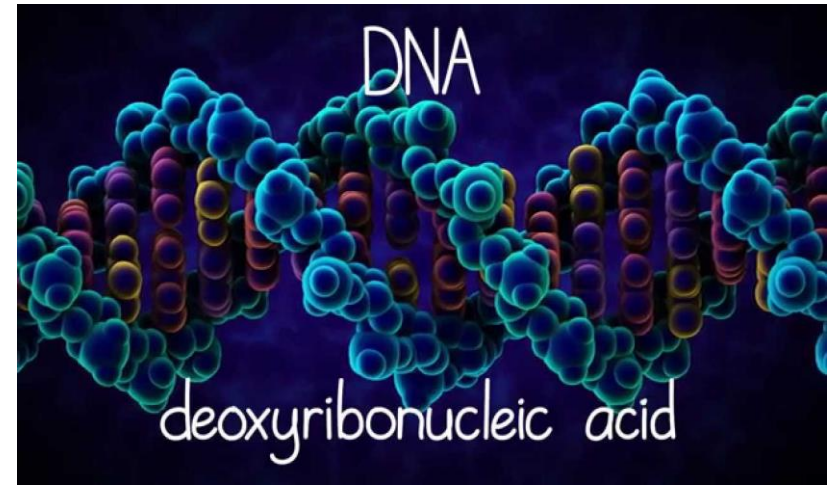
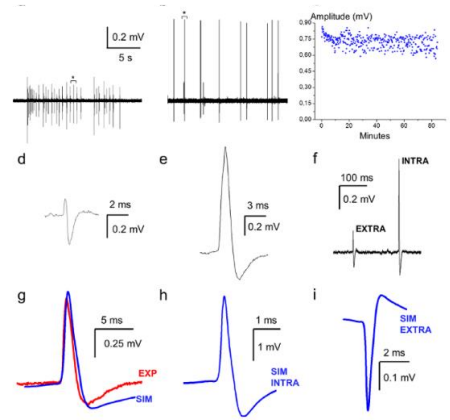
Mechanism and Consequences of Myelination

Jun Li, MD, PhD
Professor and Chair of Neurology
Houston Methodist Neurological Institute
Houston Methodist

start with a high-level of view…



DNA ensures transmission of biological traits



Action potential is a binary and rapid communication system with high fidelity

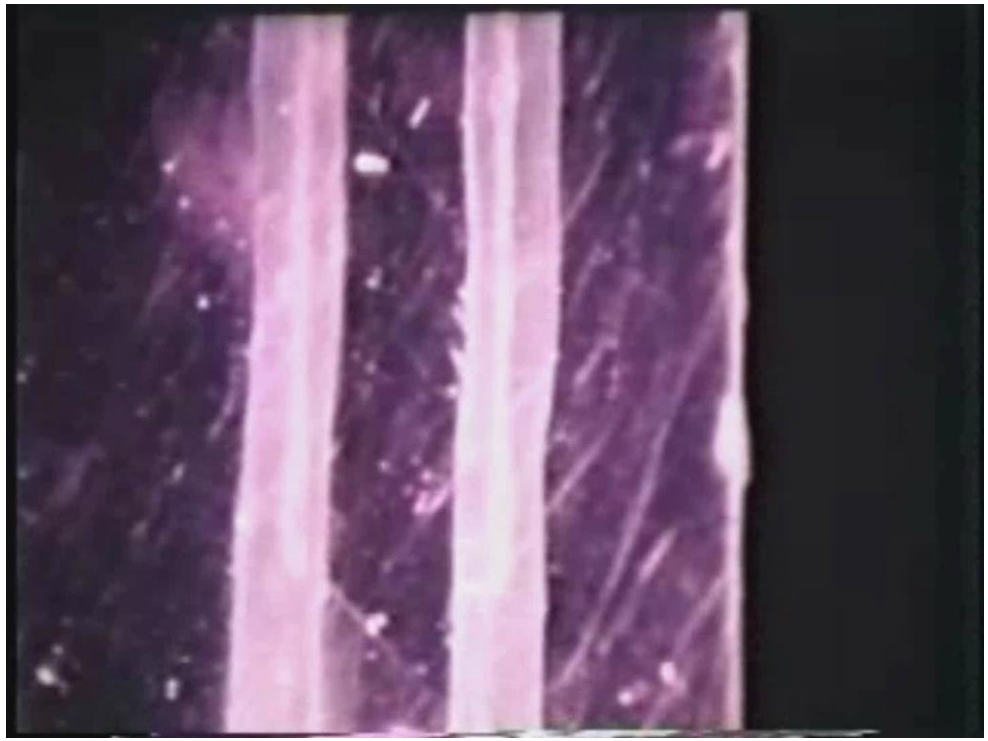
Thinking is decoded by brain-machine interface

Experiments in Squid Axons (non-myelinated) Revealed Inward Sodium Current of Action Potential

- **diameter = 1,000µM**
- **allowed a manually made silver electrode to be inserted into axon**
- **-60mV inside of axon**
- **overshot after depolarization reaches a threshold – action potential**
- **overshot disappeared after sodium ions were removed from the extracellular medium, suggesting the overshot was caused by sodium ion influx.**

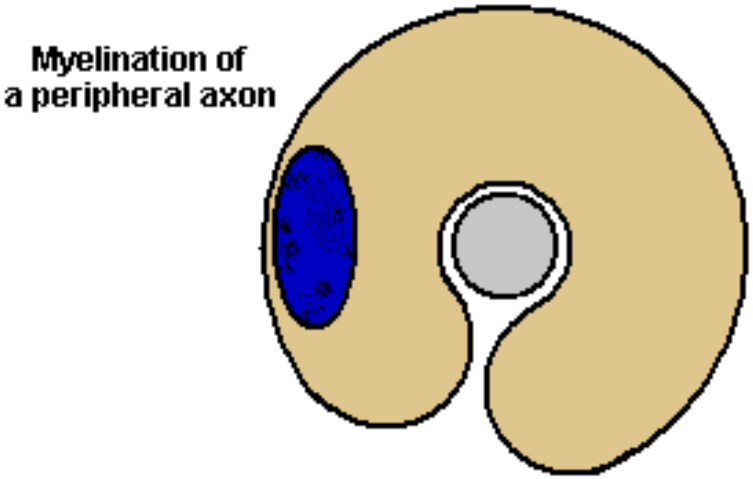
Inward or Outward Current was Isolated by Voltage-Clamp Technique

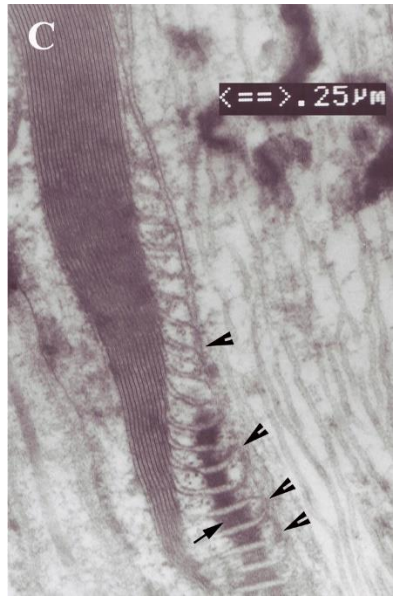
Non-myelinated Axon

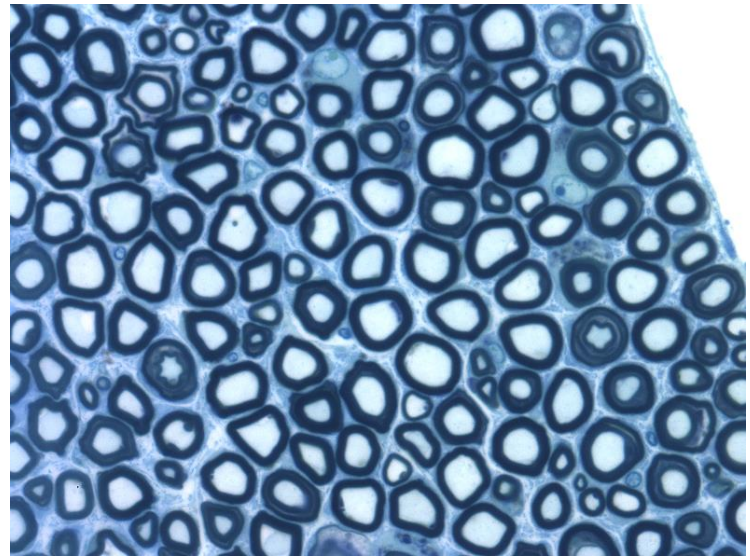


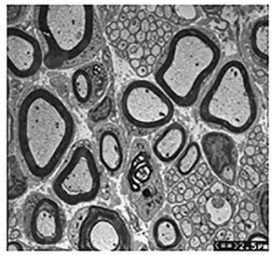
- **The overshot was brought back to the resting level by outward potassium ion current – repolarization.**
- **HH produced a mathematical equation**
- **It produced a trace that matched the actual recording of action potential.**
- **Nobel Prize in 1963**
- **Squid axon model – simple structure with no myelin, and large diameter, which made experiments feasible**

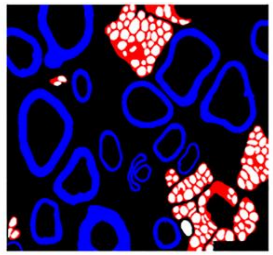
How about myelinated nerve fibers?







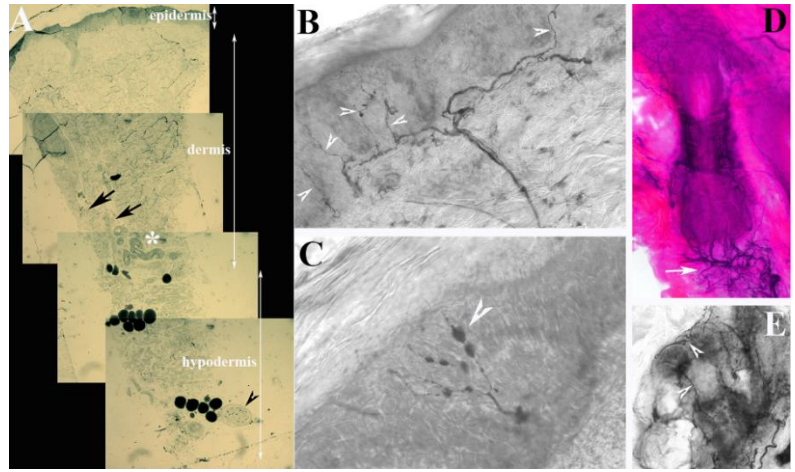


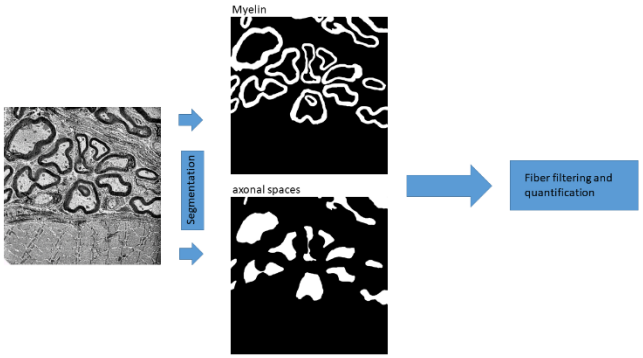


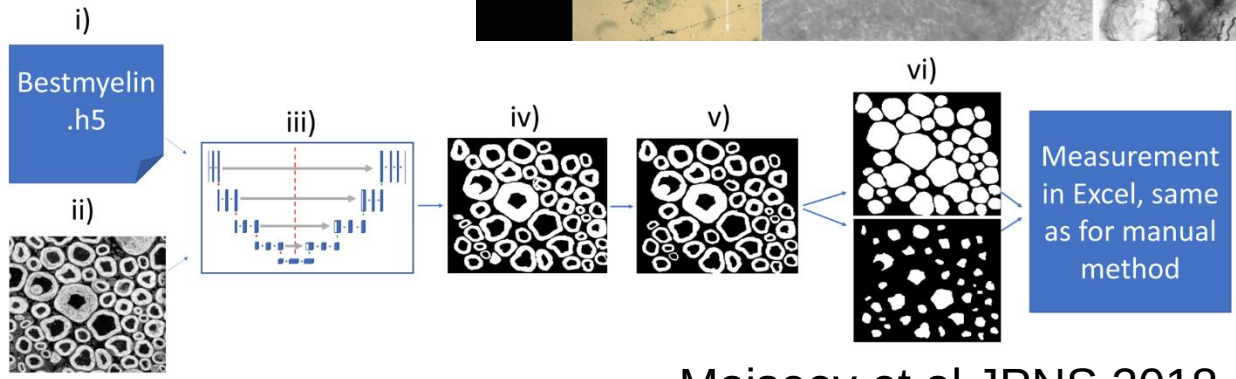
124 non-myelinated nerve fibers were segmented, while myelinated nerve fibers were also captured and segmented

Segmentation
Prediction

Quantification

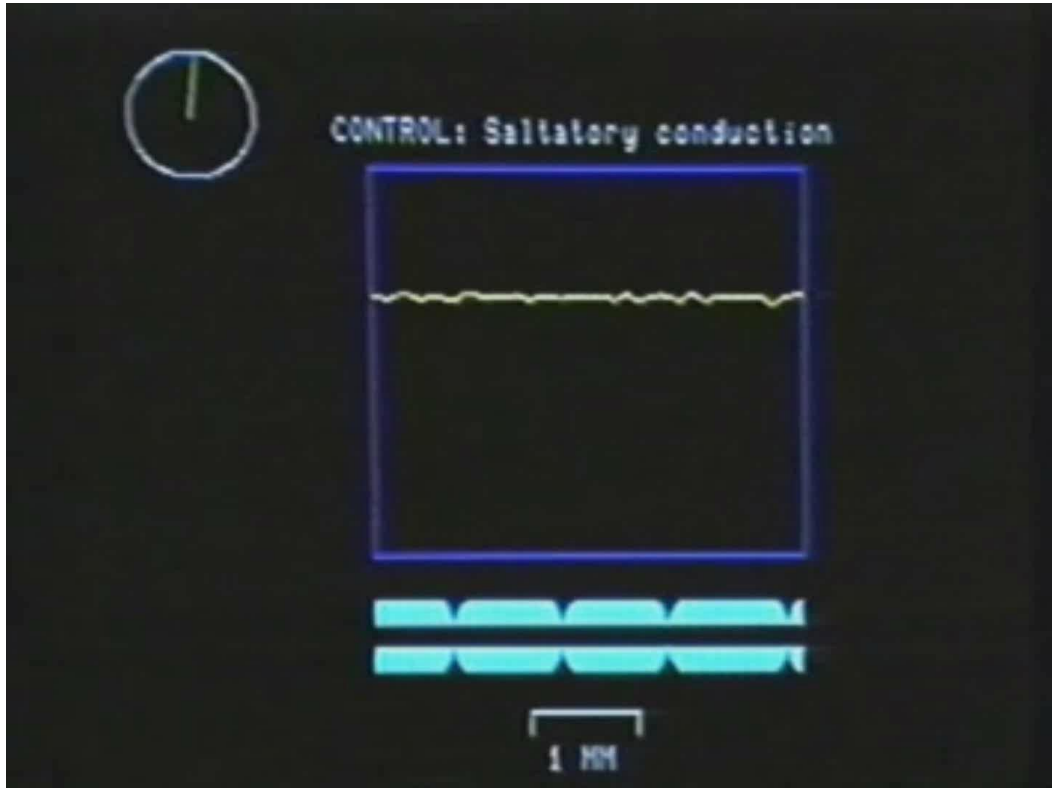






Moiseev et al JPNS 2018

Myelin enables saltatory conduction, not ***continuous*** conduction, thereby increases the velocity of action potential propagation



