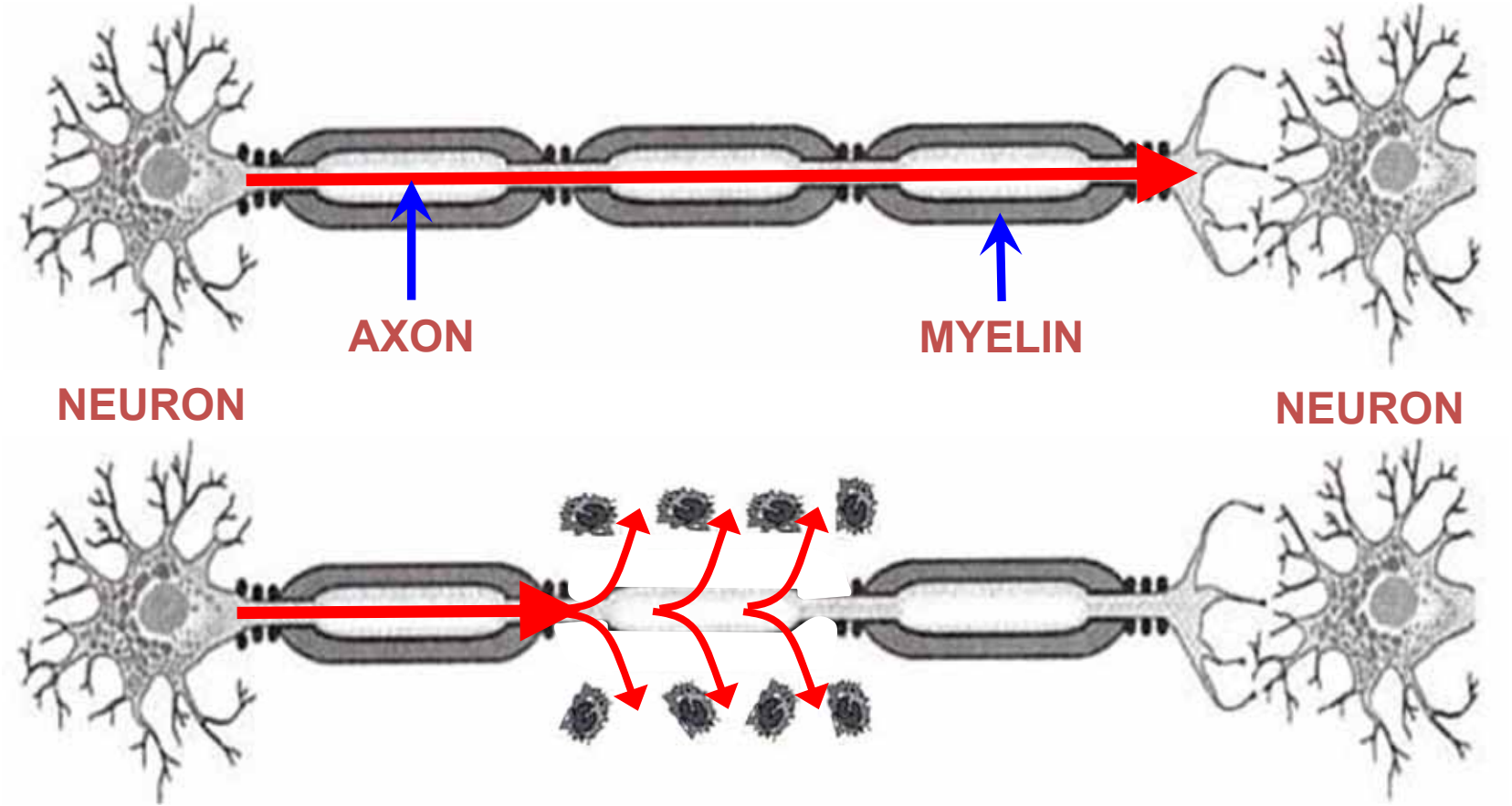
Neurodegeneration and Remyelination in MS

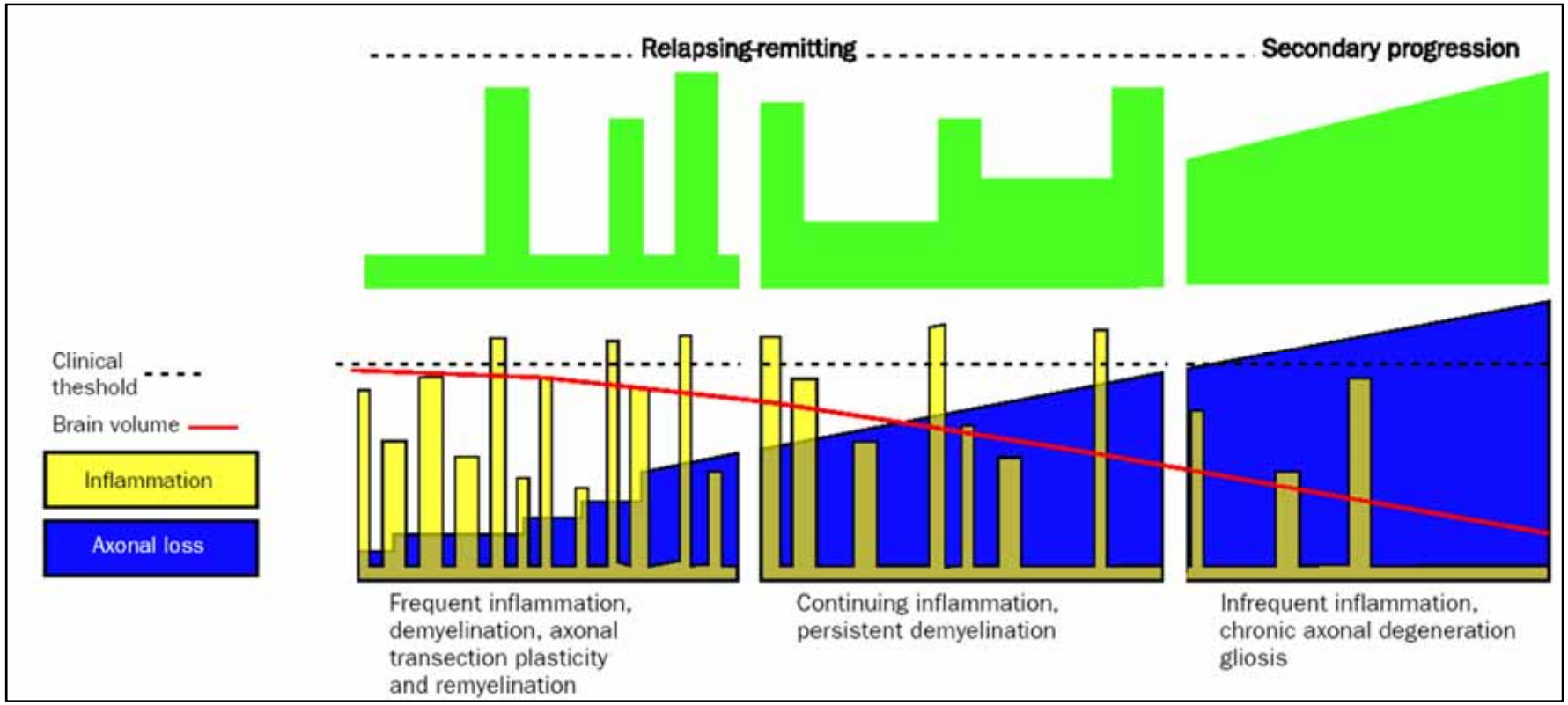
- **Axon loss**
- **Neurodegeneration**
- **Atrophy**
- **Incidence of remyelination in MS**
- **Mechanisms of remyelination**

Prof R Reynolds 2011

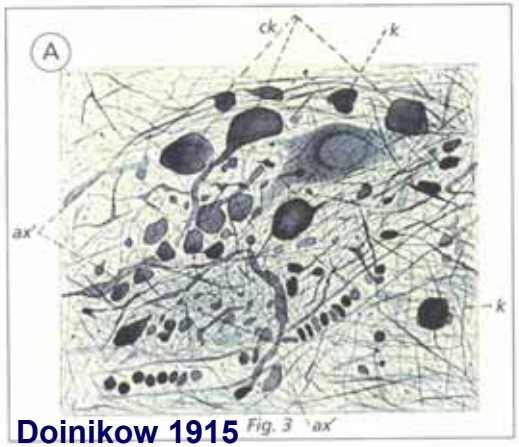
Multiple Sclerosis is an inflammatory demyelinating disease of the CNS



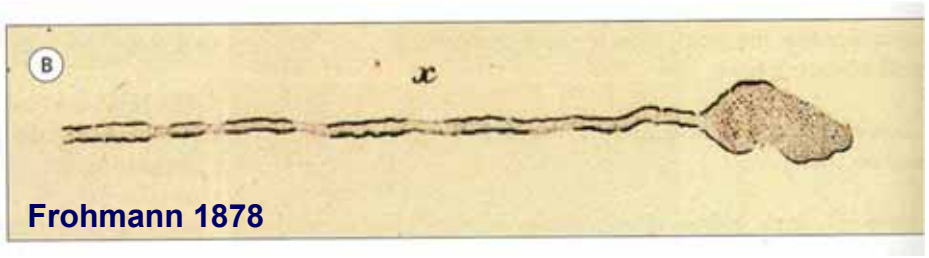
It is now thought that axonal loss is the major pathological correlate of the progressive irreversible neurological deficit in chronic MS



Axon Loss in Multiple Sclerosis



Doinikow 1915

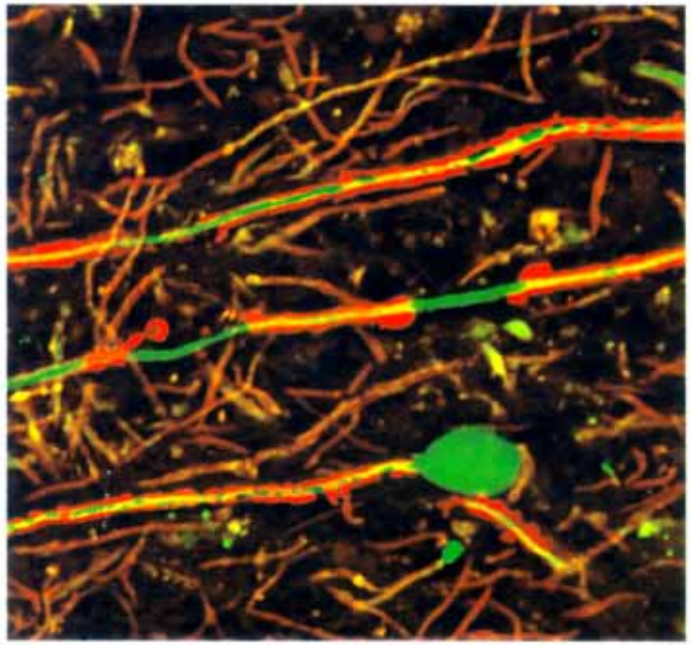


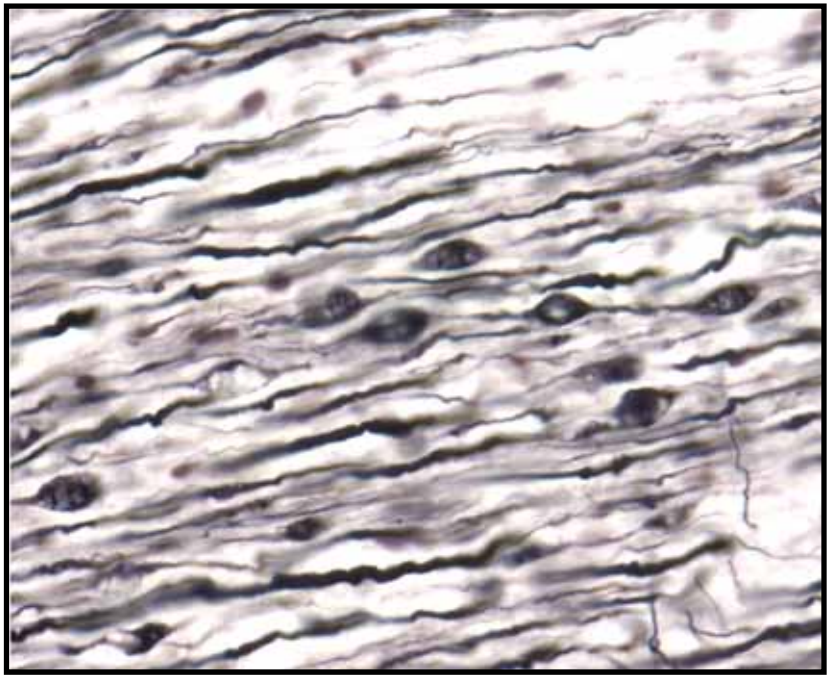
Frohmann 1878

•early studies noted axon damage and their relative but not absolute preservation.

- axonal loss is present in all MS lesions, ranging from 20% to nearly 100% loss
- 65% loss in cervical cord lesions of SP MS
- larger axons better preserved than smaller ones

Axonal damage in MS

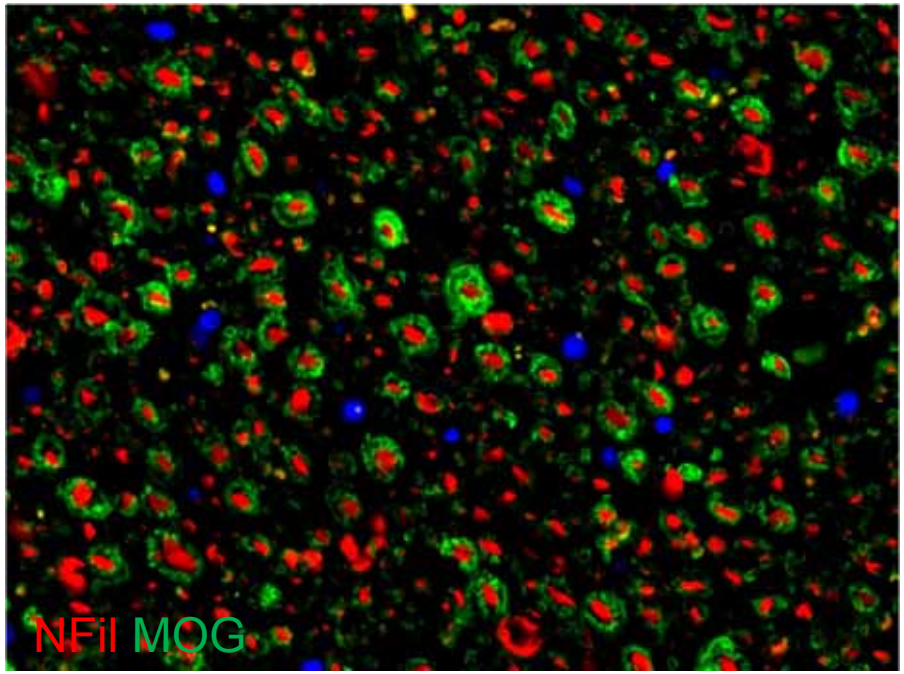




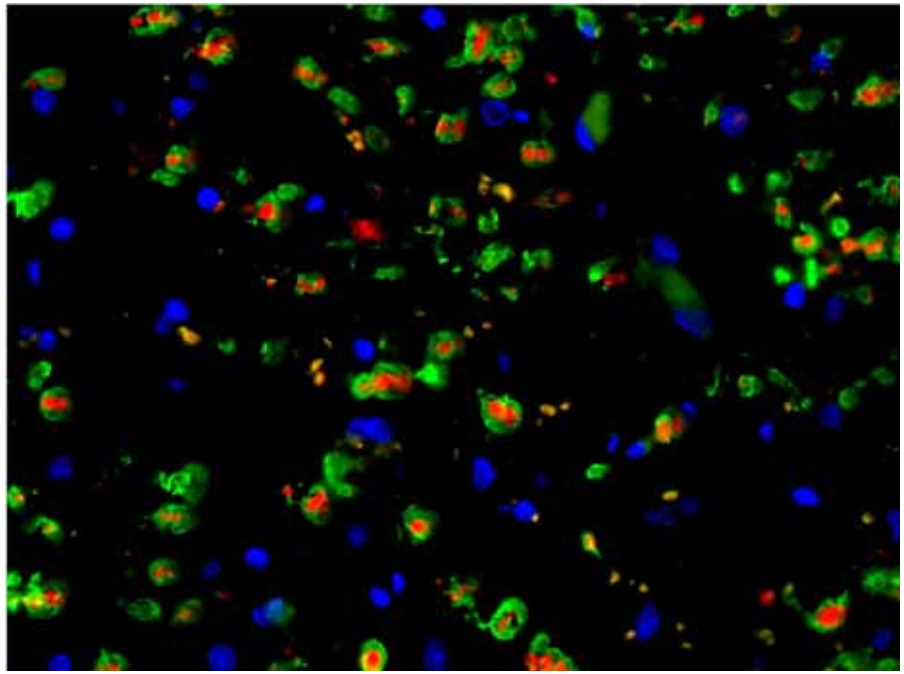
Kuhlmann et al (2002)

- axon loss occurs during early inflammatory attacks and continues throughout the course of MS
- extensive axon and neurite damage and neuronal loss seen in grey matter MS lesions
- not much evidence of direct immune attack to date

Spinal cord axon loss in MS



Control

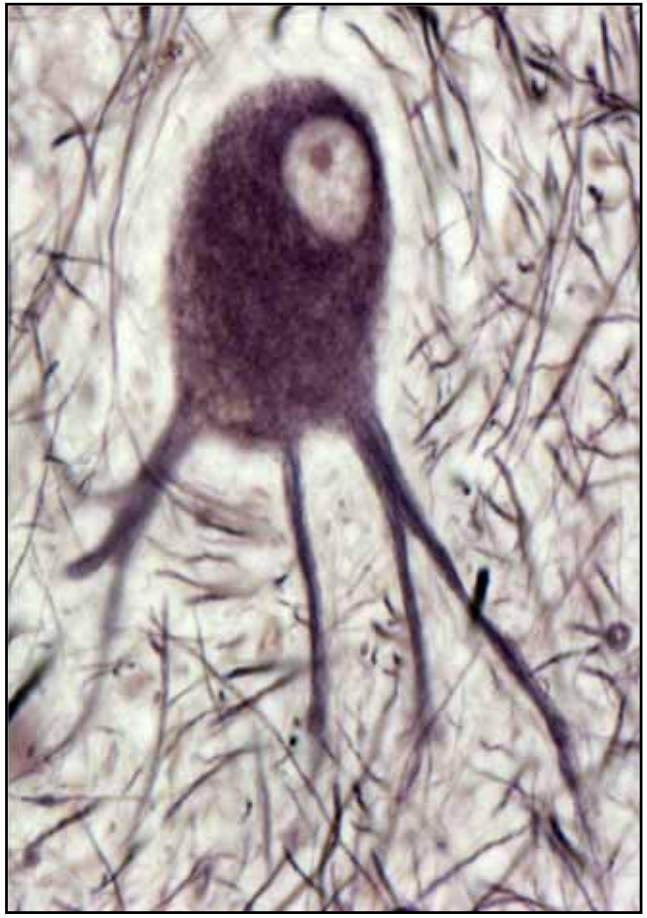


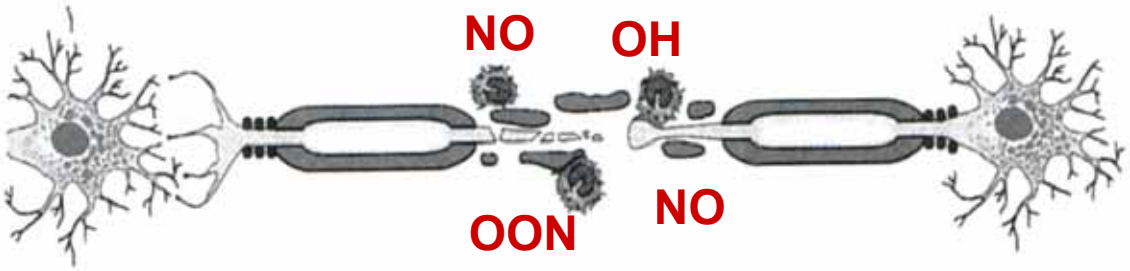
MS

Axon degeneration in MS

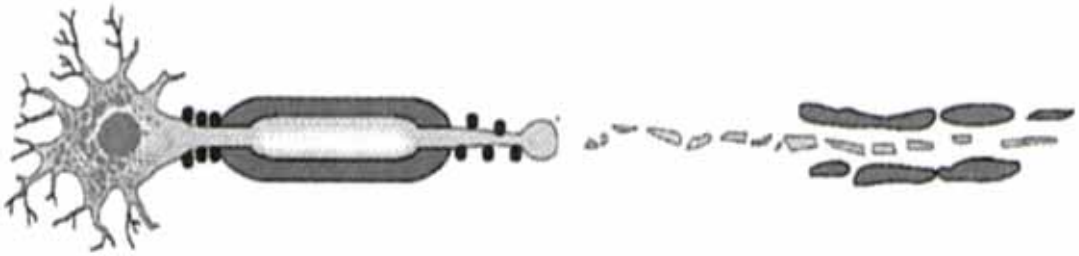
- Direct or bystander immune-mediated attack...TNFa or Fas ligand
- Energy deficiency: inherent mitochondrial defects or due to damage by inflammatory mileu (e.g free radicals)
- Glutamate excitotoxicity
- Antibodies to neurofascin, a component of the node/paranode (Mathy et al, 2007)
- **Na^+ and $Ca2^+$ overloading in electrically active/ energy depleted axons** (Trapp and Stys, 2009)
- Detected indirectly in patients by MRS for N-acetyl-aspartate (NAA). Reduction in NAA level correlates with increasing disability.

Neurodegeneration leads to chronic progression





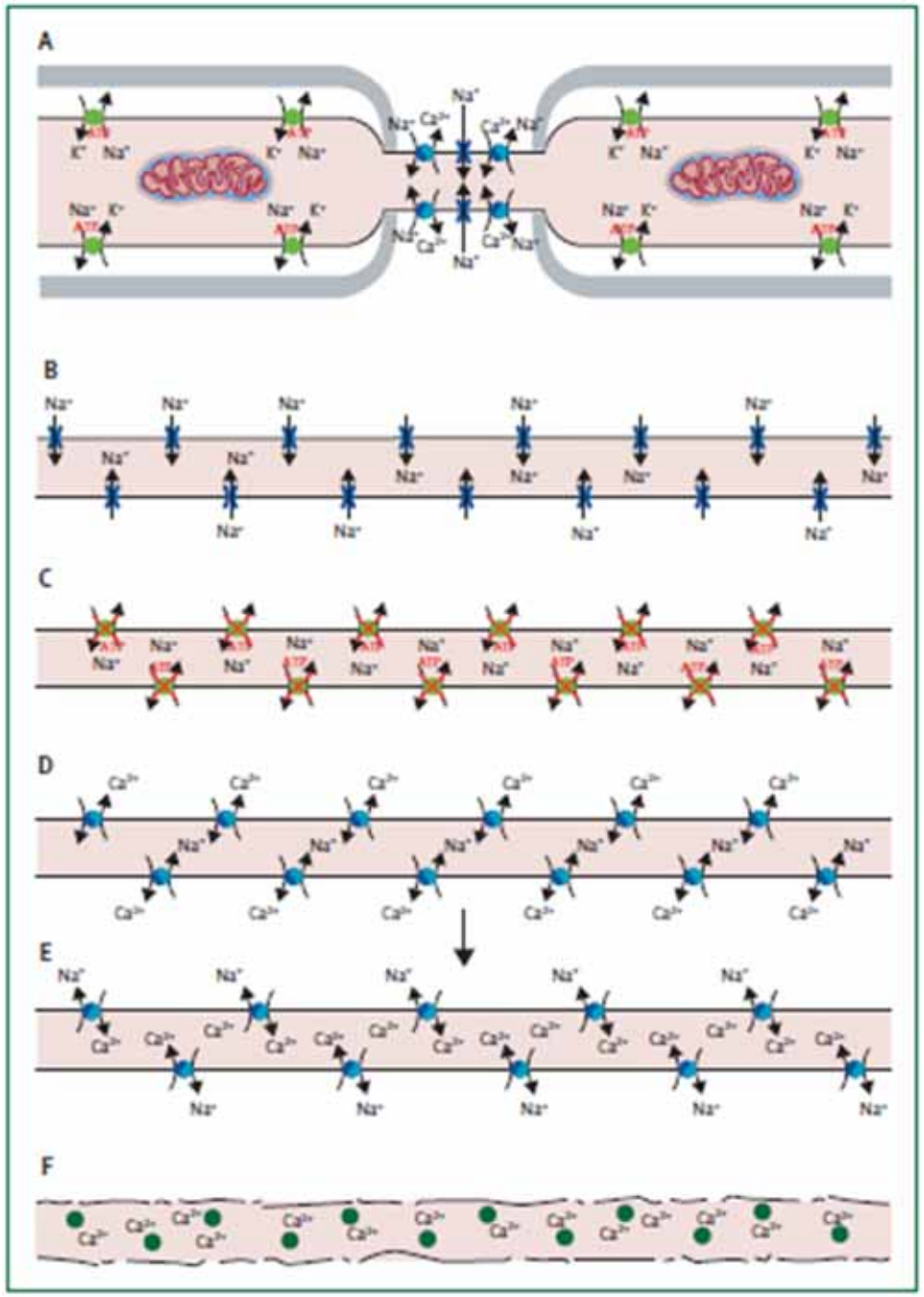
Inflammatory cells release free radicals and cytotoxic mediators



A build up of damage kills the neuron

This is irreversible but there is spare capacity

Neuroprotective treatments should stop progression



Chronically demyelinated axons degenerate due to ionic imbalance and calcium influx

(A) In normal myelinated axons, action potentials are propagated through the opening of Na^+ channels at the node. Na+ also enters through $Na^+/Ca2^+$ exchangers, which passively trade Na^+ entry for Ca2+ removal. Na^+ entry is rebalanced by removal through internodal Na^+/K^+ ATPase, which uses ATP produced by axonal mitochondria to pump Na^+ out in exchange for K^+.

