Meccanismi di danno assonale nel SNC

Corso di Residenziale di Neuroimmunologia

Bergamo, 12-15 Marzo 2003

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Axonal damage in CNS disorders

A key predictor of outcome in human diseases

(Medana and Esiri 2003)

Axonal damage in CNS disorders

Detecting damaged axons

Silver stains

Immunocyochemical staining for _-APP or non phosphorilated neurofilaments

MRI spectroscopy

Axonal damage in CNS disorders

Head trauma

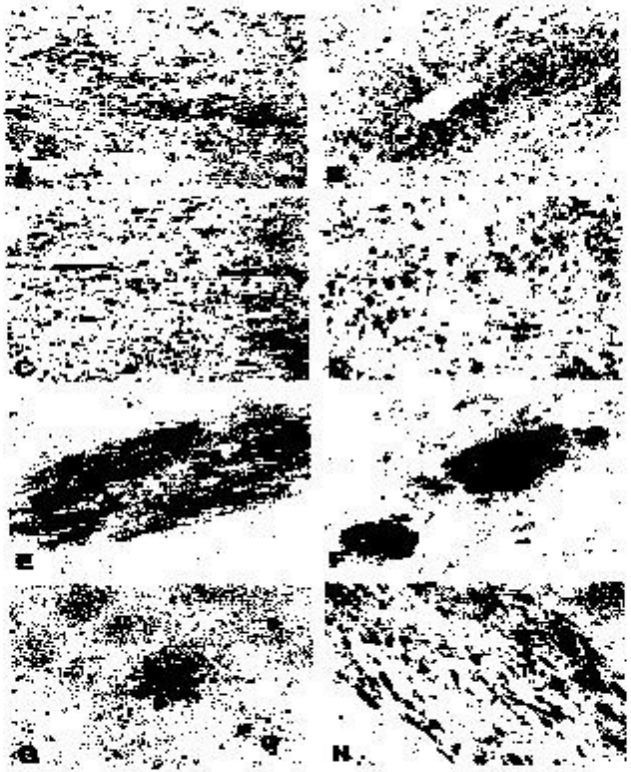
- Damage to axons is almost an universal finding in head trauma
- Extensive axonal damage is found in the brainstem of persistent vegetative state.
- Axonal damage is a constant finding in severe head trauma
- Axonal damage may underlie cognitive disturbances following head trauma

Axonal damage in CNS disorders

Spinal cord injuries

Secondary injury mechanisms may be relevant

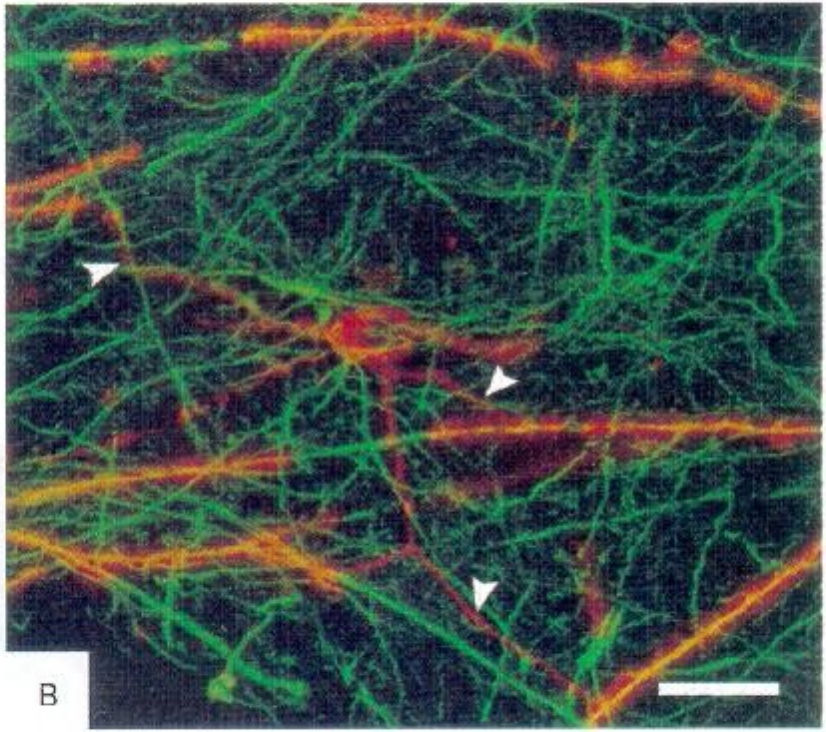
Brain sections stained for APP



Medana and Esiri 2003

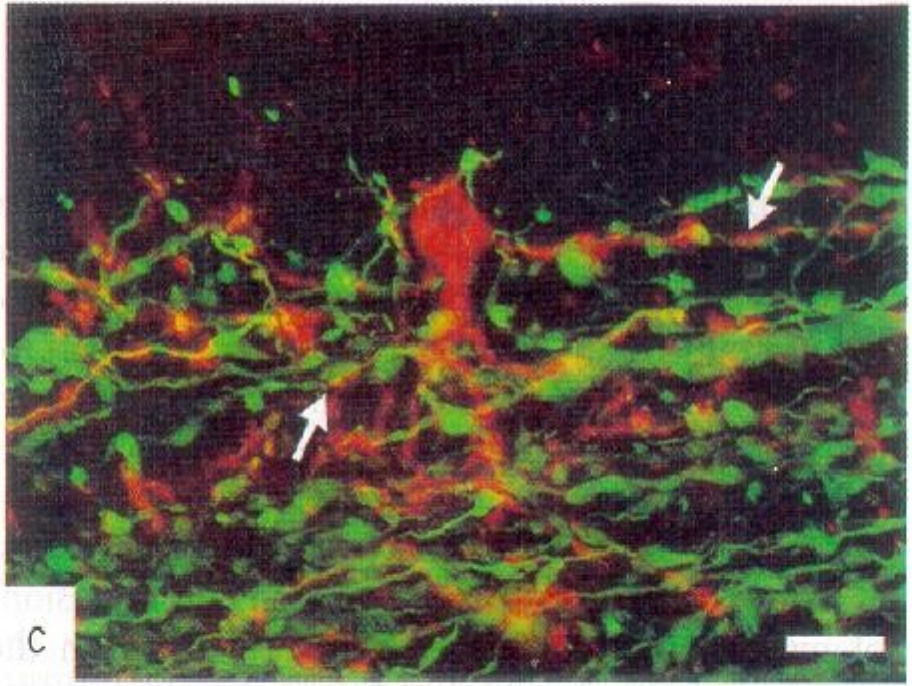
Axonal damage is the cause of permanent disability in demyelinating disorders of CNS

Remyelination in a shadow plaque



Chang et al NEJM 2002

Chronic MS lesion: oligodendrocytes spirale around dystrophic axons



Chang et al NEJM 2002

Axonal damage in demyelinating diseases of the CNS can be due to

1) acute inflammation

2) chronic degeneration of axons devoid of myelin

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Restricted immune responses lead to CNS demyelination and axonal damage