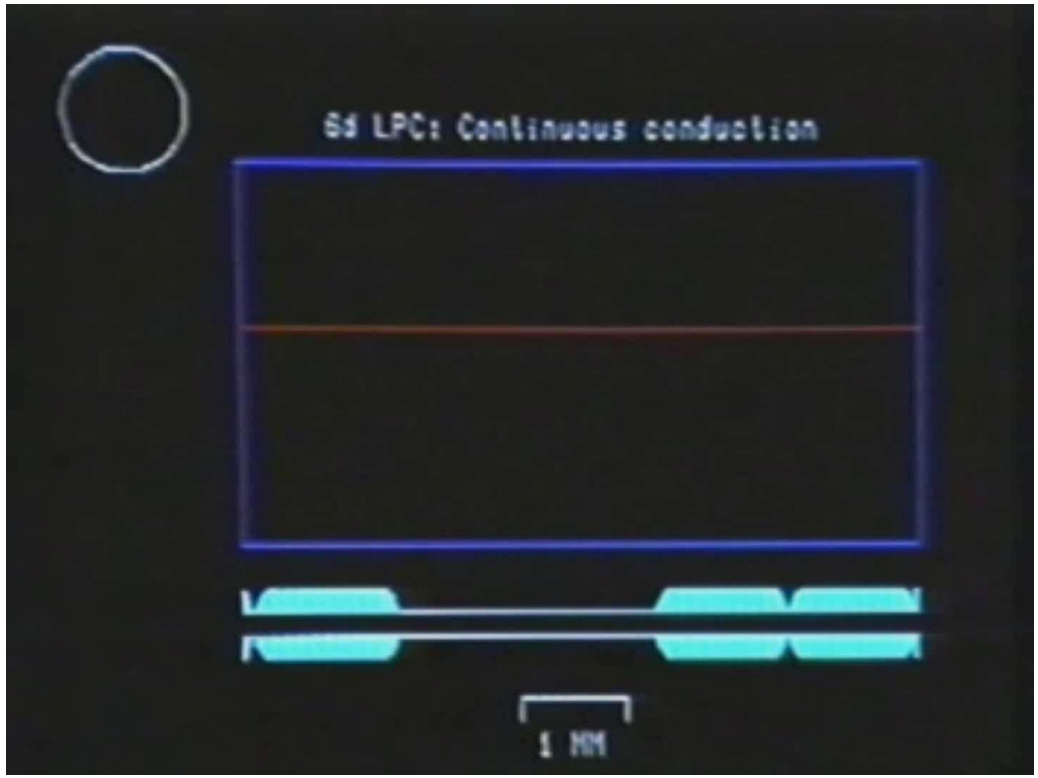
Hugh Bostock's teased nerve fiber preparation

Myelinated internode enables saltatory conduction of action potentials that is energy-efficient and faster.

Inward current at the node of Ranvier is usually five times higher than the minimum of current required to depolarize the node of Ranvier. This **surplus** is called **Safety Factor**.

This surplus of depolarization current is preserved by the excellent seal of internodal myelin, which ensures abundant current available for the depolarization at next node of Ranvier.

When the shunting (<u>increase of capacitance</u>) becomes severe, conduction block may occur.



The large outward current diminishes the safety factor by shunting out the depolarizing current.

Because action potential propagates continuously in demyelinated internode, not in saltatory fashion, it moves slowly with high energy cost. When the situation is severe, action potential may completely stop propagating, called **<u>conduction block</u>**.

- **Conduction block results in disabilities (*acute symptoms*).**

Compound Nerve Action Potential

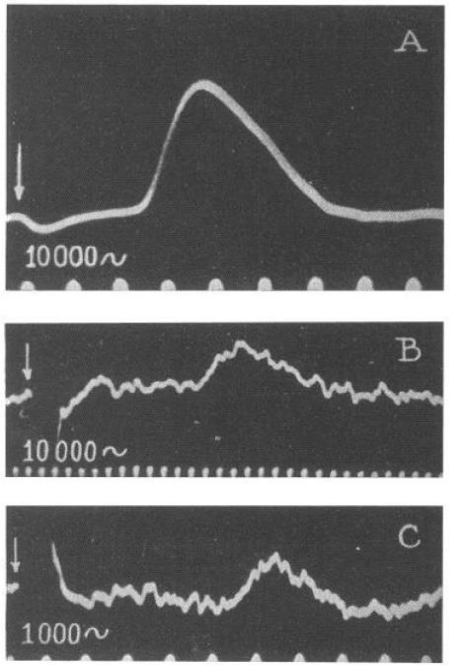


FIG. 1. Spikes of A, B and C fibers; A, potential from a single large fiber of a dorsal spinal root of the cat; B, from the cervical sympathetic nerve of the rabbit, threshold response, possibly not a single fiber (spike 25 μv.); C, from the splenic nerve of the cat, threshold response (spike 20 μv.). The fine oscillations are occasioned by the noise level.

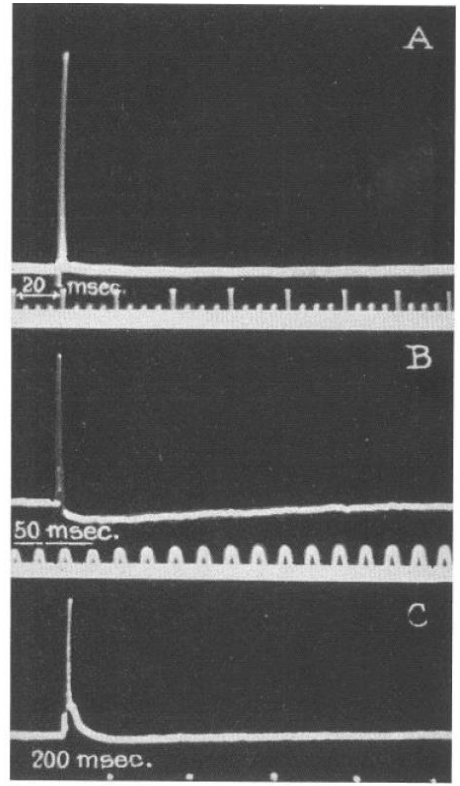
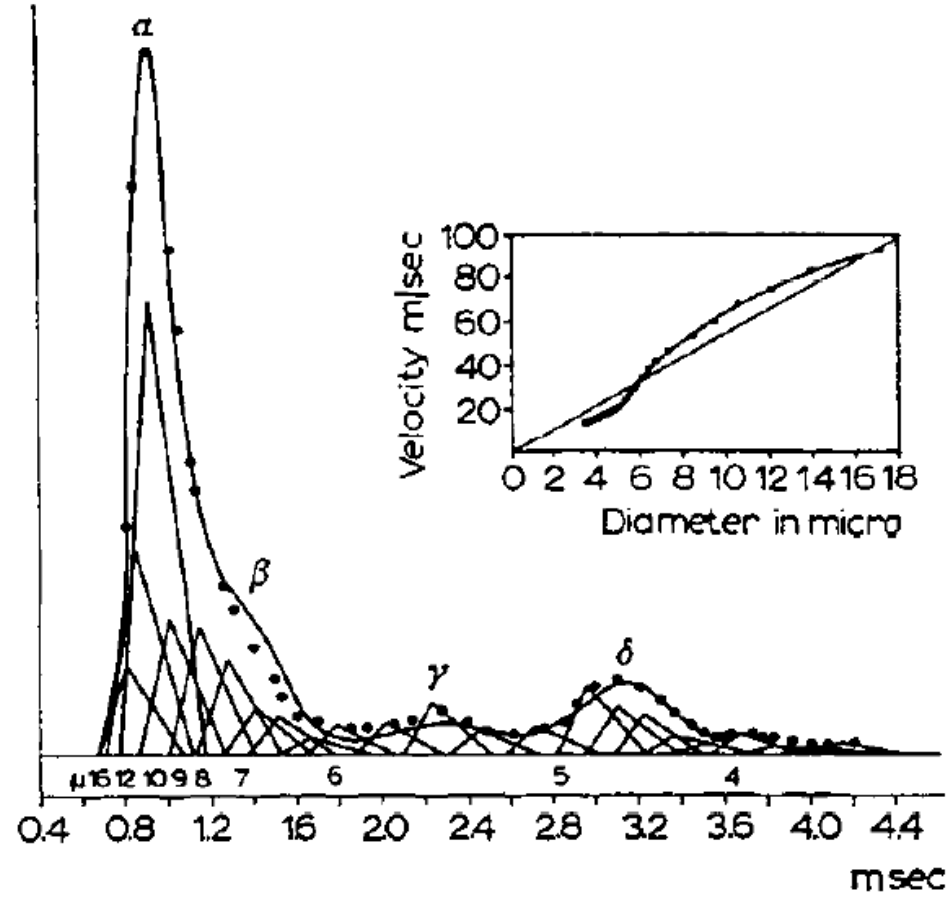


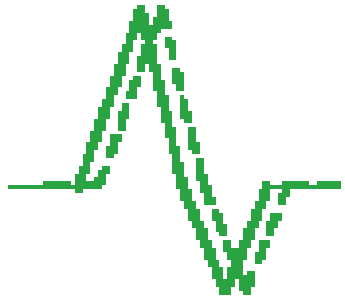
FIG. 2. Action potentials of A, B and C fibers (cat): A, from the phrenic nerve; B and C from hypogastric nerves.

Gasser and Erlanger Nobel Lecture in 1947



Compound nerve action potential recorded from a nerve bundle is equal to the summation of all action potentials from individual nerve fibers.

Normal nerve



Responses arrive at recording electrode almost together

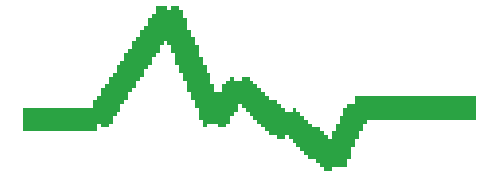
=

Demyelination

Responses arrive at different times phase cancellation

=

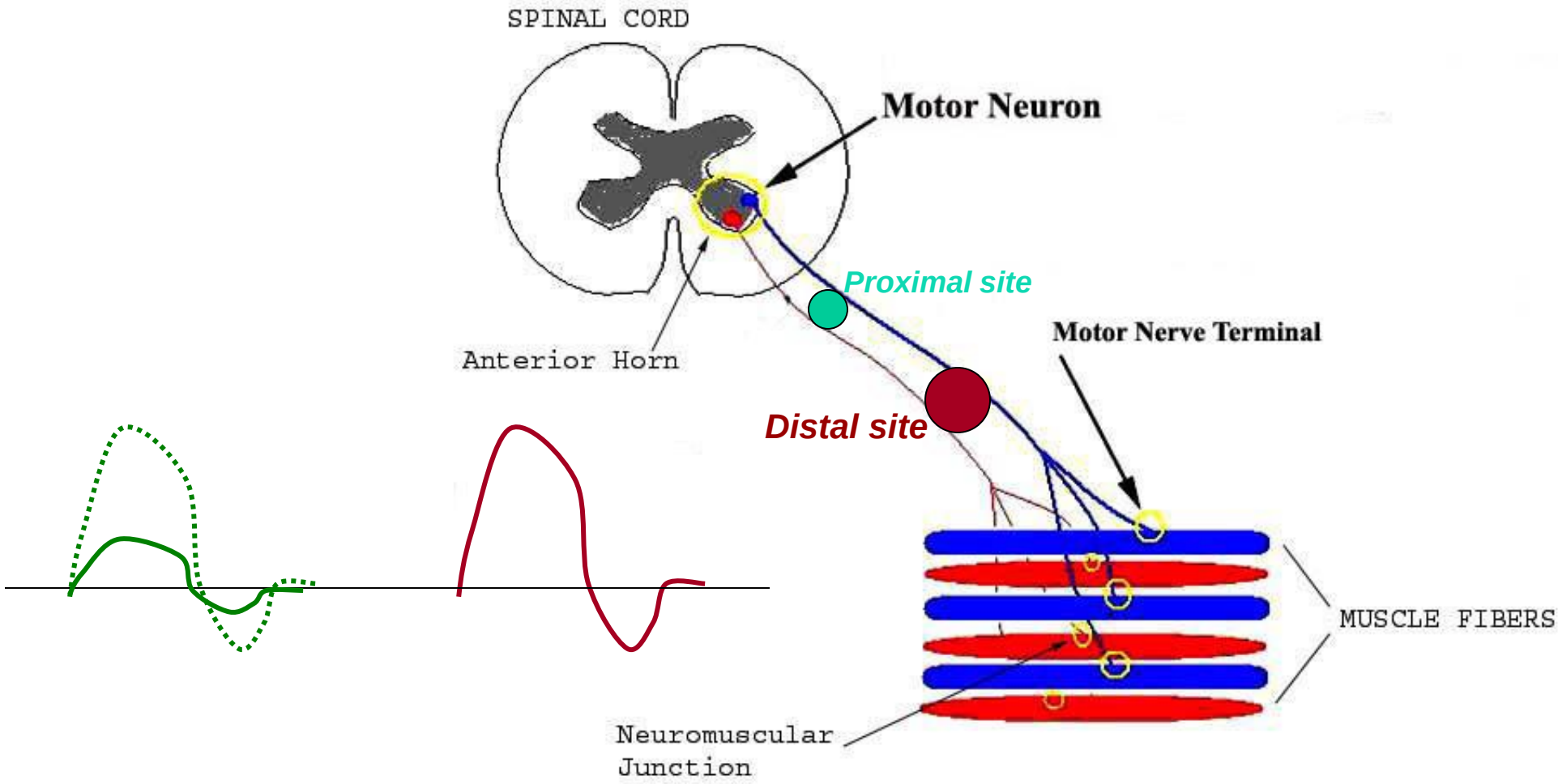
Temporal dispersion



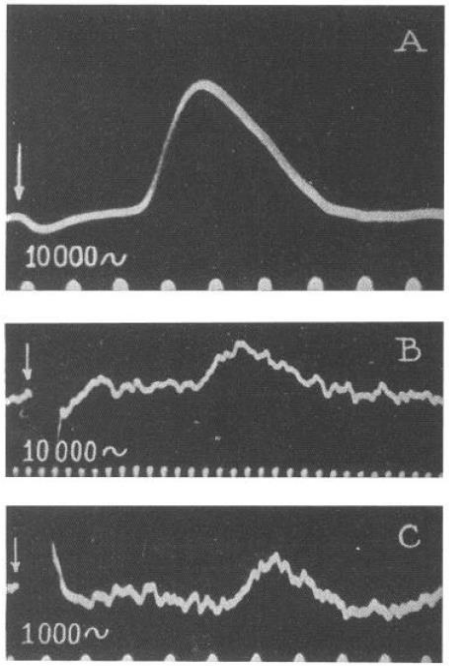
CV is measured based on the onset of CNAP, which is mainly contributed by the largest myelinated nerve fibers.