(B) Na^+ channels are diffusively distributed along demyelinated axons resulting in increased Na^+ influx during impulse transmission and increased ATP demand for operating Na^+/K^+ ATPase pumps.

(C) Alterations in ATP production reduce Na^+/K^+ exchange capacity and increase axonal accumulation of Na+.

(D) Increased axonal Na^+ reverses the $Na^+/Ca2^+$ exchanger, and increases axonal $Ca2^+$

(E) Increased Ca2+ activates proteolytic enzymes, leading to damage of axoplasmic contents (F) and
eventually to axonal death.

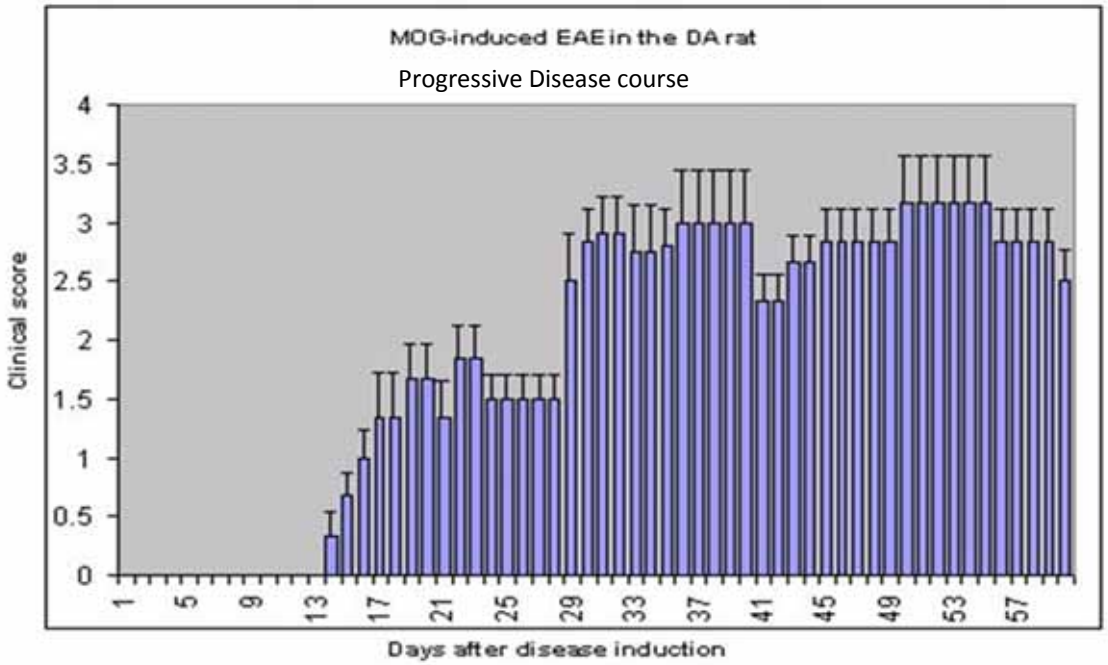
Trapp & Stys, Lancet Neurol 2009.

Does axonal loss resulting from inflammation/demyelination produce clinical progression in models of MS ?

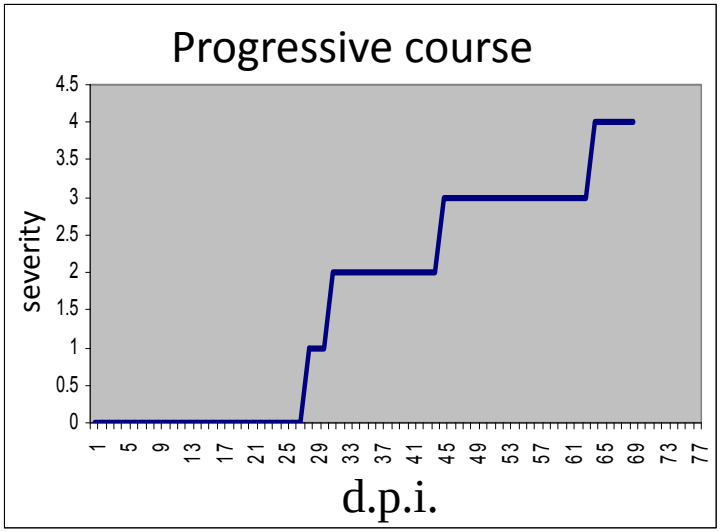
MOG-EAE in the DA rat

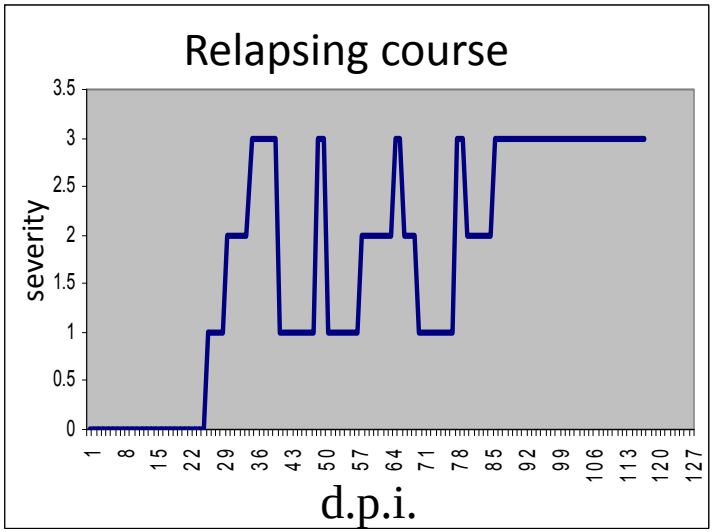
Induction of EAE

- Adult female DA rats.
- Recombinant mouse MOG (100μg) N terminal extracellular domain (1-116a.a.) in IFA.
- Single SC injection in the dorsal aspect of the base of the tail.

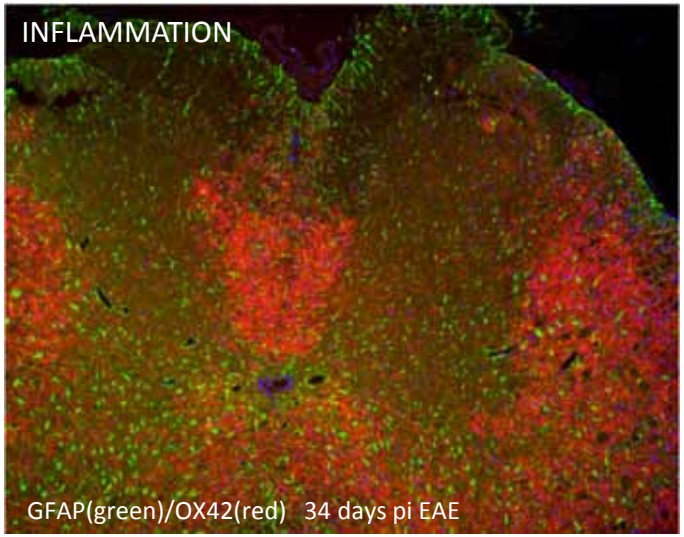


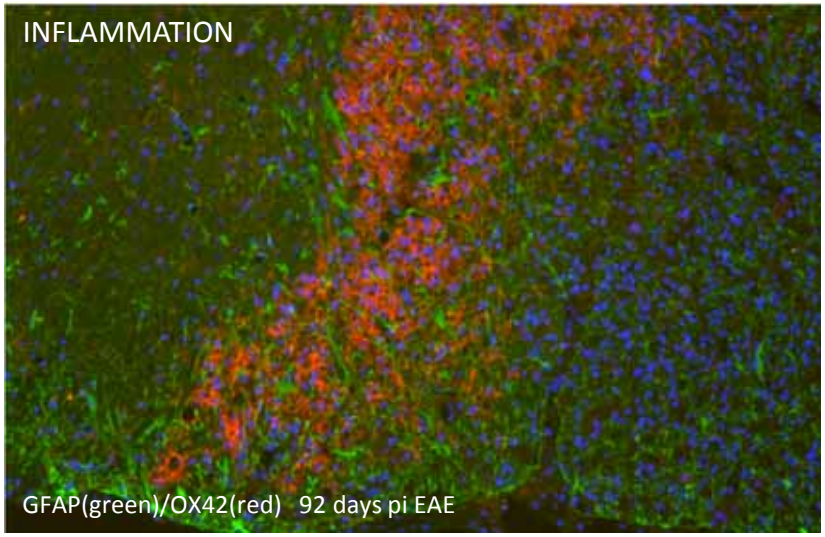
Disease courses

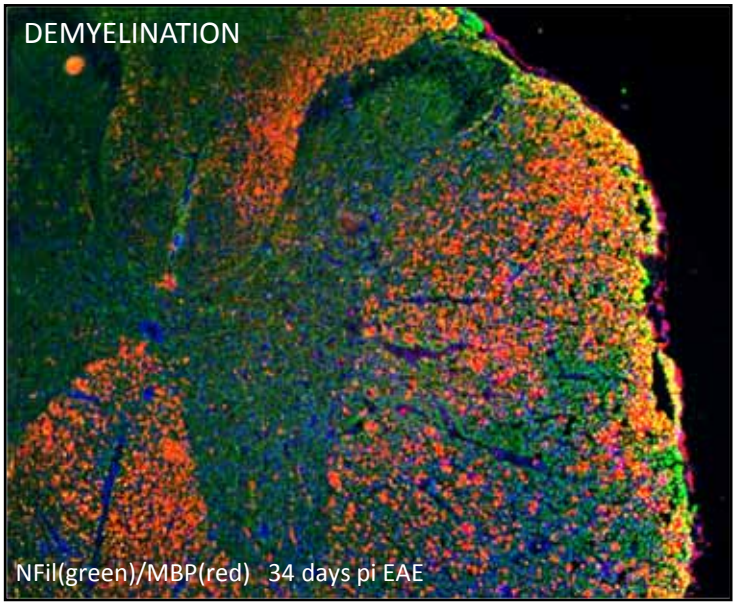


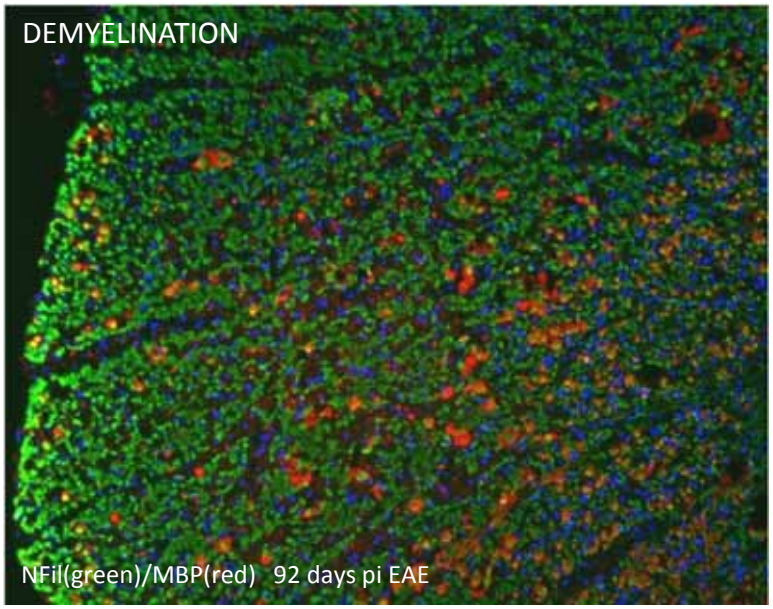


Inflammation & demyelination in MOG-EAE

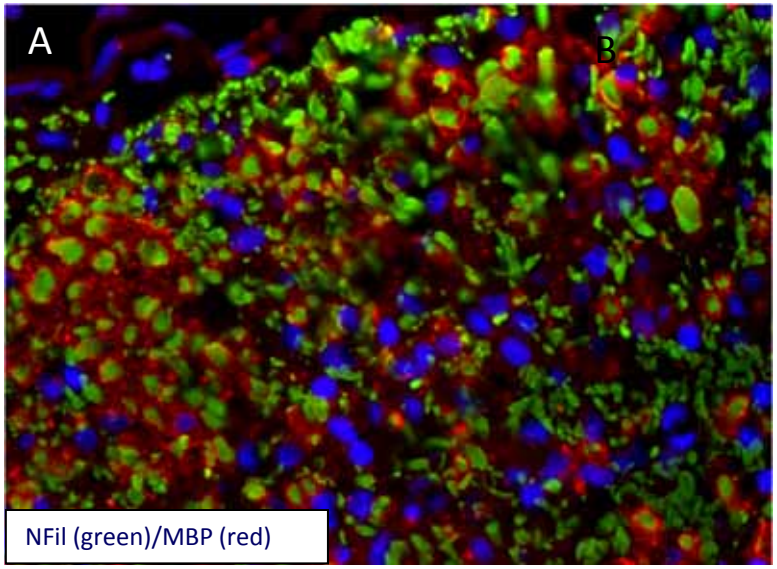


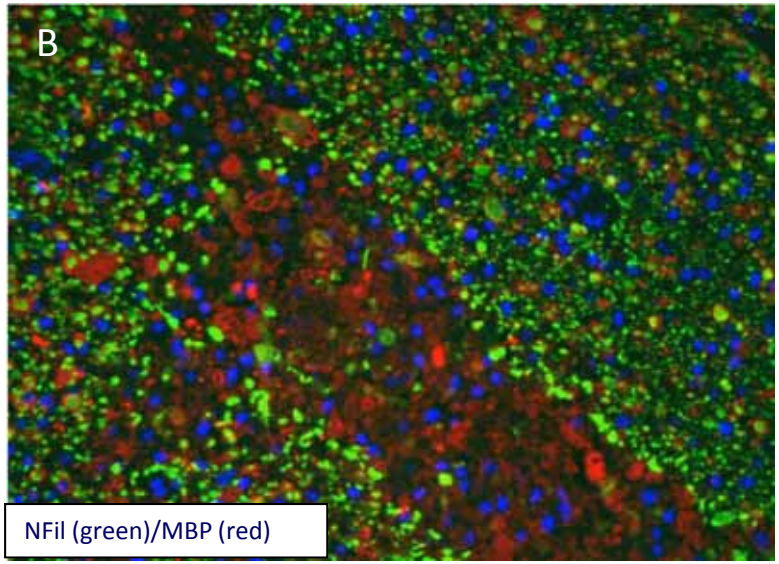


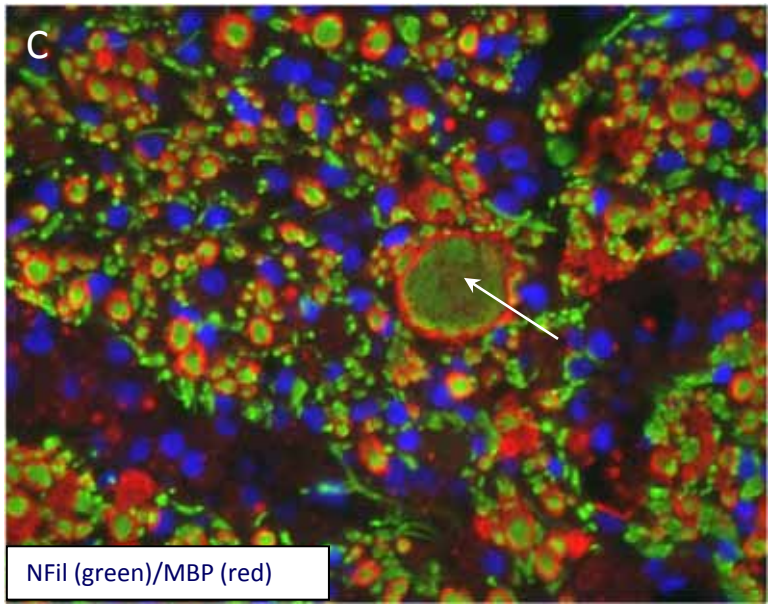




Neuronal/axonal damage and loss in MOG-induced EAE

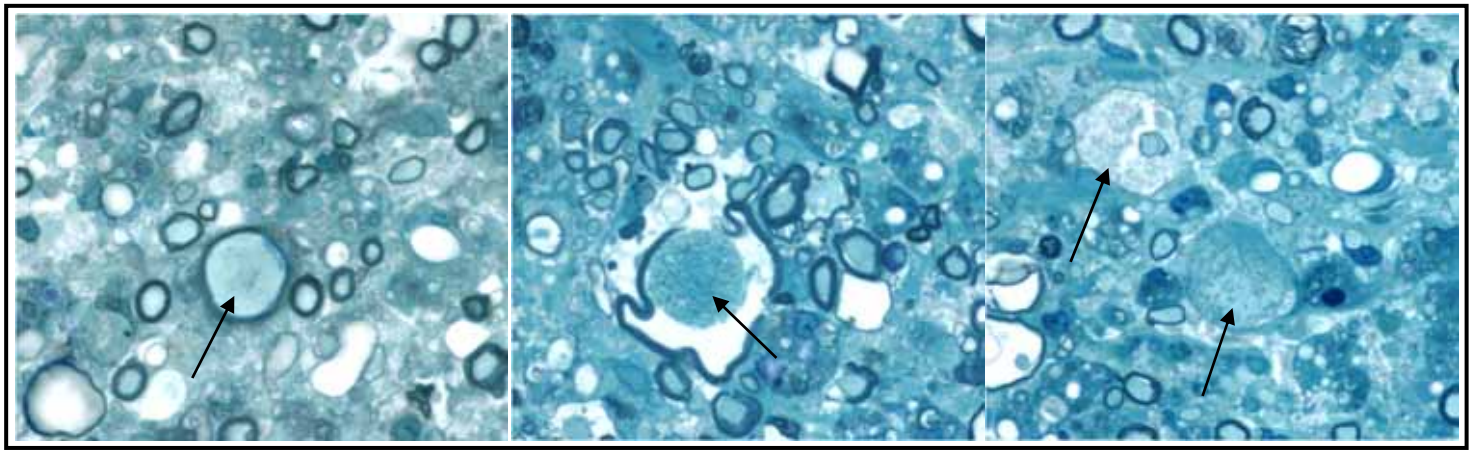




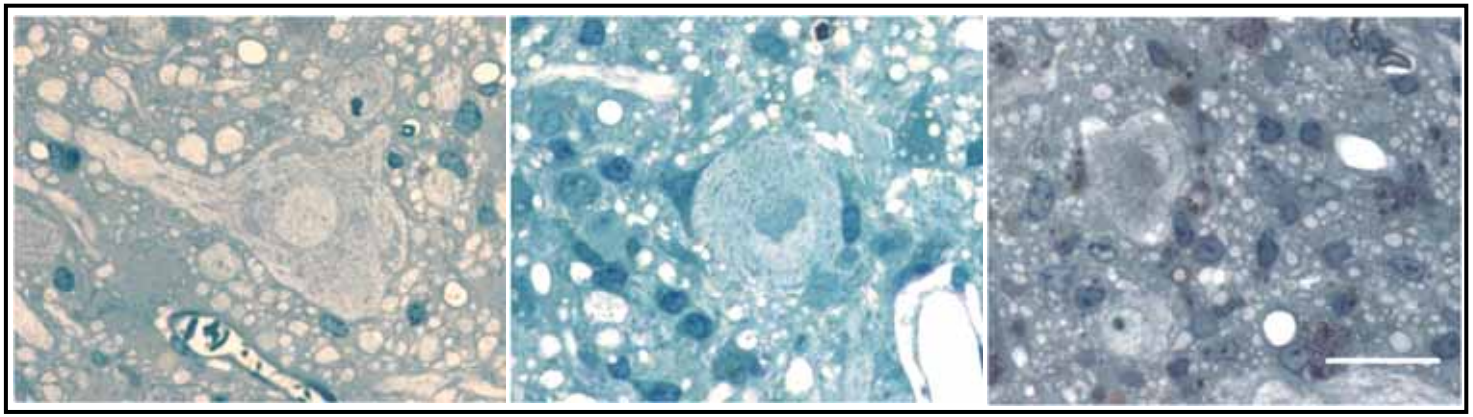


- In addition to inflammation and demyelination, injection of DA rats with recombinant MOG also results in neuronal and axonal changes in the spinal cord.
- Disorganisation of axonal fascicles and reduction in axonal density is seen in areas of demyelination (A).
- MBP/Neurofilament double immunostaining reveals areas of Wallerian degeneration (B) and axonal dystrophy (C).

Neuronal and axonal changes in MOG-EAE

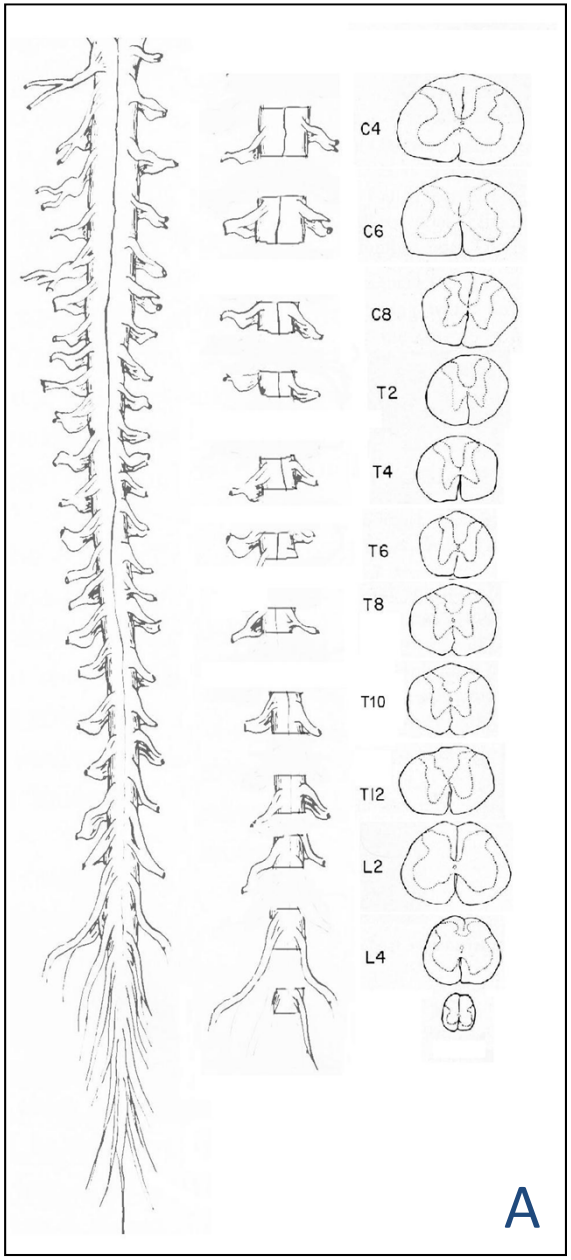


Swollen and degenerating axons (granular disintegration of cytoskeleton) are frequently seen in areas of inflammation and demyelination

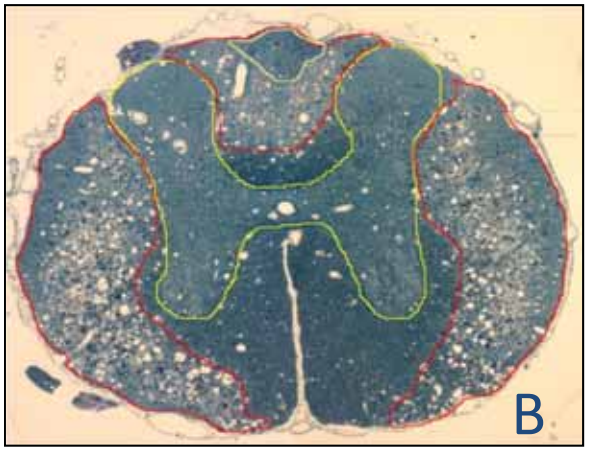


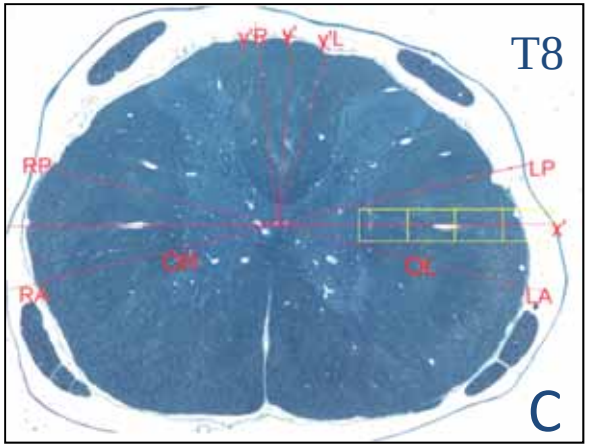
Degenerative changes to spinal motoneurons are seen in areas of inflammation in the grey matter and close to white matter lesions. Loss of motoneurons is seen at some levels.