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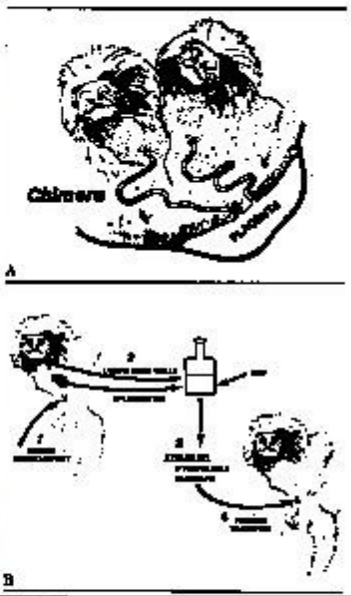
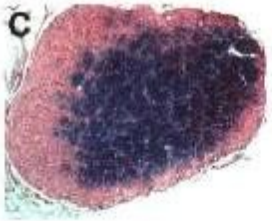
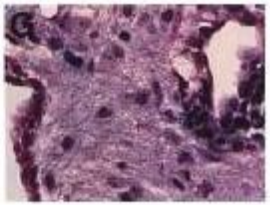
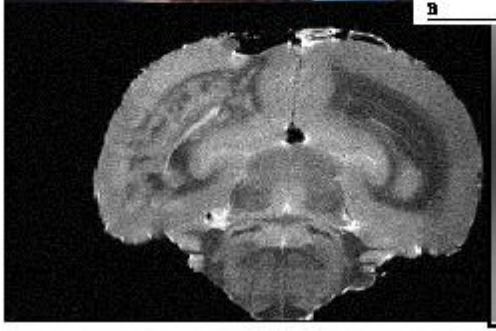
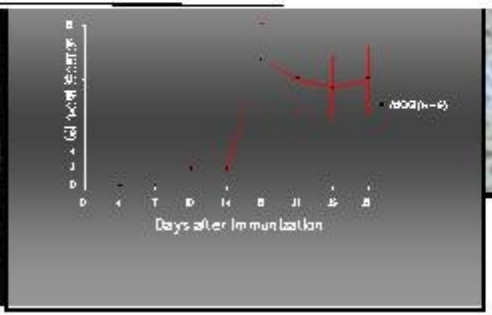
EAE IN THE MARMOSET