Weir et al JNNP 2005

Conduction Block



Compound Nerve Action Potential



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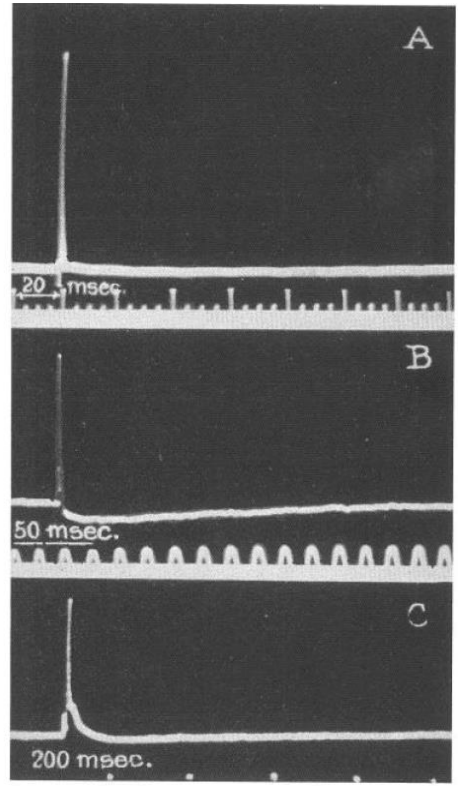

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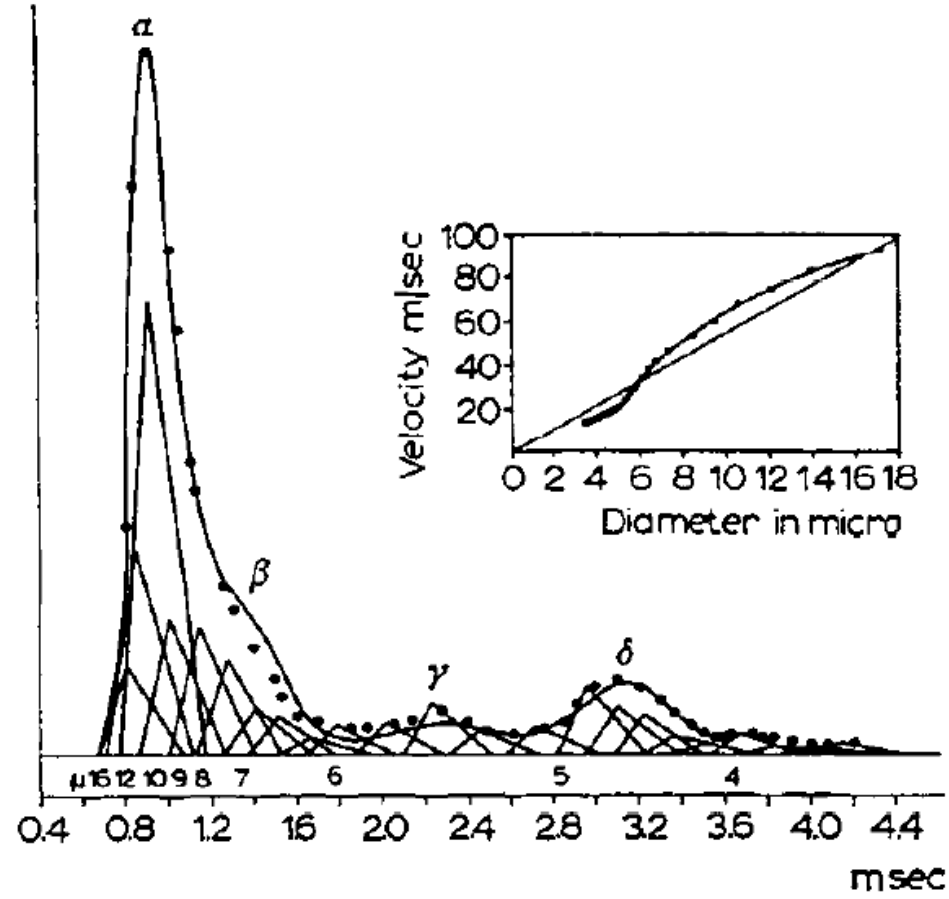

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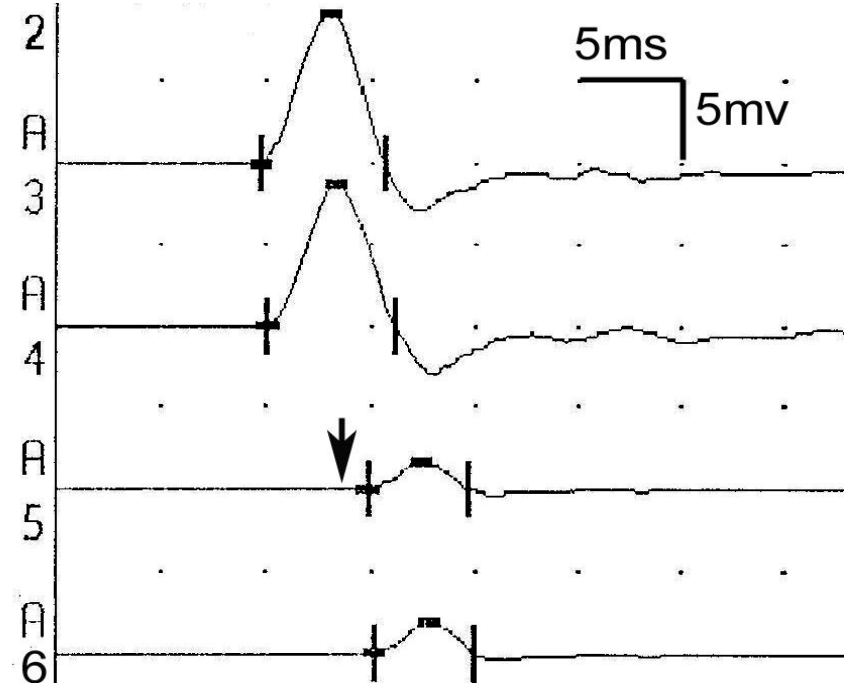
Electrophysiological signatures of segmental demyelination

- ***Slowed conduction velocity***
- ***Temporal Dispersion***
- ***Conduction block***



Bai et al J Neurosci 2010

Demyelinating Diseases:

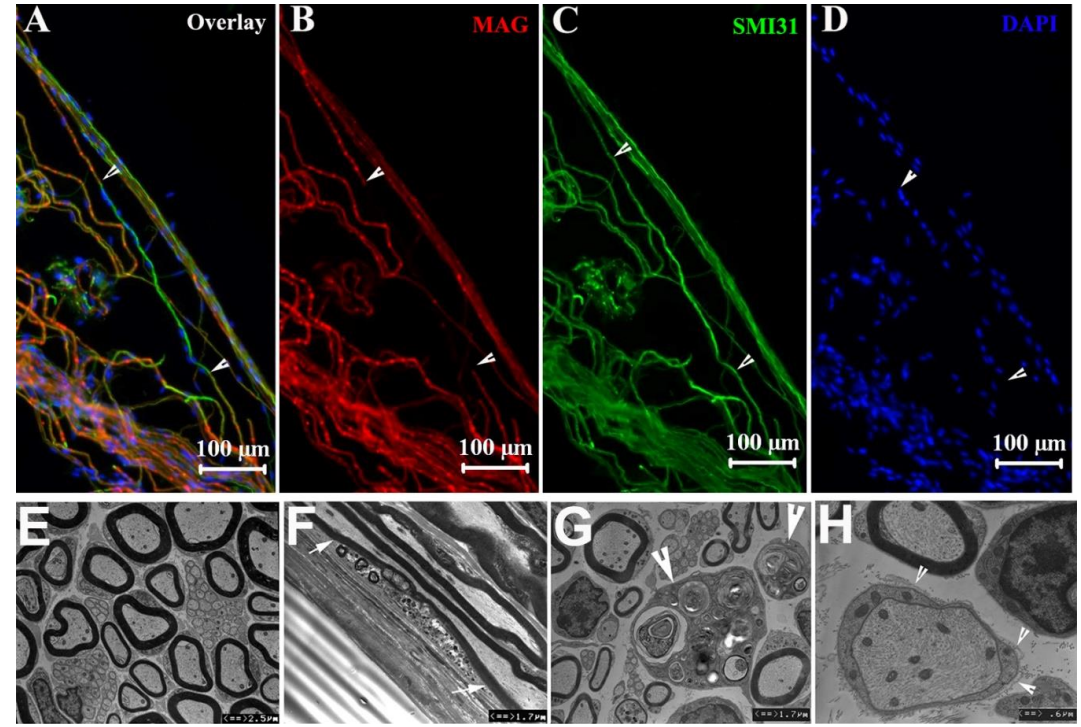
PREMARY
Guillain Barre syndrome; Chronic inflammatory demyelinating polyneuropathy; Multifocal motor neuropathy; a sub-group of inherited neuropathies

SECONDARY
- **Diabetic neuropathy**
- **Neuropathies after certain infections**

Dysmyelinating Diseases:

- **Charcot-Marie-Tooth type-1A**
  - *uniform slowing of conduction velocity*
  - *non-uniform slowing of CV in subtypes of CMTs, such as CMTX1, CMT4J, HSN1C*

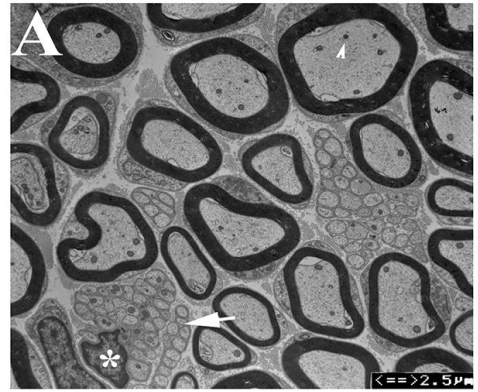
These inherited neuropathies now provide many new insights into mechanisms of nerve conduction.

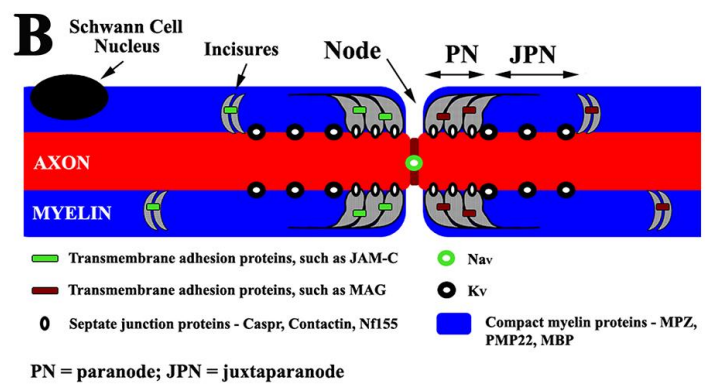


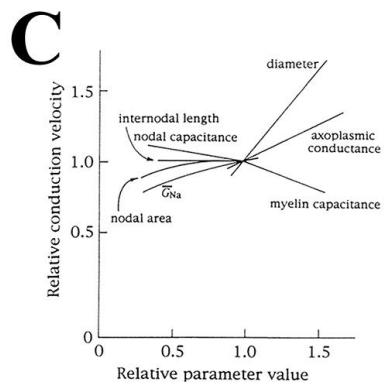
Zhang et al, Brain 2008

Inherited Neuropathy and genetic-manipulated animal models provide additional insights

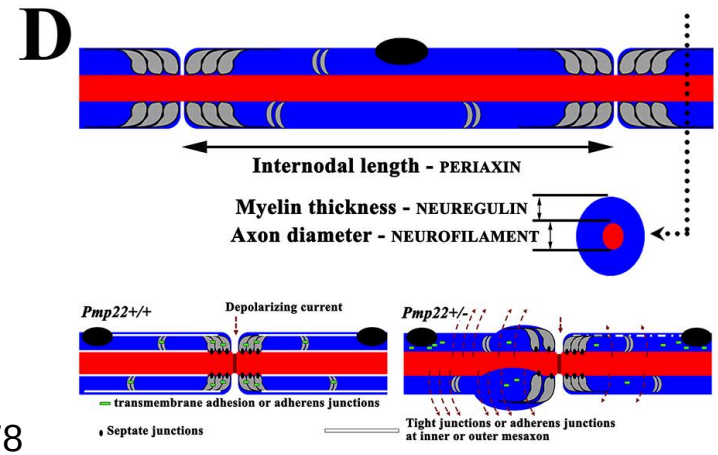
- Early simulation studies showed a variety of nerve metrics affecting nerve conduction.
- Many of these predictions have been re-substantiated in genetic manipulated animal models or persons with inherited peripheral nerve diseases.