Methodology for Quantification of Spinal Cord Pathology

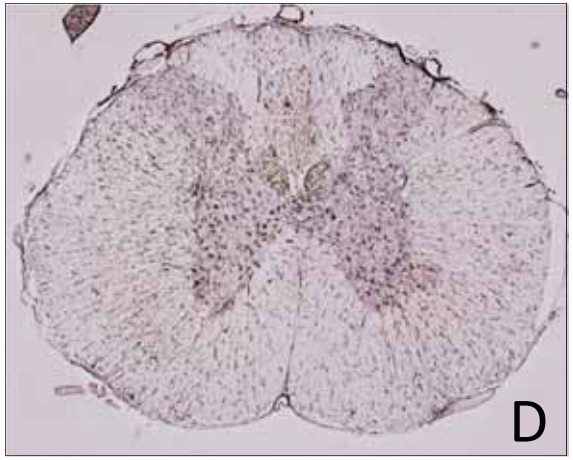


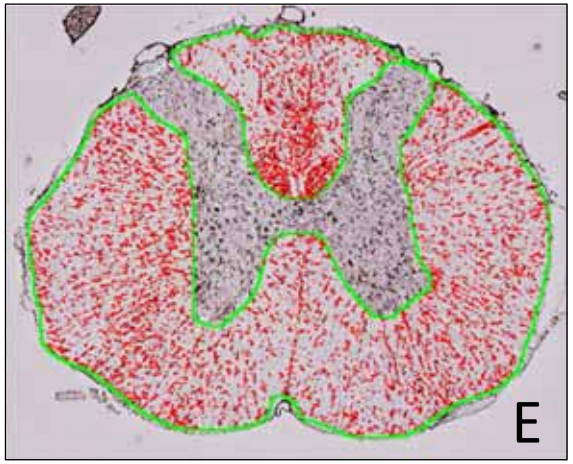
Toluidine blue stained semi thin sections



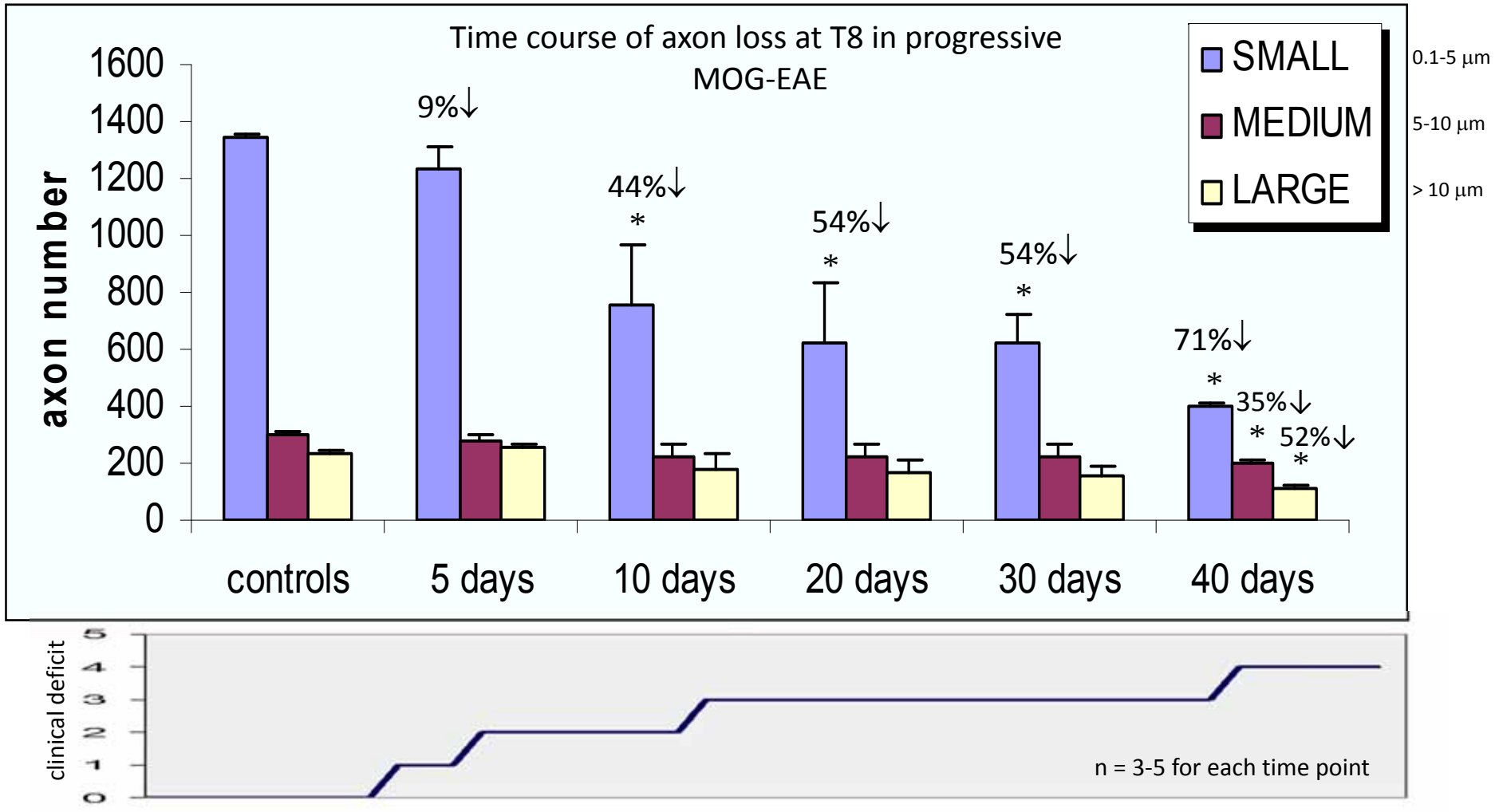


OX-42 immunohistochemistry



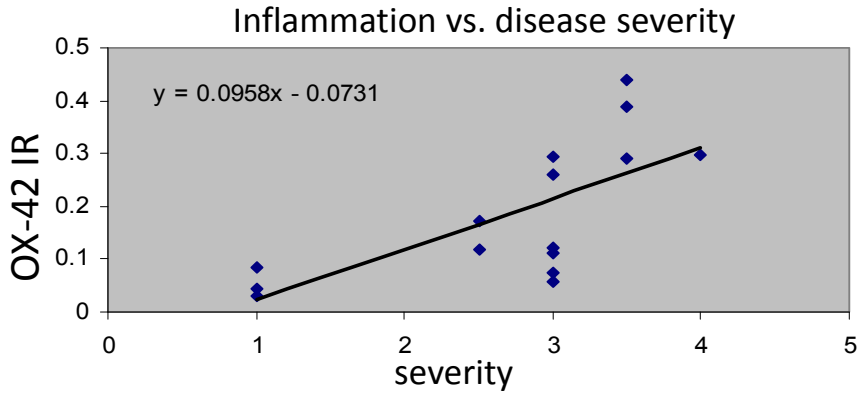


Axonal loss occurs early after disease onset with small axons most affected

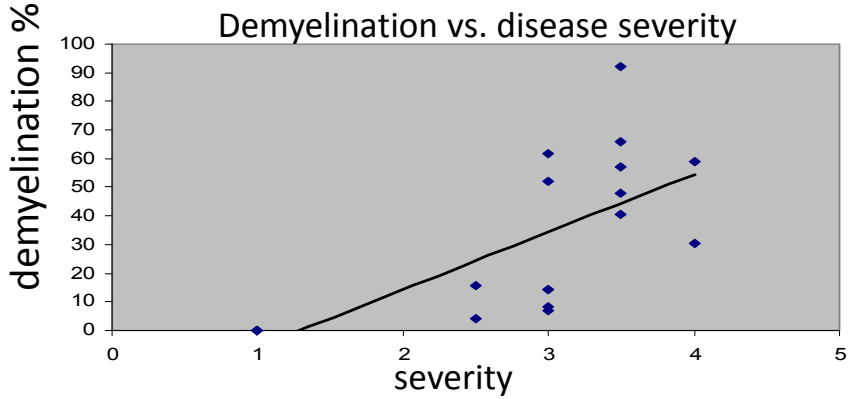


Papadopoulos et al, Exp Neurol 2006

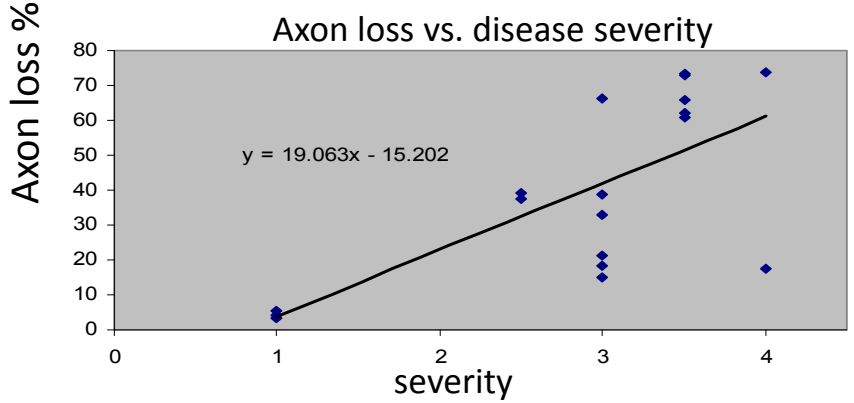
Demyelination, Inflammation & Axon loss determine disability in progressive EAE



OX-42 cell density correlates significantly with disease severity in progressive EAE (r=0.695, p<0.01).

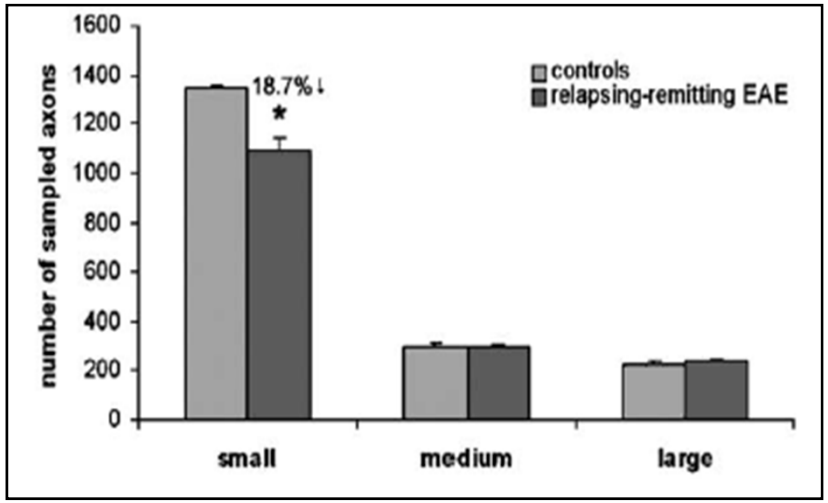


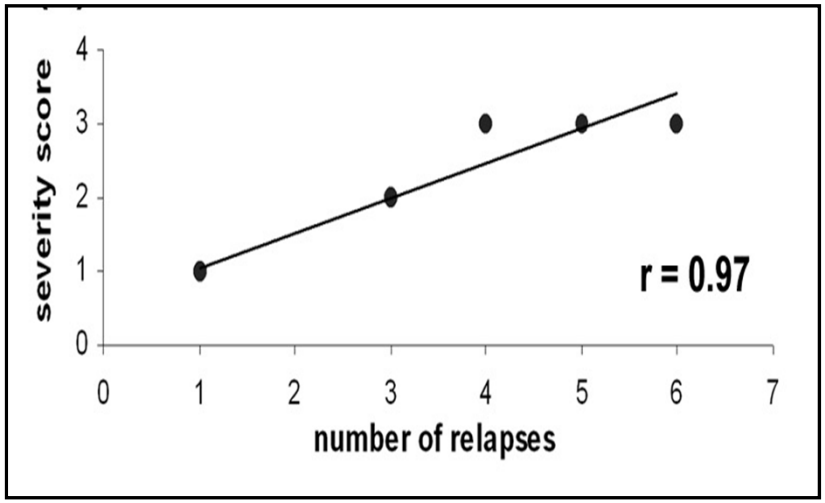
Demyelination correlates significantly with disease severity (r =0.676, p<0.01).

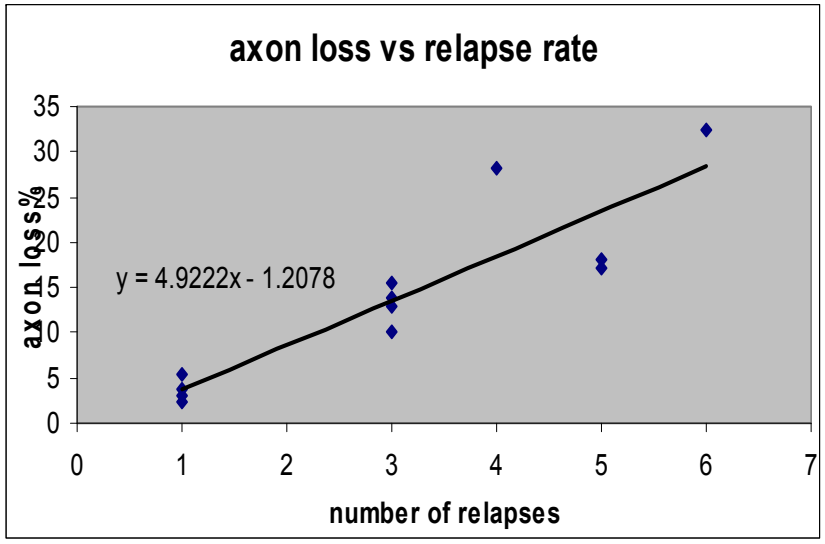


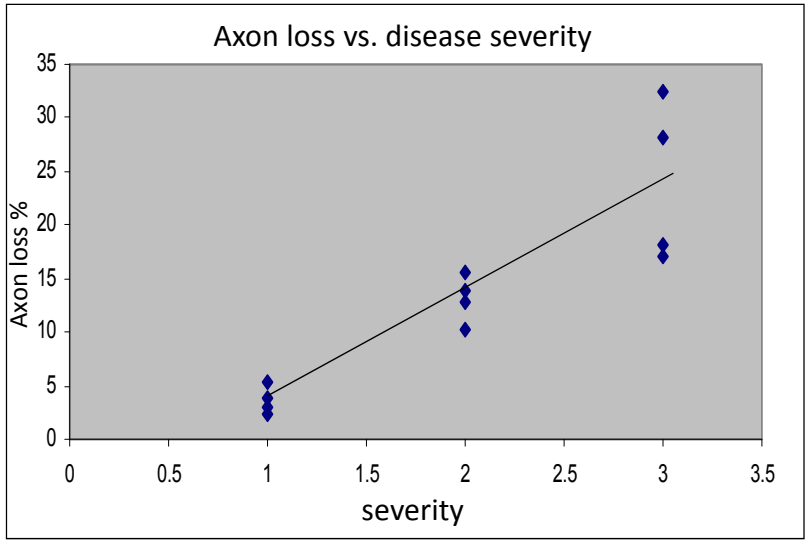
Axon loss correlates significantly with disease severity in progressive EAE (r=0.69, p<0.01).

Fibre loss is episodic in relapsing EAE corresponding to the number of relapses and correlates with disability.









The percentage of axon loss correlates significantly with the number of relapses (r Pearson=0.89, $p<0.001$)

In relapsing EAE, axon loss exhibits a significant correlation with disease severity ($r=0.90$, $p<0.01$).

AXON LOSS - CONCLUSIONS

1. There is significant axonal loss (~ 44-65% total), which is comparable to the numbers reported by neuropathological studies of axonal loss in MS spinal cord and may account for the accumulating disability observed in the model.

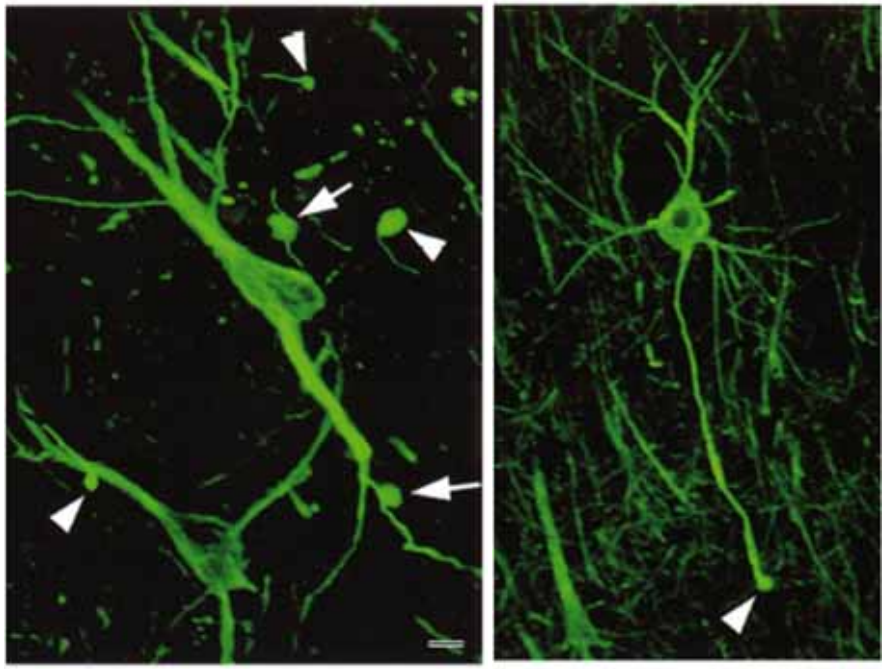
2. Significant loss of axons occurs early in the disease process but the exact mechanism remains to be elucidated

3. Small caliber fibres are more vulnerable to the pathogenetic mechanisms of MOG-induced EAE which is consistent with what has been observed in MS lesions.

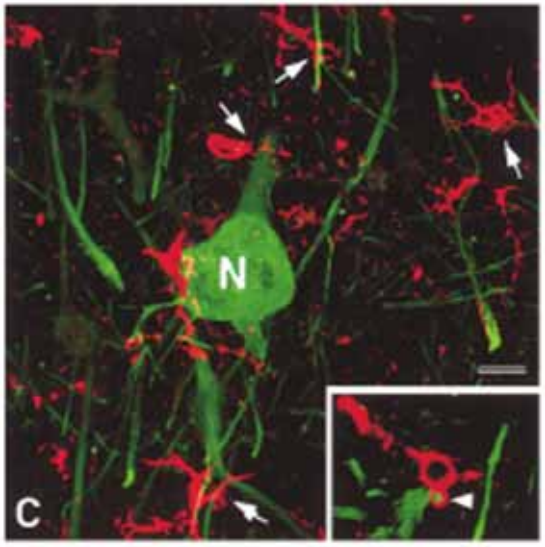
4. Only axon loss correlates with increasing chronic clinical disability in relapsing-remitting MOG-EAE

5. May occur via mitochondrial insufficiency, ATP reduction, increased Na+ loading, reversal of Na/K ATPase & subsequent calcium loading of axons (Trapp & Stys, 2009)

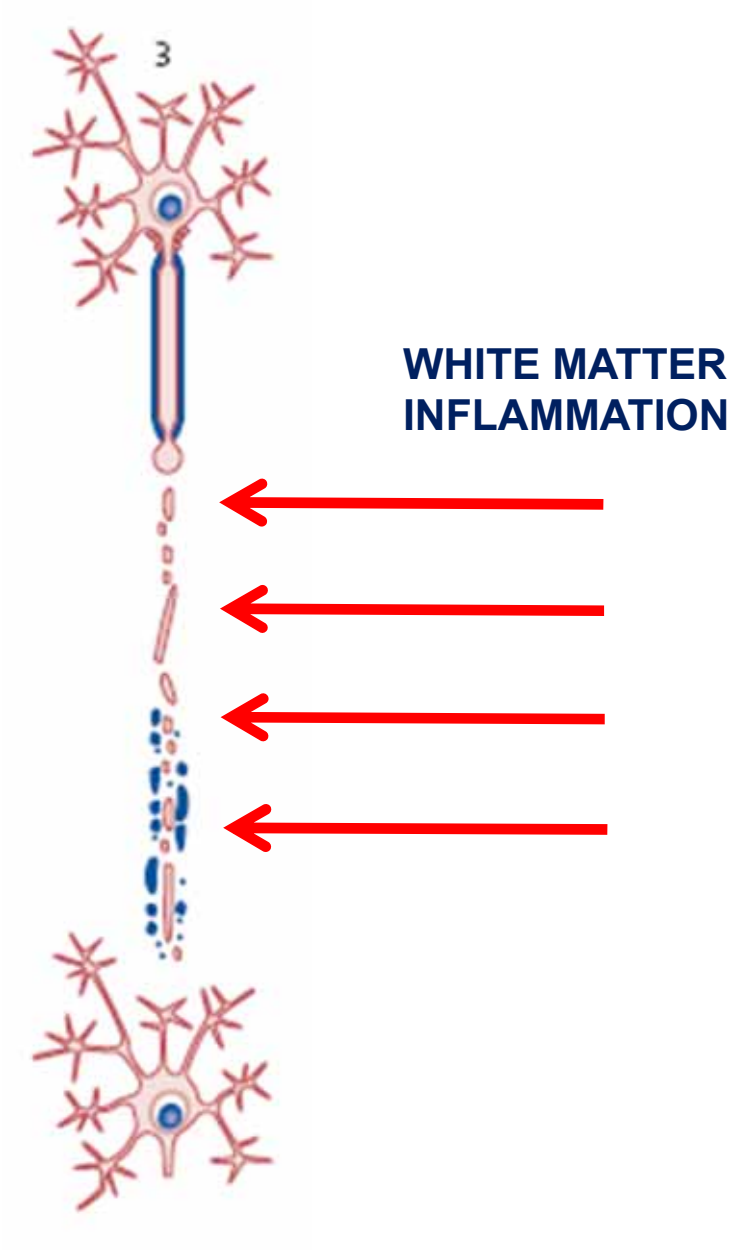
Evidence for neurodegeneration in MS



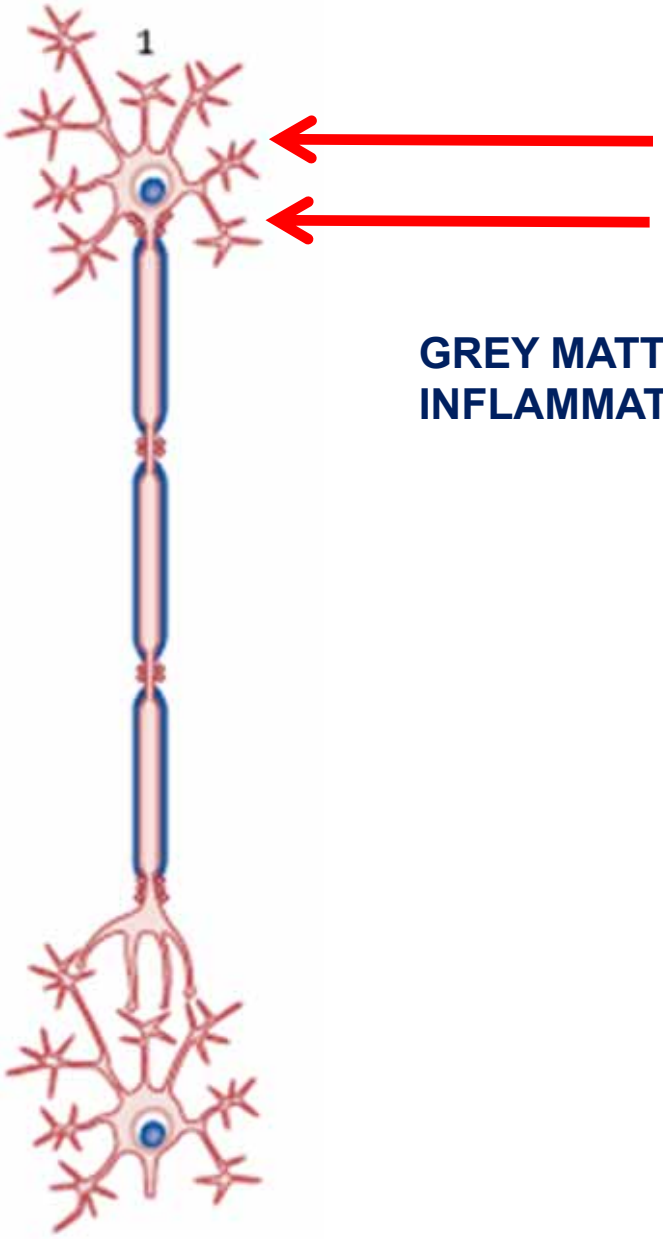
- 20-30% of total cortical grey matter is demyelinated in SPMS and PPMS
- damage to axons and dendrites in grey matter lesions *(Peterson et al Ann Neurol 2001)*



- cell loss in grey matter lesions
- degenerating neurons in grey matter lesions
- neuronal loss in cerebral cortex
- 35% reduction in total neuronal numbers in mediodorsal thalamus