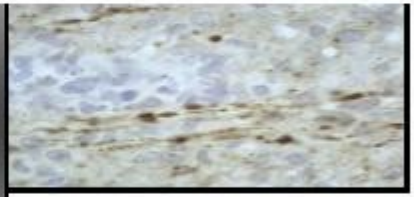




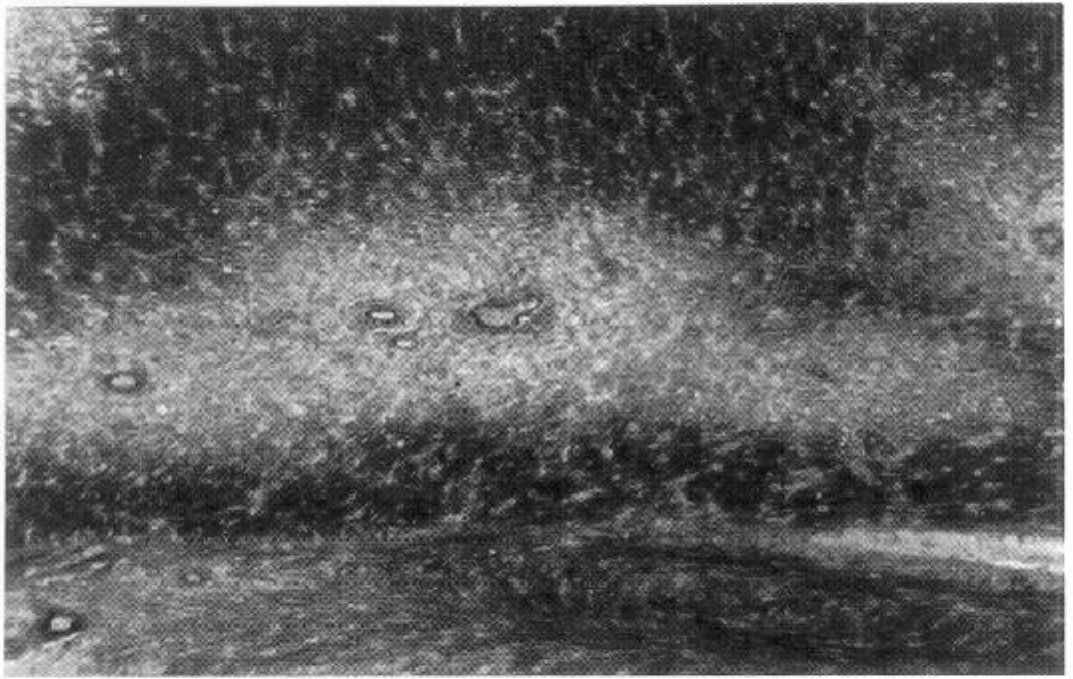






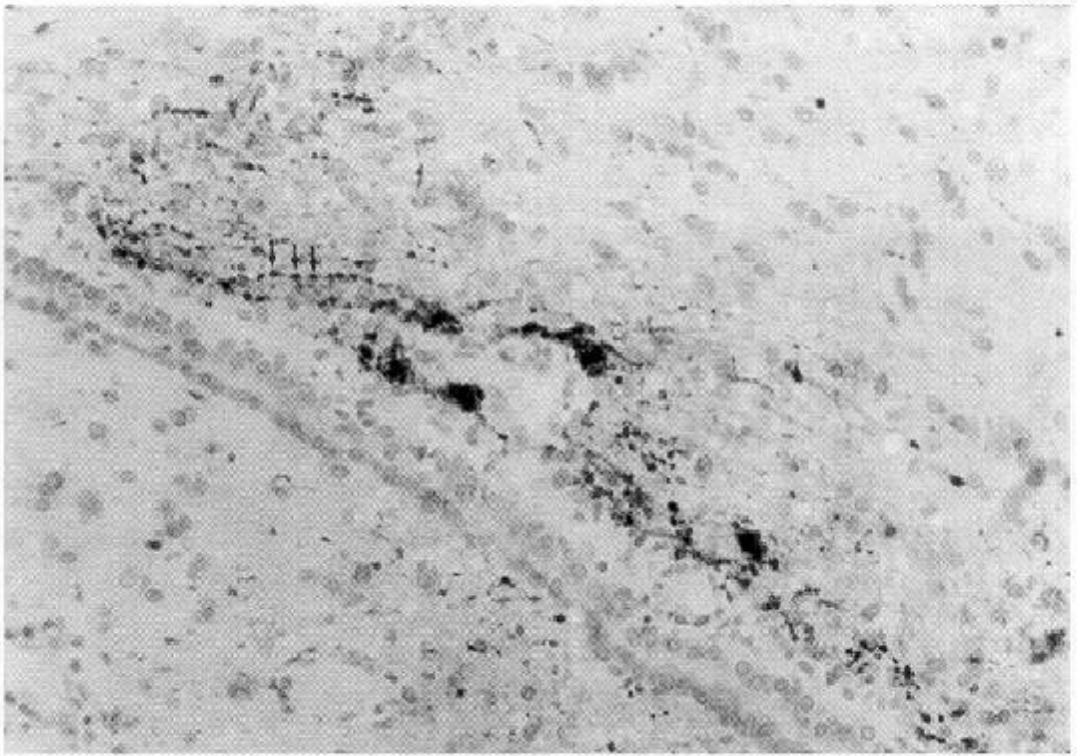


Early active demyelinating lesion in the marmoset



Mancardi et al J Neuroimmunol 2000

APP positive axons in early active demyelinating lesion in the marmoset



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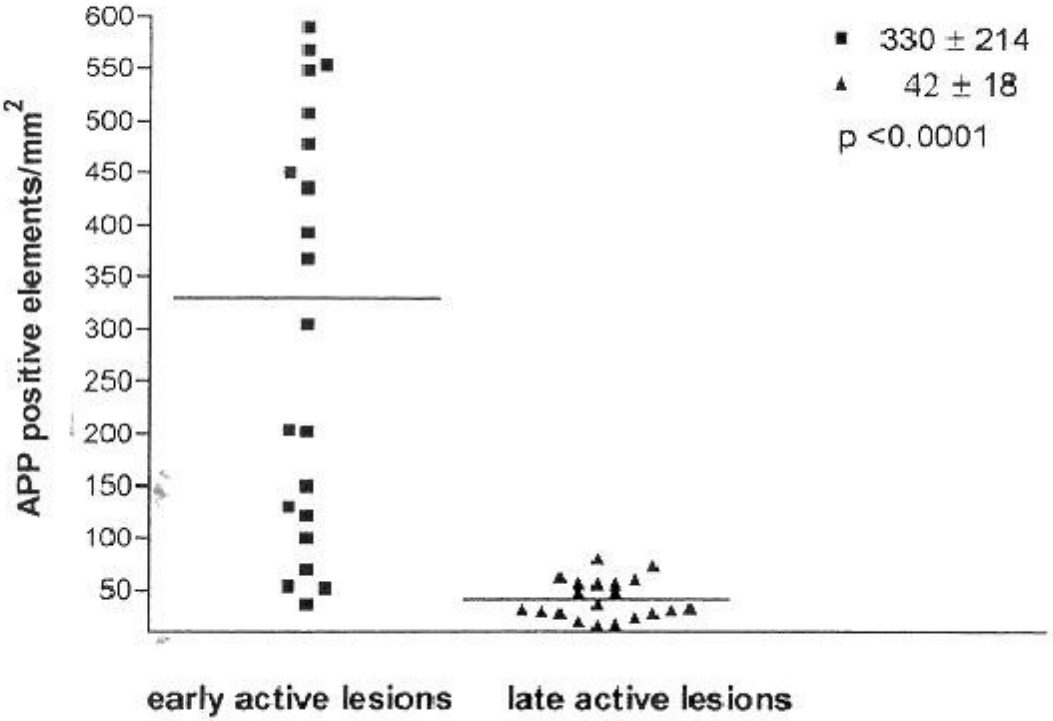
Number of APP positive axons /mm3



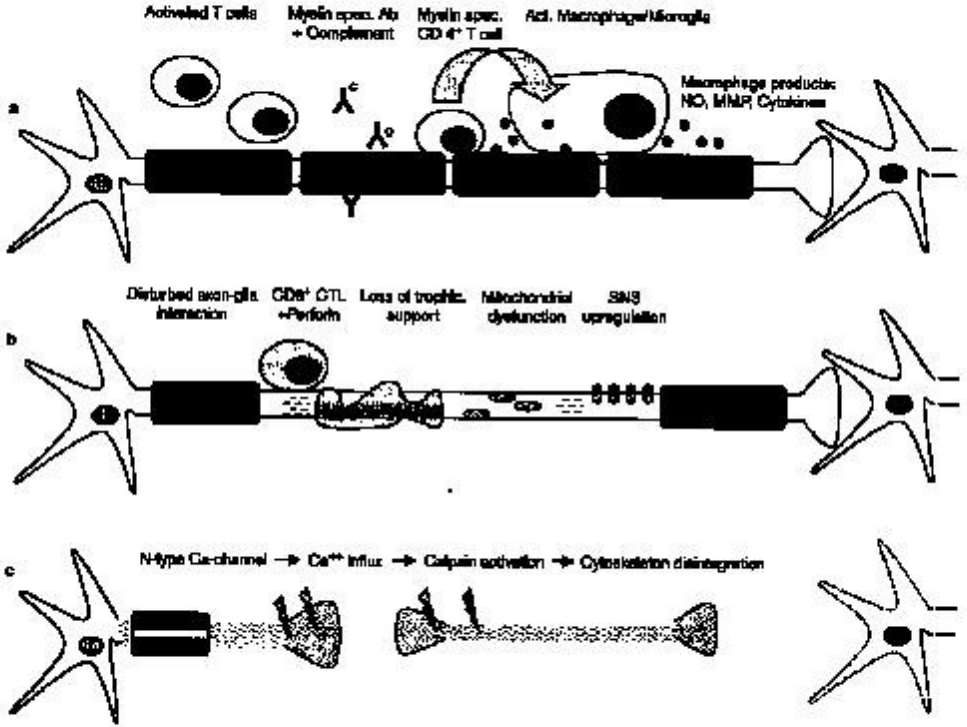
Mancardi et al J Neuroimmunol 2000

Inflammatory mediated damage to axons

Macrophage, microglia and CD8 T cells

- Glutamate
- NO
- Activated T cells
- Complement
- Metalloproteinases
- Cytokines (TNFalfa)
- Accumulation of neuronal type voltage gated calcium channels
- SNS channels upregulation

Inflammatory mediated axonal damage



Rieckmann and Maurer 2002

Inflammatory mediated damage to axons

Acute axonal damage in MS is most extensive in early disease stages and decreases over time

(Kuhlmann et al 2002)

Potential strategies for reduction inflammatory axonal injury

Inflammatory mediated axonal damage	Potential treatment
Immune cell traffic across the blood–brain barrier	Natalizumab, CCR-1 antagonist
Impaired axon–glial interaction	Neurotrophic factor (e.g. CNTF), restorative approaches (Schwann cell transplantation)
Macrophage/microglia products	
Proinflammatory cytokines (e.g. TNF-α)	Glucocorticosteroids, interferon β, phosphodiesterase IV inhibitors, TNFR p55 blockade
Nitric oxide	Nitric oxide synthase inhibitor, phosphodiesterase IV inhibitors
Metalloproteinases	Specific matrix metalloproteinase inhibitors
Calcium-mediated axolemma disintegration	Calcium antagonists, oestrogen, progesterone, cannabinoids, tacrolimus (FK506)
SNS channel upregulation	Sodium channel blocker
T-helper-1 phenotype	Glatiramer acetate, interferon β
CD8-mediated CTL	Specific neutralization (anti-CD8 antibodies), blockade of perforin

CCR, chemokine receptor; CNTF, ciliary neurotrophic factor; CTL, cytotoxic T lymphocyte; SNS, sensory neurone-specific sodium channel; TNF(R), tumour necrosis factor (receptor).

Rieckmann and Maurer 2002

Axonal damage and axonal loss

There are evidences of axonal loss in NAWM and in the cervical spinal cord of MS cases (Evangelou et al 2000; Bjartmar et al 2000; Wujek et al 2002).