Moore et al Biophys J, 1978



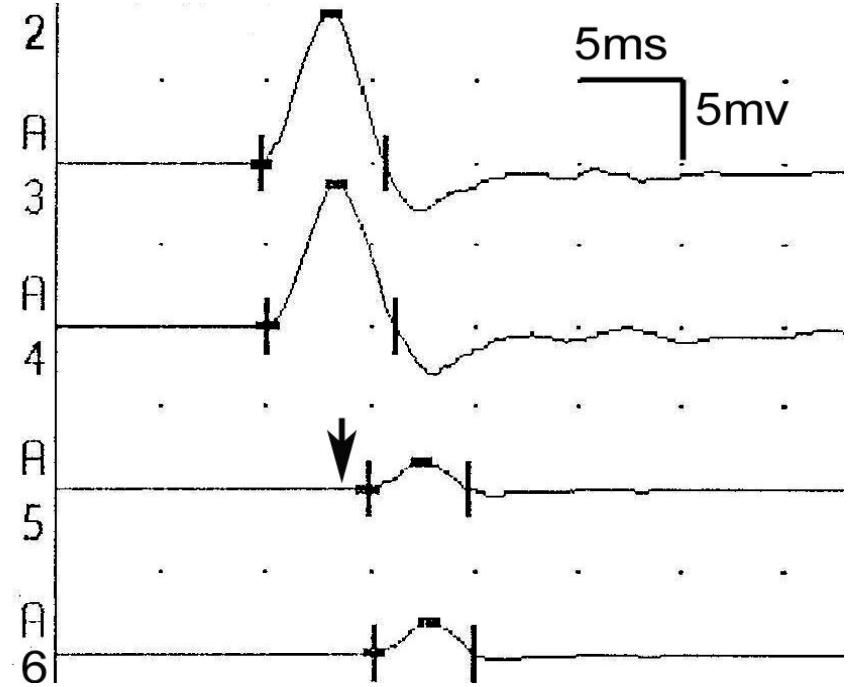
Li, Experimental Neurology, 2015

Slowed conduction velocity ≠ always segmental demyelination

Concept of Functional Demyelination

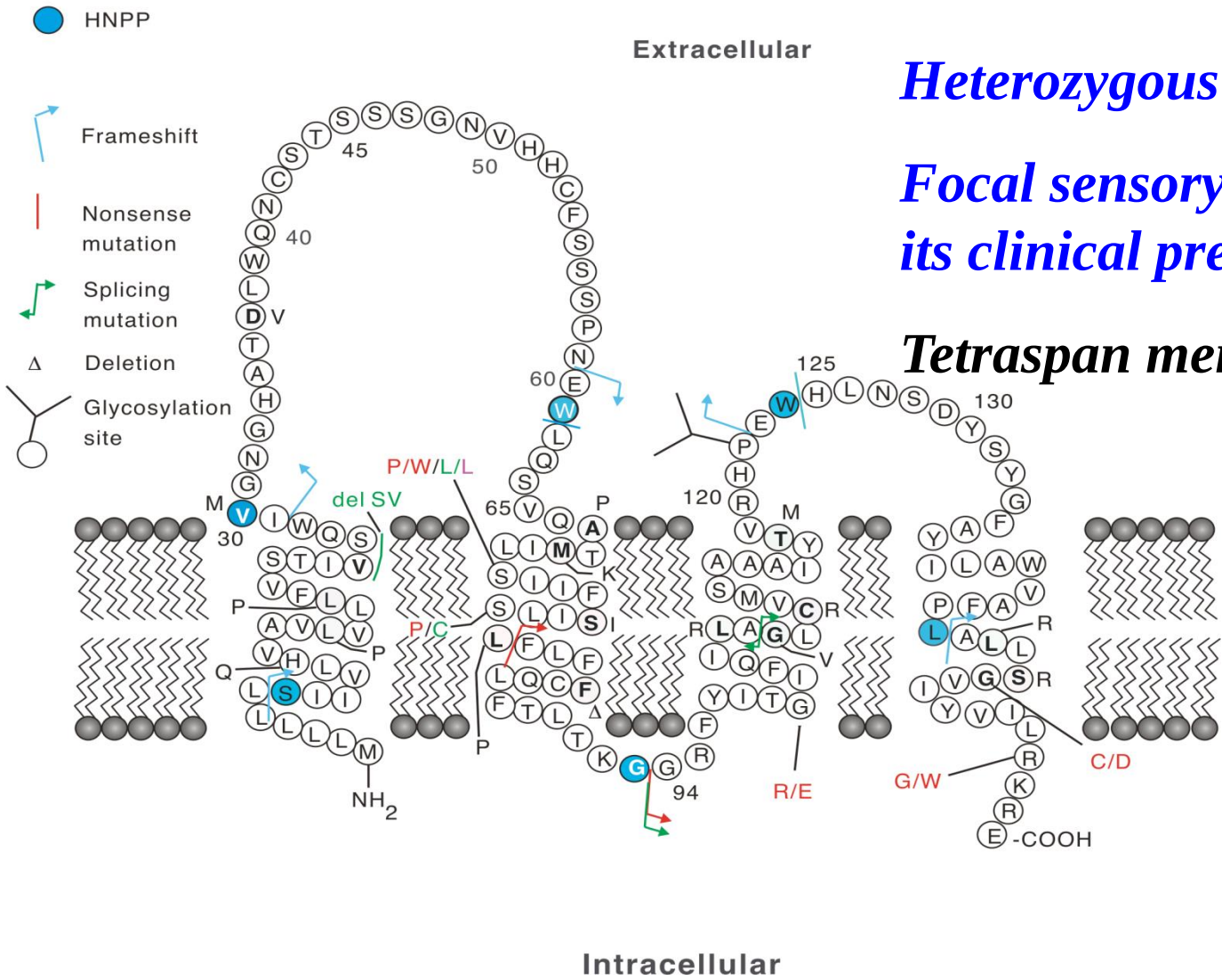
- *signatures of segmental demyelination have been observed in the absence of segmental demyelination using a HNPP mouse model*

- ***Slowed conduction velocity***
- ***Temporal Dispersion***
- ***Conduction block***



Bai et al J Neurosci 2010

PMP22 PROTEIN (Heterozygous deletion of *PMP22* gene causes hereditary neuropathy with liability to pressure palsies)

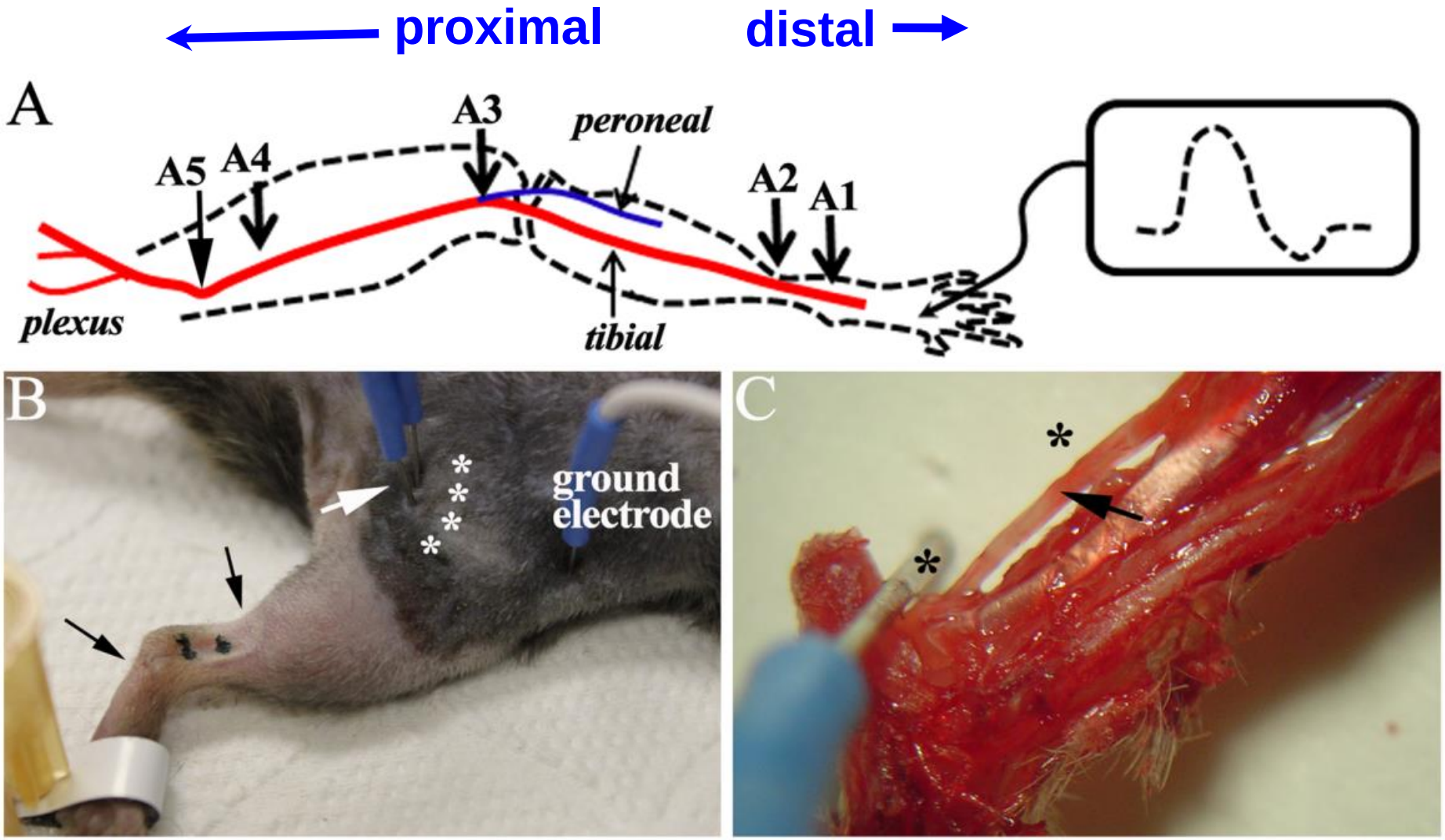


Heterozygous Deletion – HNPP

Focal sensory and motor deficits in its clinical presentation

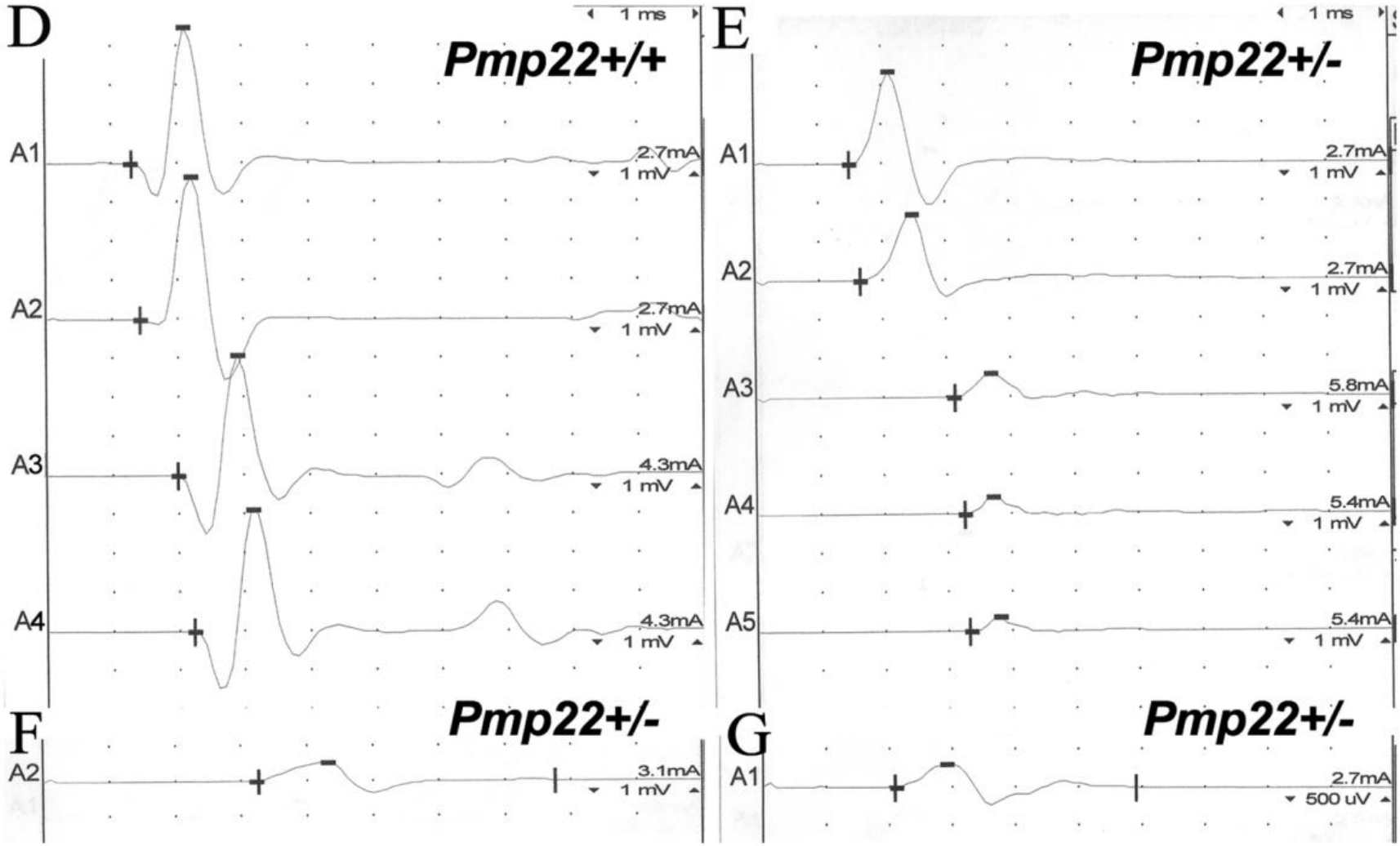
Tetraspan membrane protein

Primarily express in myelinating Schwann cells



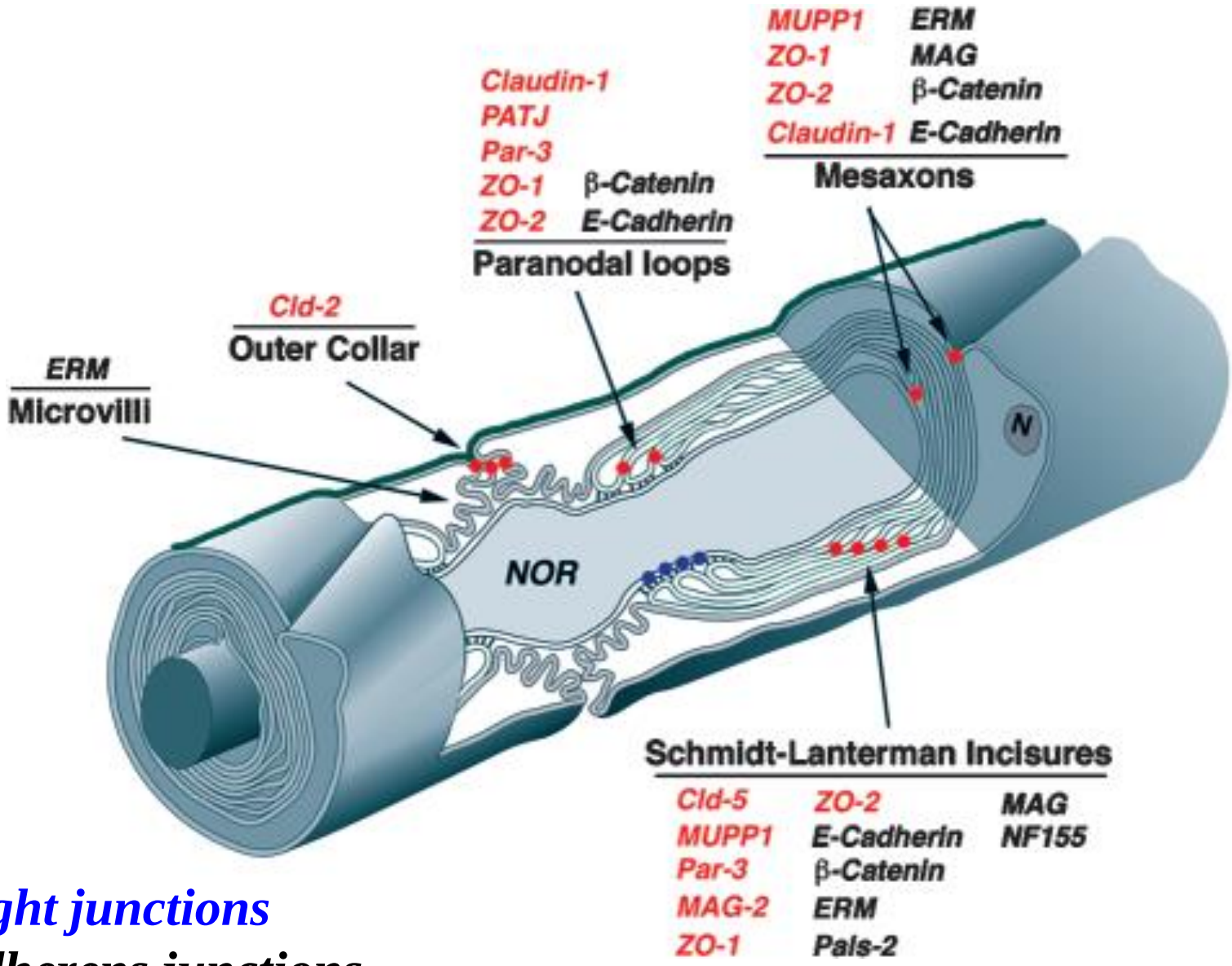
Electrophysiological features of demyelination are also observed in HNPP mouse nerve conduction studies. One of the two *Pmp22* copies was deleted from the mouse genome – heterozygous knockout mouse.