WHITE MATTER INFLAMMATION



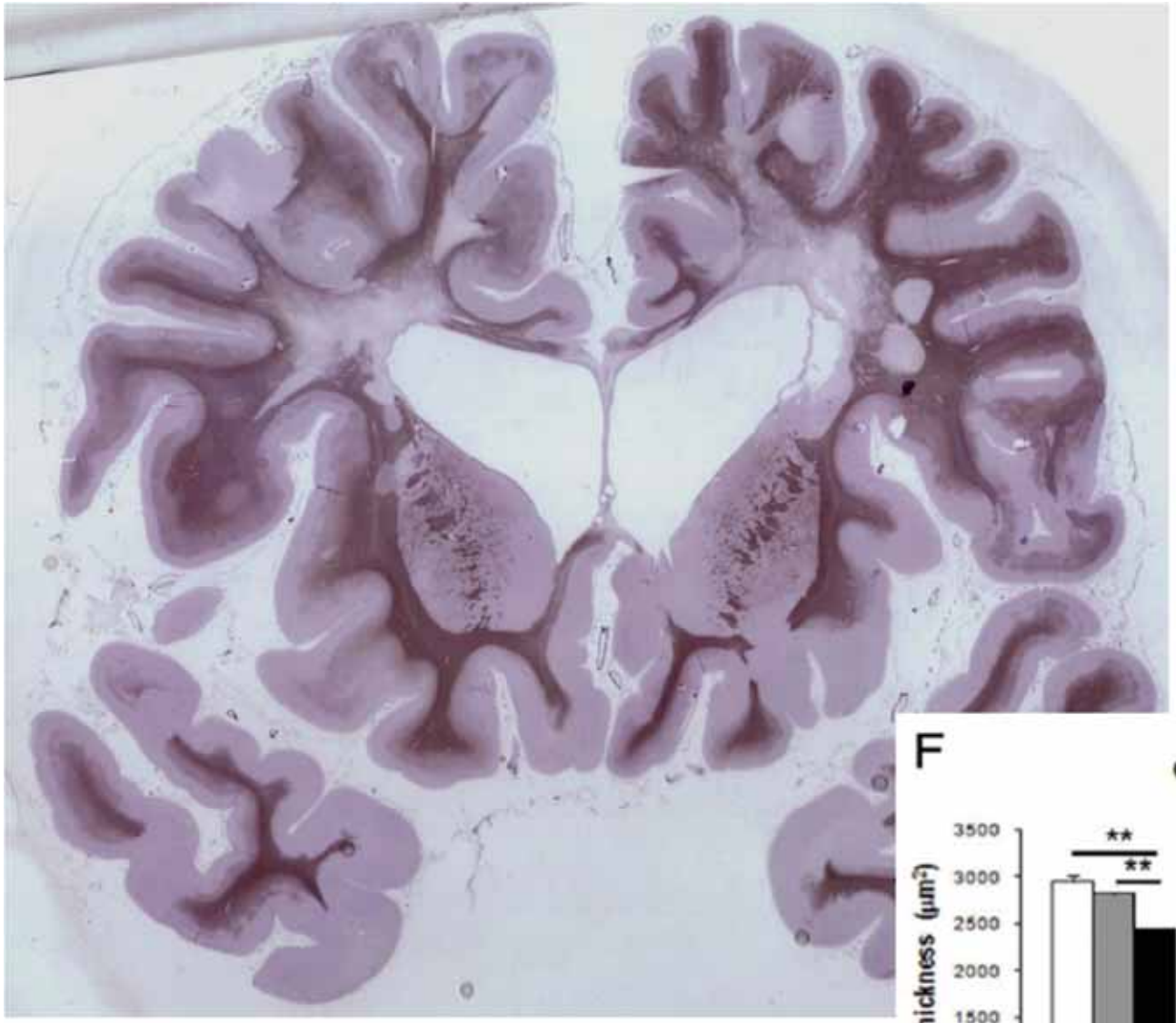
GREY MATTER INFLAMMATION

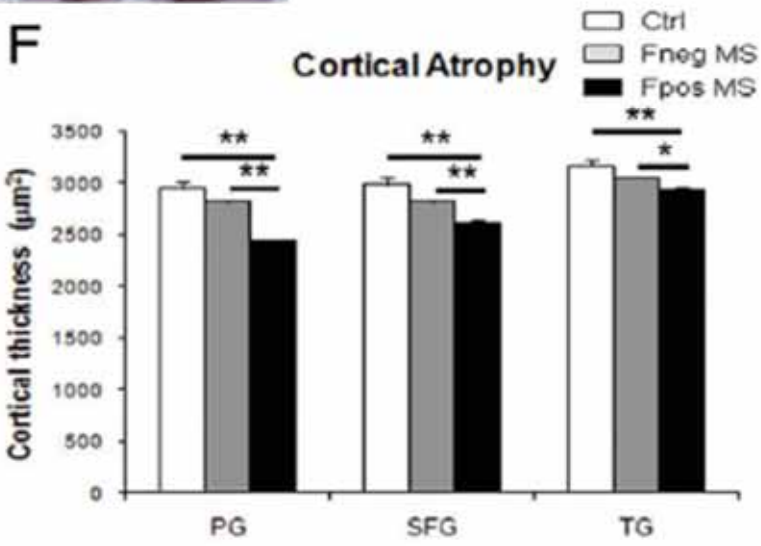
Gray Matter Atrophy Is Related to Long-Term Disability in Multiple Sclerosis

Leonora K. Fisniku, MRCP,[1,2] Declan T. Chard, PhD,[1,2] Jonathan S. Jackson, MSci,[1,2] Valerie M. Anderson, BSci,[1,2] Daniel R. Altmann, PhD,[1,3] Katherine A. Miszkiel, MRCP,[4] Alan J. Thompson, PhD,[1,5] and David H. Miller, MD[1,2]

- there was significantly more GM, but not WM atrophy, in secondary-progressive MS versus relapsing-remitting MS (*p* 0.003), and relapsing-remitting MS versus clinically isolated syndrome (*p 0.001).*

- GM, but not WM, fraction correlated with expanded disability status scale (*rs 0.48; p 0.001) and MS Functional Composite scores (rs 0.59; p 0.001).*

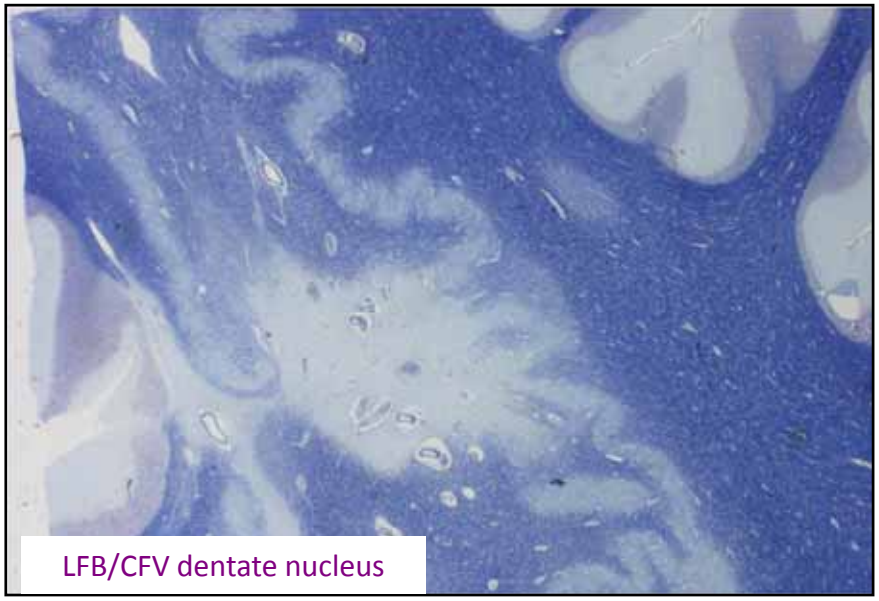


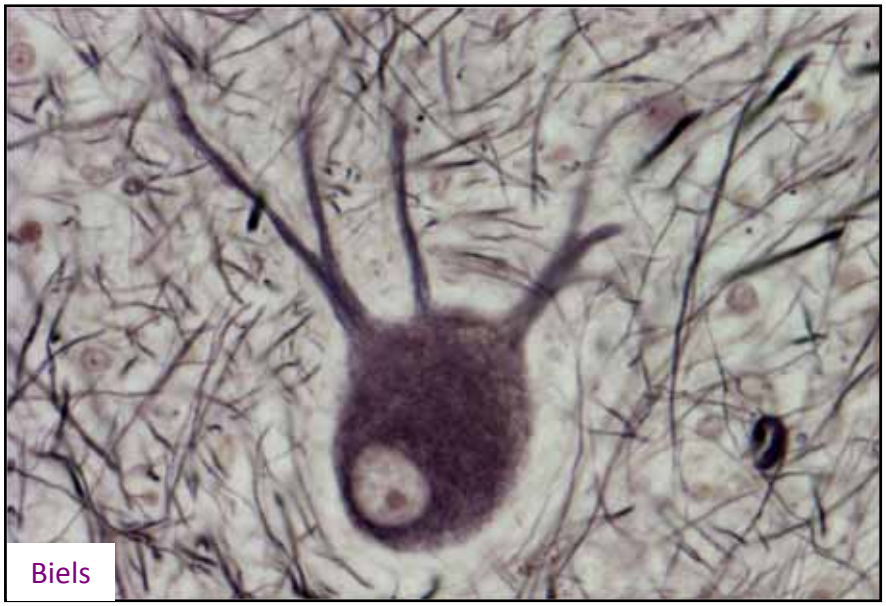


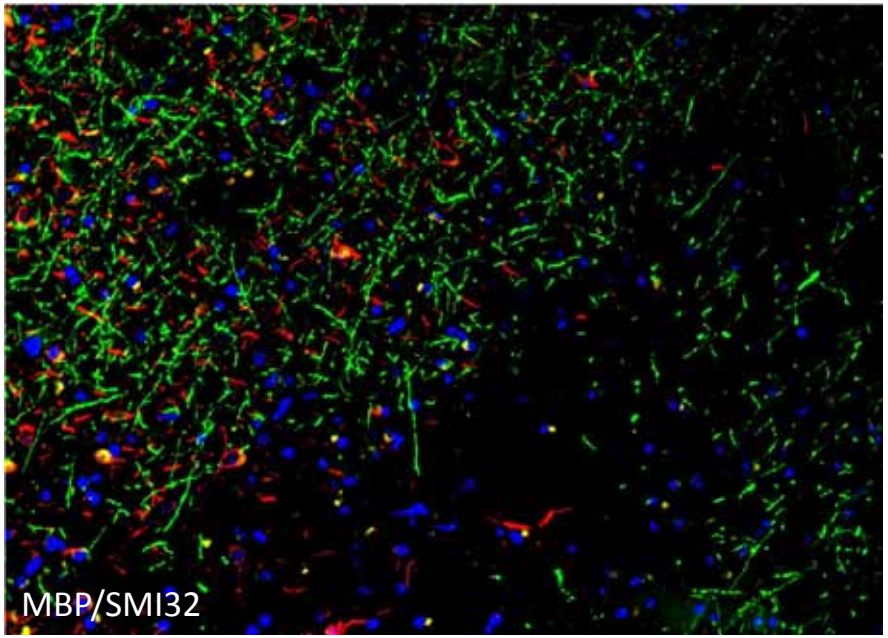
Up to 20% reduction in cortical GM thickness

Magliozzi et al, 2010

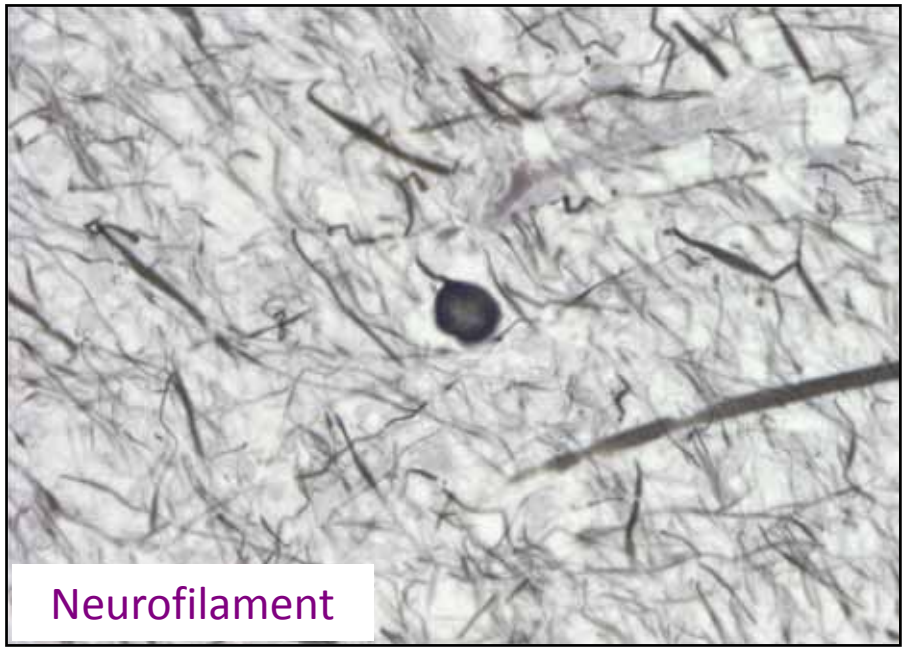
Axonal loss and neurodegeneration

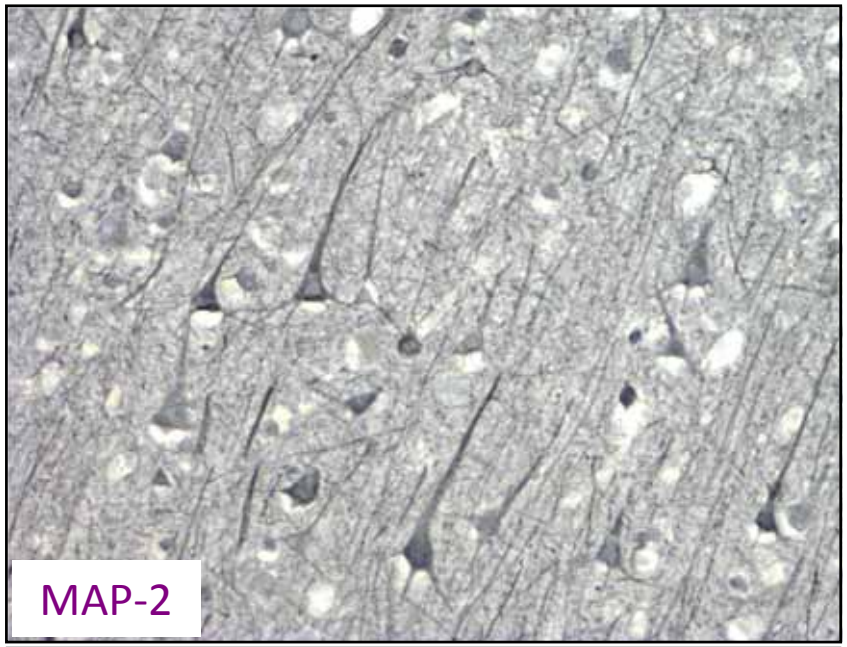


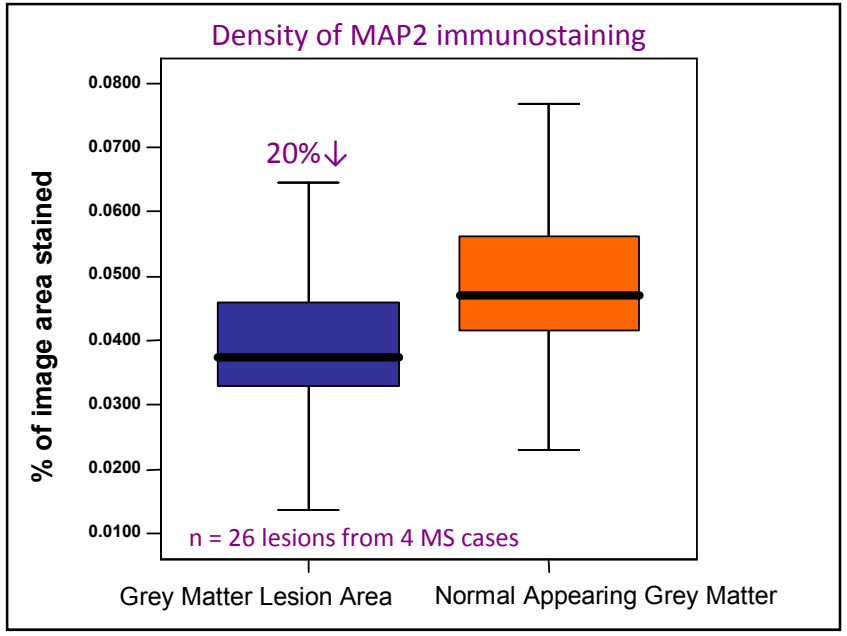




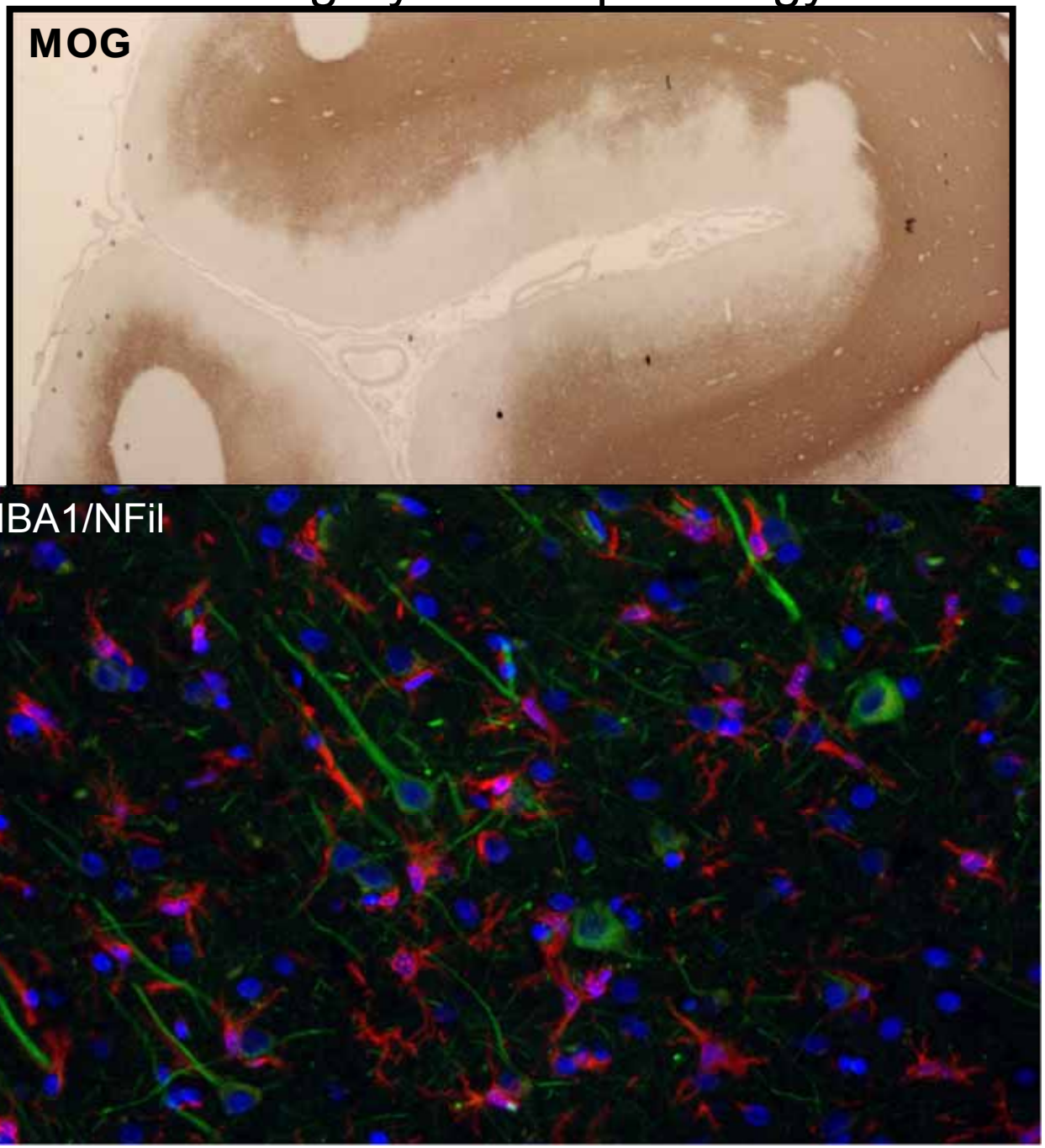
Neuronal changes in grey matter lesions



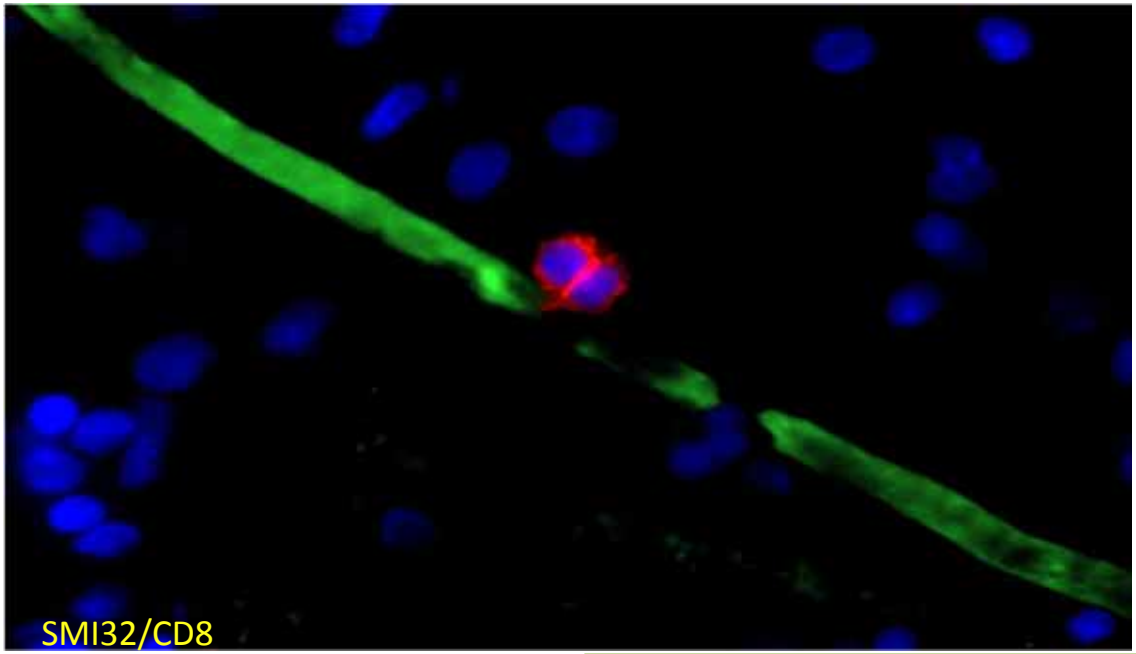


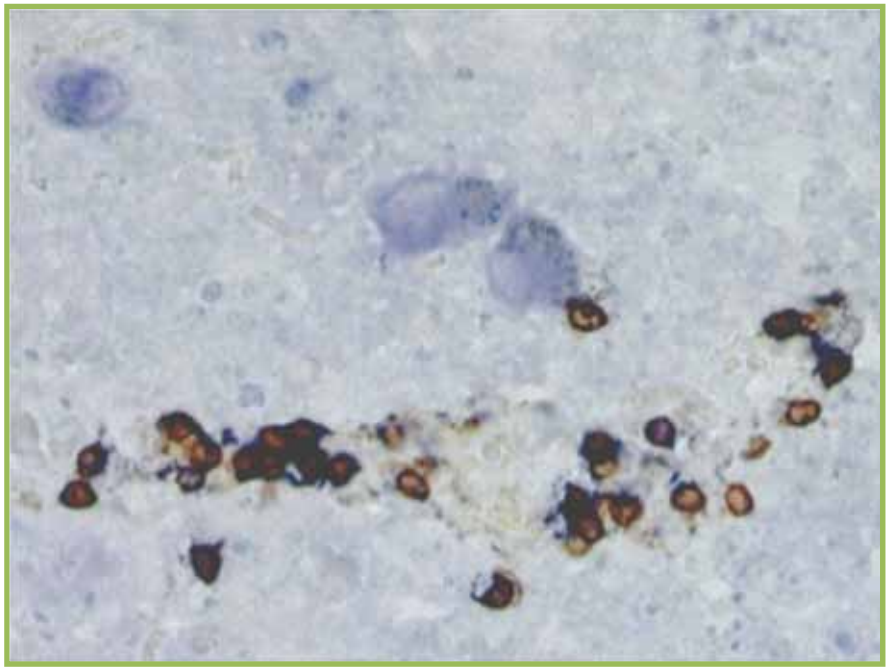


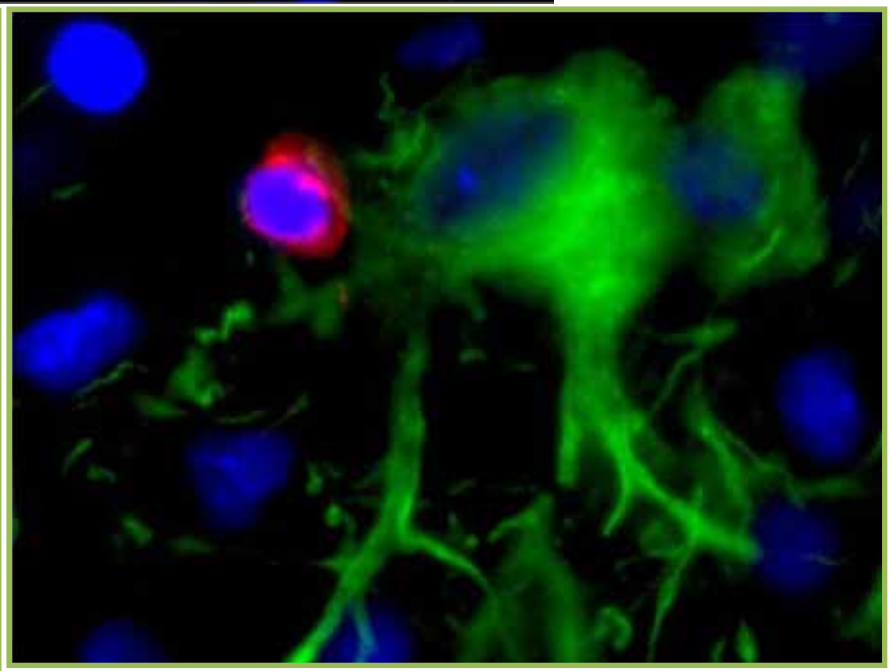
MS grey matter pathology:

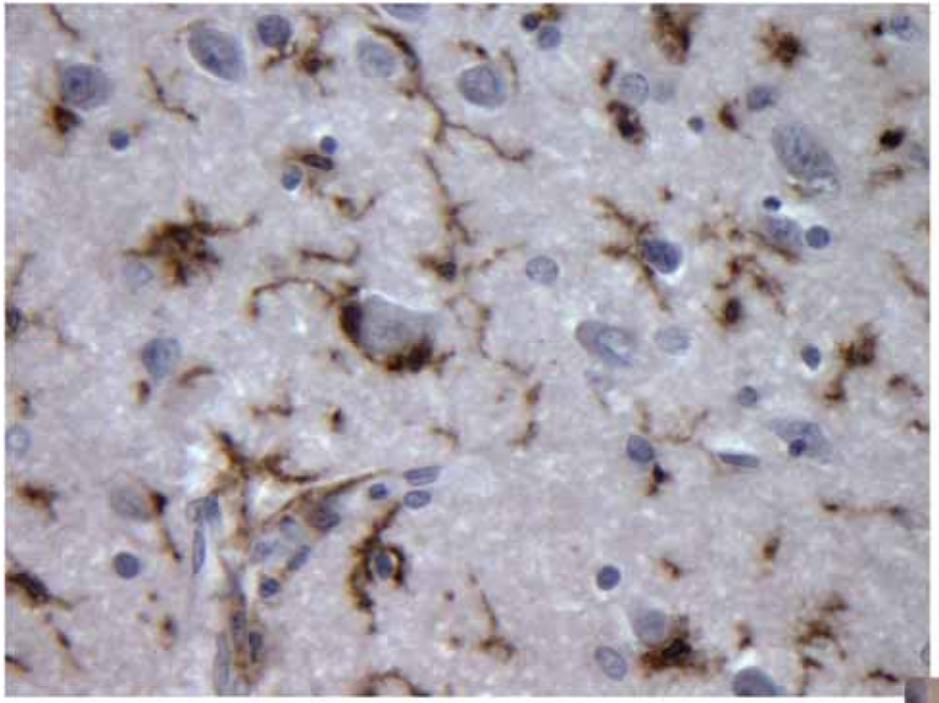


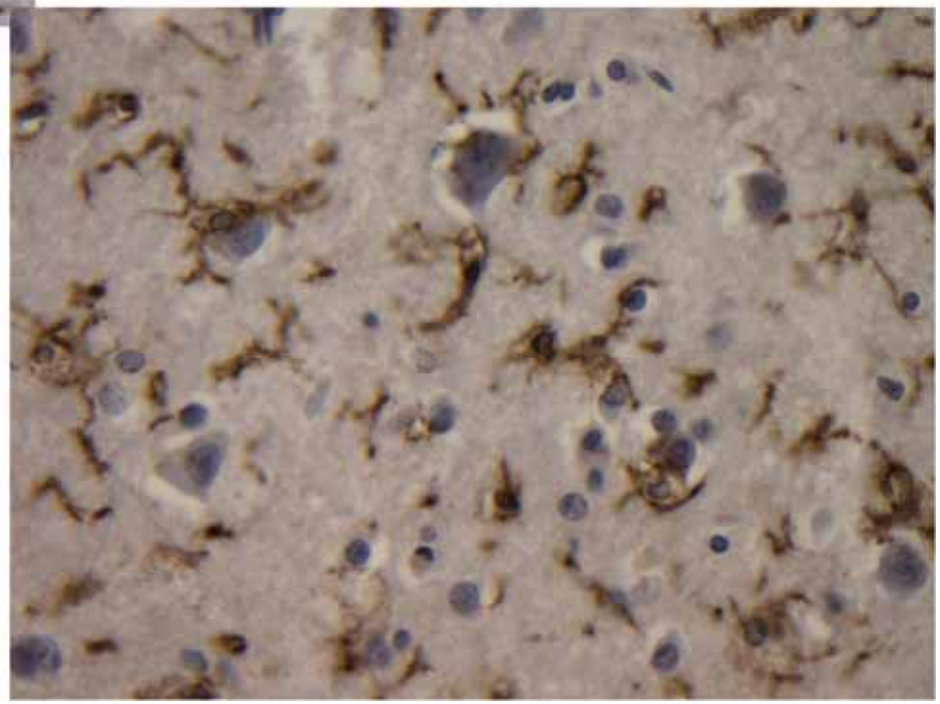
Neuronal pathology in normal appearing grey matter



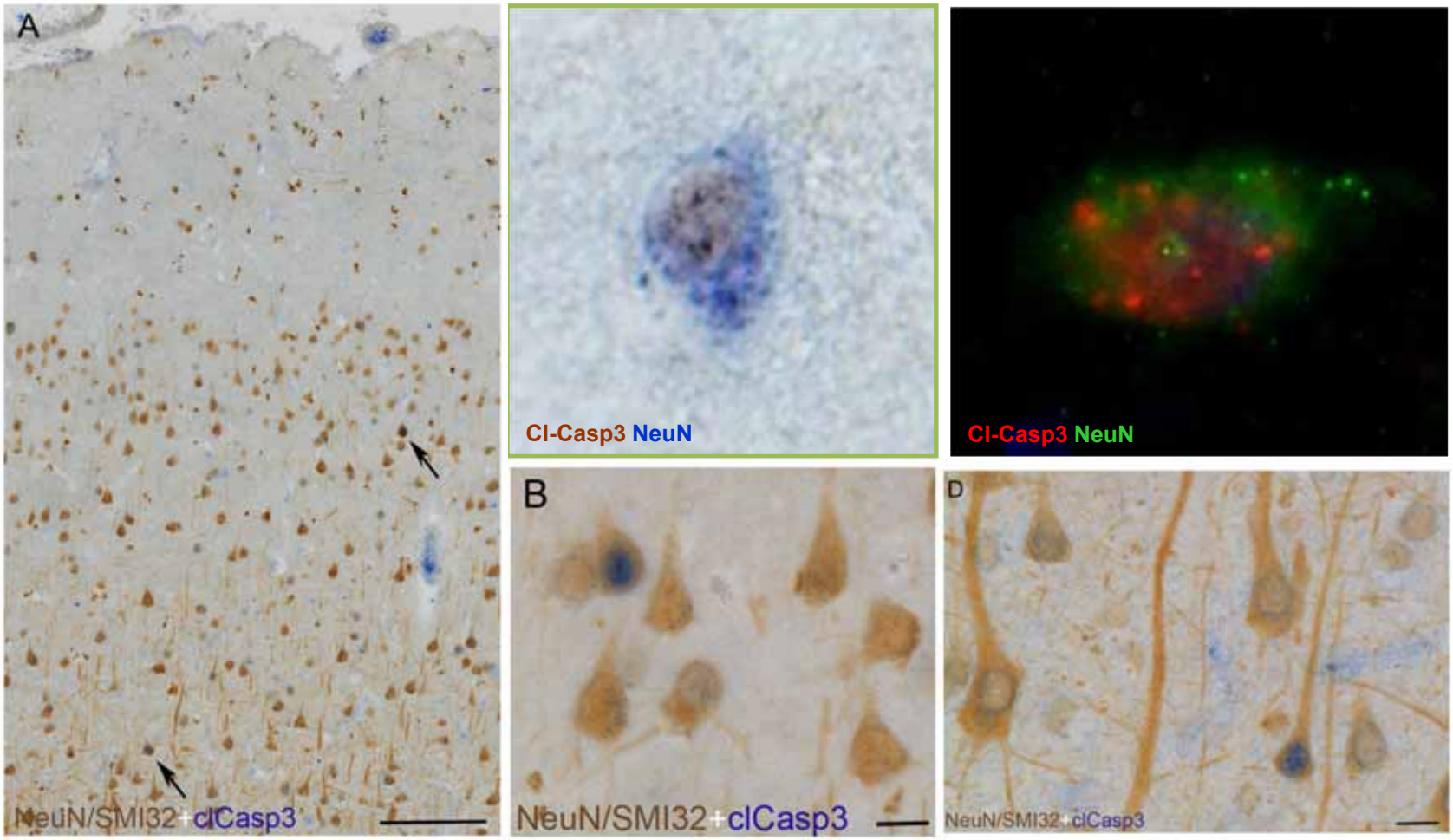








Neuronal pathology in MS cases

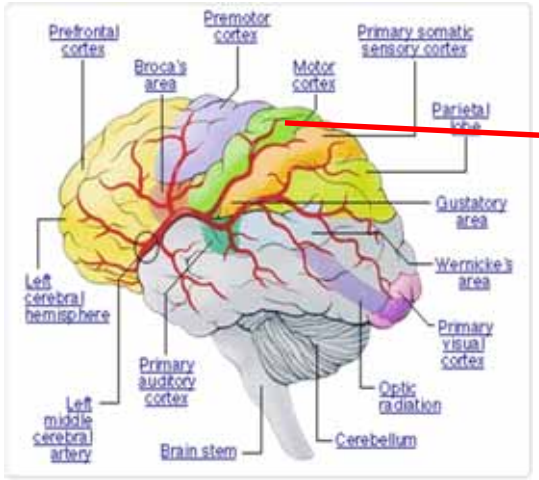


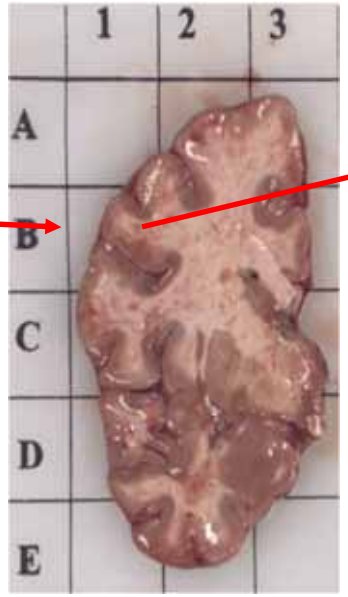
Caspase 3 expressing neurons at the early stages of apoptosis are seen throughout the cortical GM in MS, but not in large numbers

Magliozzi, Ann Neurol 2010

Cellular pathology of GM lesions

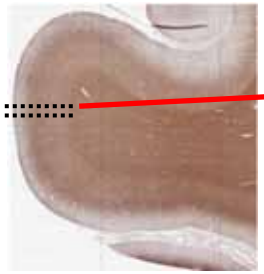
Precentral gyrus: motor cortex

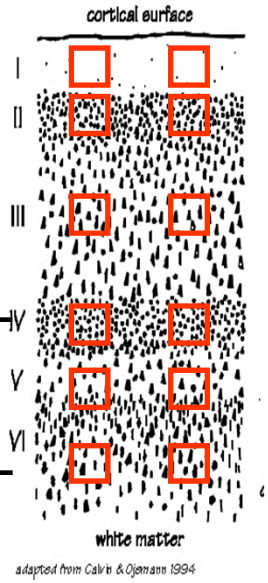




Snap frozen tissue

(*UK MS Tissue Bank*)

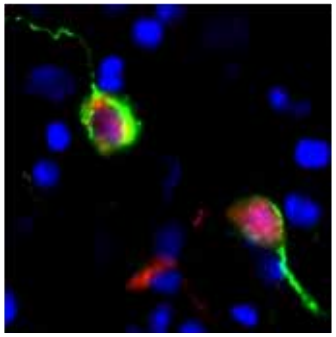




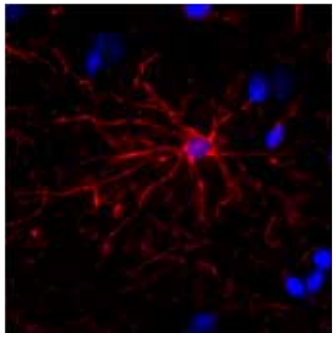
10 F+SPMS { **Ch A Type III GML**, **NAGM**

10 F-SPMS { **Ch A Type III GML**, **NAGM**

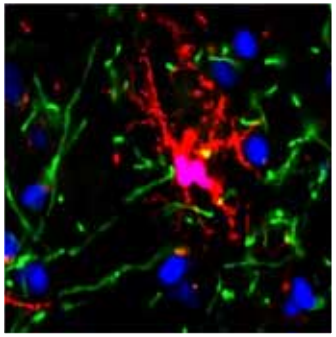
10 Controls **NAGM**



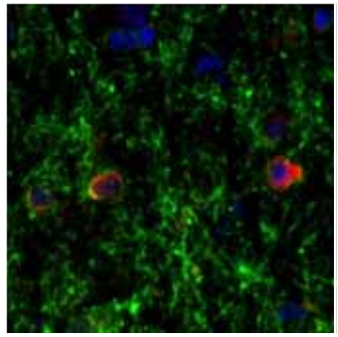
NeuN + MAP2



GFAP

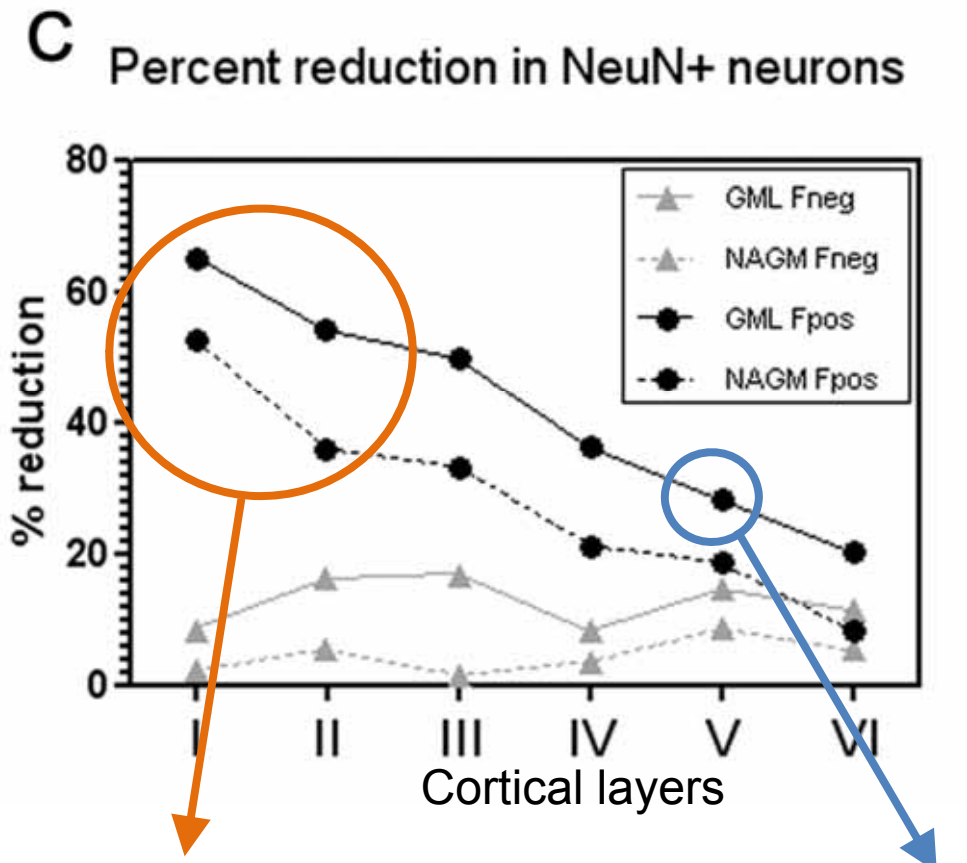


MHC-II + MBP



NogoA + CNP

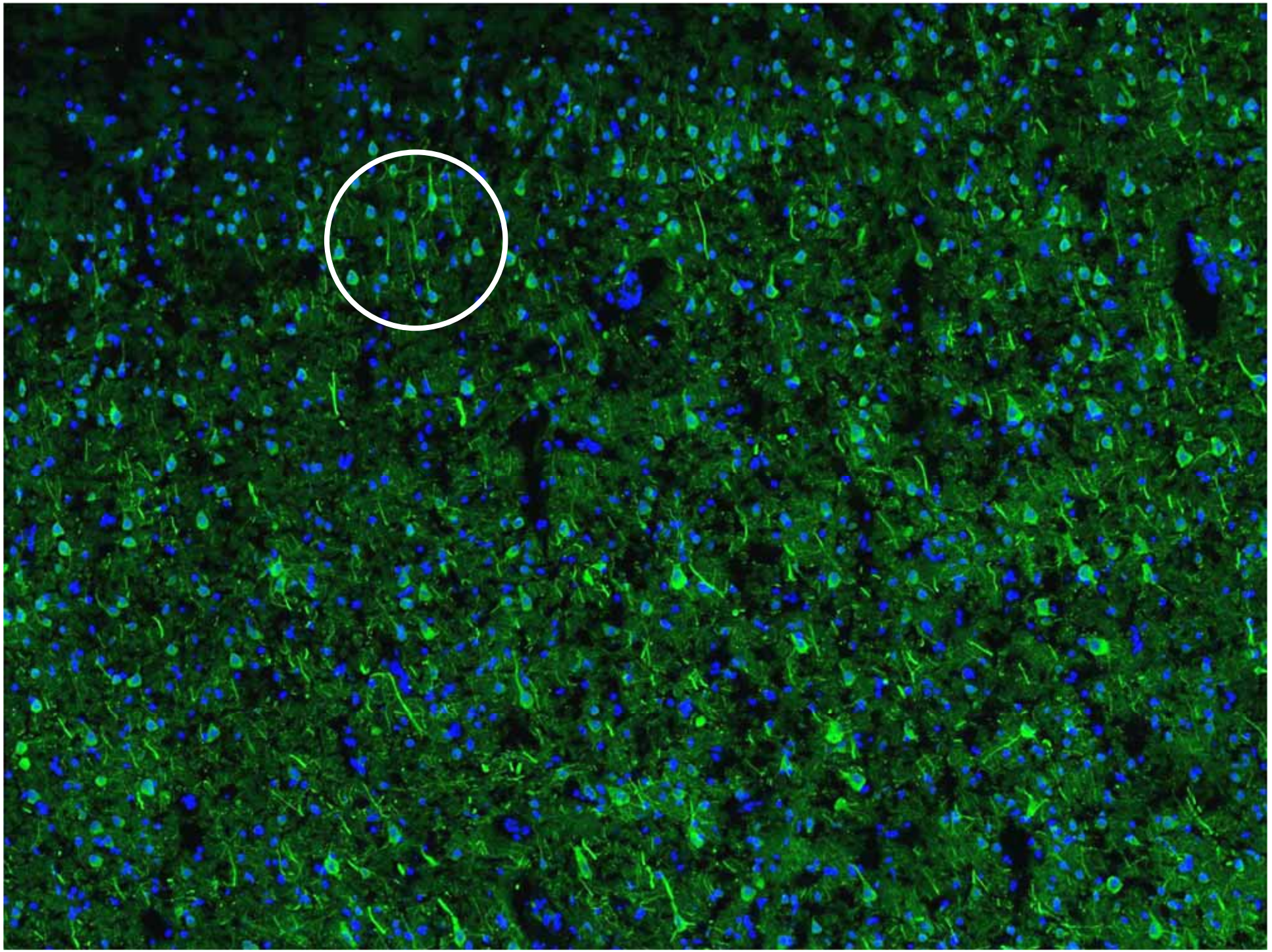
Decreased neuron density
(NeuN+ nuclei)
Clear evidence of a gradient of neuronal loss

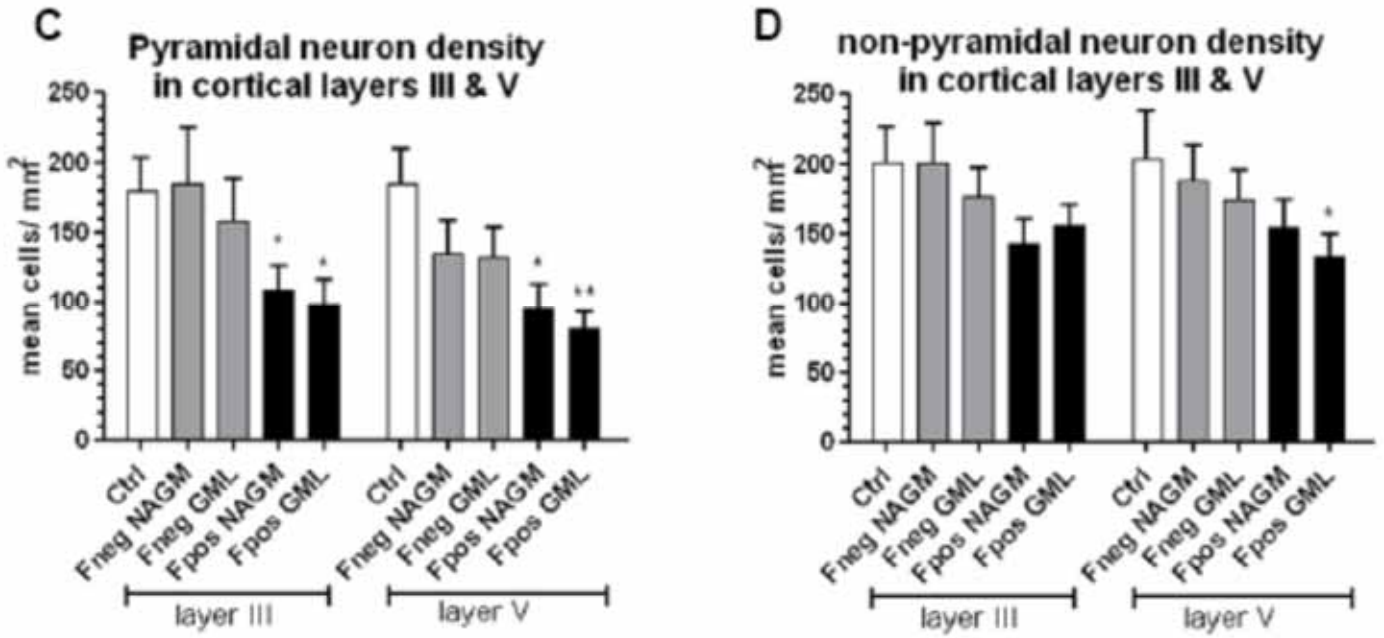


Greatest neuronal density decrease in layer I & II of F+SPMS in GML (>60%) and NAGM (>50%)

40% reduction in layer 5 neurons

All the data for F+ cases are statistically significant ($p<0.0001$) compared to controls

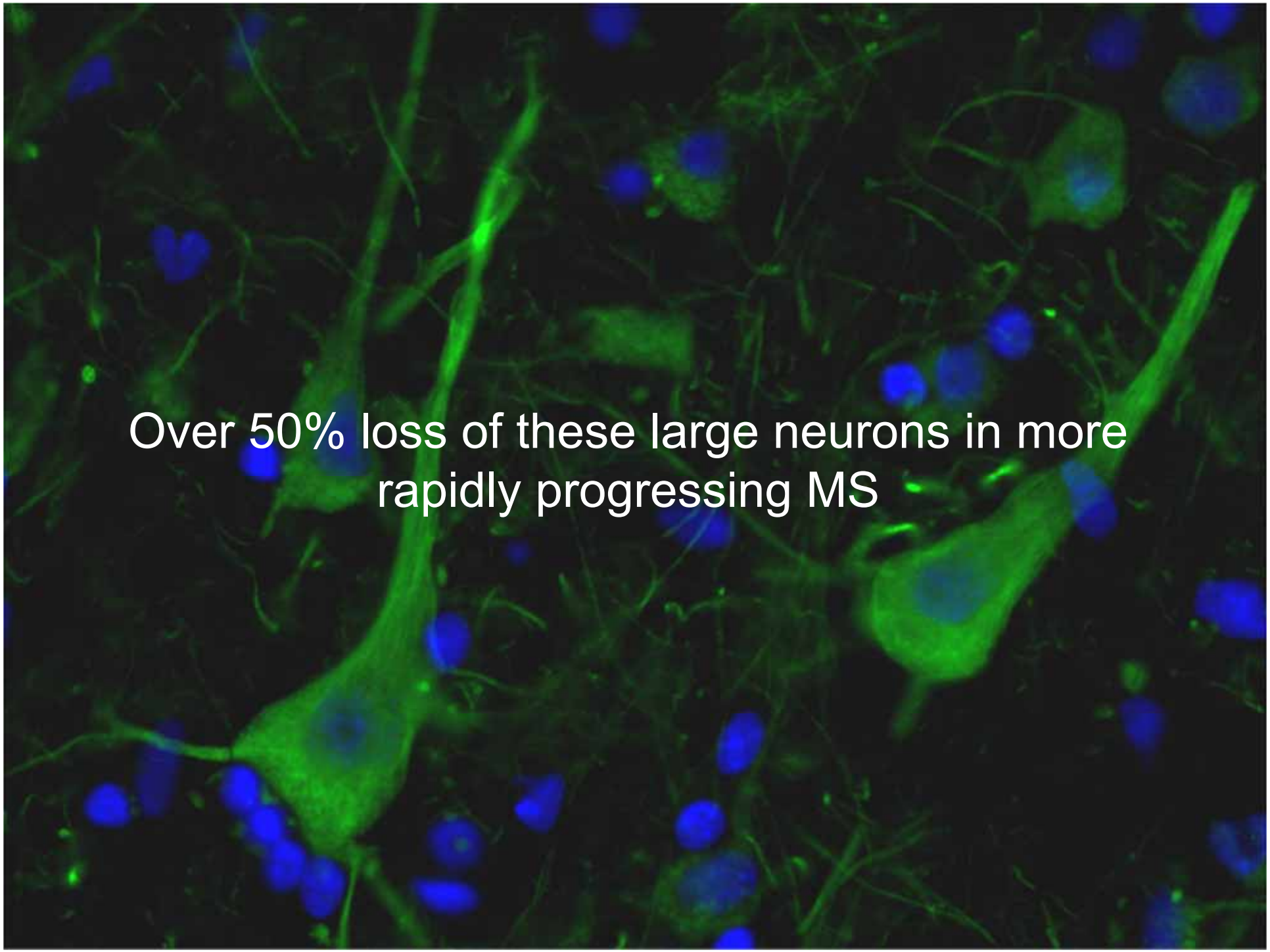


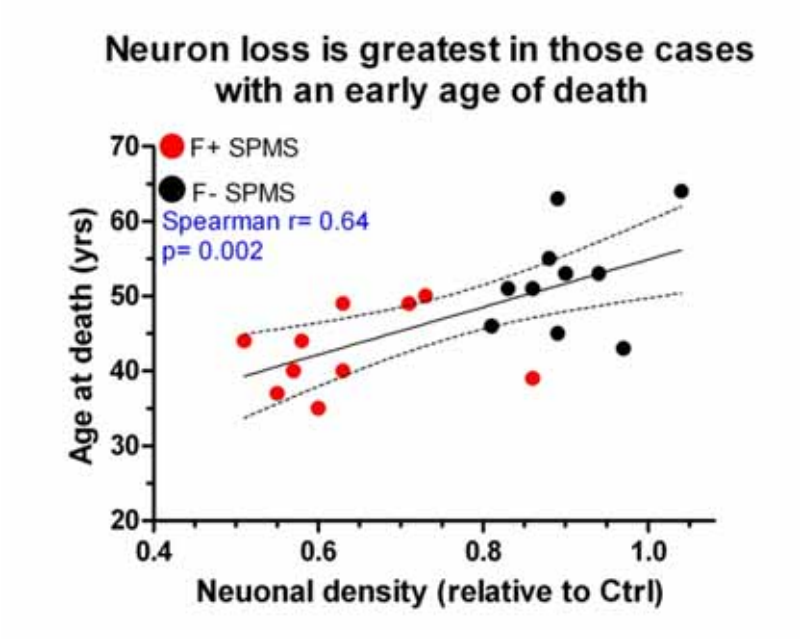


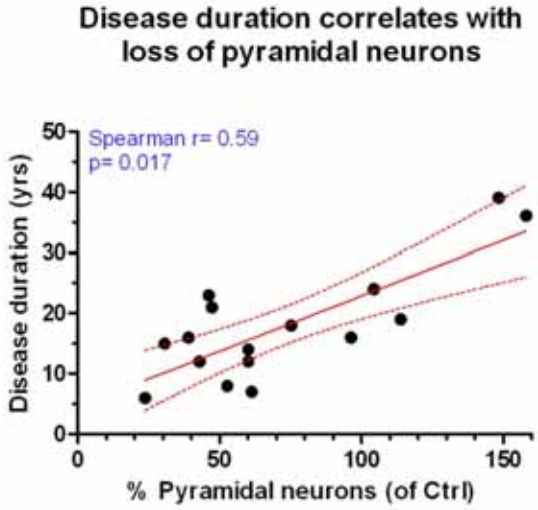
Cells were co-labelled with NeuN/ smi32 and pyramidal neurons defined by shape, relative size, a primary dendrite extending towards pial surface as well as DAPI+ nucleus (n=10+10+10)

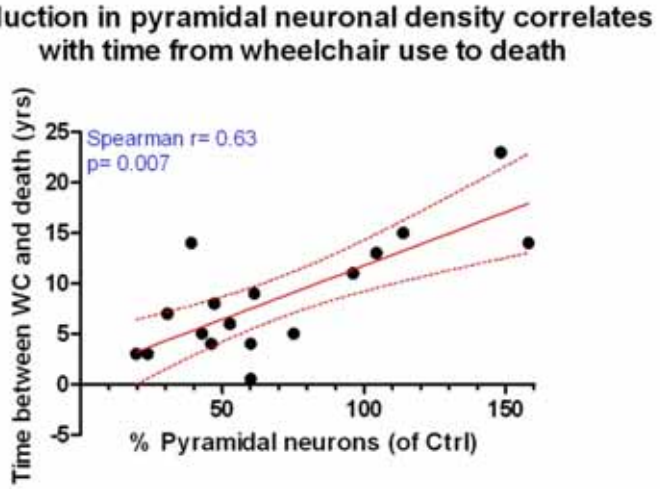
65% loss of layer V pyramidal neurons in GMLs associated with B-cell follicles !!