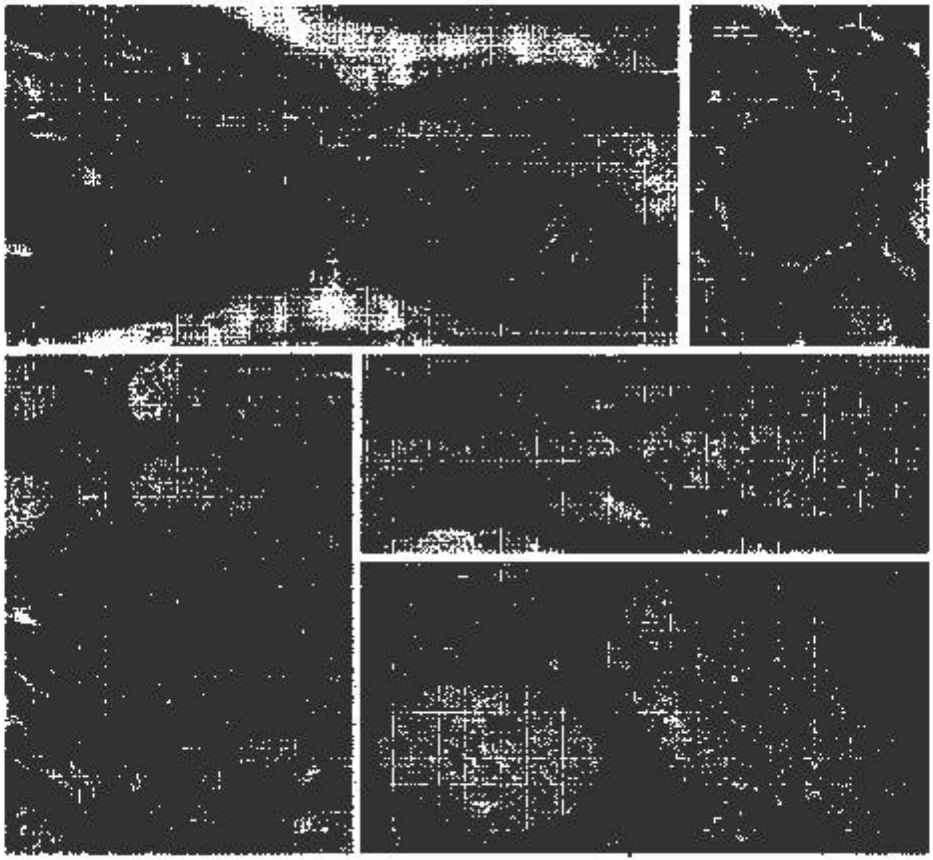
Glial cell-neuron interactions

Axonal damage can be due to a chronic process of axonal sufferance secondary to the loss of myelin

Axonal damage in mice lacking MAG or PLP

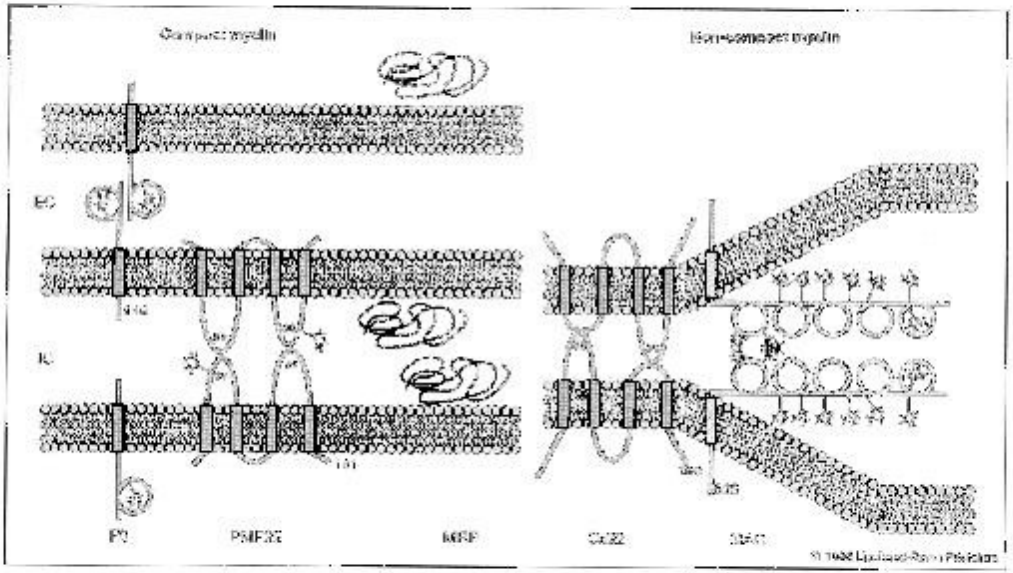
Axonal damage and degeneration is a consequence of chronic demyelination