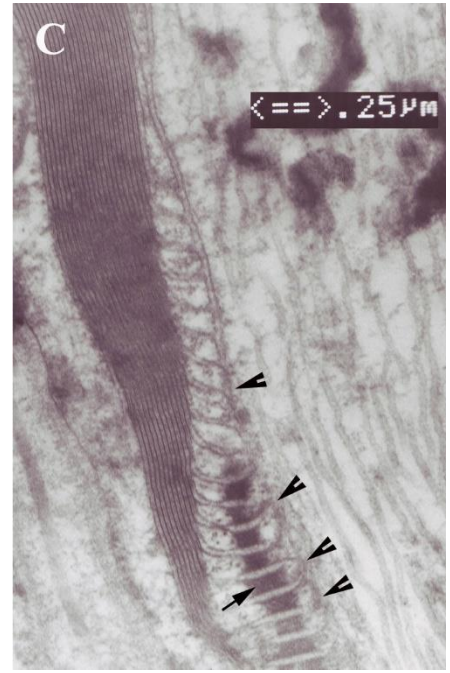
Hu et al, PLOS Genetics 2016



- **Morphometric analysis & teased nerve fiber: no axonal loss or segmenal demyelination**

Hu et al, PLOS Genetics 2016



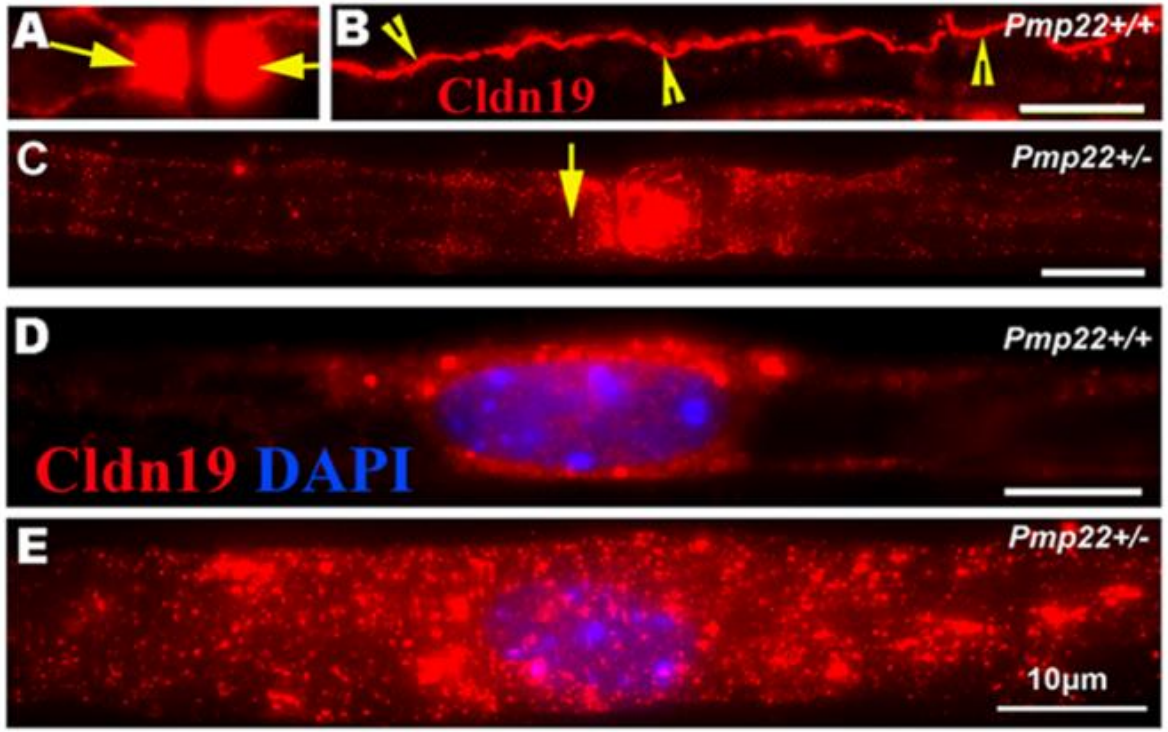


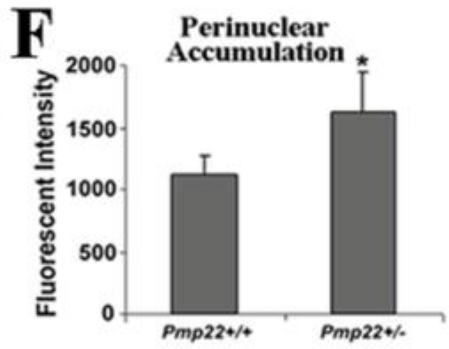
Tight junctions

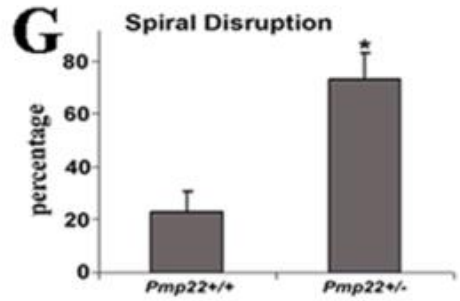
Adherens junctions

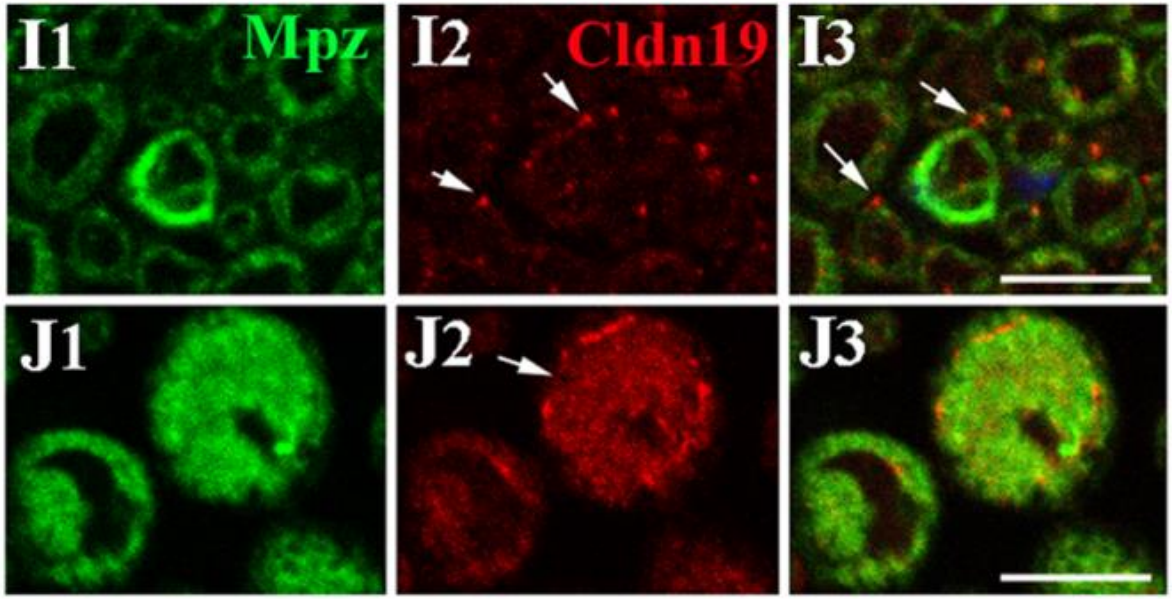
Septate junctions

Poliak et al J Neurosci 2002









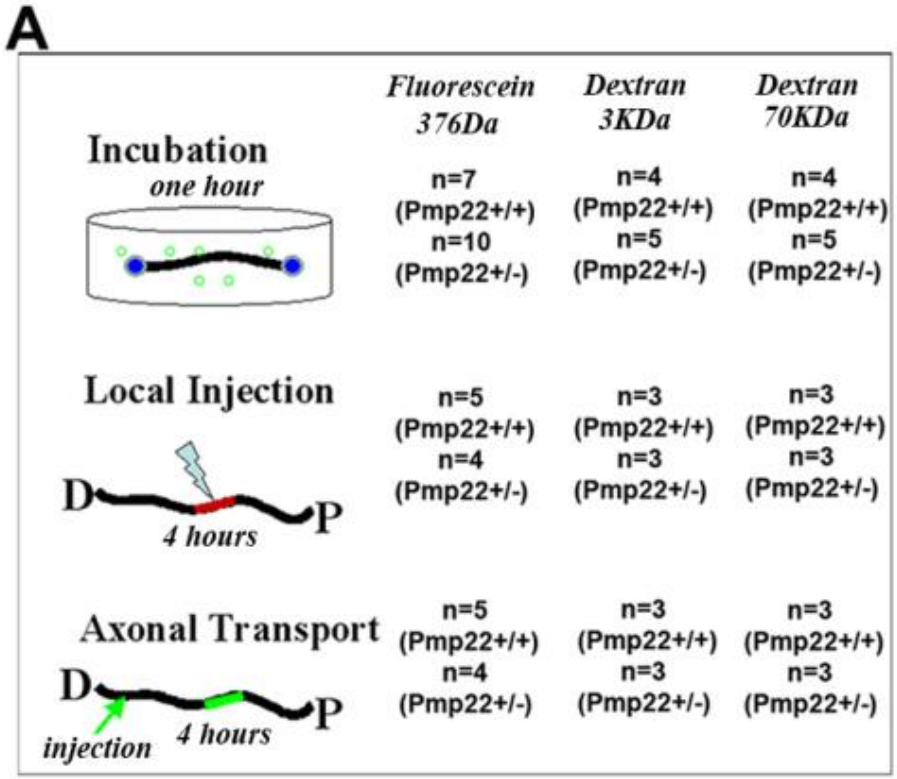
H

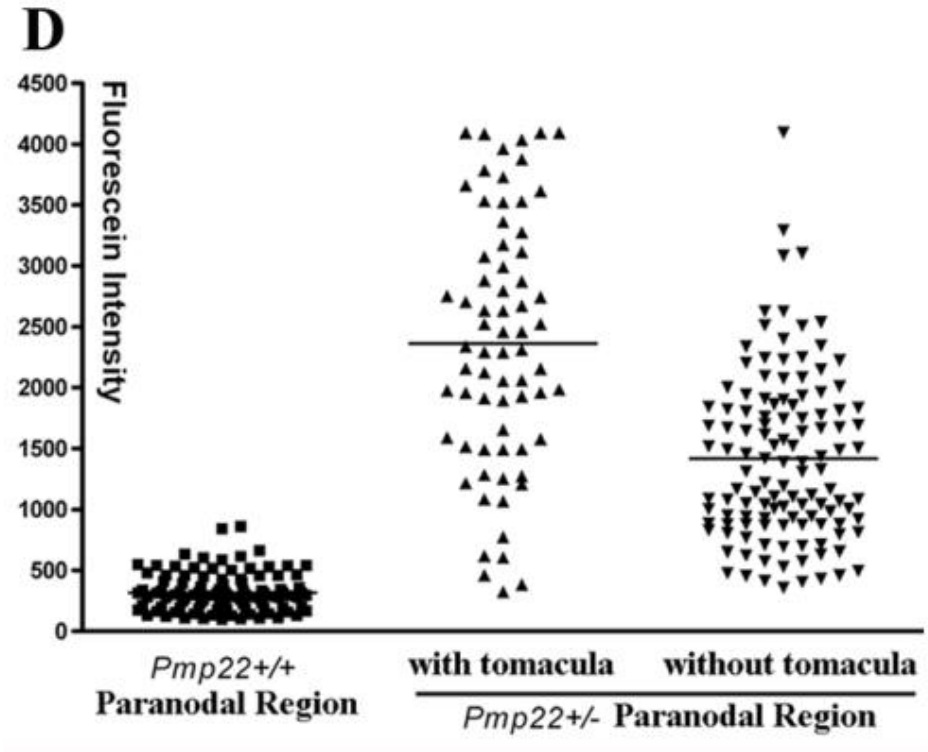
Quantification of Abnormal Paranodes in *Pmp22*+/- Nerves

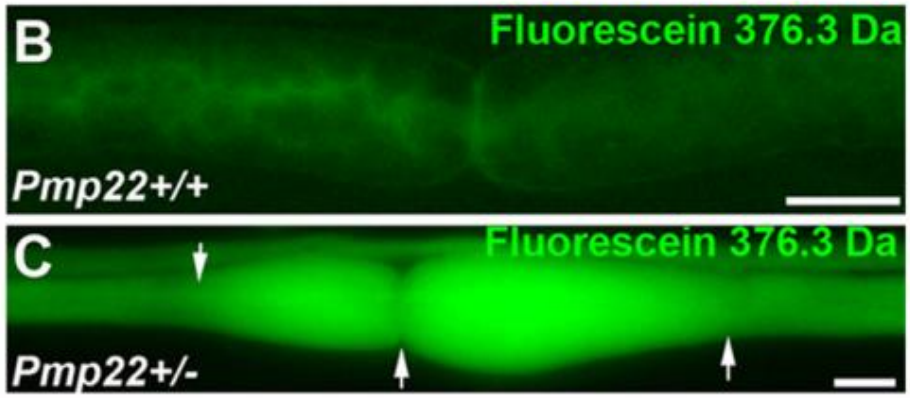
number of counted PN	tomacula	% abn PN
256	yes	65±10%
832	no	12±4%

