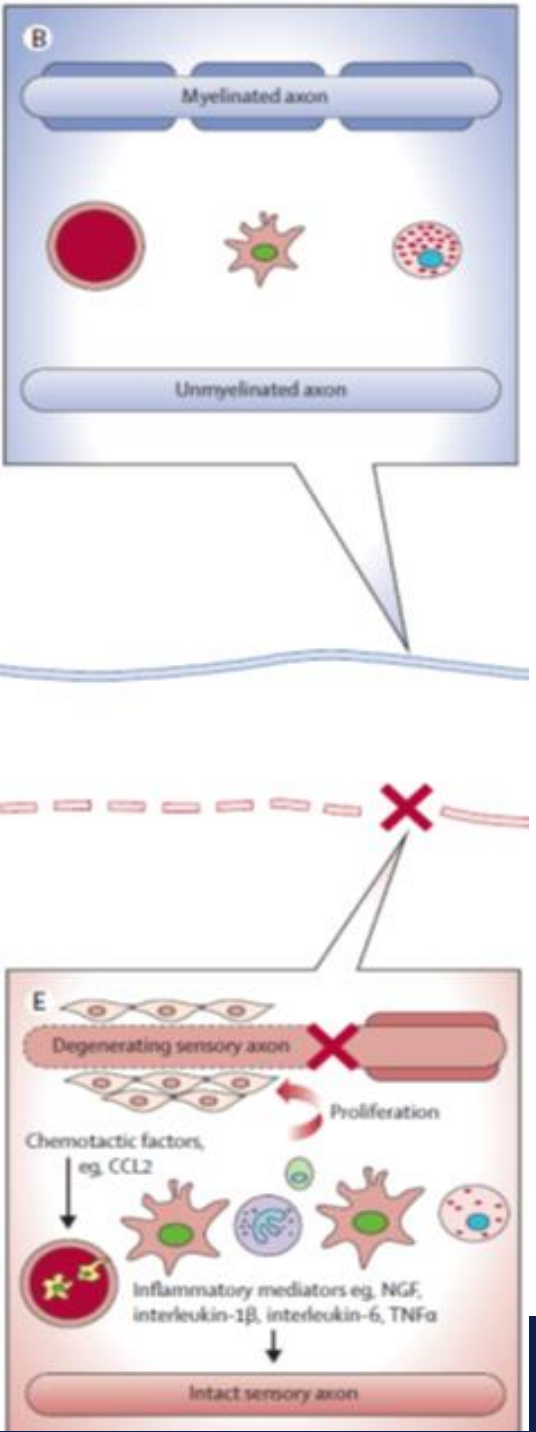

Skin
Nerve
Dorsal root ganglion
A
B
C
Myelinated axon
Unmyelinated axon
Uninjured
Langerhans cell
Mast cell
Macrophage
Neutrophil
T lymphocyte
Schwann cell
Chemokine receptor
Satellite cell
Sensory neuron cell body
Keratinocyte
Blood vessel
D
E
F
Injured
Inflammatory mediators?
Growth factors eg, NGF
Degenerating sensory axon
Proliferation
Chemotactic factors, eg, CCL2
Inflammatory mediators eg, NGF, interleukin-1β, interleukin-6, TNFα
Intact sensory axon
Inflammatory mediators
Increased cytokine and chemokine receptor expression eg, TNFR, CCR2