Axonal pathology in myelin disorders



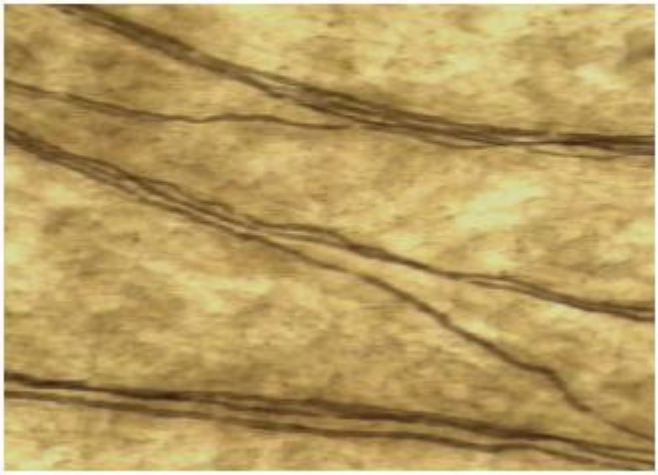
Bjartmar et al 1999

Myelin proteins of the PNS

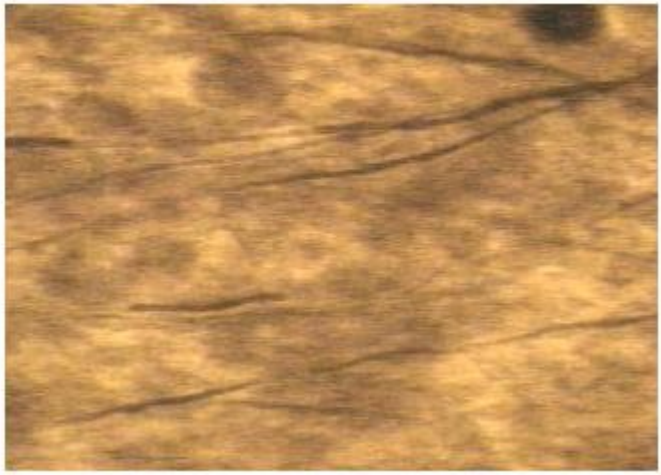


From Steck et al 1998

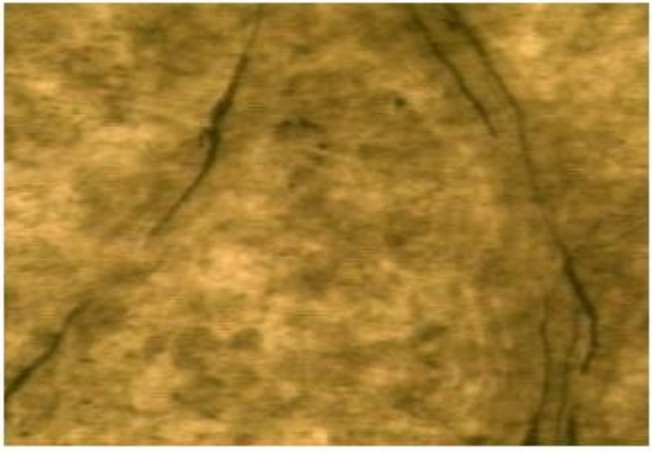
Normal DRG culture. 250x



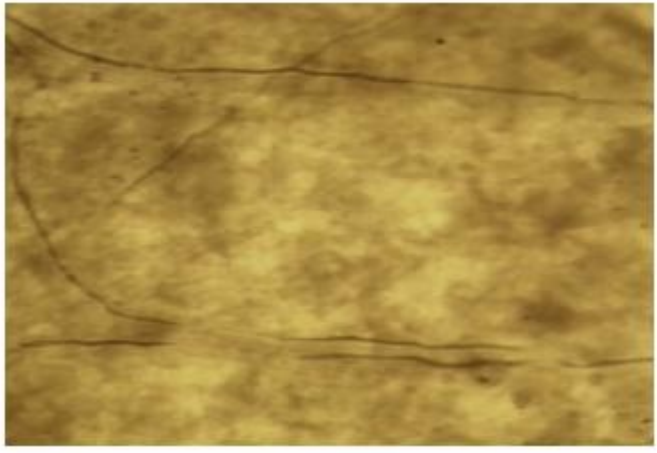
PMP22-transgenic DRG culture. 250x



PMP22-transgenic DRG culture.250x

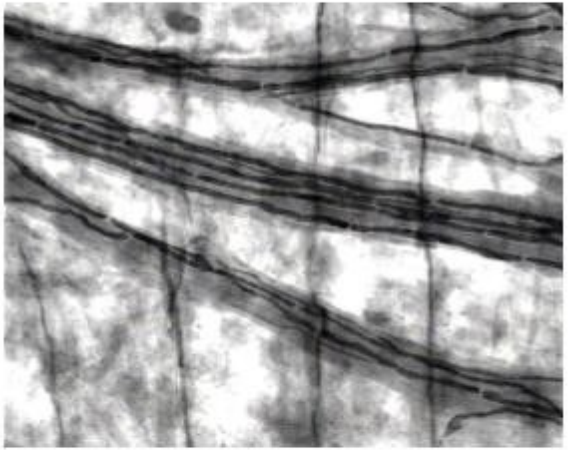


PMP22-transgenic DRG culture. 250x

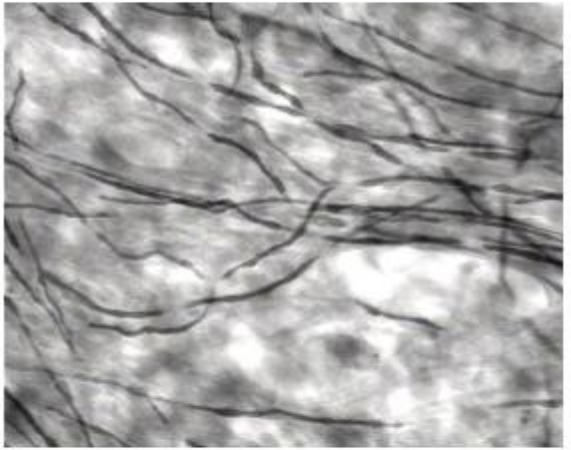


Organotypic DRG cultures

Three months old

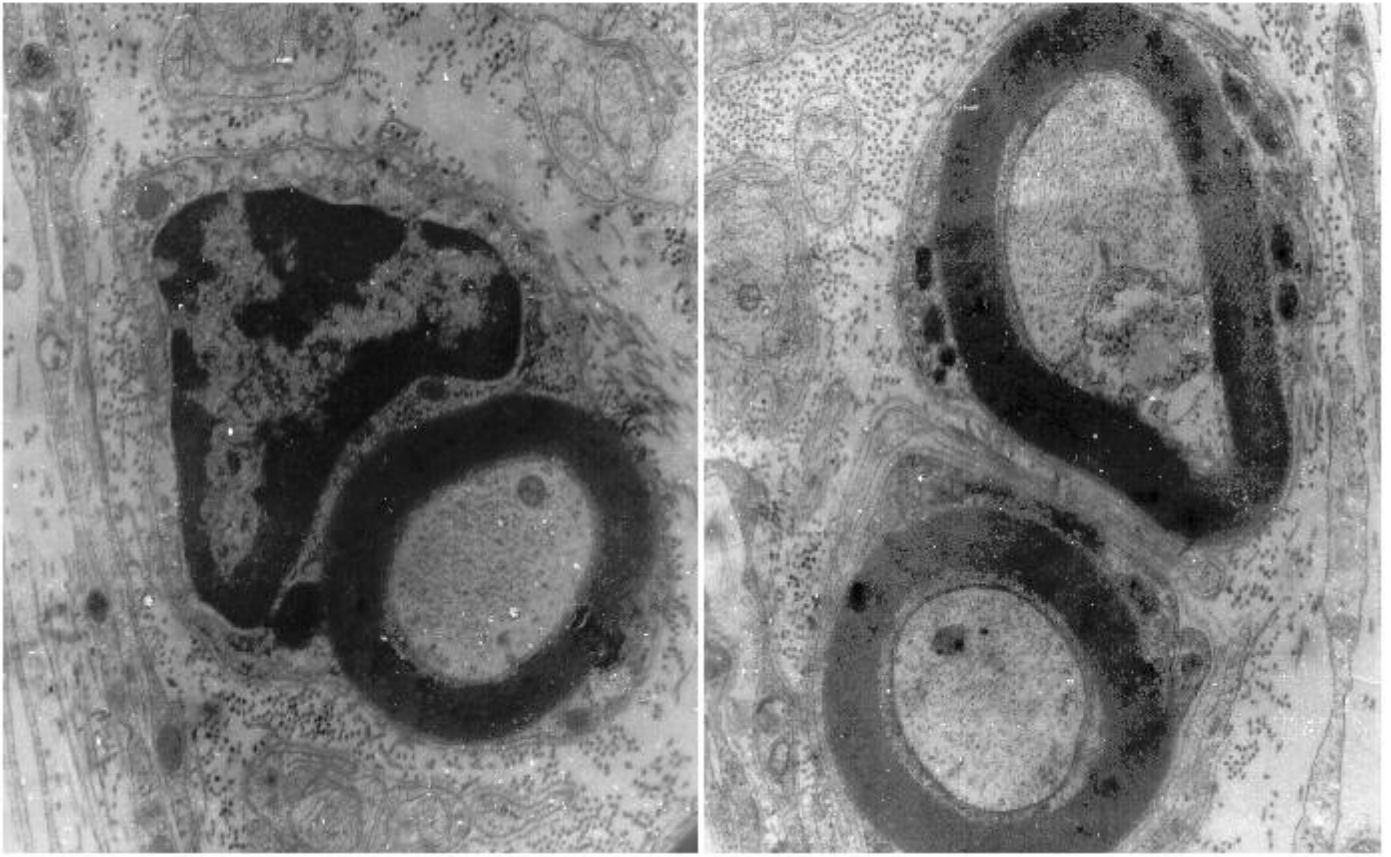


Normal control

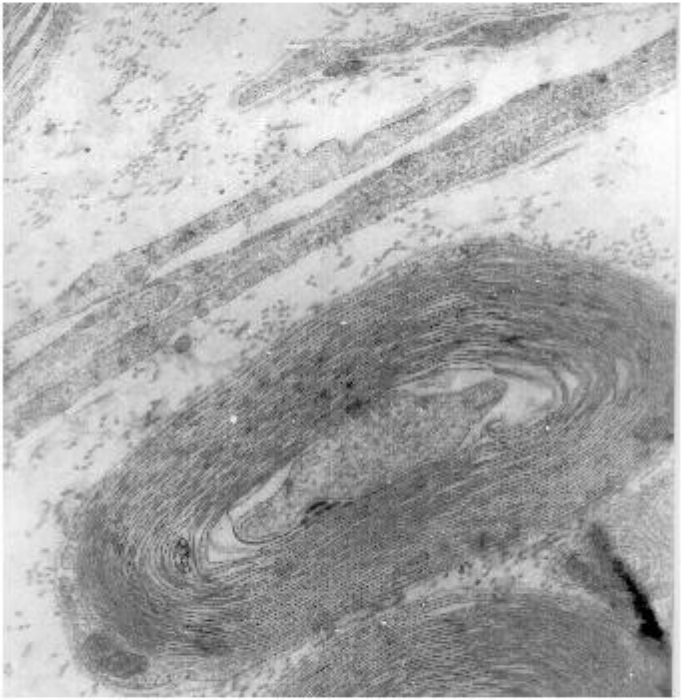