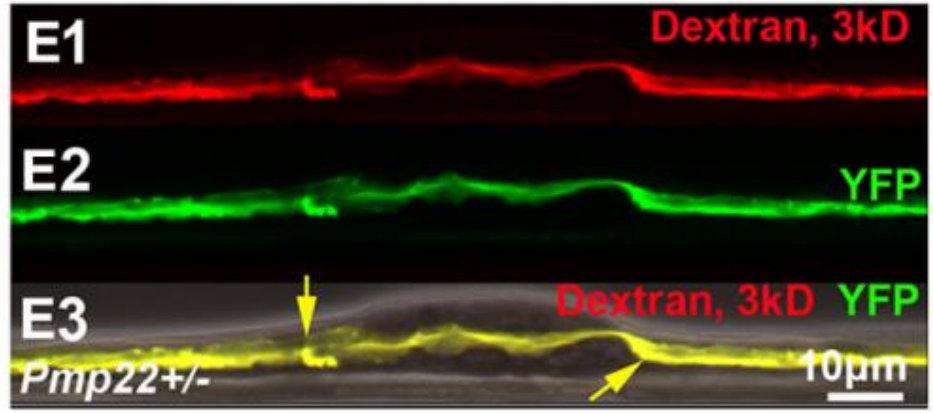
Guo et al Annals of Neurology 2014

ABNORMAL MYELIN PERMEABILITY



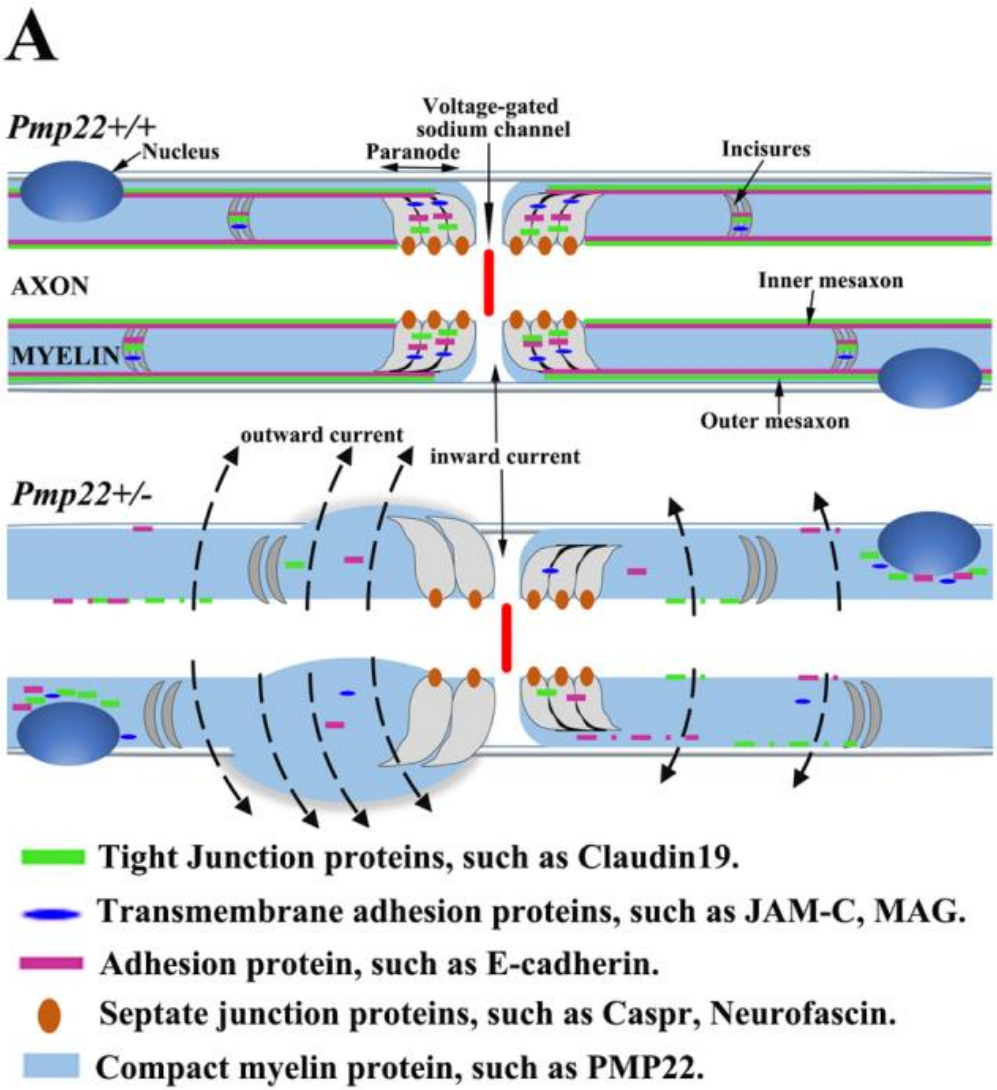






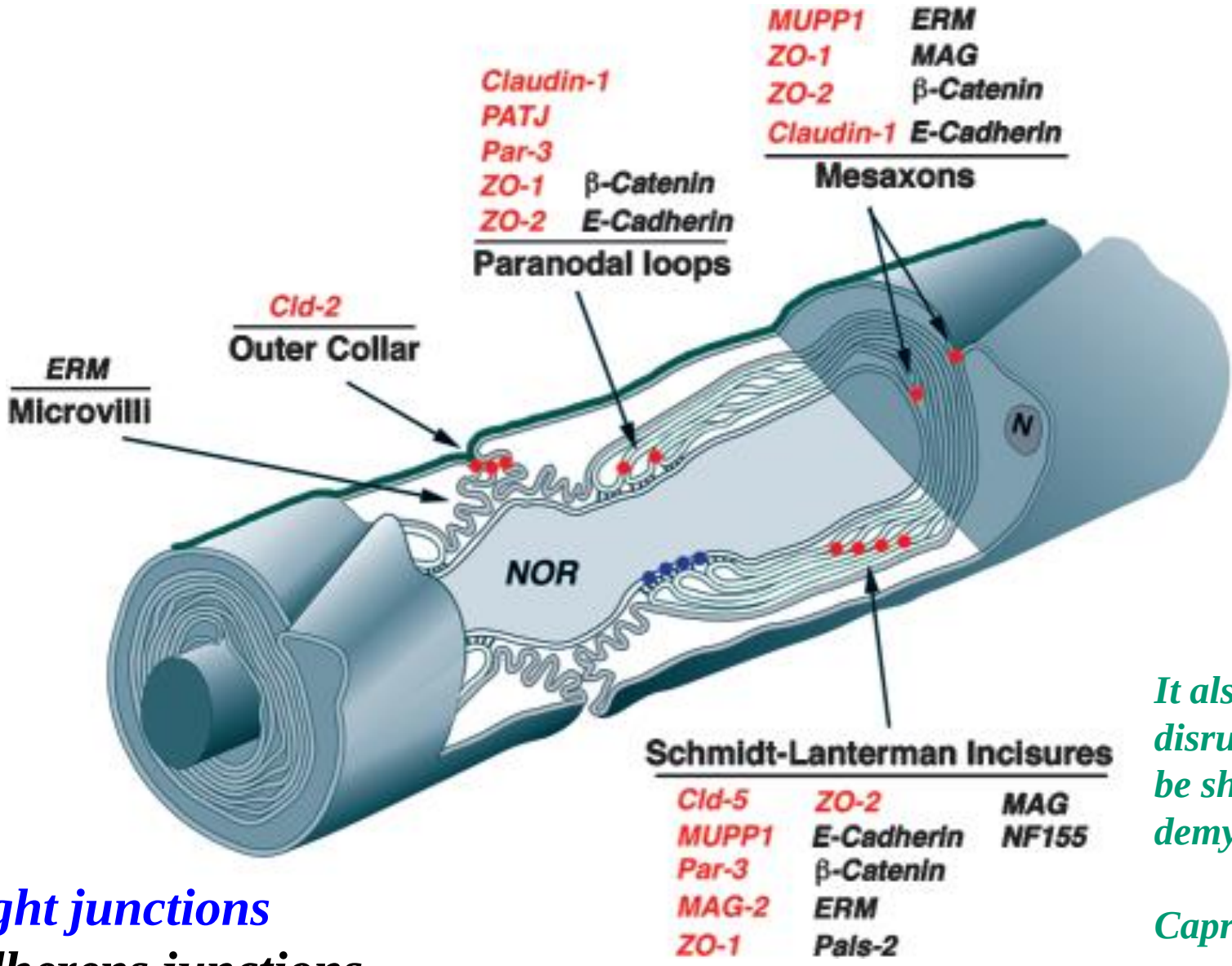
Guo et al Annals of Neurology 2014

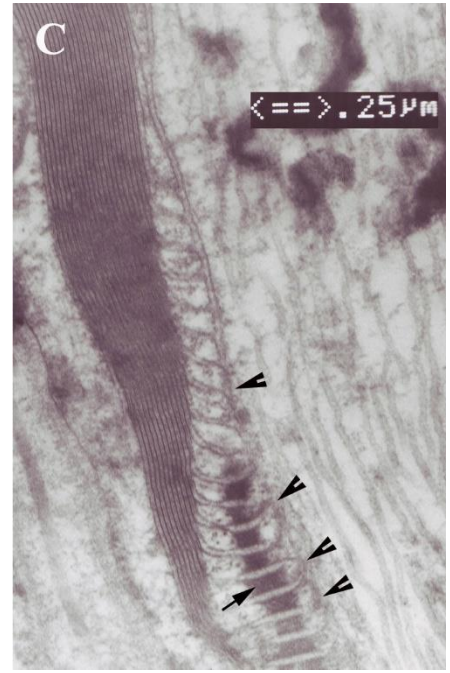
Functional Demyelination



- This mechanism denotes pathological processes that alter myelin permeability without physically stripping off the myelin sheath.
- Functional demyelination was detectable at one-week old HNPP mice, but actual segmental demyelination (strip myelin off axon) did not occur until 10 months of age in HNPP mouse model
- Upstream mechanism matters.

Guo et al Annals of Neurology 2014





It also makes sense the disruption of myelin junction be shared by many types of demyelinating diseases?