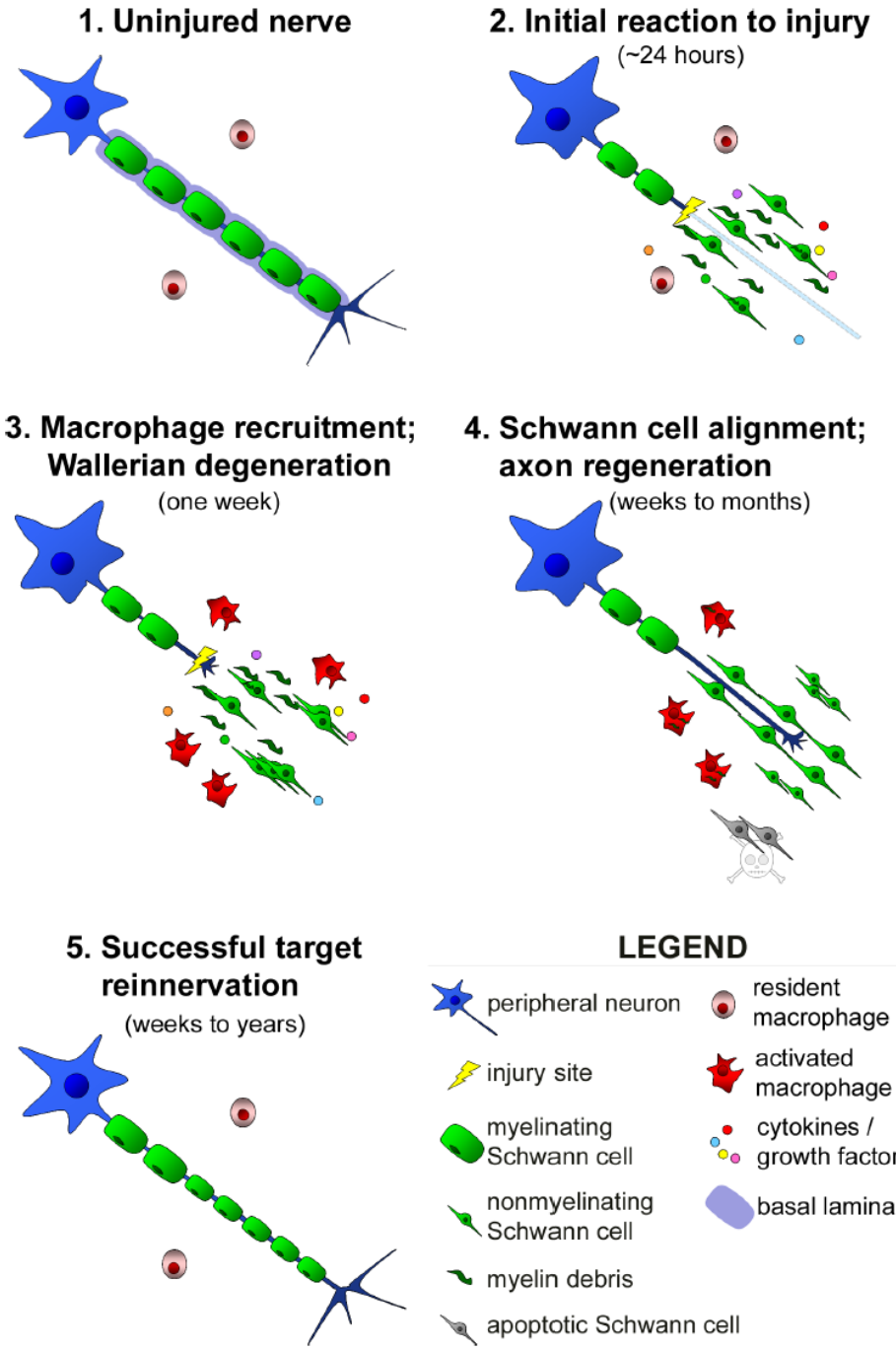


Events in the skin after nerve injury

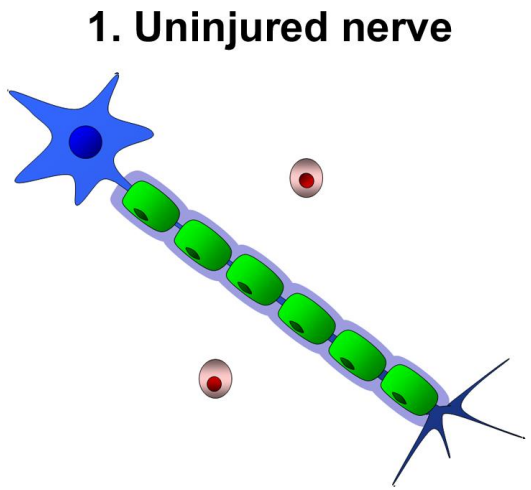
- Terminals of damaged nerve fibre degenerate
- Mast Cell degranulation
- Activation and numerical increase of Langehans cells -> initial secretion of proinflammatory cytokines, release of NO -> initial sensitization of nociceptive terminals
- Keratinocytes themselves can release inflammatory mediators, ATP, GFs
- In CRPS and post-herpetic neuralgia Nav-channels and CGRP is upregulated (possibly neuropeptide regulated);
  Higher concentration of TNF-a, IL 1b, 6, 8, chemokines are found

→ Note that the changed micomillieu also acts on the uninjured, none degenerating nerve fibres!


B
Myelinated axon
Unmyelinated axon
E
Degenerating sensory axon
Proliferation
Chemotactic factors,
eg. CCL2
Inflammatory mediators eg. NGF,
interleukin-1β, interleukin-6, TNFα
Intact sensory axon


1. Uninjured nerve
2. Initial reaction to injury
(~24 hours)
3. Macrophage recruitment;
Wallerian degeneration
(one week)
4. Schwann cell alignment;
axon regeneration
(weeks to months)
5. Successful target
reinnervation
(weeks to years)
LEGEND
peripheral neuron
resident macrophage
injury site
activated macrophage
myelinating Schwann cell
cytokines / growth factors
nonmyelinating Schwann cell
basal lamina
myelin debris
apoptotic Schwann cell