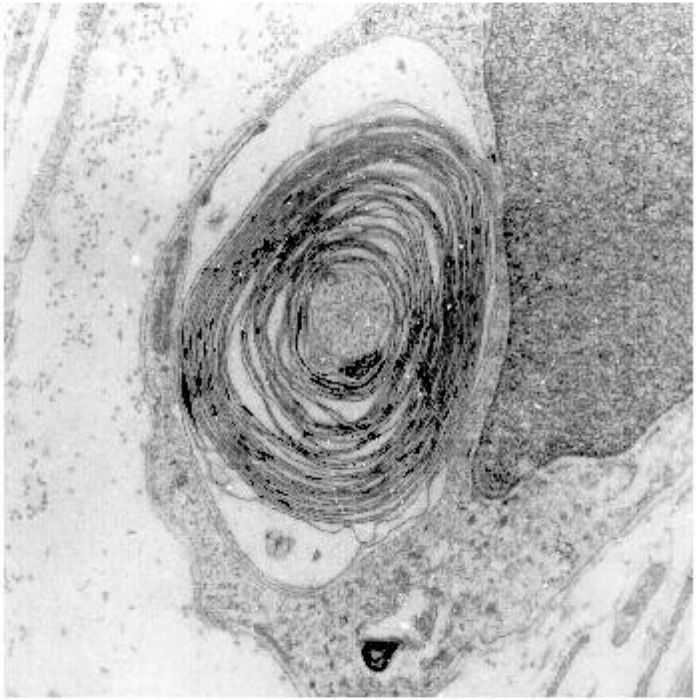
Transgenic cultures

DRG cultures: normal control

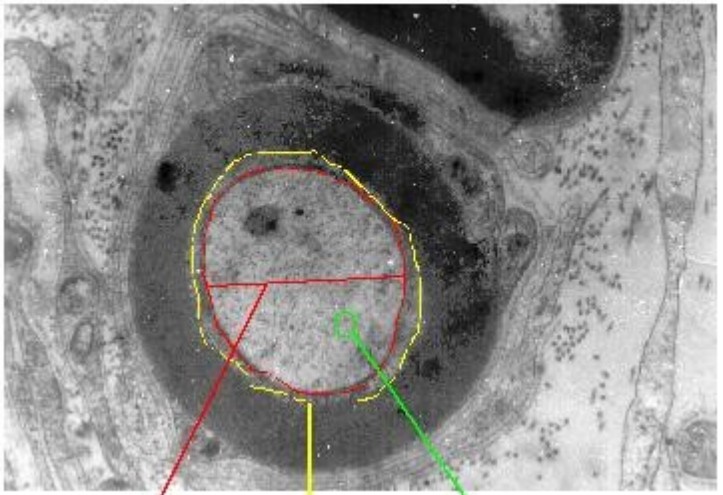


DRG cultures: transgenic rats

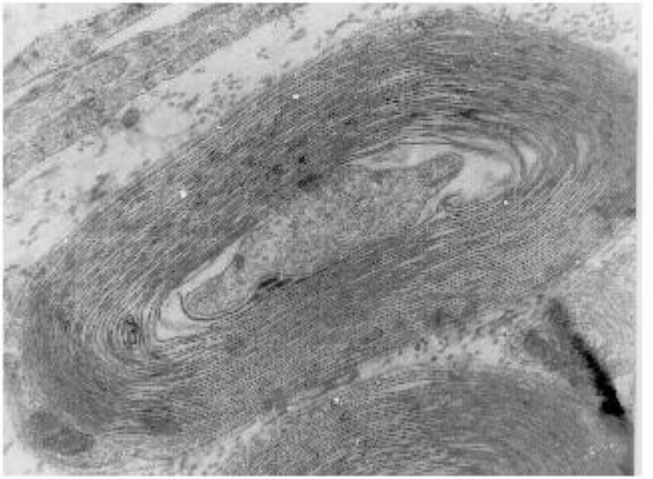




Wild type



CMT1A heterozygous

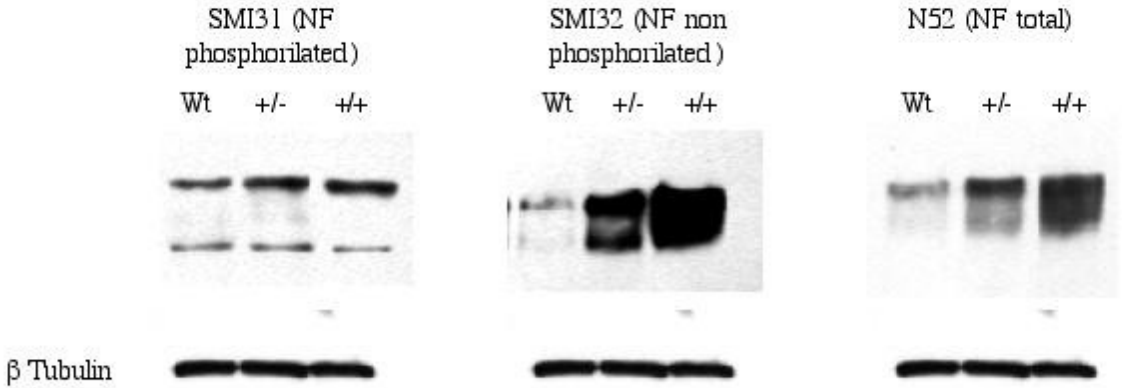


Axon Diameter *	Periaxonal Area	NF Density
1.17 μm	0.08 μm	684.7 μm2

Axon Diameter *	Periaxonal Area	NF Density
0.89 μm	0.13 μm	766.9 μm2

* $P < 0.001$

Western blot of phosphorilated and non phosphorilated neurofilaments in long term (3 months) DRG cultures



A model of axonal damage following a primary demyelinating process has been established.