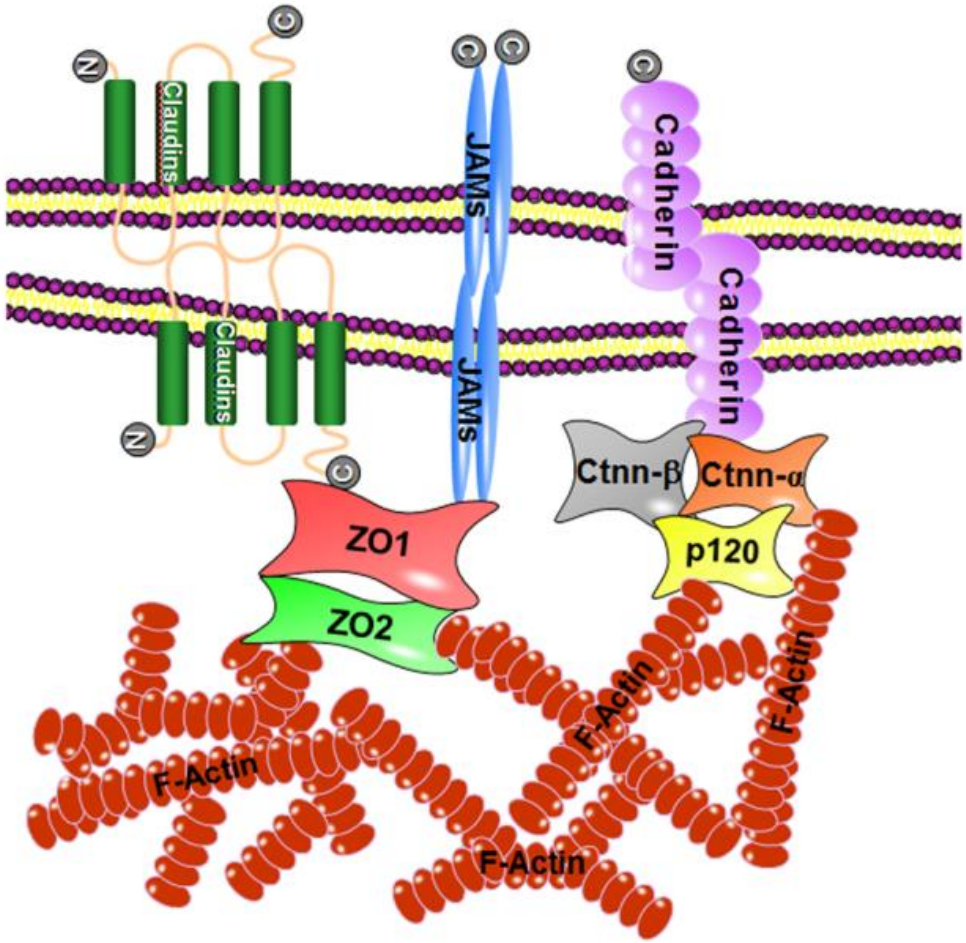
Caprariello et al PNAS 2018

Tight junctions

Adherens junctions

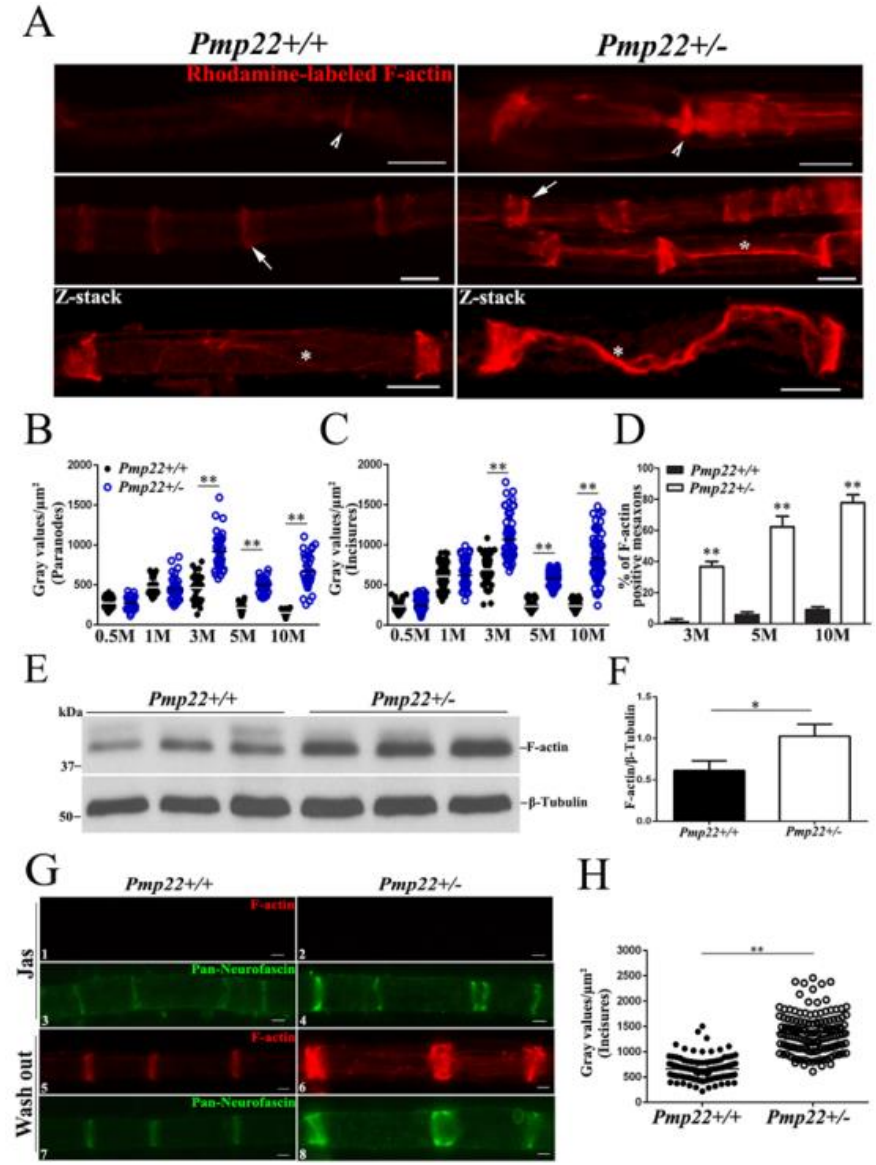
Septate junctions

Poliak et al J Neurosci 2002

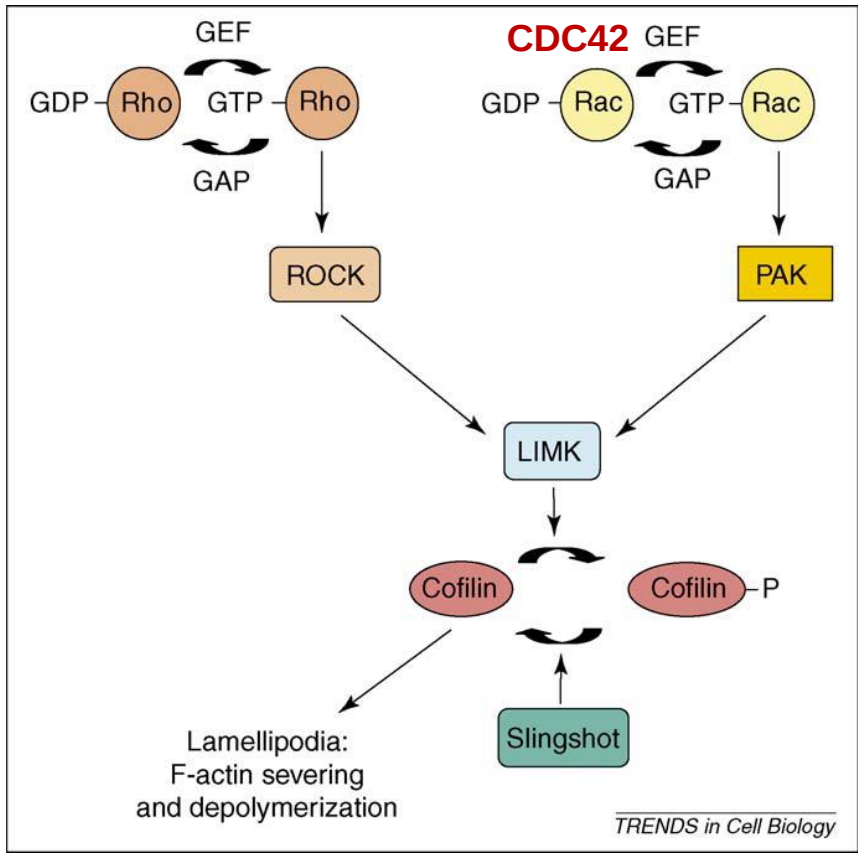


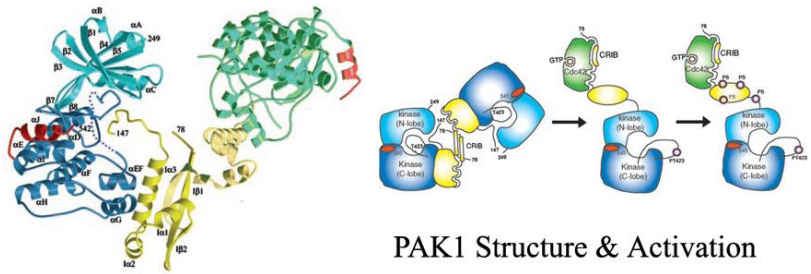
EQUILIBRIUM

Actin polymerization – depolymerization of F-actin

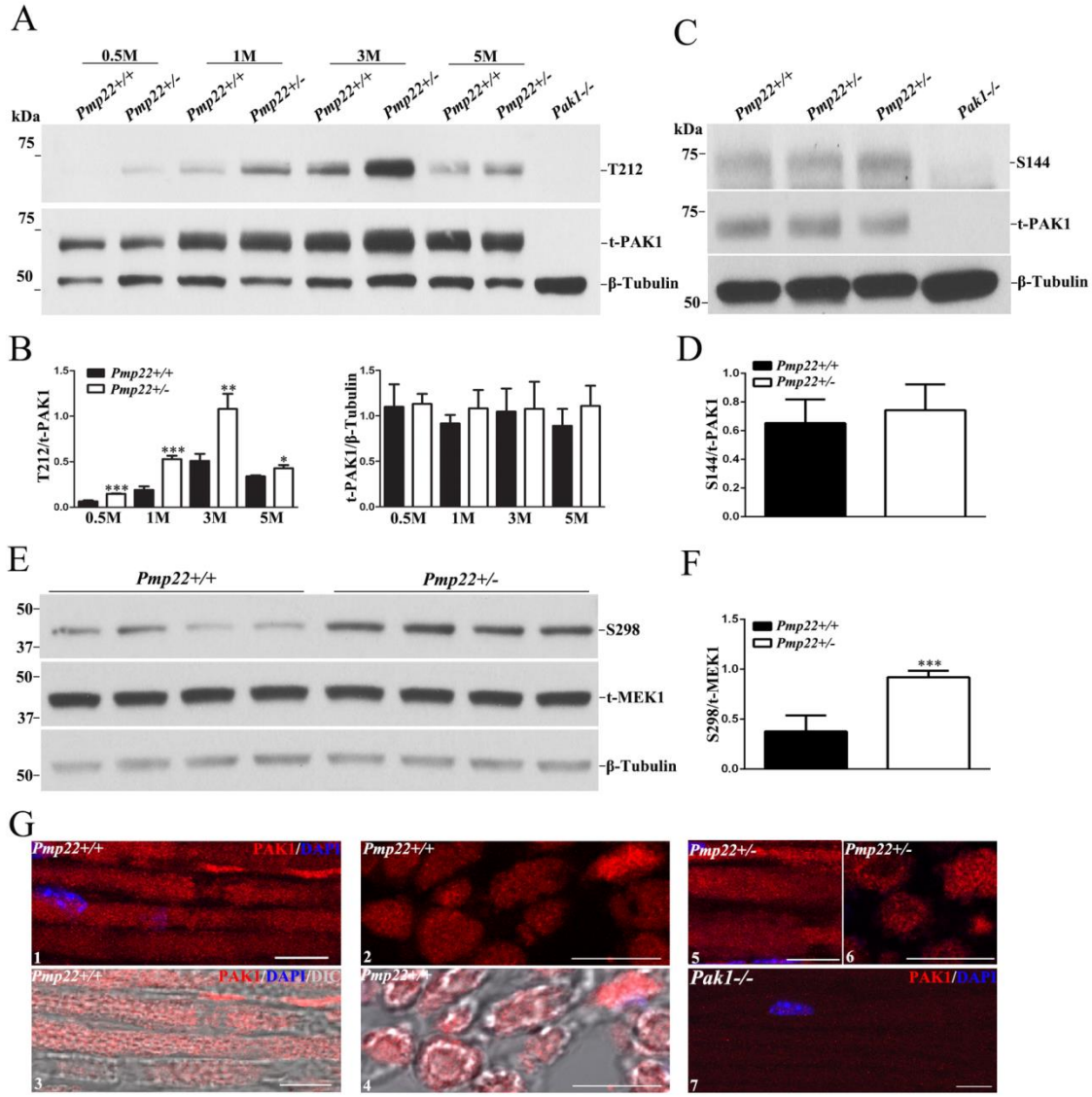


Hu et al, PLOS Genetics 2016

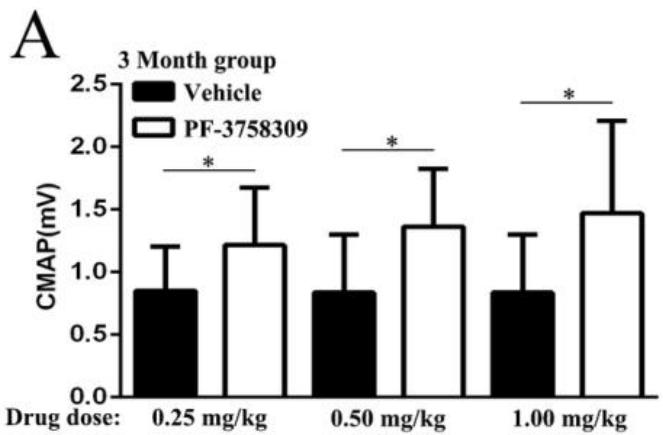


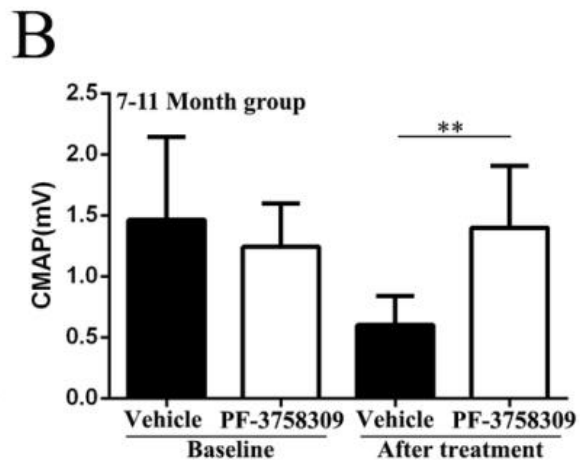


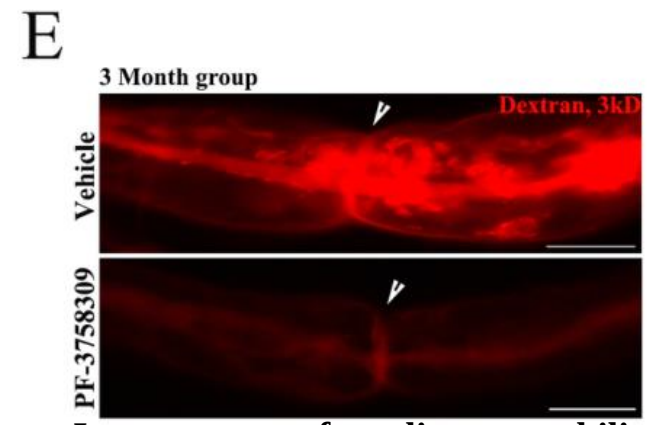
PAK1 Structure & Activation



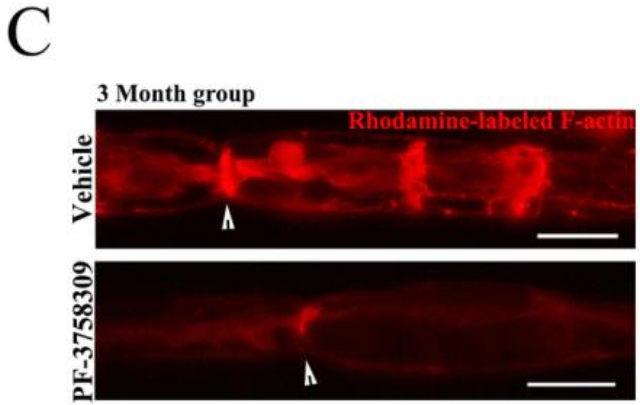
Hu et al, PLOS Genetics 2016



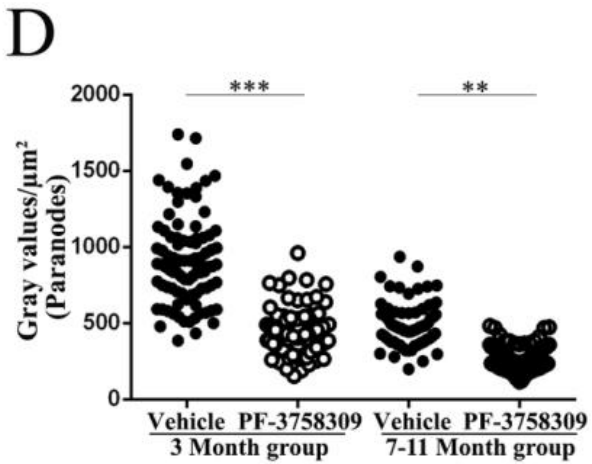




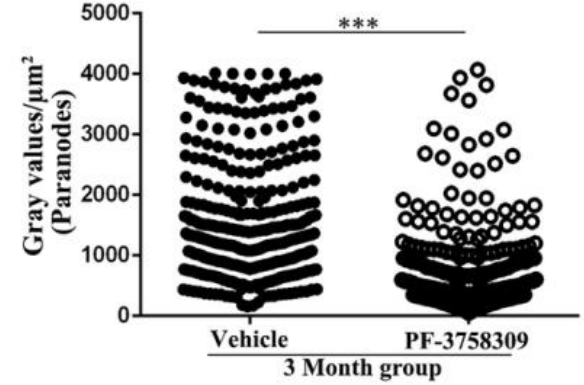
Improvement of myelin permeability



Reversal of F-actin increase

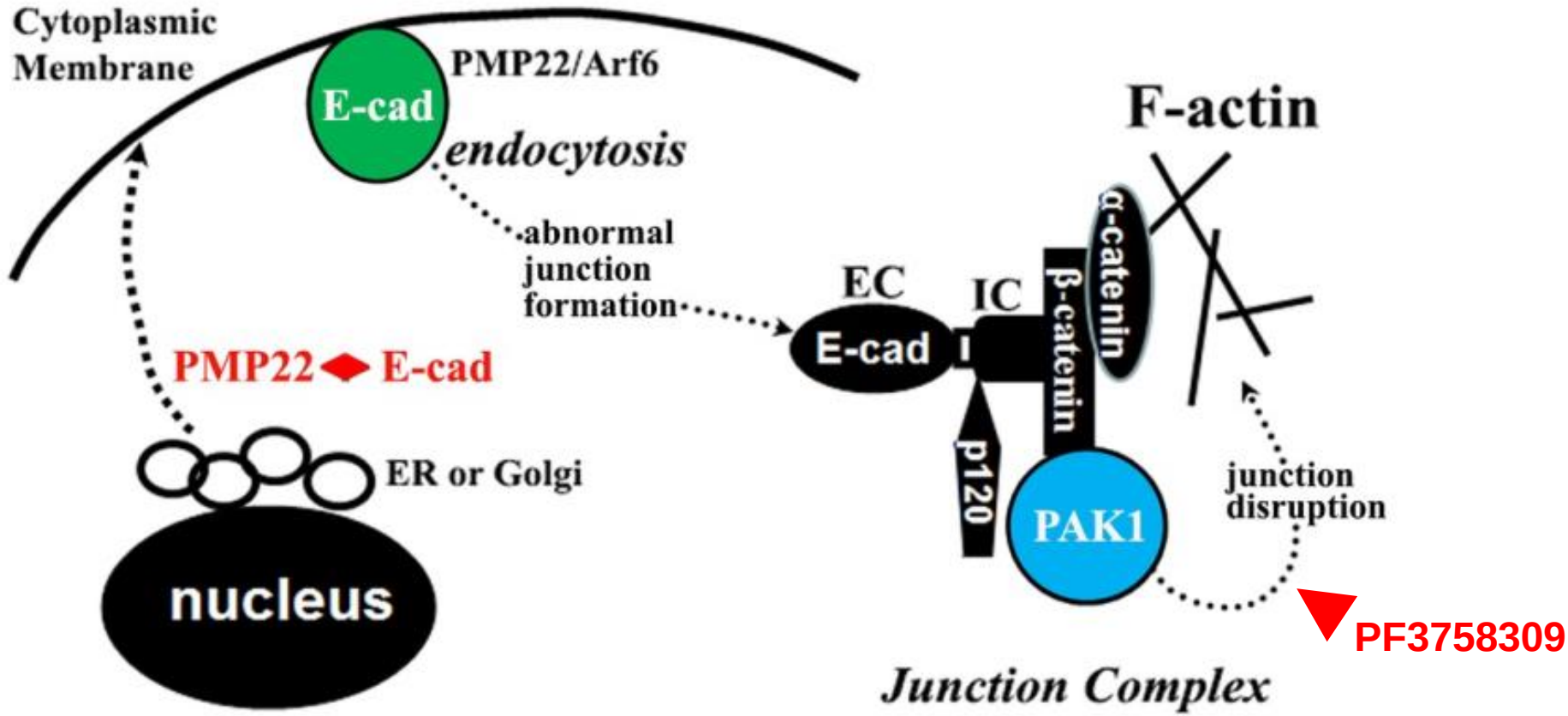


Improvement of myelin permeability



Reversal of F-actin increase

Hu et al, PLOS Genetics 2016



INITIATION

PERPETUATION

PATENT: VU17002US1 LI Appl. No. 15/651,931
Title: Therapeutic Development for HNPP

independently replicated by Poitelon et al; J Neurochem 2018

Hu et al, PLOS Genetics 2016

***Summary-I*: Action potential propagation functions as a binary informatic system with high fidelity. Disruption of this signal transduction results in disabilities in a variety of demyelinating diseases. Emerging monogenic inherited neuropathies enable us to appreciate specific molecules in regulating nerve conduction.**

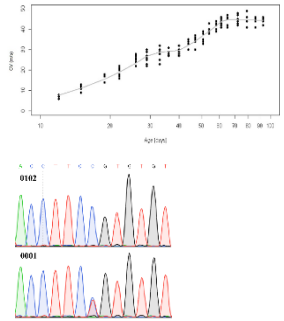
Hyperactive PAK1 disrupts myelin junctions that appear to be an upstream mechanism before the internodal myelin is removed in demyelination.

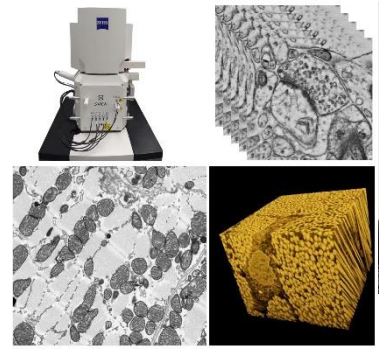
This mechanism may operate not only in HNPP but also in other types of demyelinating diseases.