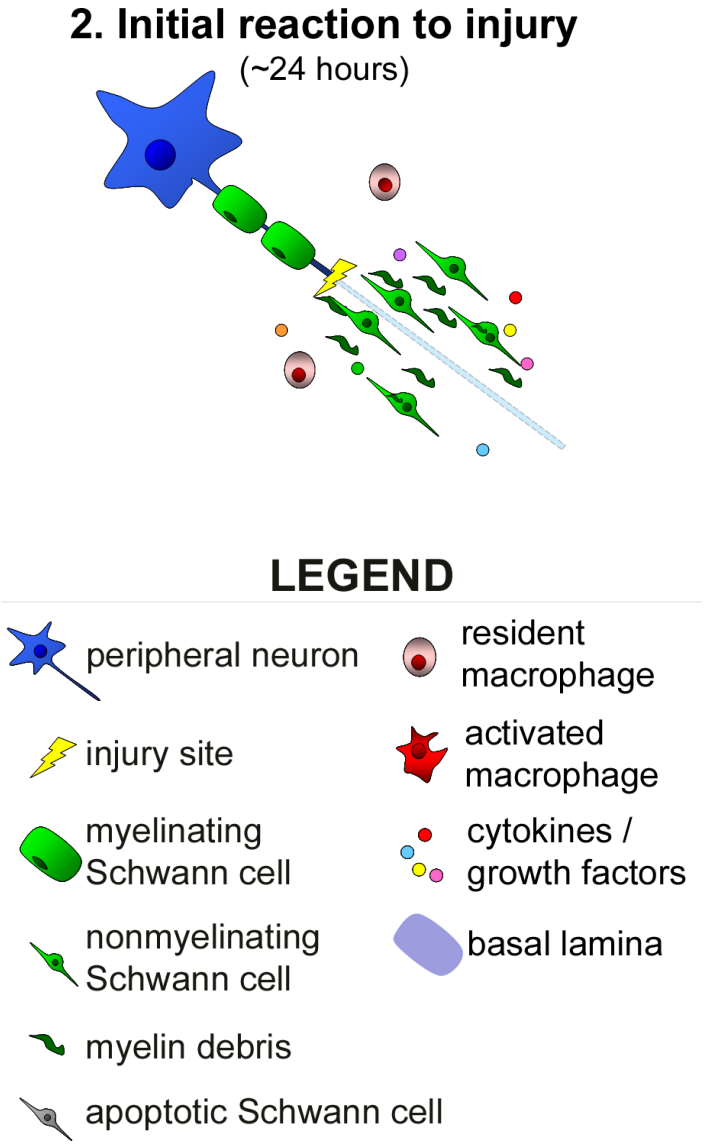
The uninjured nerve



- The endoneurium of an uninjured nerve consists of axons, associated Schwann cells (myelinating and nonmyelinating), and resident, inactivated macrophages

Gaudet, A. D., et al. (2011). "Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury." Journal of neuroinflammation 8: 110-110.

Wallerian degeneration a brief overview



PNI -> Degeneration of axon, distal to lesion (24h to several days in primates -> the severed nerve still shows excitability!

-> The Calcium influx -> Calpain and Ubiquitin-proteasom activation is essential for the next phase:

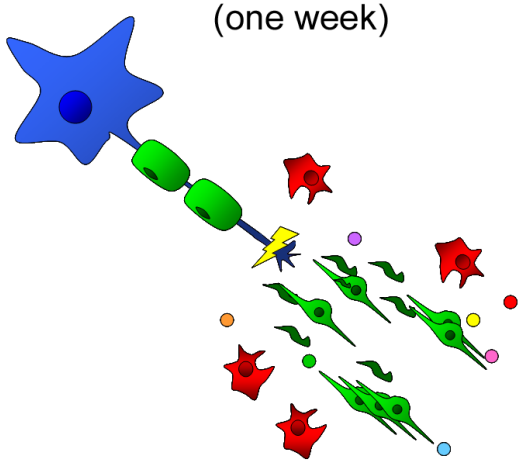
Axons bead and swell -> catastrophic granular disintegration of the cytoskeleton occurs (within 24h)

- Within 4-7 days the blood nerve barrier (bnb) permeability double, coinciding with peak inflammation, the bnb tightens and second, sustained increase in permeability starting ~4 weeks after transection becomes permeable again (homeostasis?)

Schwann cells – First responders in PNI

3. Macrophage recruitment; Wallerian degeneration

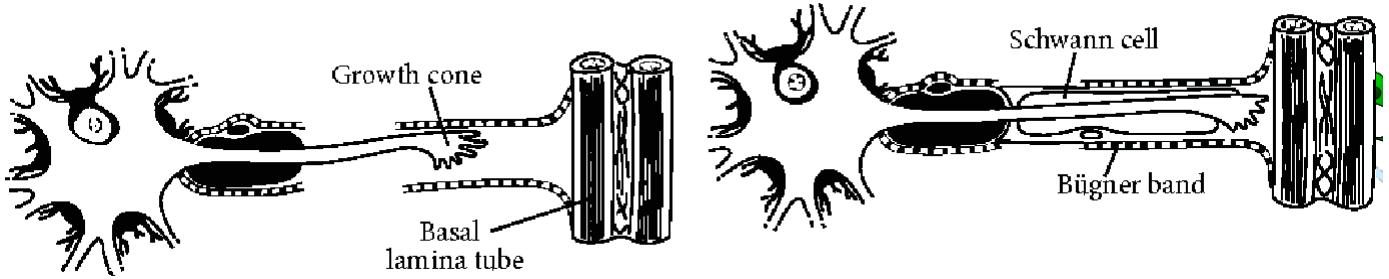
(one week)