The mechanisms of axonal damage (and eventually the treatment strategies) still need to be established

Axonal damage following demyelination can be due to different mechanisms such as:

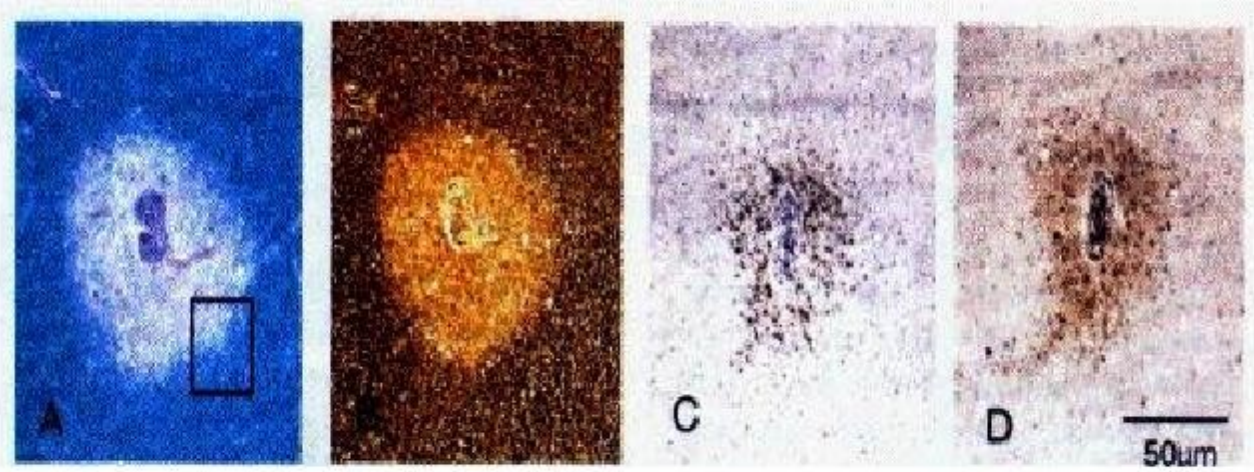
- Ectopic distribution of calcium channels, with increased calcium influx and axonal degeneration via activation of neutral proteases

- Enhanced expression of Na 1.8 channels and aberrant electrical activity in neurons.

- Abnormal expression of myelin proteins at Schwann-axon interface such as MAG

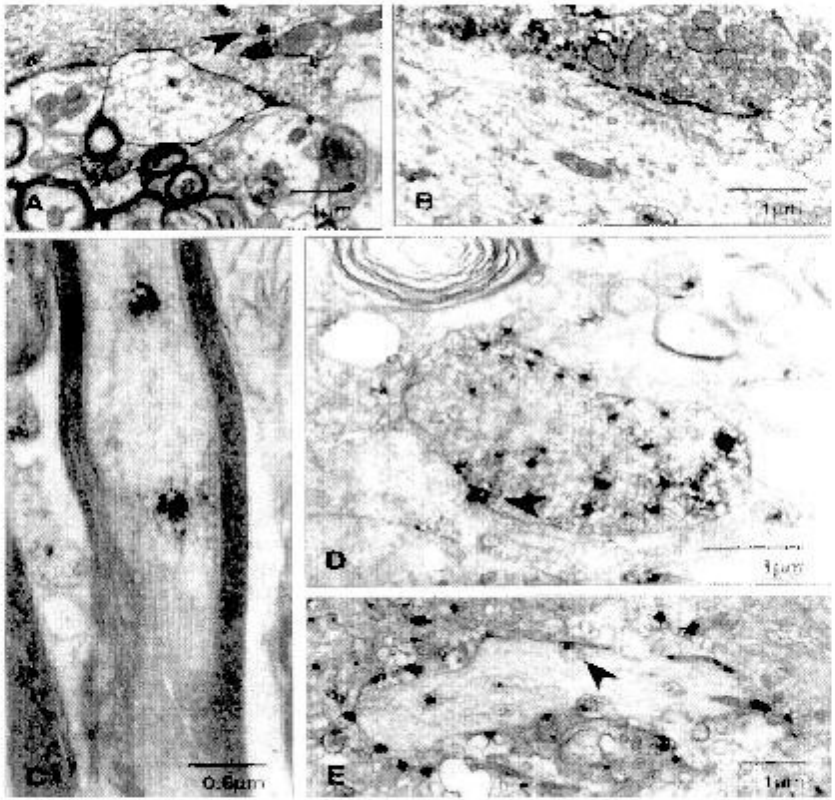
- Deficiency of neurotrophic factors

Distribution of a calcium chanel subunit in EAE and MS



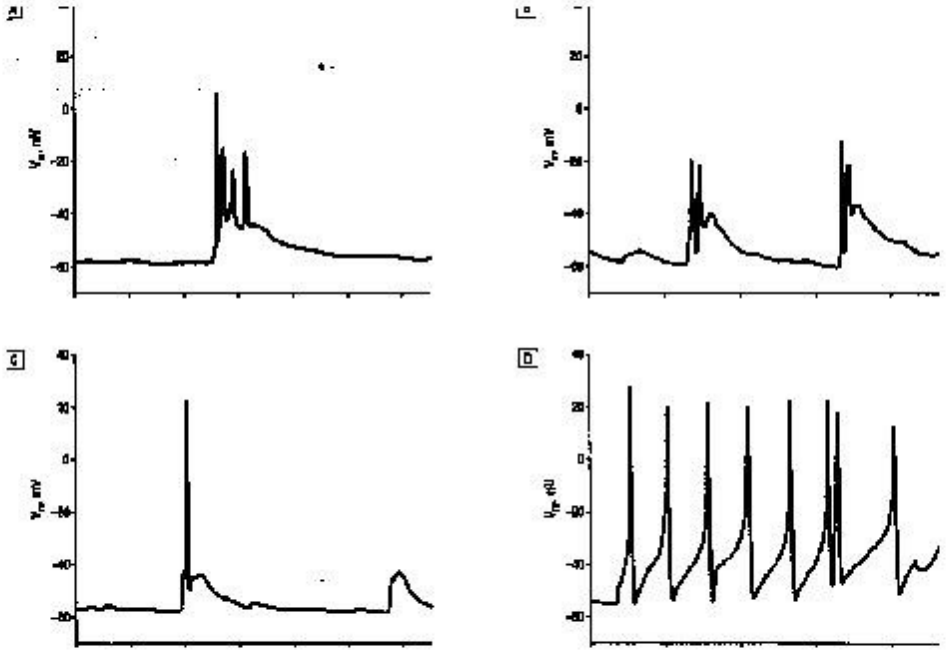
Komek et al 2001

Distribution of a calcium chanel subunit in EAE and MS



Kornek et al 2001

Expression of Na1.8 sodium channels perturbs signalling in cultured Purkinje cells