Major consequences for motor function


Over 50% loss of these large neurons in more rapidly progressing MS

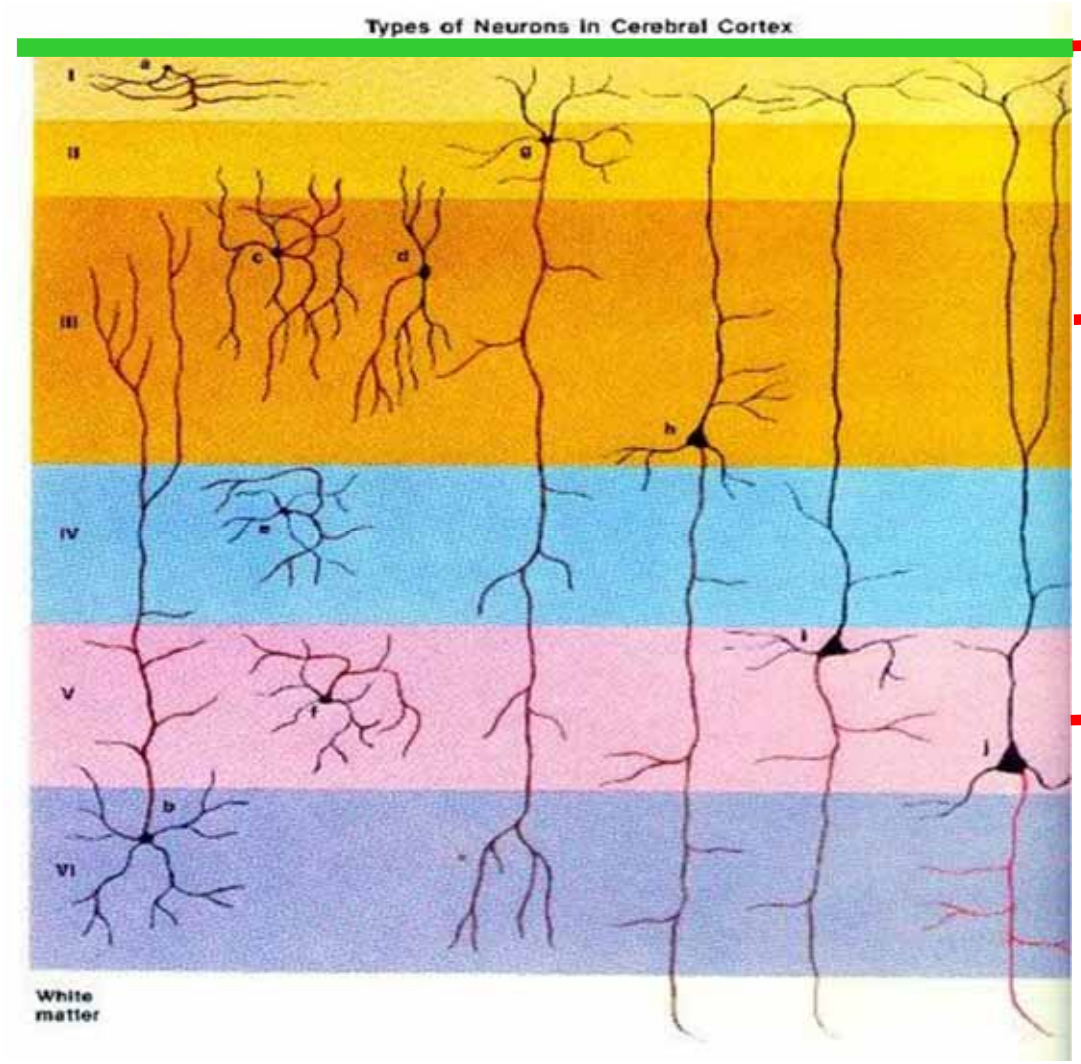

Neuron loss is greatest in those cases with an early age of death
F+ SPMS
F- SPMS
Spearman r= 0.64
p= 0.002
Age at death (yrs)
70
60
50
40
30
20
0.4
0.6
0.8
1.0
Neuonal density (relative to Ctrl)


Disease duration correlates with loss of pyramidal neurons
Spearman r= 0.59
p= 0.017
Disease duration (yrs)
50
40
30
20
10
0
0
50
100
150
% Pyramidal neurons (of Ctrl)


Reduction in pyramidal neuronal density correlates with time from wheelchair use to death
Spearman r= 0.63
p= 0.007
Time between WC and death (yrs)
25
20
15
10
5
0
-5
50
100
150
% Pyramidal neurons (of Ctrl)

B-cell follicle (B-cells, CD8+ T-cells, NK cells)

CHRONIC MICROGLIAL ACTIVATION

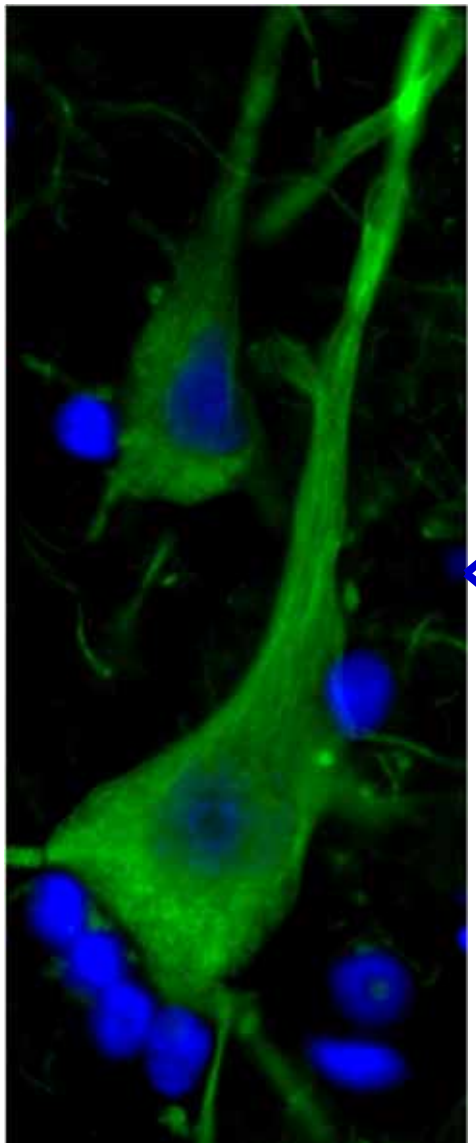


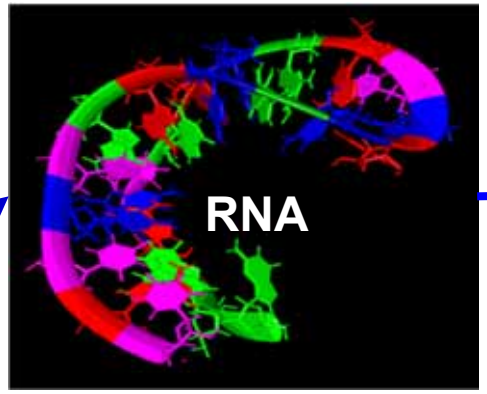
Damage to glia limitans

50% loss of pyramidal neurons

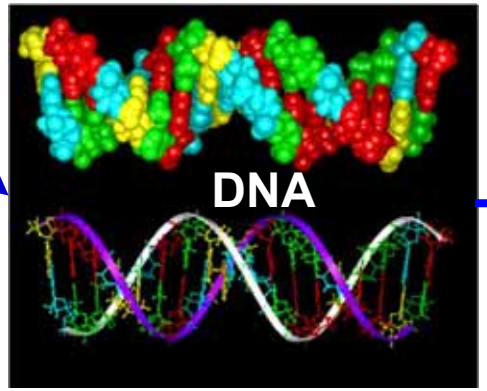
65% loss of pyramidal neurons

Why are the neurons dying in MS ?



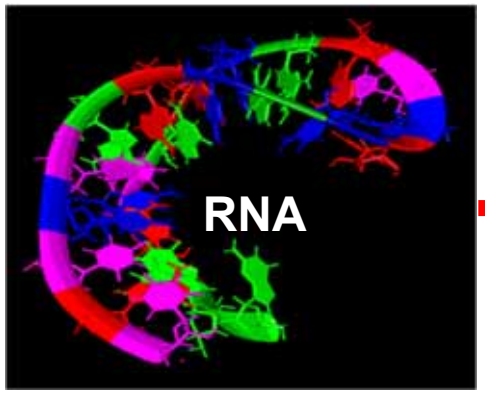


Which genes have been turned on and which have been turned off?

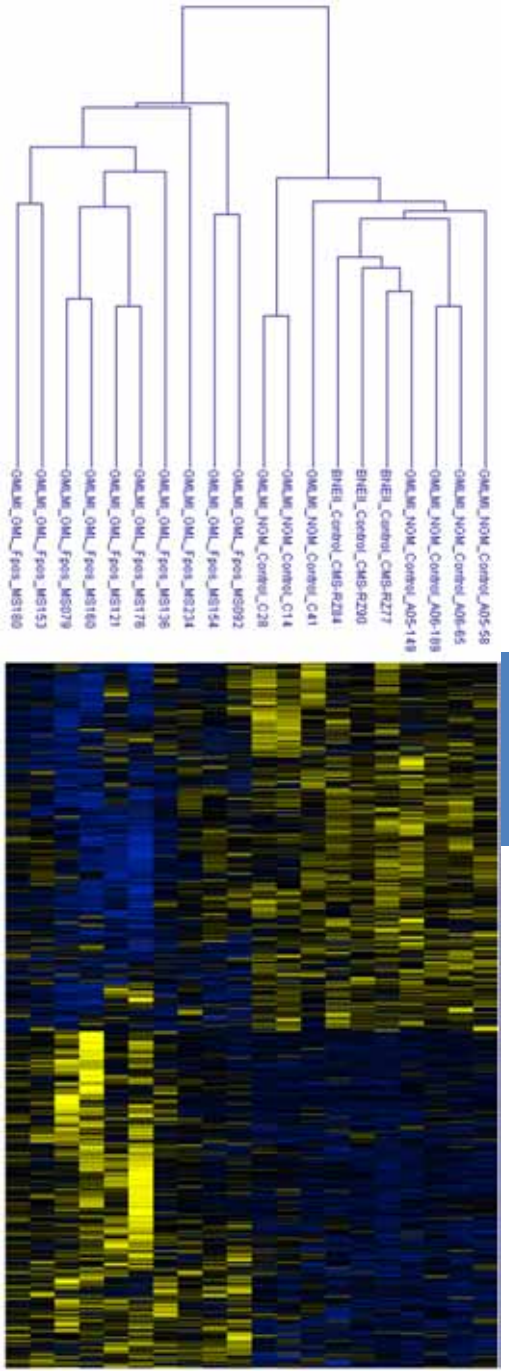


Do gene variations confer susceptibility to mild or aggressive disease

24,000 genes
(30,000)



Gene
chip

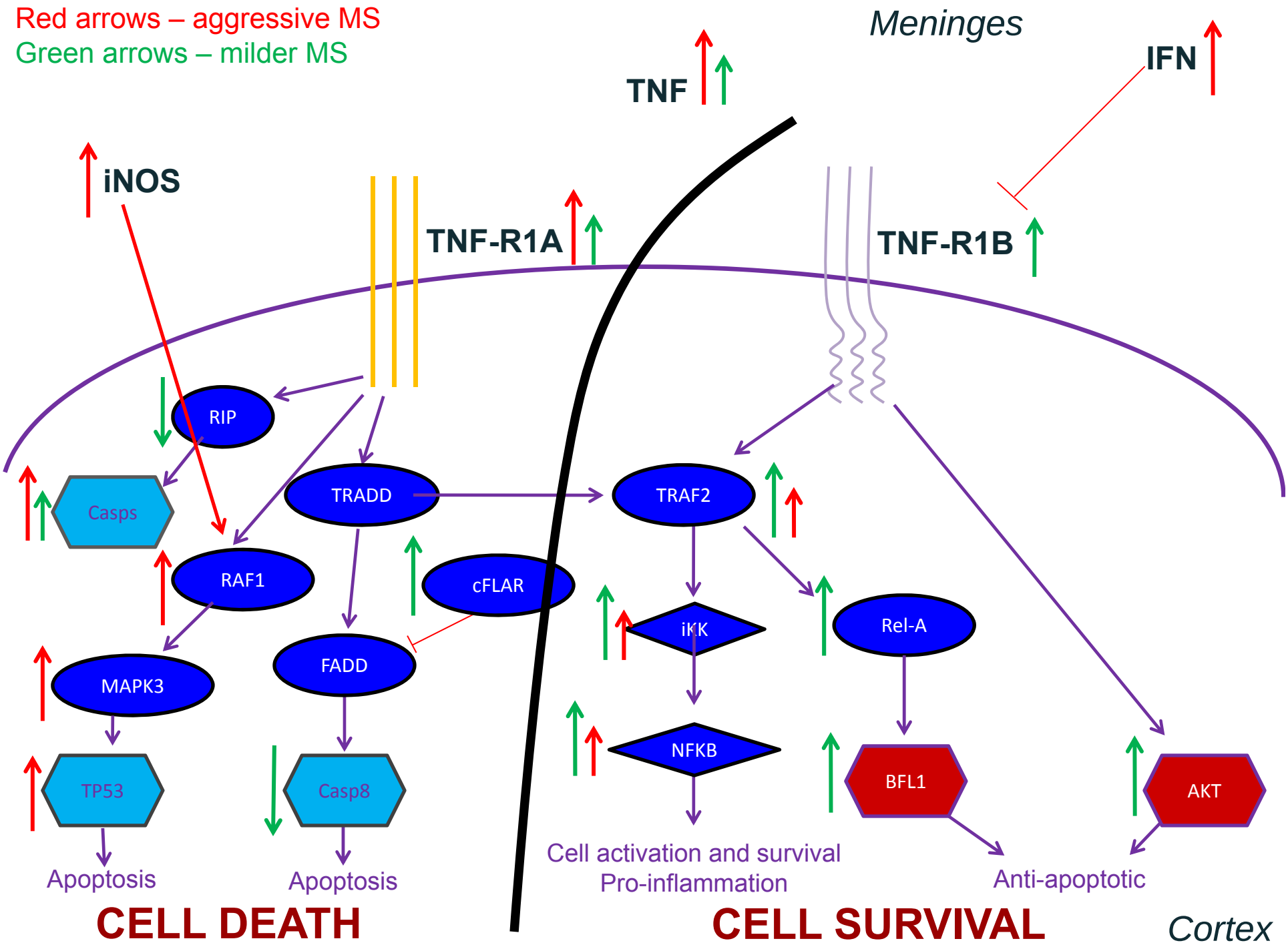


282 up

182 down

Neuronal markers

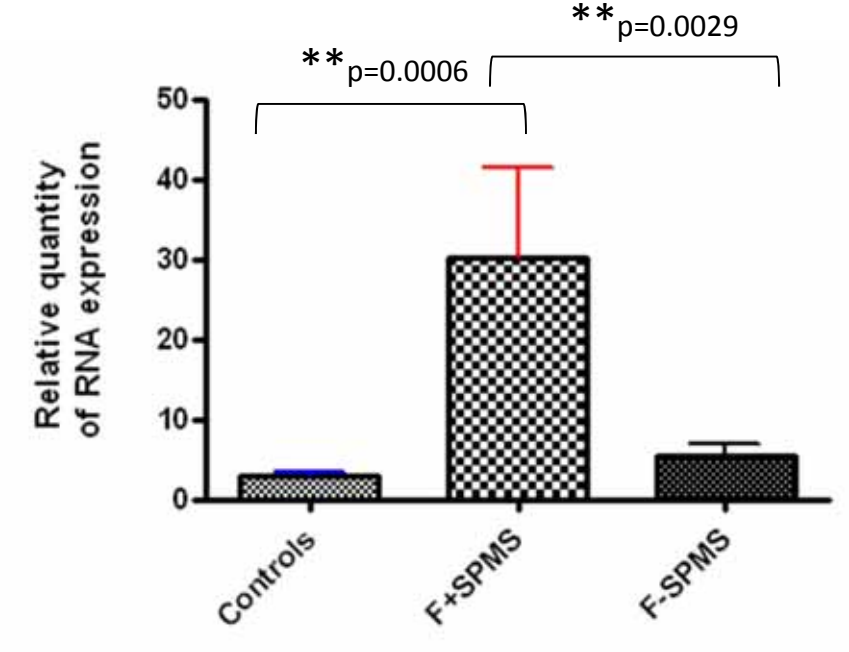
Neurofilament H	2.2 fold down
Neurofilament M	2.1 fold down
Neurofilament L	1.5 fold down
Parvalbumin	3.7 fold down
GAD67	1.7 fold down
Presenilin	1.6 fold down
NRG1	2.0 fold down
FGF22	1.8 fold down
Synaptobrevin	2.7 fold down
Snap 25	1.7 fold down
Neuroligin 4	2.3 fold down
GABA-A α1	1.6 fold down
NMDA 2A	1.6 fold down
AMPA 3	1.6 fold down
Na+v1β	3.0 fold down
K+ shaker 1	2.0 fold down
K+v KQT5	2.2 fold down
Ca2+v β4	1.7 fold down
Cl channel 4	1.7 fold down


Red arrows – aggressive MS
Green arrows – milder MS
Meninges
TNF
IFN
iNOS
TNF-R1A
TNF-R1B
RIP
Casps
TRADD
TRAF2
RAF1
cFLAR
iKK
Rel-A
MAPK3
FADD
NFKB
BFL1
AKT
TP53
Casp8
Apoptosis
Apoptosis
Cell activation and survival
Pro-inflammation
Anti-apoptotic
CELL DEATH
CELL SURVIVAL
Cortex

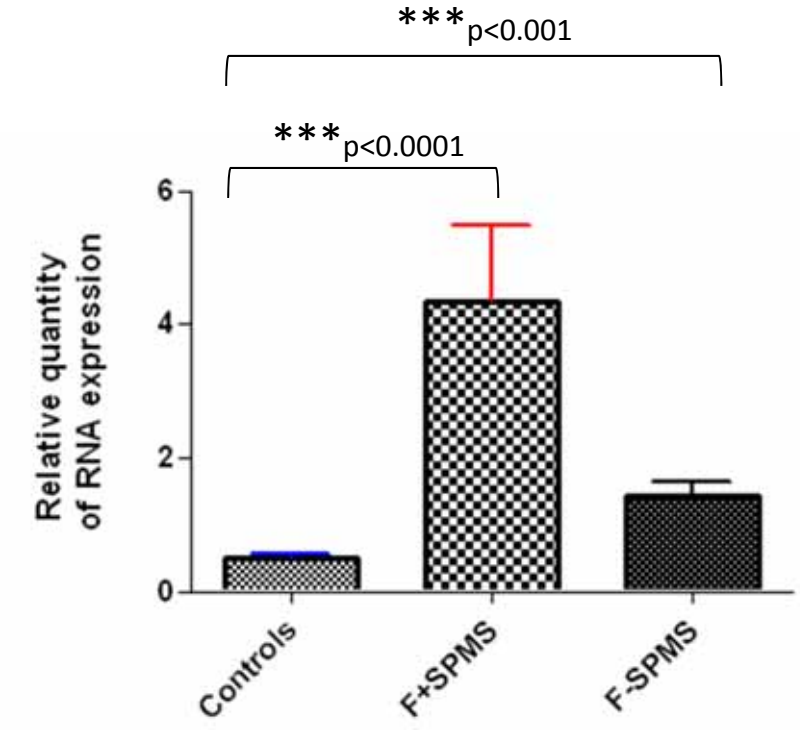
Relative quantity of IFNg and TNF mRNA in the meninges

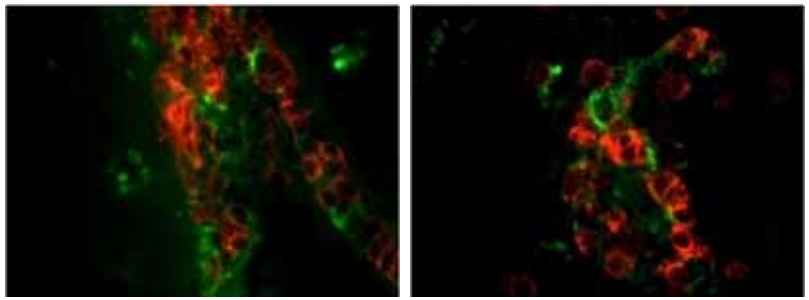
(5 F+MS, 5 F-MS, 5 Ctrl)

IFNγ

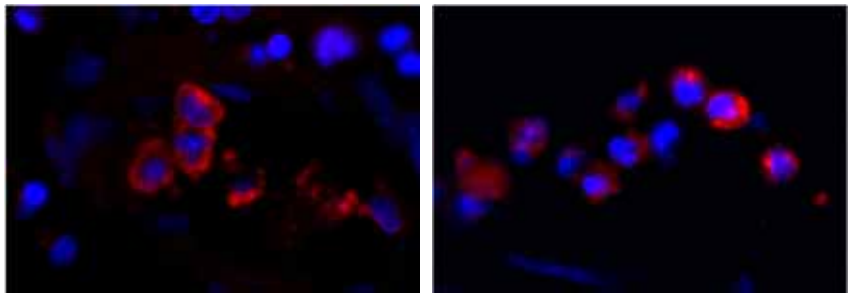


TNFα





CD3+IFN in the meninges of 2 F+MS

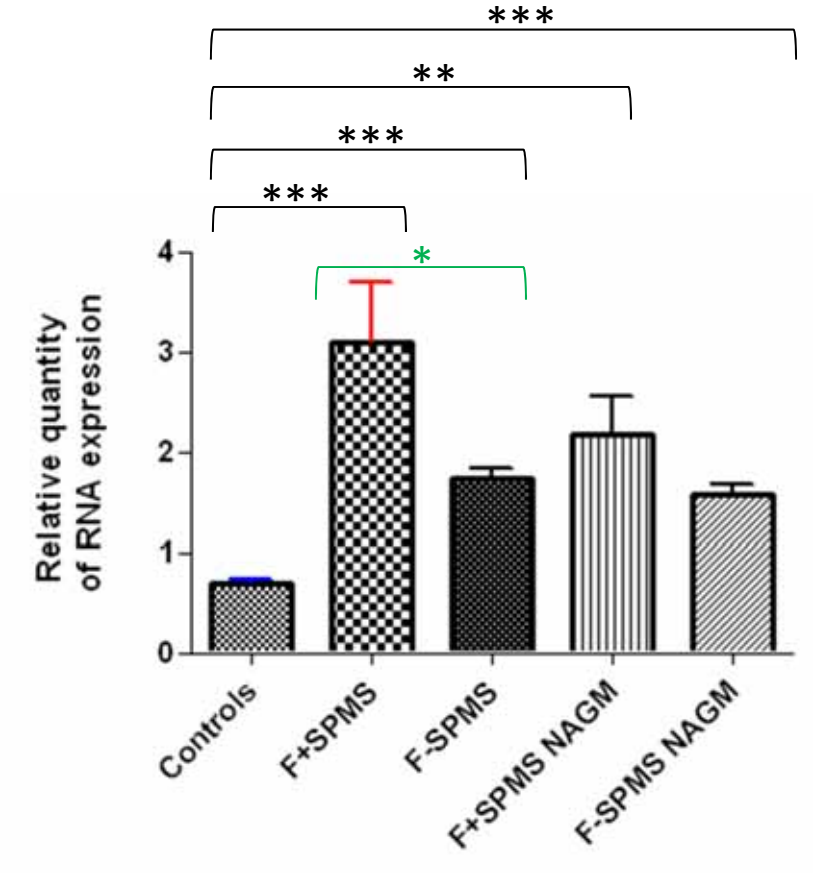


TNF in the meninges of 2 F+MS

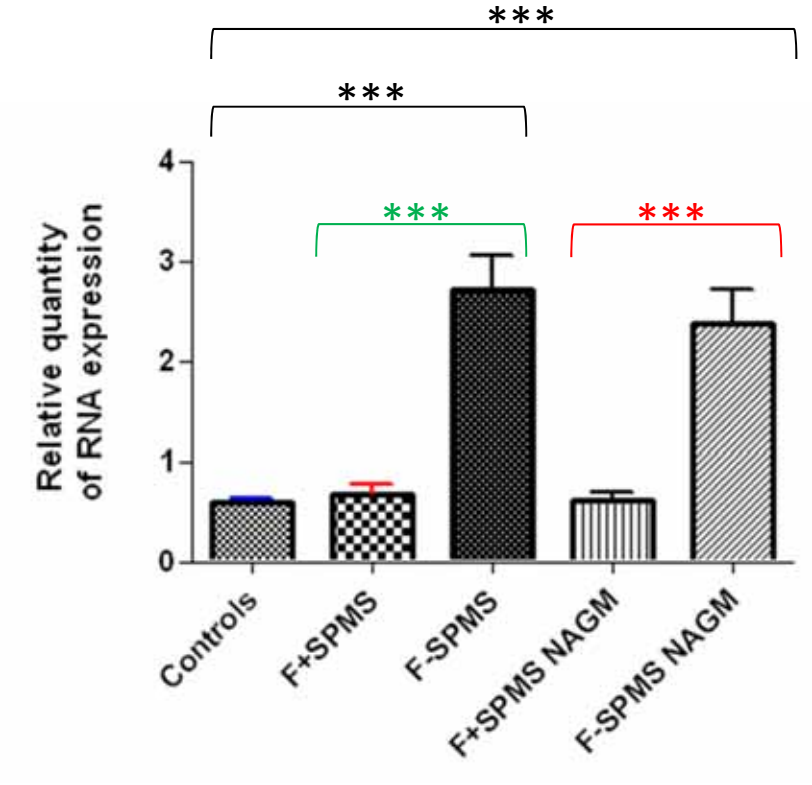
Relative quantity of TNFRs in the cortex