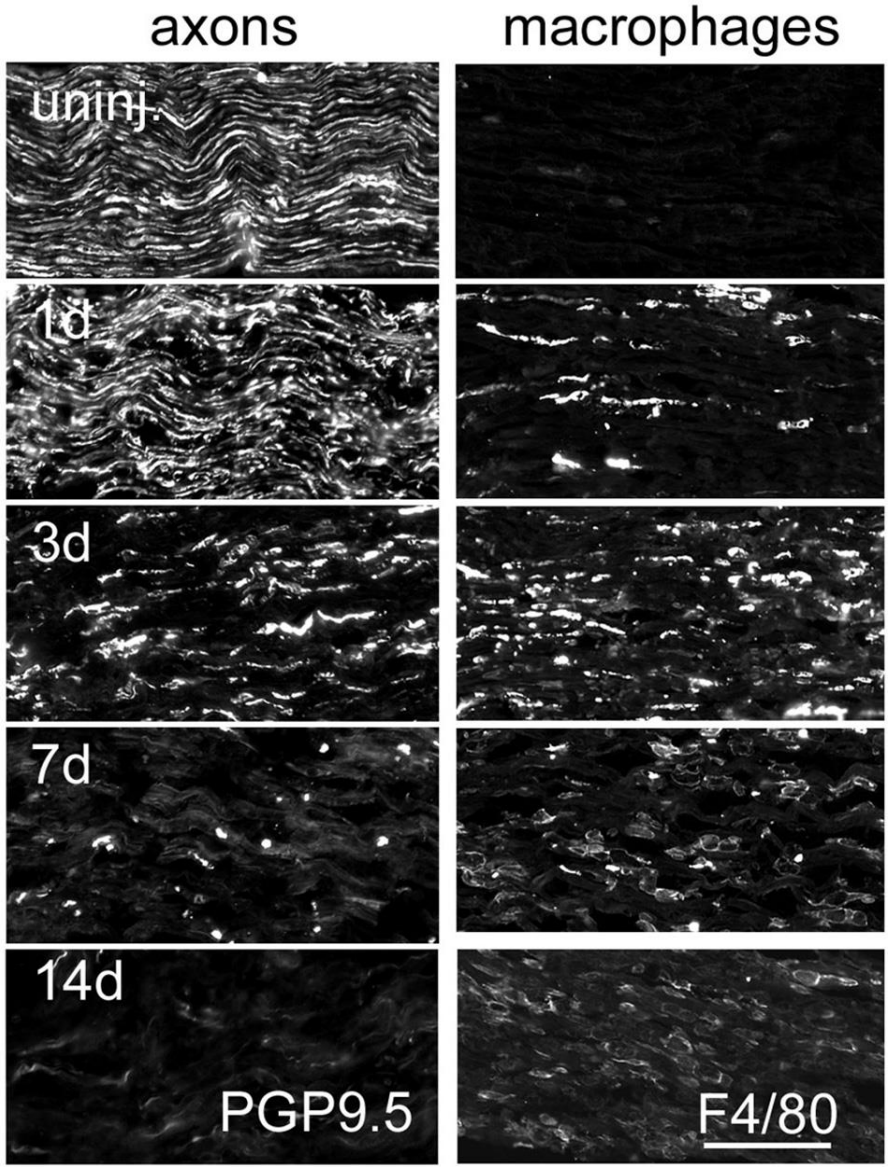


- Soon after PNI, denervated myelinating Schwann cells release their myelin (ubiquitin proteasome dependent)
- **Schwann cells** then **proliferate** within their basal lamina tubes, produce cytokines/trophic factors (upregulate regeneration-associated genes GAP-43, neurotrophic factors and their receptors, neuregulin and its receptors) **phagocytosis of detached debris** by macrophages and schwann cells themselves!

-> The proliferation takes place in **Bügner bands**

- Reaction within the neuron cell body begins: cell soma hypertrophy, displacement of the nucleus to an eccentric position, and dissolution of Nissl bodies.