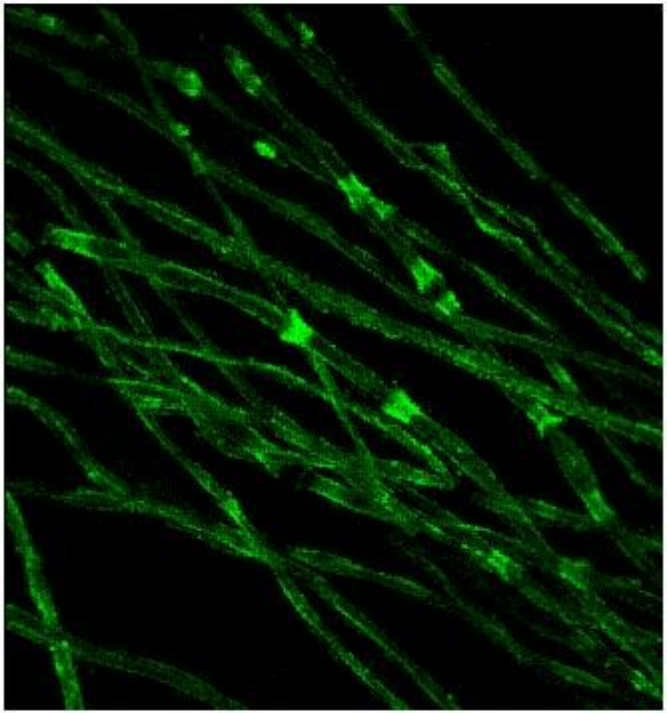


Waxman 2002

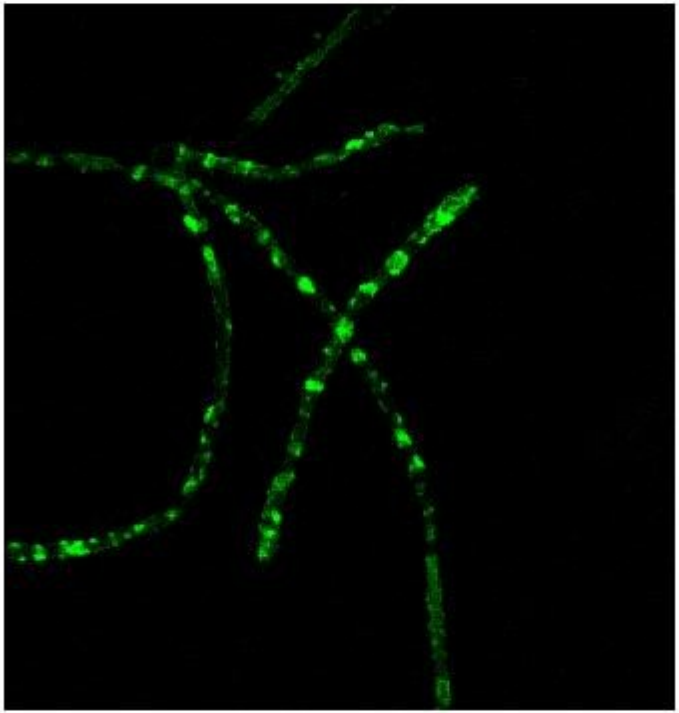
Inflammatory mediated damage to axons

Blockers of Sodium and Calcium entry protect axons from NO mediated degeneration (Kapoor et al 2003)

Confocal Microscopy

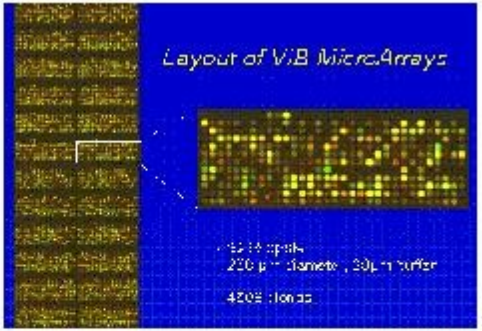


MAG: Normal DRG cultures

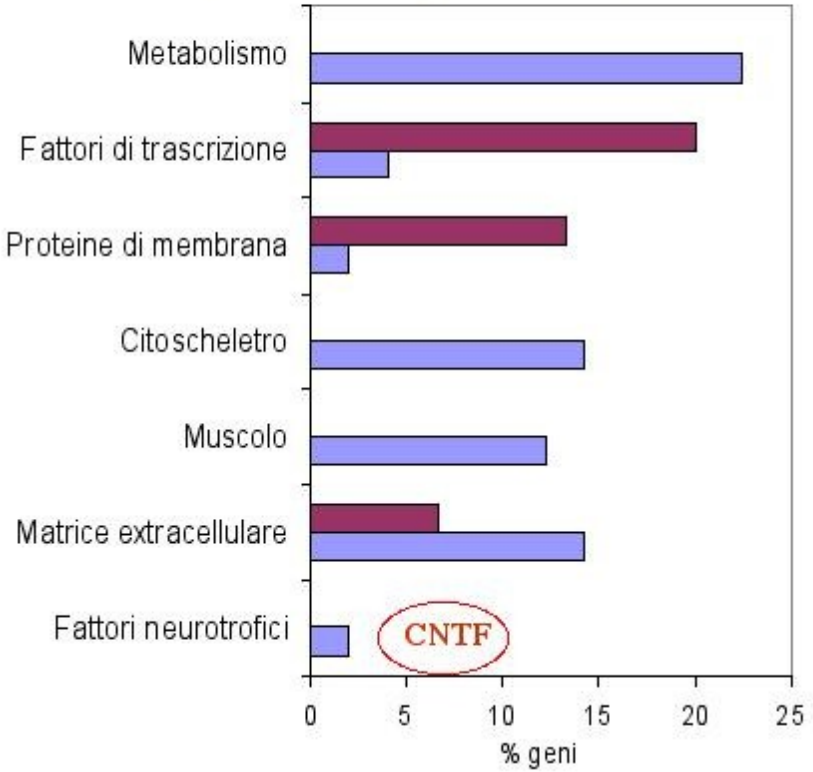


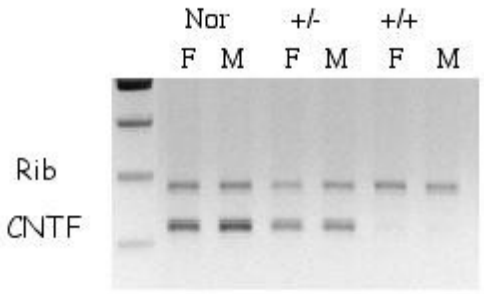
MAG: Transgenic DRG cultures

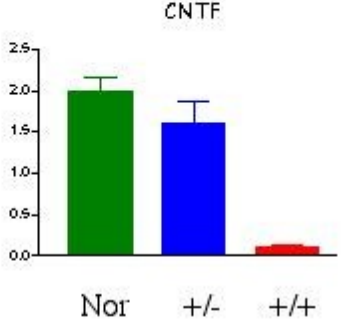
cDNA microarrays



Sequenze up-regolate 18 geni - 20 EST
Sequenze down-regolate 62 geni - 33 EST







Axonal damage and axonal loss in CNS disorders

- Is due to acute events, such as head or spinal injury or inflammation
- May be also due to a chronic degeneration following demyelination or a previous CNS hit

Axonal damage

Axonal damage and demyelination are related processes

Axonal damage is an early event, not necessarely related to demyelination or inflammation

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