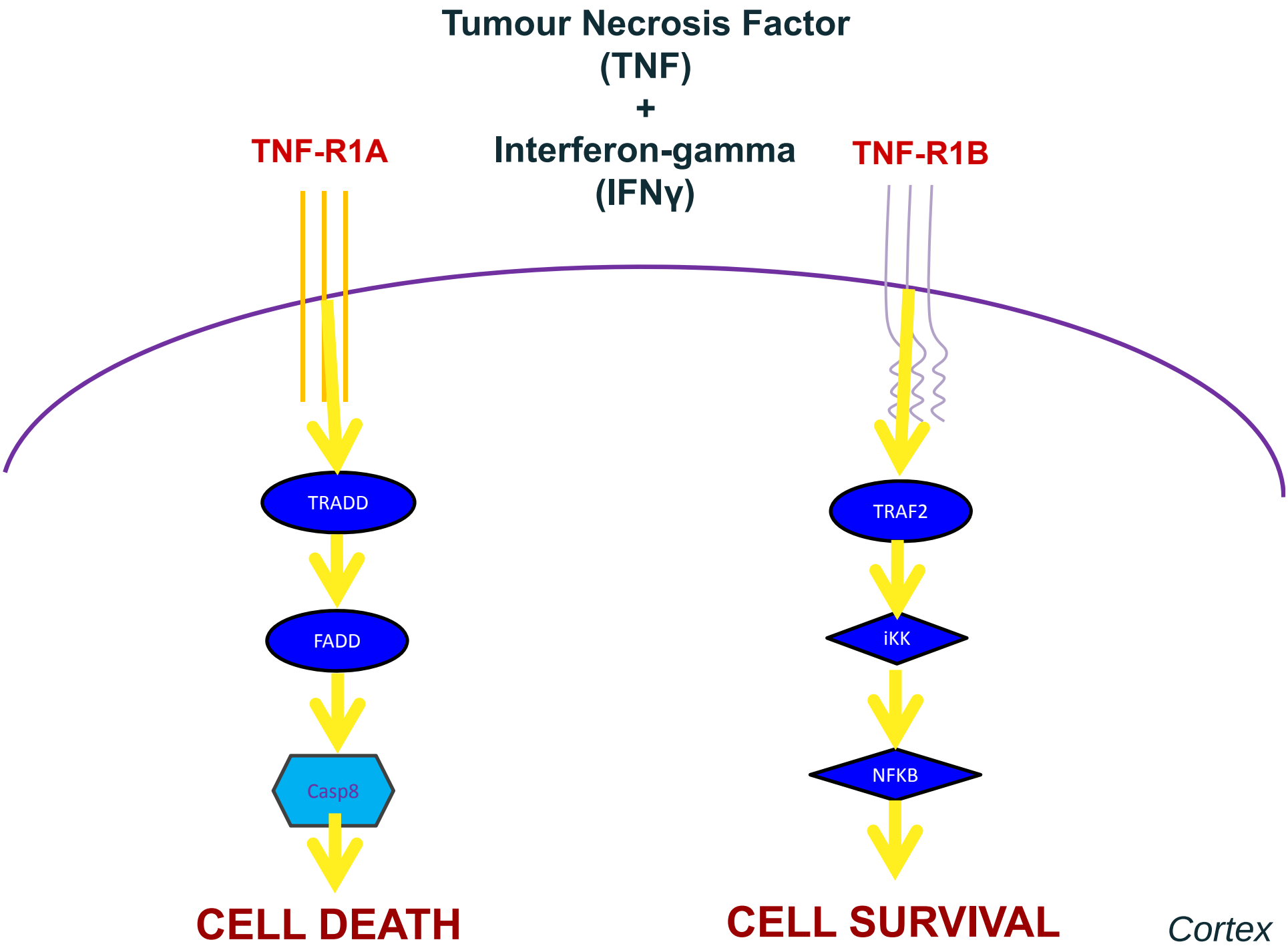
(6 F+MS, 6 F-MS, 6 Ctrl)

TNF-R1A



TNF-R1B




Tumour Necrosis Factor
(TNF)
+
Interferon-gamma
(IFNγ)
TNF-R1A
TNF-R1B
TRADD
FADD
Casp8
TRAF2
iKK
NFKB
CELL DEATH
CELL SURVIVAL
Cortex

Is multiple sclerosis a neurodegenerative disease ?

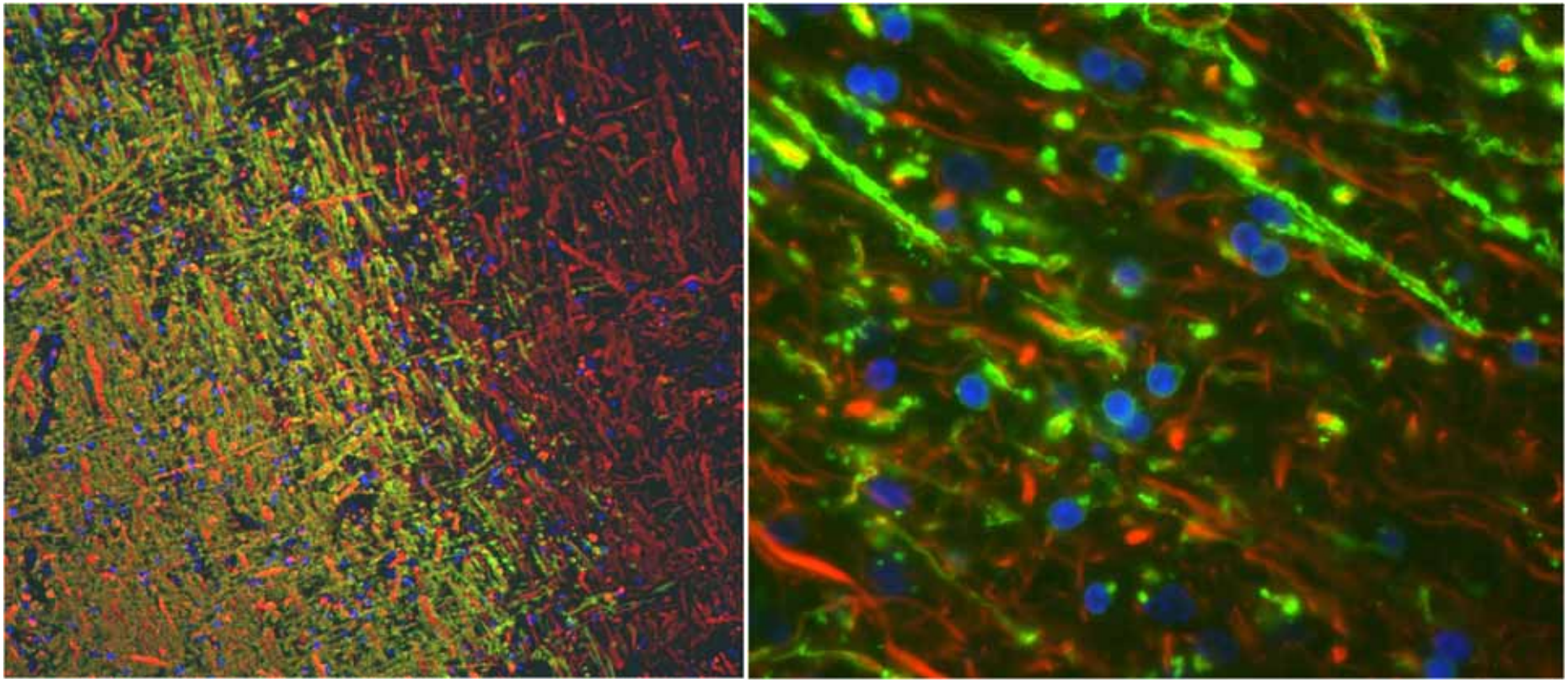
- Cortical pathology has a major impact on clinical progression in MS
- Presence of meningeal B-cell follicles leads to more extensive pathology and loss of neurons
- Cytotoxicity mediated by factors released by B-cells and/or CD8 T-cells and/or microglia
- How early during the disease course does this start?
- Different pathogenetic mechanisms may be involved in WM & GM pathology, suggesting novel treatment options

Multiple sclerosis is an inflammatory neurodegenerative disease

Does the brain try to repair the damage caused by MS ?

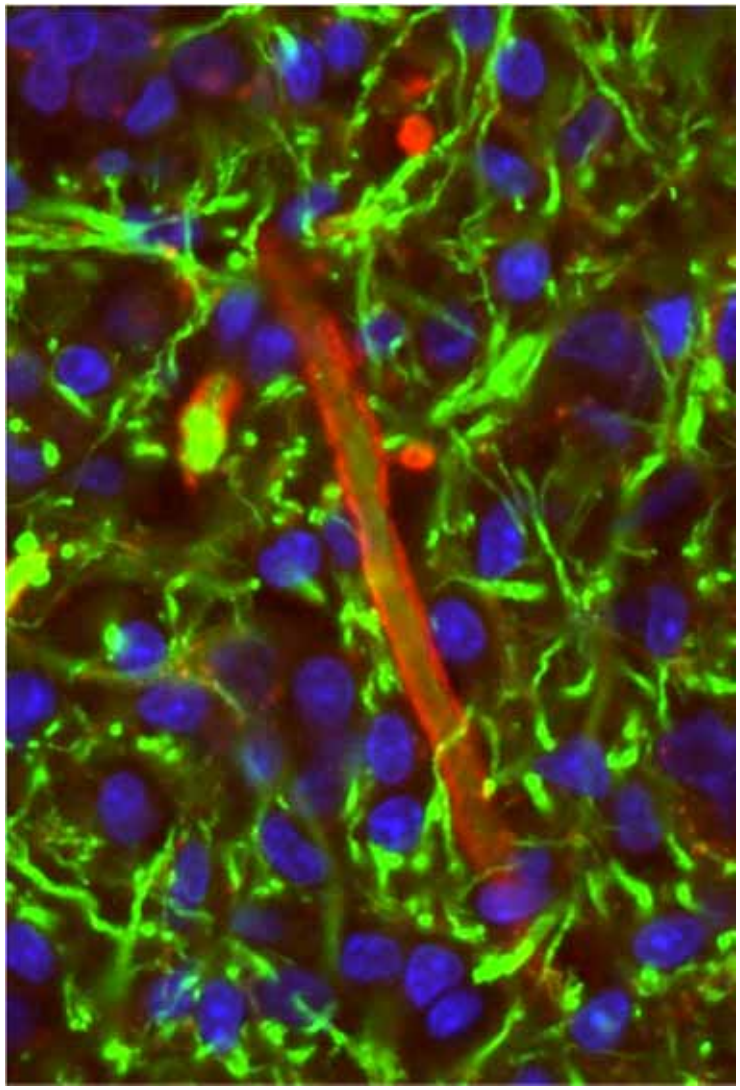
Demyelination in MS

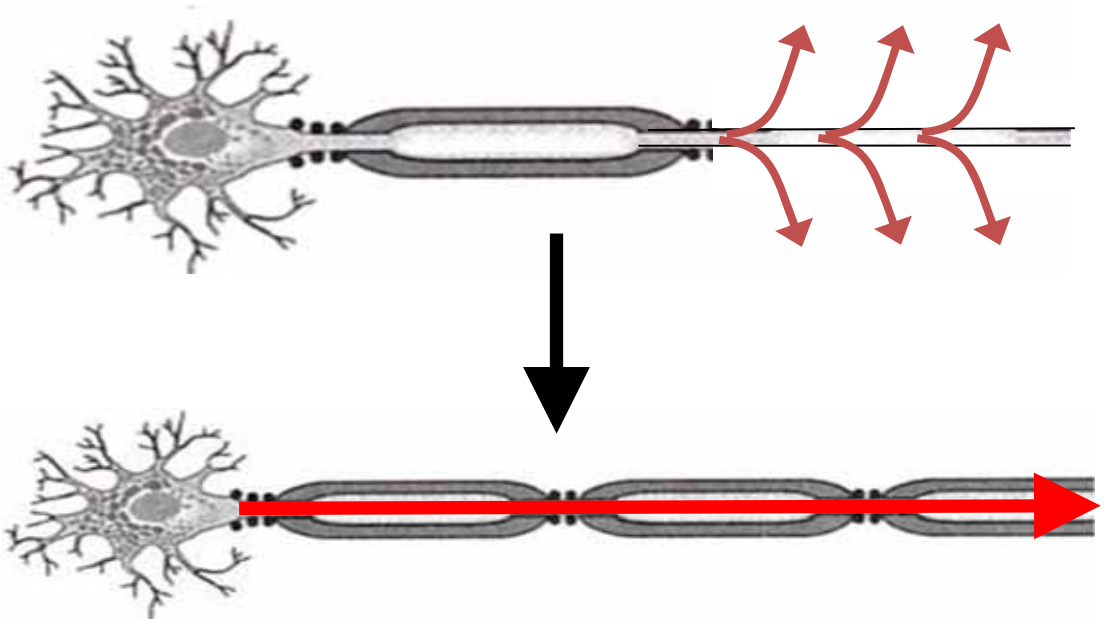
Oligodendrocytes in MS lesions



Oligodendrocytes are generally absent from the centre of chronic lesions although increased numbers are often seen at the lesion edge. MBP-expressing cells were found in large numbers at the edge of some MS lesions.

Demyelination can be repaired





REMYELINATION IS A NATURAL REPAIR PROCESS THAT SHOULD OCCUR IN MS

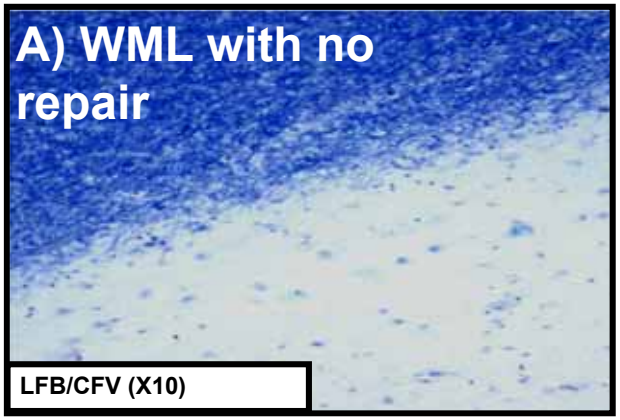
It can be stopped by ongoing inflammation and by accumulating damage to the axons

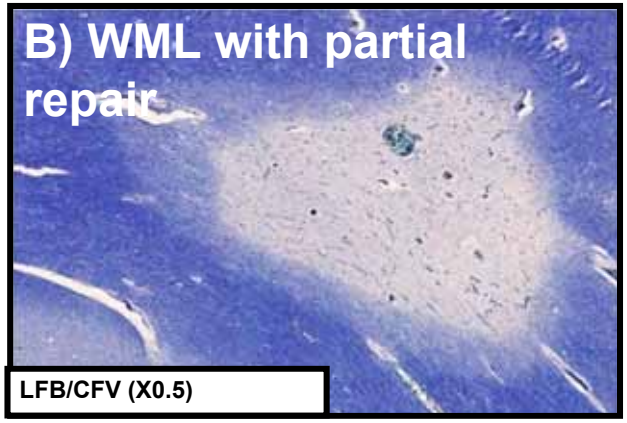
Remyelination restores conduction and protects axons

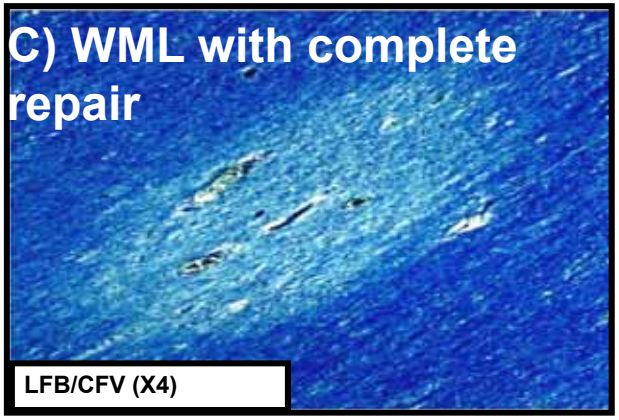
Remyelination in MS

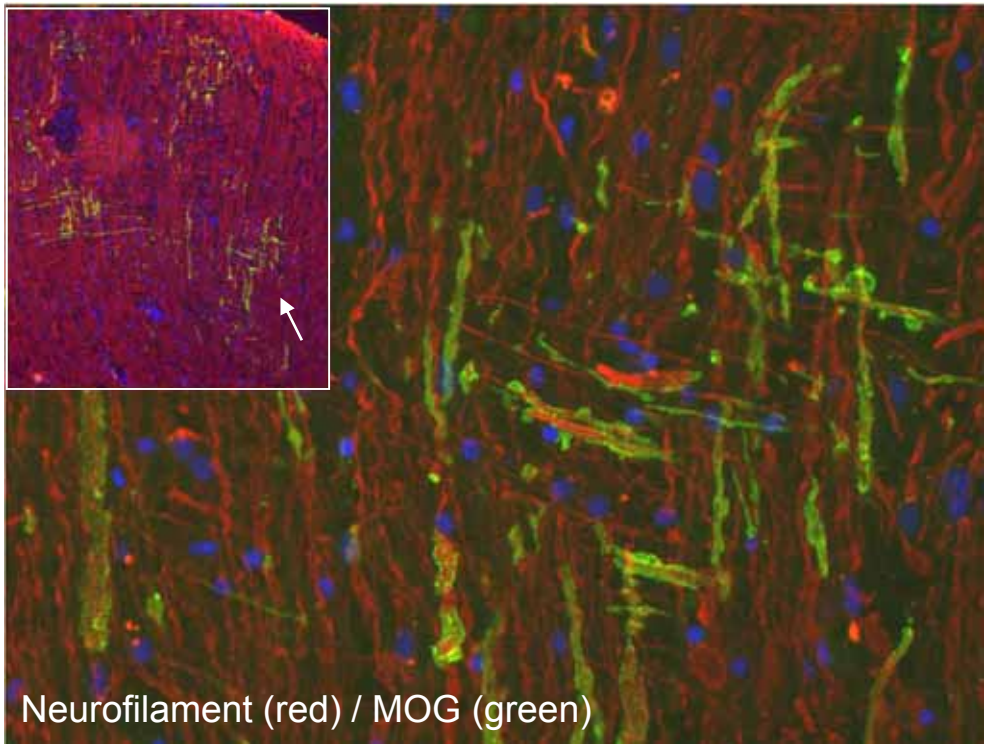
- repair in MS is indicated by the presence of thinly remyelinated sheaths.
- remyelination is a frequent finding at the edge zones of inactive plaques.
- in the early stages of MS rapid and extensive remyelination may be the rule.
- complete remyelination of lesions can occur.
- Schwann cell remyelination is found in the spinal cord.
- if remyelination is effective during the early stages of MS why then does it fail as the disease progresses?