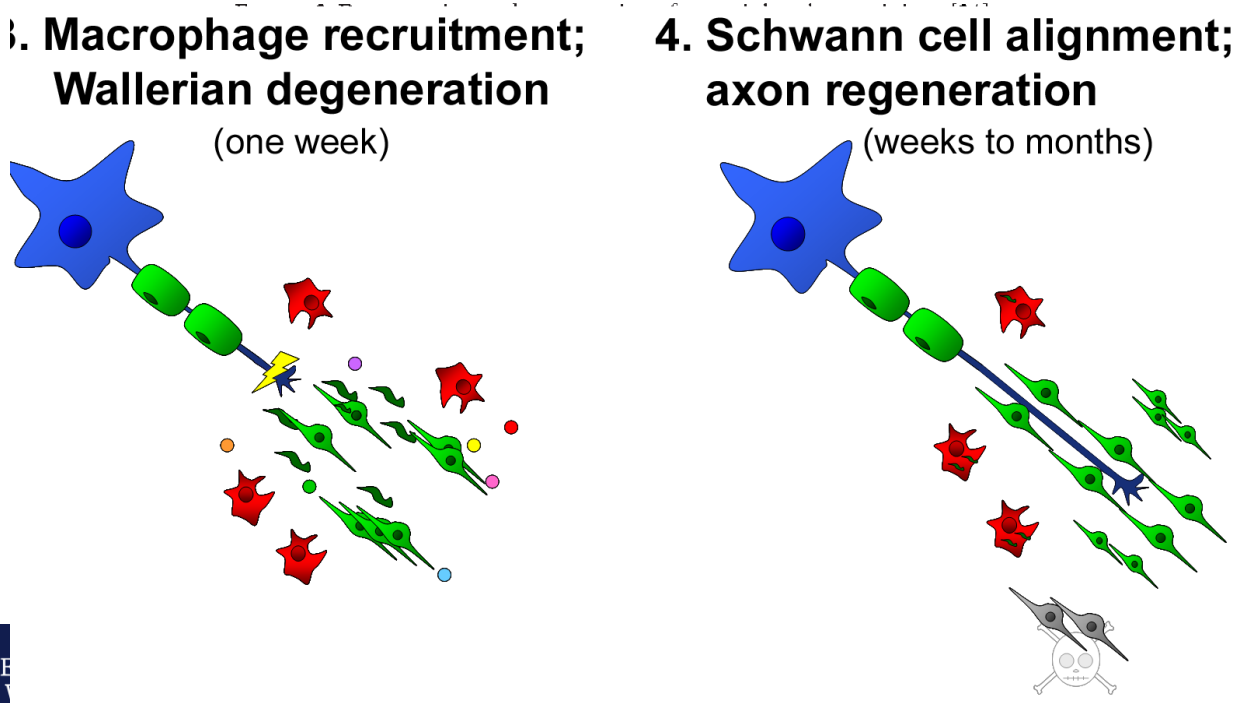




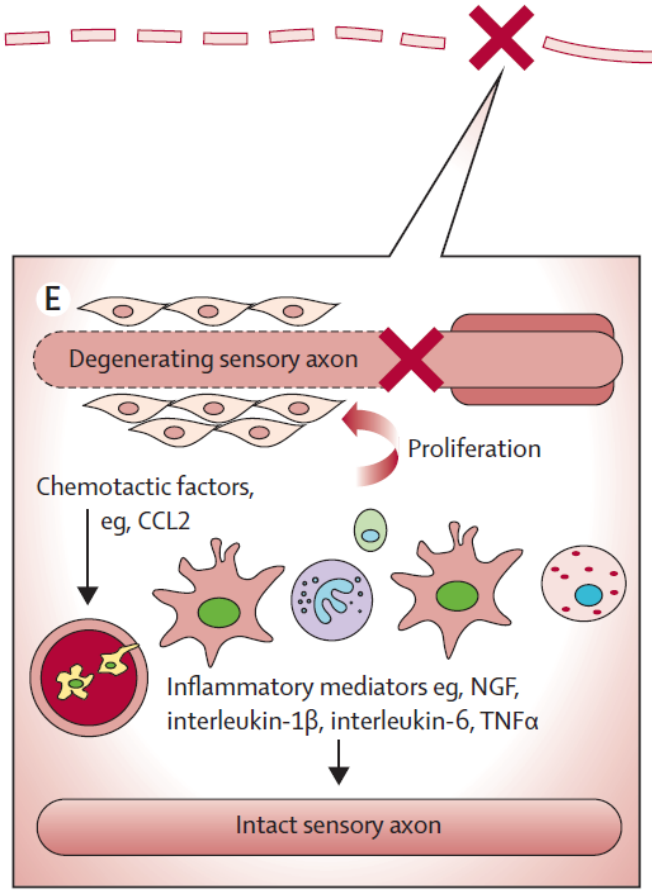
Immunohistochemistry to visualize axons (PGP9.5) and macrophages (F4/80) in sciatic nerves of rats, after PNI, at the site of injury

Gaudet, A. D., et al. (2011). "Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury." Journal of neuroinflammation 8: 110-110

- Activated Schwann cells initiate cytokine/chemokine cascades that amplify and fine-tune the inflammatory response after PNI.
- Macrophages take over debris, and produce factors that facilitate Schwann cell migration and axon regeneration.
- After a lag period, injured axons form a growth cone and begin to regenerate along bands of Büngner formed by Schwann cells.
- Schwann cells that have been chronically denervated (e.g., for a few months) are less supportive of regrowth and are more likely to undergo apoptosis