***Summary-II*: We have identified a model of dysmyelination via a different PAK signaling. This finding will allow us to design PAK activators or inhibitors to regulate or repair dysmyelination in neurological diseases.**

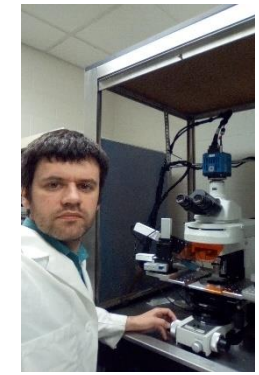
Our Recent Team:

Sadaf Saba

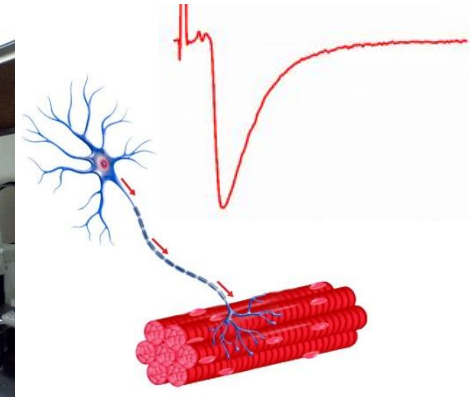


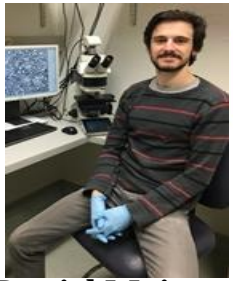


Chris Dunham

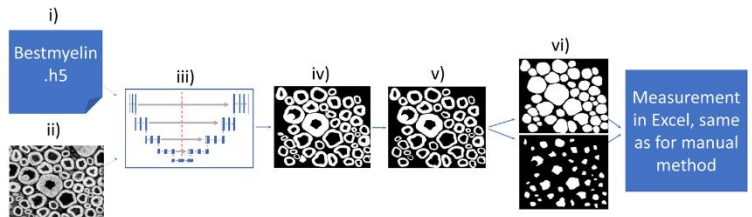


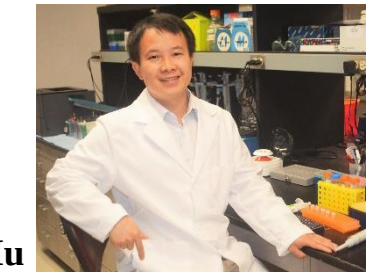
Alex Vasin



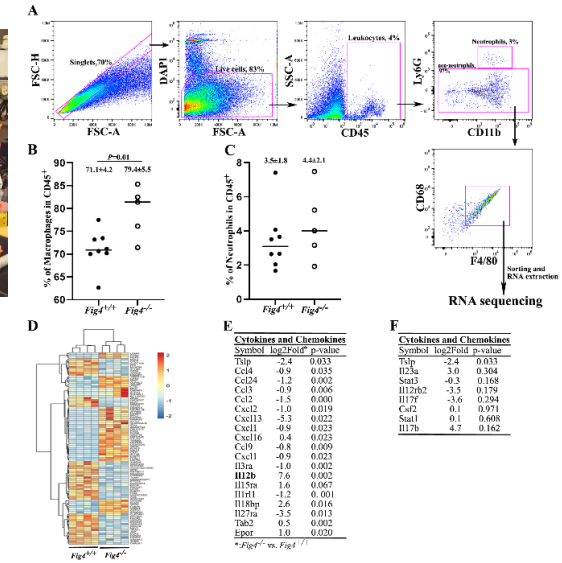


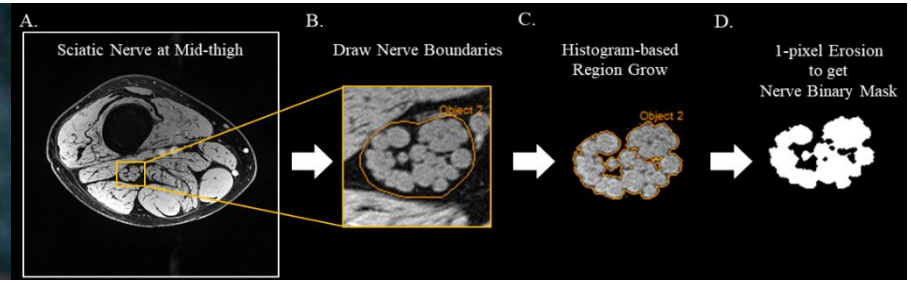
Daniel Moiseev





Bo Hu





Yongsheng Chen

Neurology, Vanderbilt

***Research Assistants*:**
Sezgi Arpag (Ex-RA)
Megan Simmons (Ex-RA)
Daniel Moiseev
Qing Yan (Ex-RA)

***Research Fellows*:**
Bo Hu, PhD
Ryan Castoro (Ex-Fellow)

***Undergrad Students*:**
Vignesh Ravi
Michael Wang
Margaret Tugend
Perry Veras

***CMT Clinic*:**
Robin Yawn

Dept Neurology, Wayne State University

***Research Assistants*:**
Daniel Moiseev, Jibraan Rashid
Chris Dunhan, Melody Hackett

***Medical Student*:**
Mark Wilhelm, Arielle Best

***PhD Students*:**
Sadaf Saba

***Research Assistant Professor*:**
Alexander Vasin, PhD
Bo Hu, PhD
Yongsheng Chen, PhD

Collaborators:

Swiss Federal Institute of Technology, Switzerland
Ueli Suter

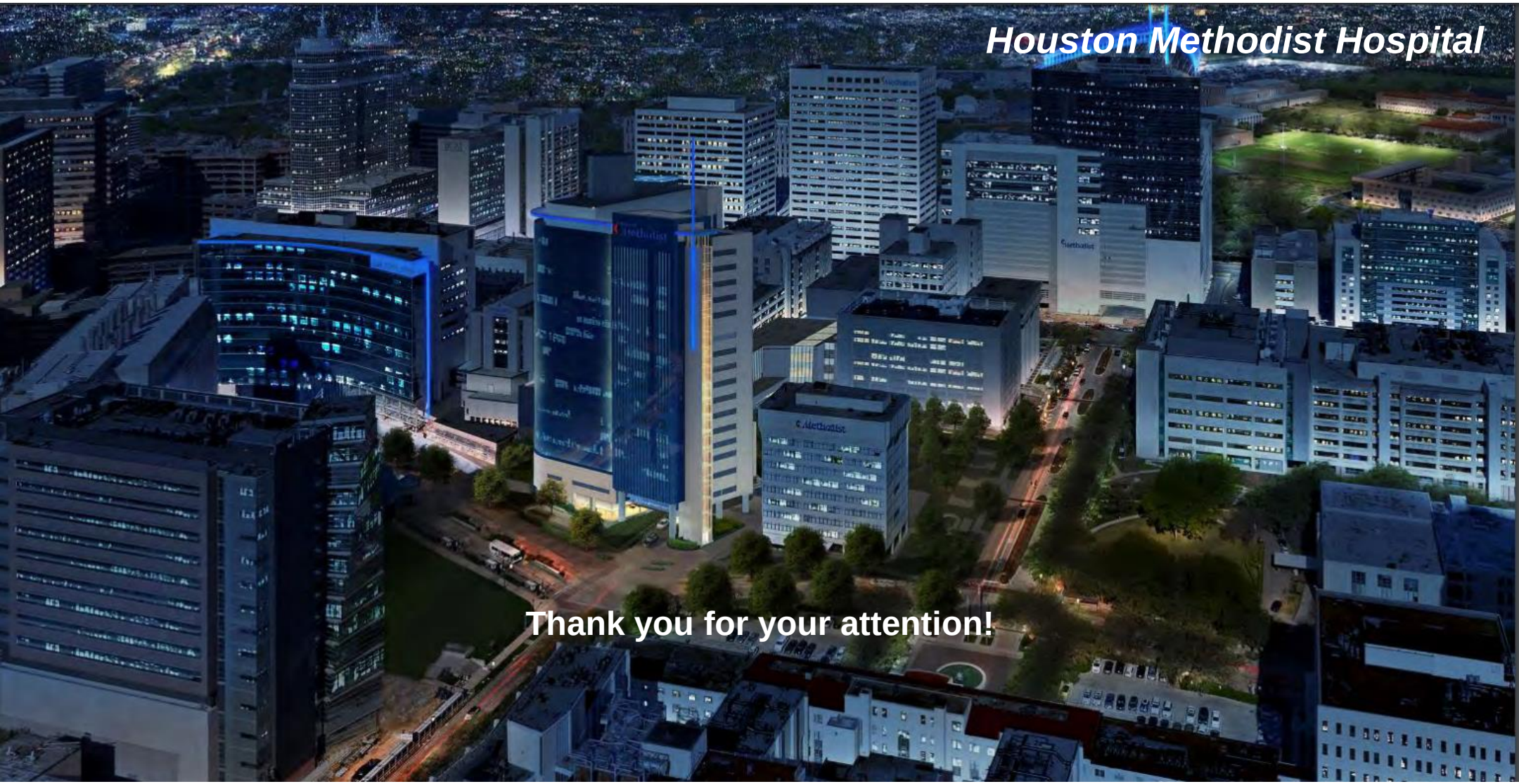
Fox Chase Canter Institute, Philadelphia
Jonathan Chernoff

Marx Plank Institute, Goettingen, Germany
Hauke Werner
Wiebke Moebius

The National Center for Microscopy and Imaging Research, University of California at San Diego
Mark Ellisman
Gina Sosinsky

Cleveland Clinic Foundation, Ohio
Cameron McIntyre

SUPPORTED BY -
NINDS (R01NS115748; PI: Li, J; MPI: Dortch, R)
NINDS (R21 TR003312; PI: Li, J)
NINDS (R61 NS119434; PI: Li J; MPI: Chen, YS)


Houston Methodist Hospital
Thank you for your attention!