Schwann cells as sentinels in orchestrating inflammation and tissue repair - a double edged sword



- Schwann cells sense DAMPs via TLRs -> trigger the build up of an inflammatory millieu necessary for regeneration

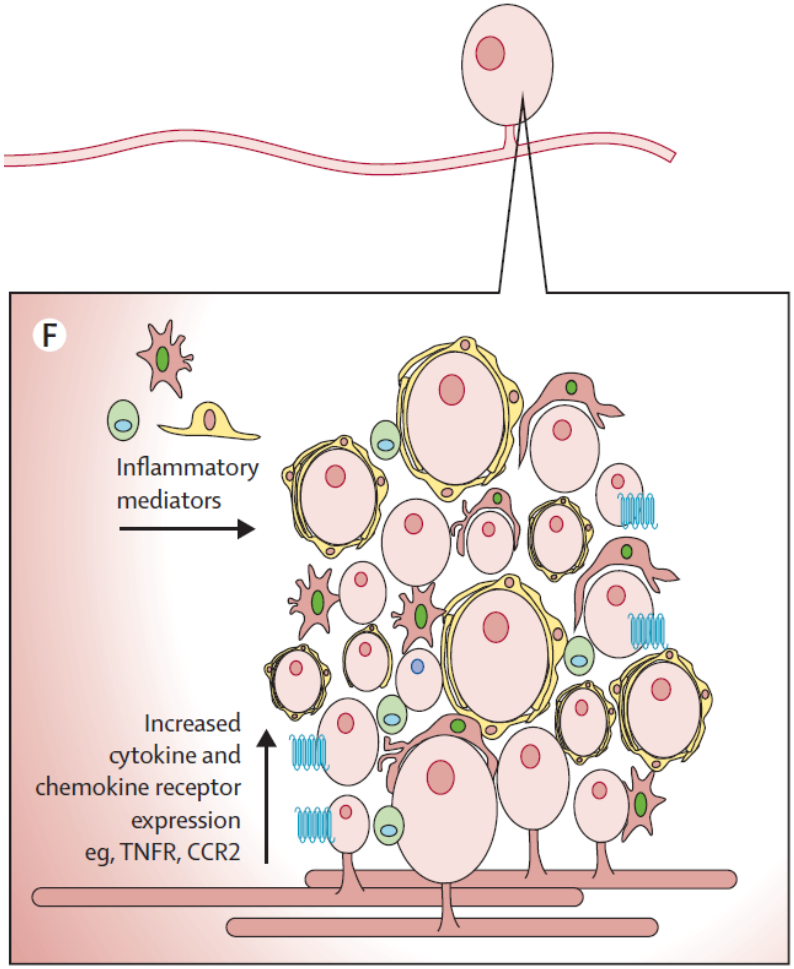
-> This micromillieu also influences intact nerve fibres!

Evidence for the involvment of peripheral inflammation in neuropathic pain

- Depletion of macrophages reduces hypersensitivity after PNI
- Higher percentage of M1 macrophages in CRPRs
- Athymic rats (T-cells depelted) show reduced hypersensitivity
- Many proinflammatory cytokines (such as TNF-a) directly stimulate nerve terminals, their respective receptors are upregulated after injury
- Injecting such mediators leads to hypersensitivity
- Note that agressive supression of the inital inflammatory response impairs regeneration!

…

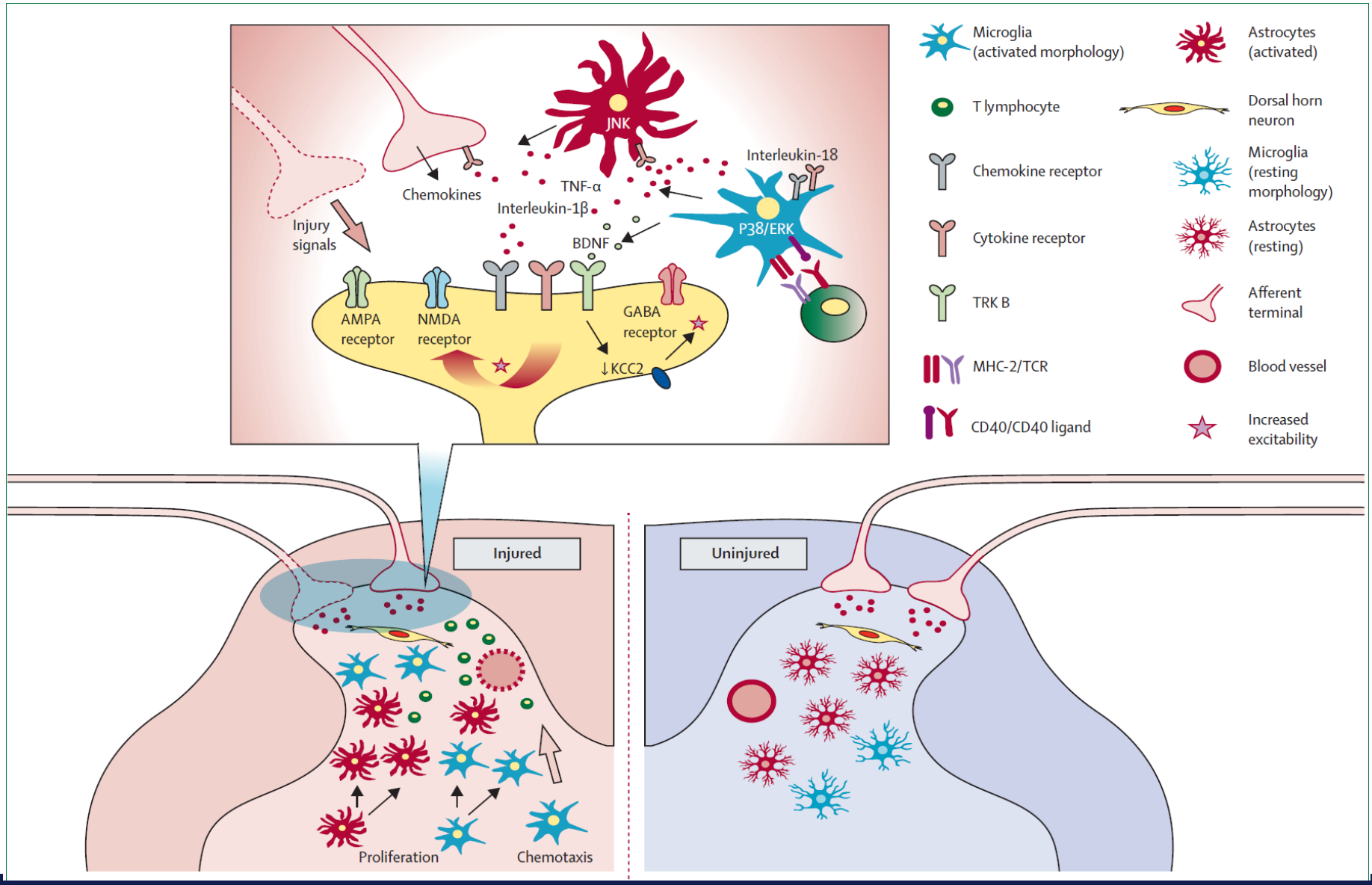
Enhanced dorsal root ganglion (DRG)-excitability

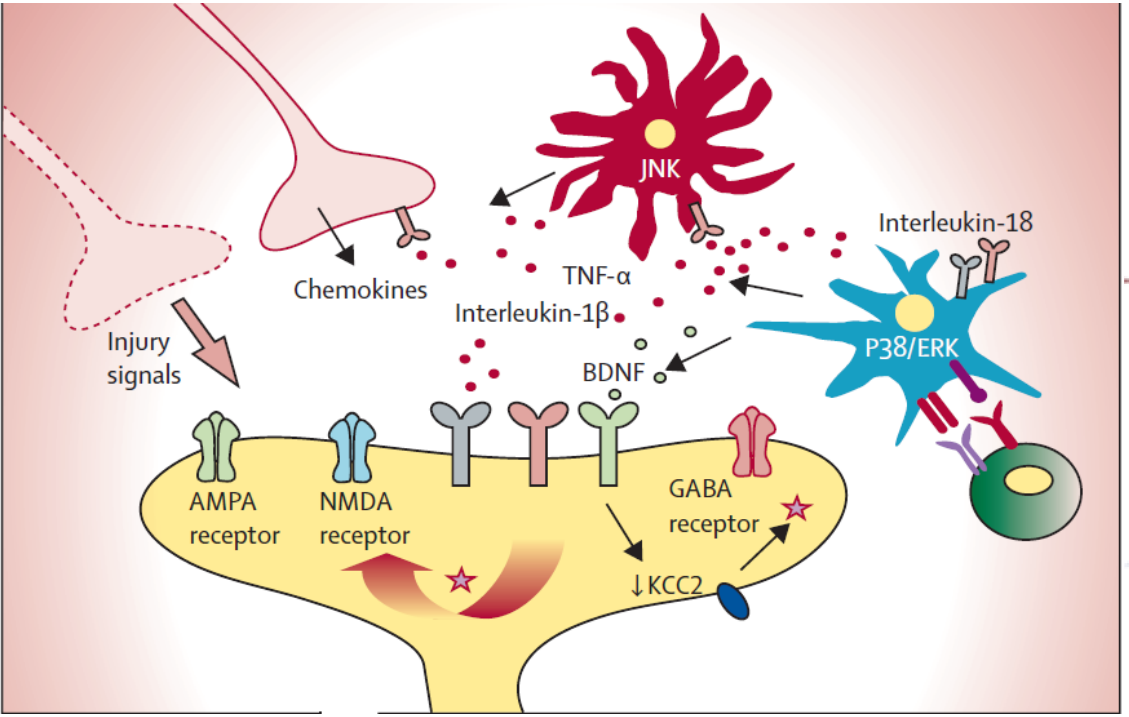


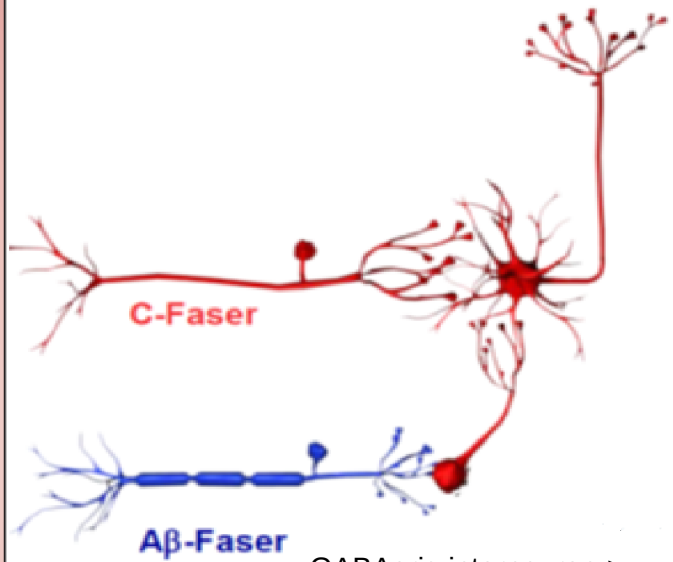
- Proliferation of satellite cells, recruitment of PBMCs, general upregulation of cytokines and their receptors in all participants
- Enhancement of excitability, recruitment of TRPV1, A1, M8 and Nav channels to the DRG-membrane
- Note that nerve cells can express TLR and release pro-inflammatory mediators themselves!

IV Neuroimmunology in the CNS

Central nervous neuroimmune interactions







GABAerig interneuron->
Becomes functionally excitatory

- DAMPs, neuregulin-1, MMP-9, CCL2, other pro-inflammatory mediators -> Microgliosis -> Immune response as distant as thalamus!
- Enhances glutamatergic wind up, e.g. GABA-Current polarity reverse, by BDNF!
- Some Anti-neuropathic drugs, with proven efficacy (e.g. Pregabalin) dampen microgliosis

V Translational considerations

Translation in to treatment

- Preclinical models show remarkable effects in alleviating neuropathic pain with the help of immunomodulatory drugs.

- However…
- Clinical evidence is sparse and inconclusive

- **General problems:**

→ Defining adequate subpopulations that will benefit from antinflammatory/immunomodulatory treatment

→ Pain, regardless of origin is multifactorial

→ Treatment in clinical scenarios is often delayed, immunomodulation in animal models is most effective when administered after injury

→ Cytokine and chemokine signalling is redundant! Just blocking TNF-a (e.g. Etarnecept, Infliximab….) might not overrule general inflammation

Table 1

Comparison of cytokine targets in treating neuropathic pain in animal models and their clinical correlates.

Cytokine	Location of action	Animal model	Clinical trials for neuropathic pain	Clinical drugs
Pro-inflammatory				
TNF-α	Periphery	CCI[a], DM[b], Disc herniation	Sciatica, spinal stenosis, lumbosacral radiculopathy	Infliximab, etanercept adalimumab, certolizumab pegol, golimumab
IL-1β	Periphery, brain, spine	Knock-out mice, peripheral nerve injury	–	Canakinumab
IL-6	Periphery, spine	CCI, peripheral nerve injury, knock-out mice	Disc herniation, chronic regional pain syndrome, Sciatica	Tocilizumab
IL-17	Periphery	CCI, peripheral nerve injury, chemical injection, knock-out mice, arthritis	–	Secukinumab
Anti-inflammatory				
IL-4	Periphery	CCI, partial nerve injury	–	Glatiramer acetate
IL-10	Periphery, brain	CCI, partial/complete nerve injury, neuritis	–	Calcineurin, uliastatin
TGF-β	Periphery, brain	CCI, partial nerve injury	–	Flexibilide

[a]CCI = chronic constriction injury.
[b]DM = diabetes mellitus.

Corticosteroid only effective when given intrathecal in post zoster neuralgia, no valid clinical data on MTX, Minocycline, Propentofylline

“Results from those studies have been mixed, and limited by small sample size and patient heterogeneity. Other approaches may include using a single agent to target multiple aspects of the immune pathway to treat neuropathic pain.”

Hung, A. L., et al. (2017). "Targeting cytokines for treatment of neuropathic pain." Scandinavian journal of pain 17: 287-293.

VI Q&A and References

References

Ropper, A. H., Adams, R. D., Victor, M., Brown, R. H., & Victor, M. (2014). *Adams and Victor's principles of neurology*. New York: McGraw-Hill Medical Pub. Division.

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