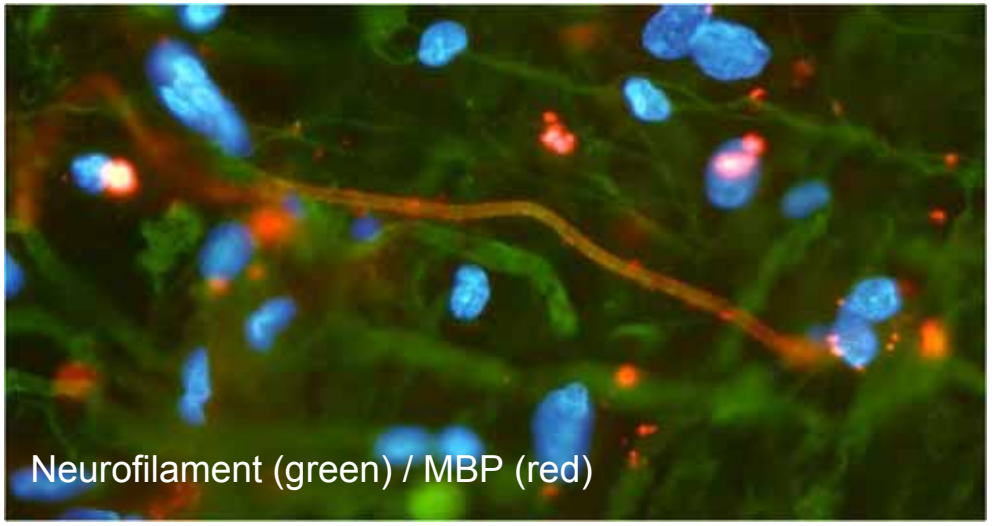
Remyelination in MS



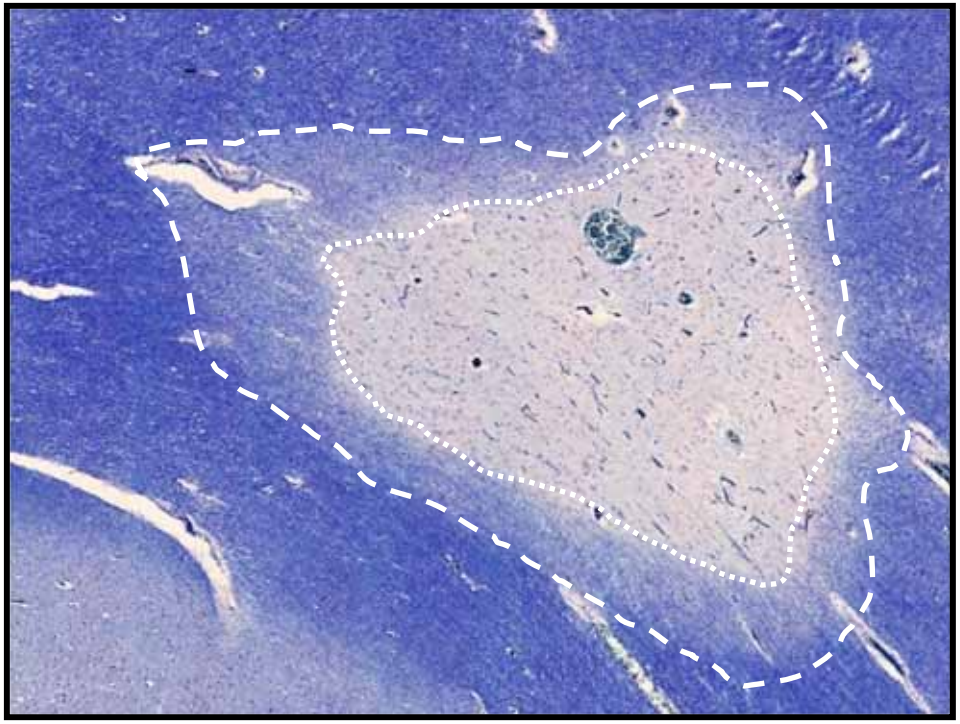




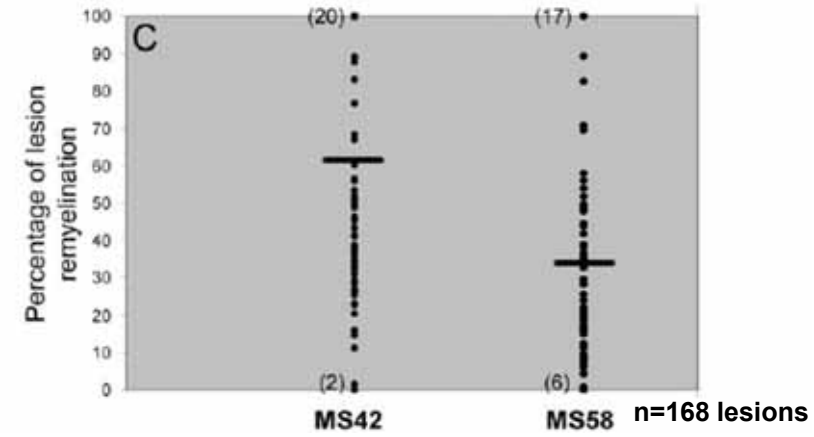




Myelin repair is a frequent finding in MS and may continue for a long time

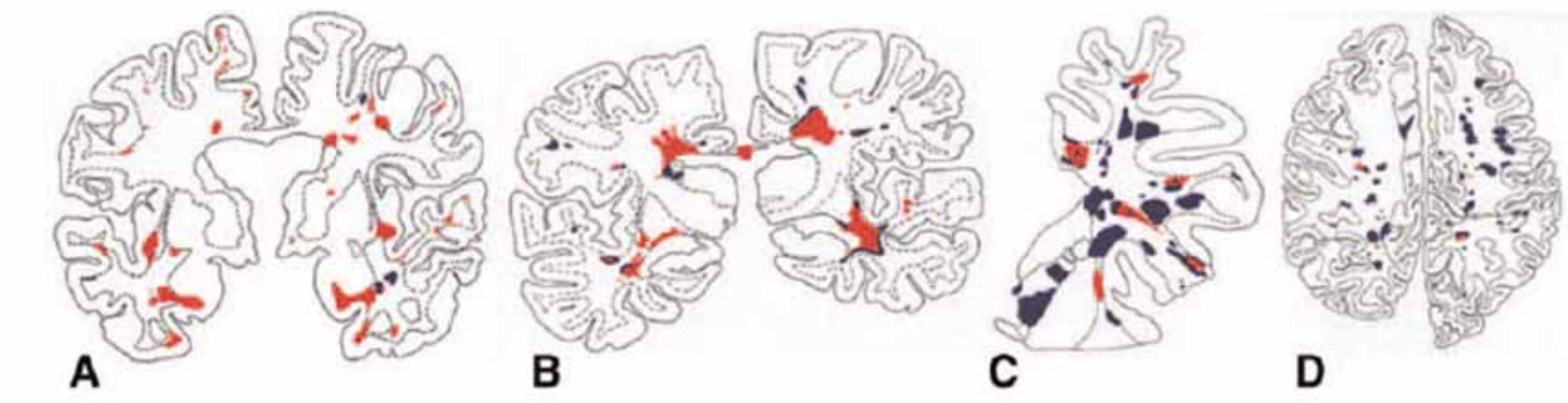


MS42	MS58
51 yrs	*51 yrs*
20 yrs MS	*21 yrs MS*
% REMYELINATION	
60%	**35%**



The extent of remyelination of individual lesions is highly variable

Patani et al, 2007



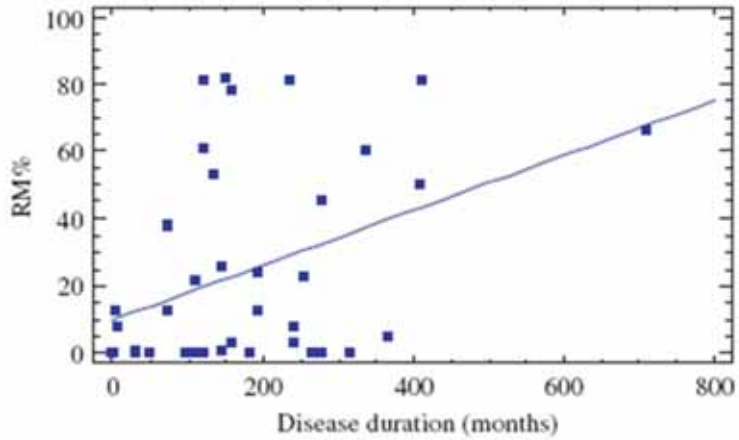


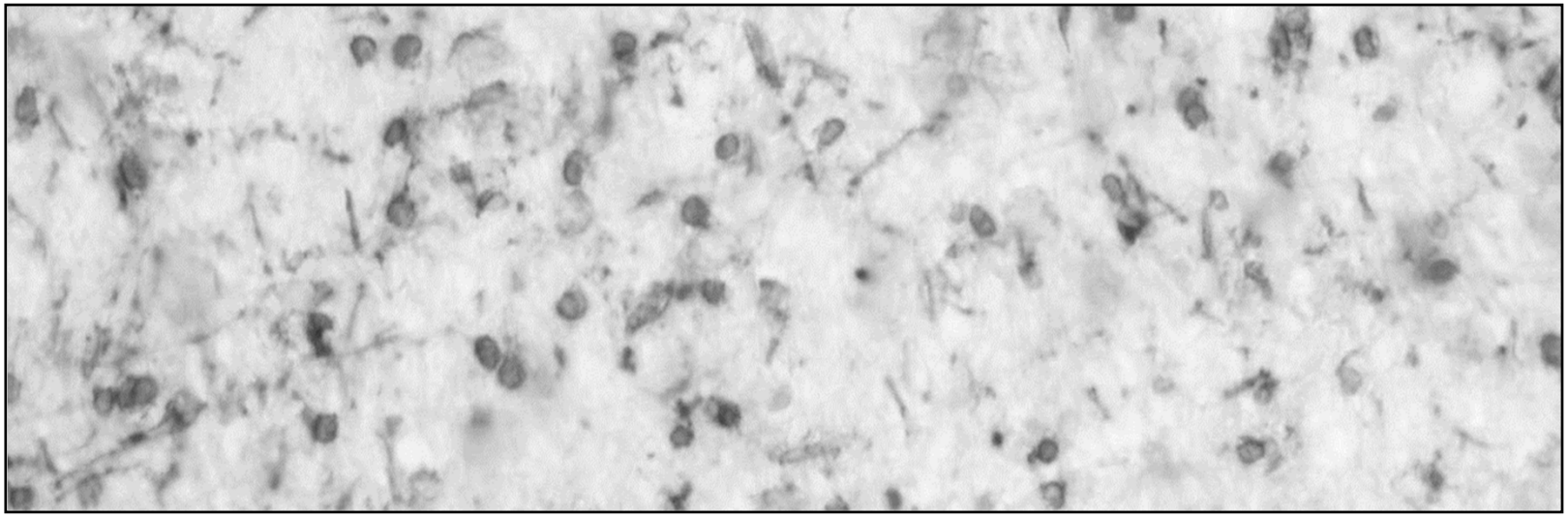
Fig. 3 Graph showing the statistically significant correlation between remyelination and disease duration (global sample; $n = 39$; $P = 0.021$; $r = 0.374$).

Patrikios et al, 2006

Oligodendrocytes in MS lesions

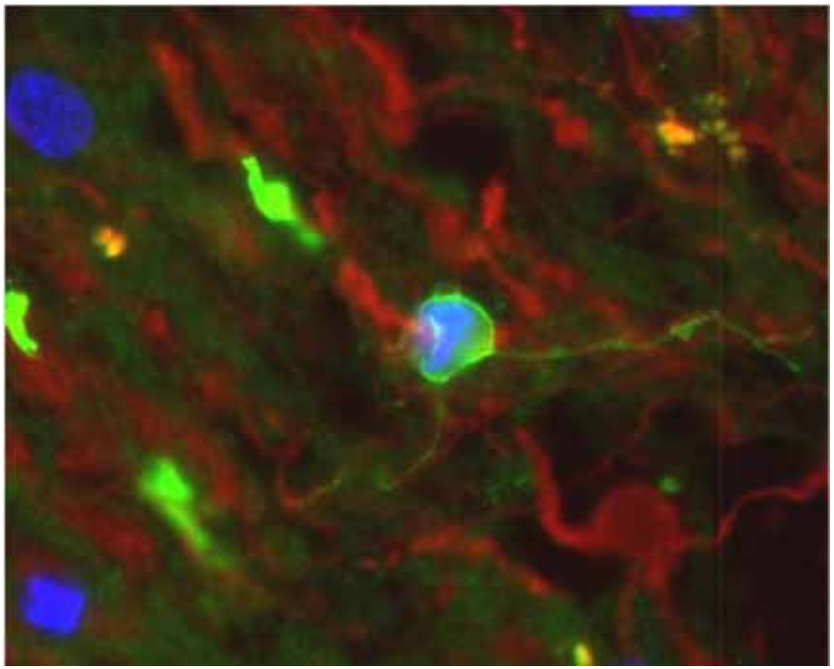
- Recent studies of large series of autopsied and biopsied cases with few exceptions confirm that oligodendrocytes are largely lost in areas of active demyelination

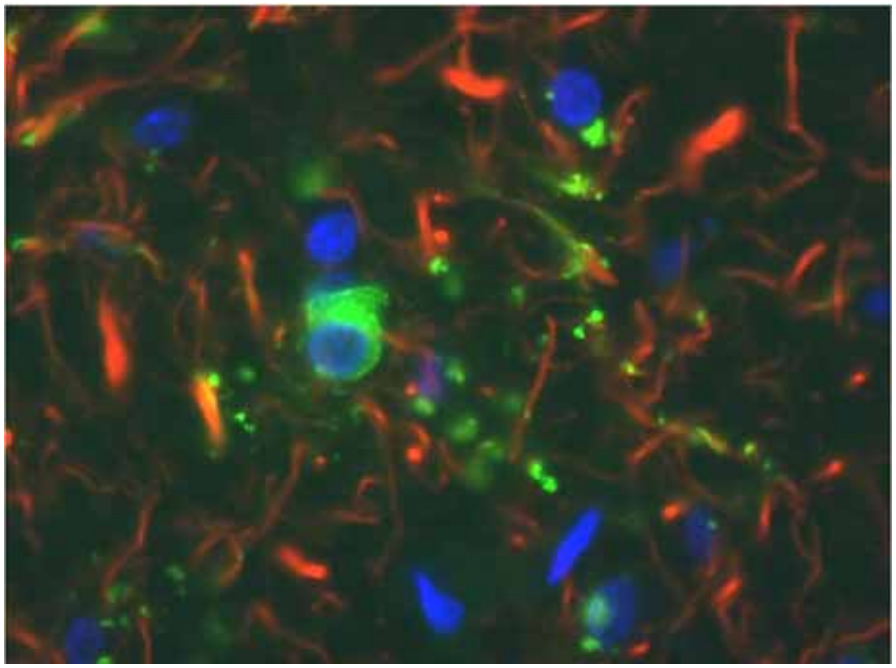
- Oligodendrocytes frequently reappear in large numbers in recently demyelinated tissue



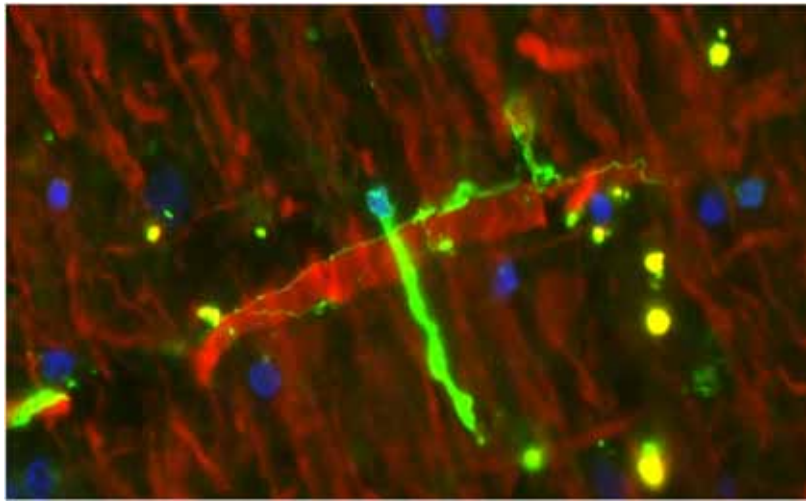
- Oligodendrocytes are present in only small numbers in chronic inactive lesions

Oligodendrocytes in MS lesions





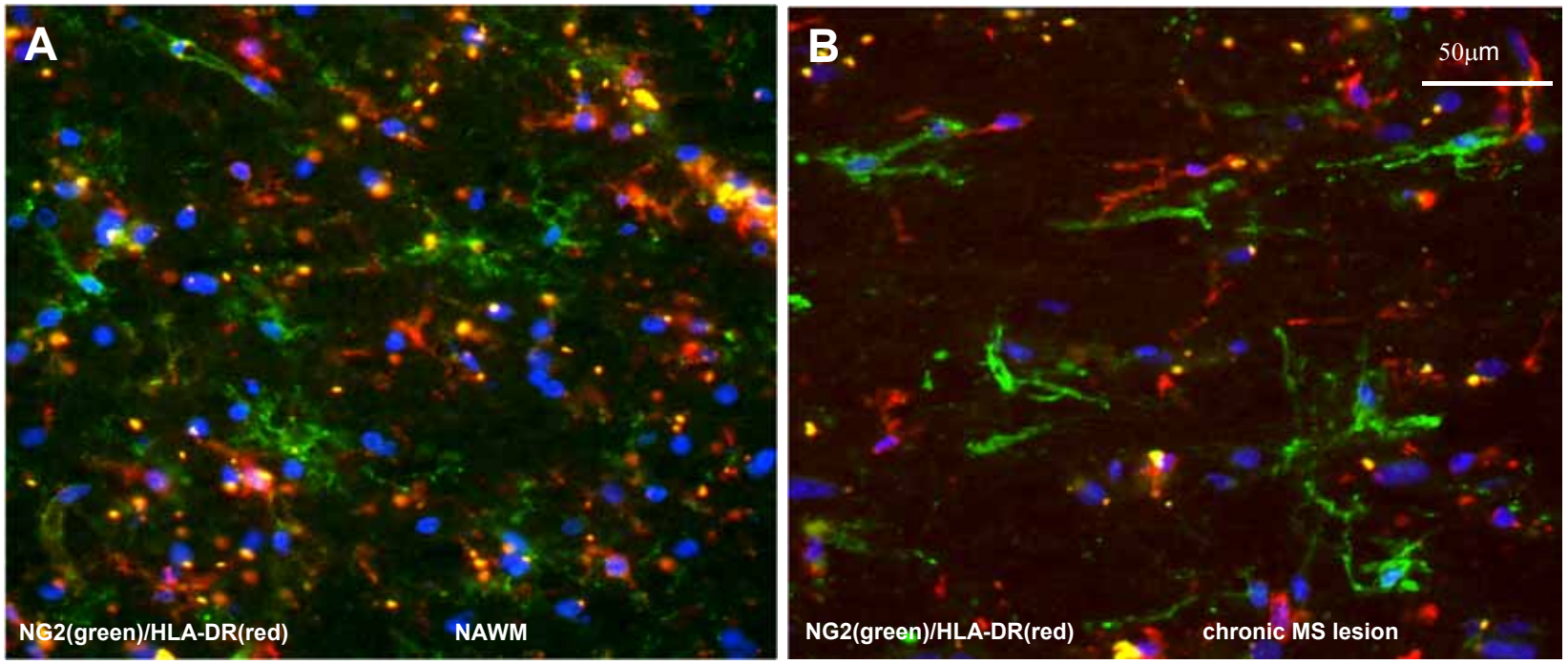
MOG-expressing oligodendrocytes are found in MS lesions but are infrequent



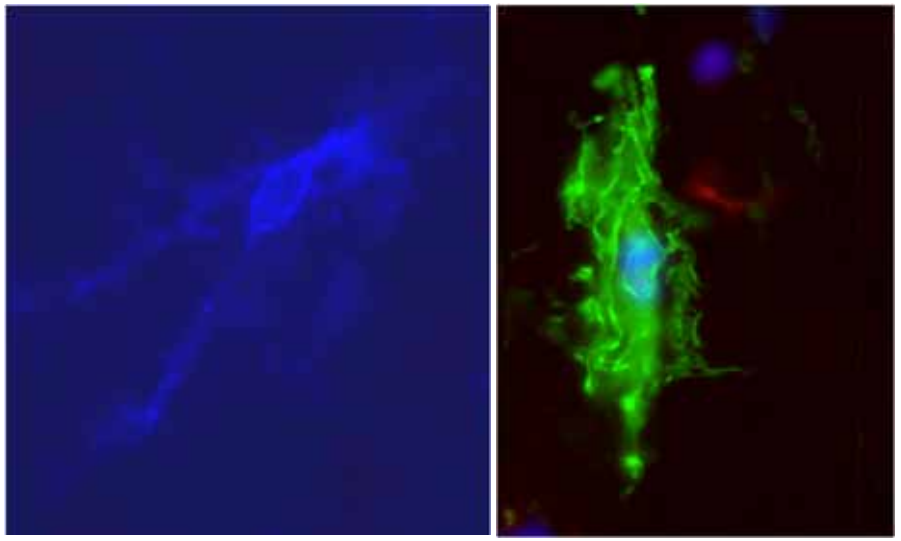
Oligodendrocyte progenitors in MS lesions

- some progenitor cells are present in most chronic plaques, in the complete absence of remyelination
- progenitor numbers are highly variable
- progenitor numbers are highest in lesions which still contain macrophages
- virtually nothing is known about the fate of progenitors in the acute lesion

Oligodendrocyte progenitors in MS lesions



Numbers of progenitors in chronic MS lesions are highly variable, from none to the same as the NAWM.



Myelin repair in MS

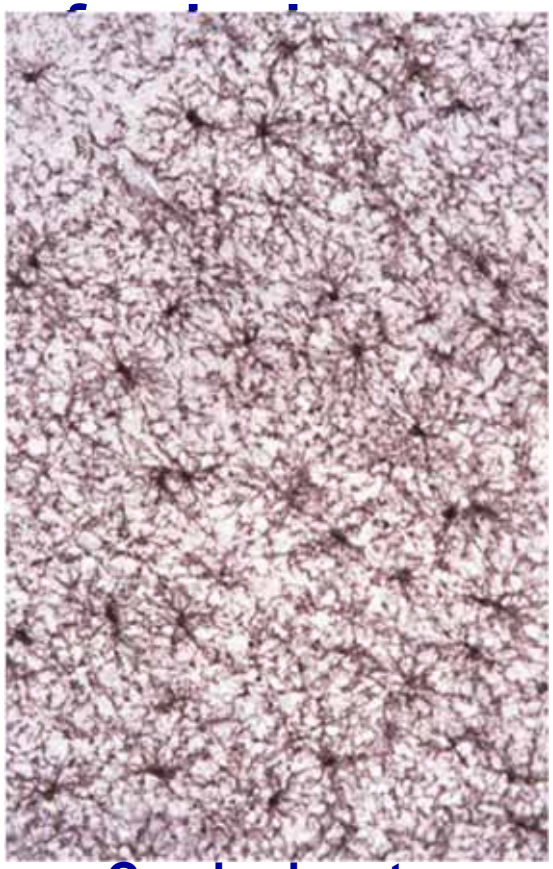
- **remyelination leads to restoration of neurological function and may protect axons against damage**
- ***The adult mammalian brain and spinal cord has an enormous capacity for repairing myelin damage***

Myelin repair in the mature CNS

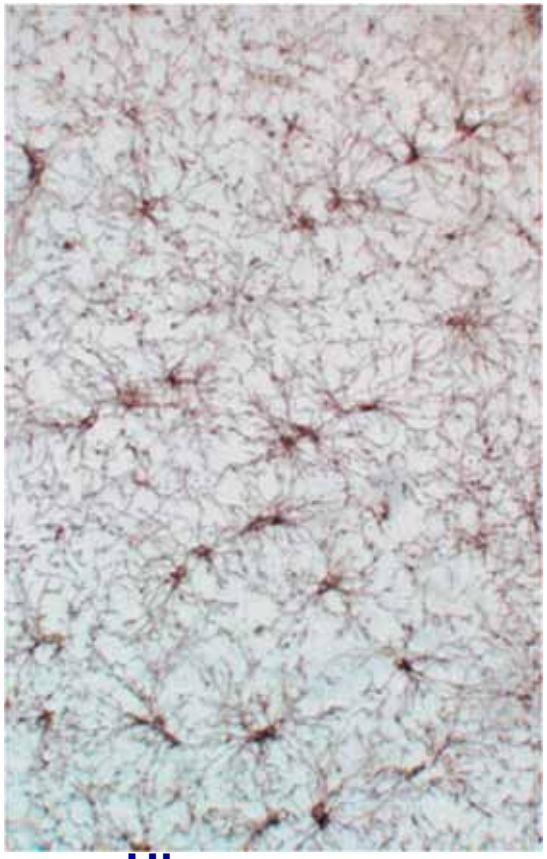
- **Adult mammalian brain contains a widespread and numerous population of oligodendrocyte precursor cells.**
- **Glial progenitors in the adult CNS are cycling.**
- **When isolated into culture they differentiate into oligodendrocytes.**
- **Glial progenitor cells are thought to be responsible for oligodendrocyte replacement following demyelination. Evidence?**

Dawson et al, 2003

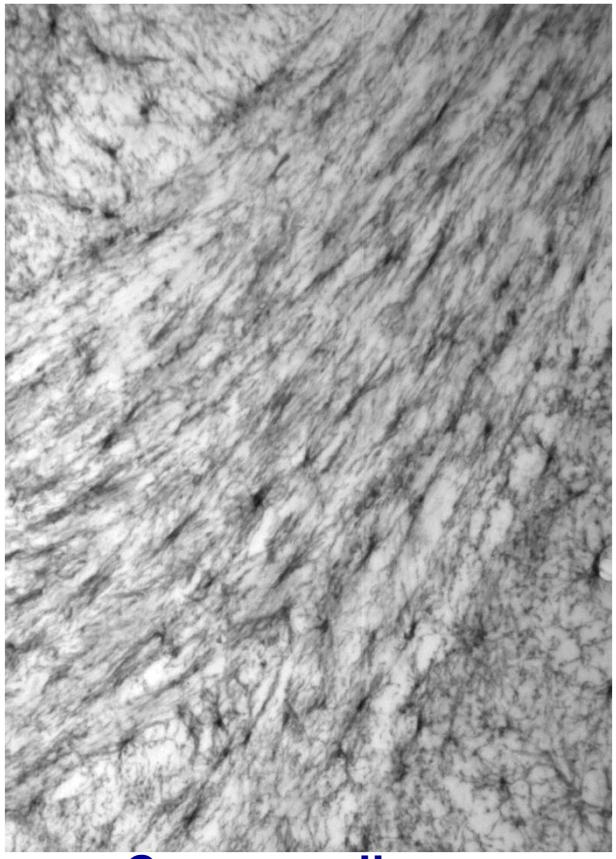
Distribution of glial progenitors in the adult rat



Cerebral cortex



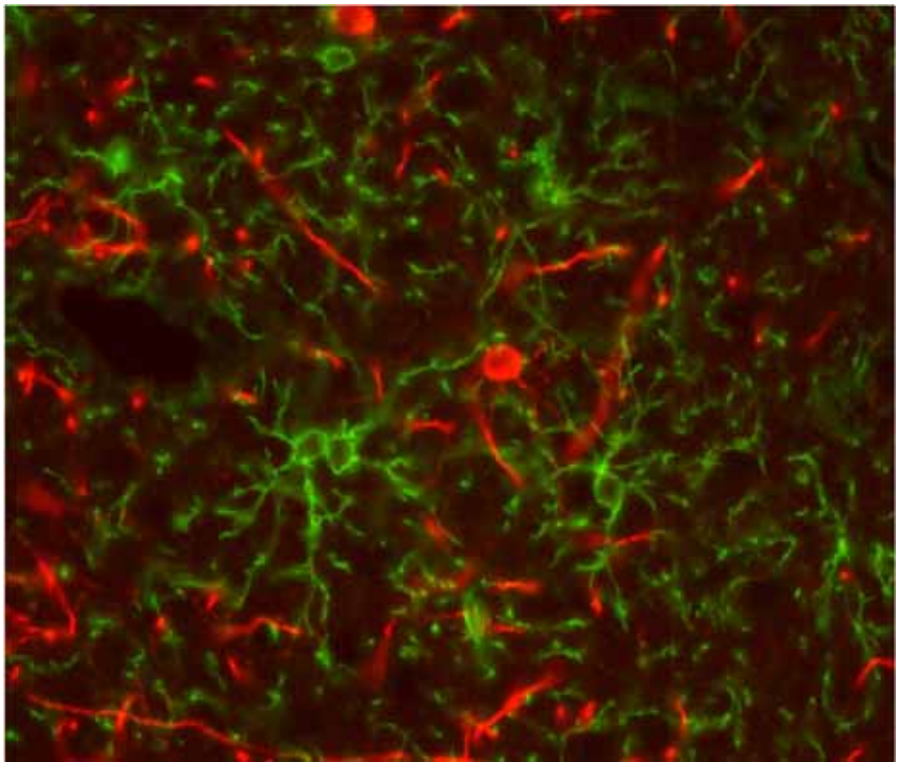
Hippocampus



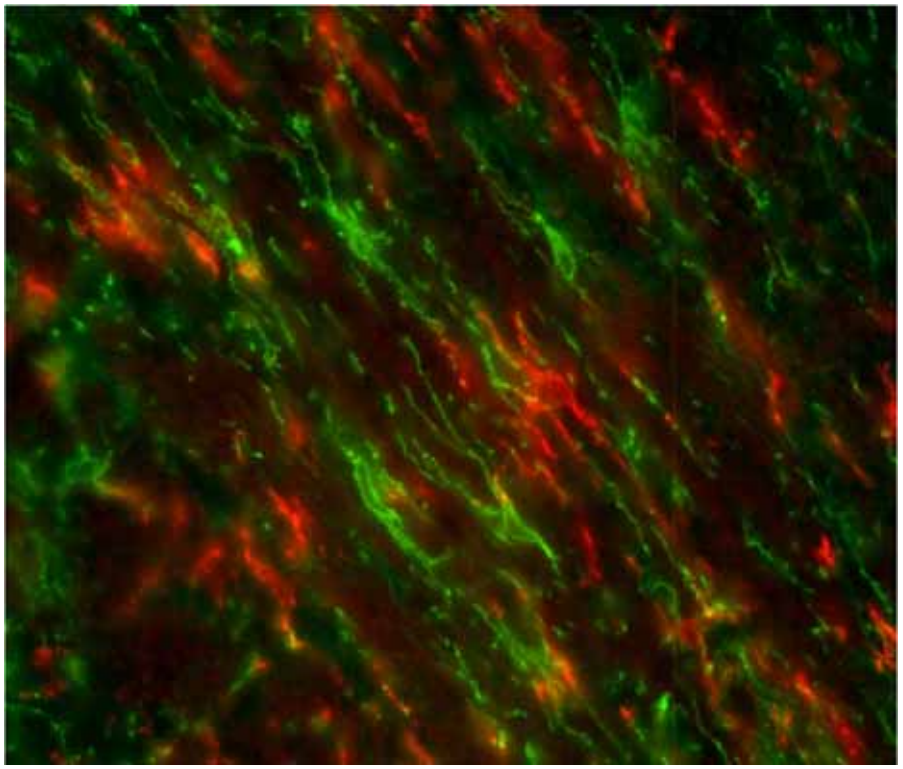
Corpus callosum

NG2-expressing glial progenitors are ubiquitously distributed throughout the adult mammalian CNS. Their morphology is varied according to their environment.

Ratio of oligodendrocytes:microglia:progenitors

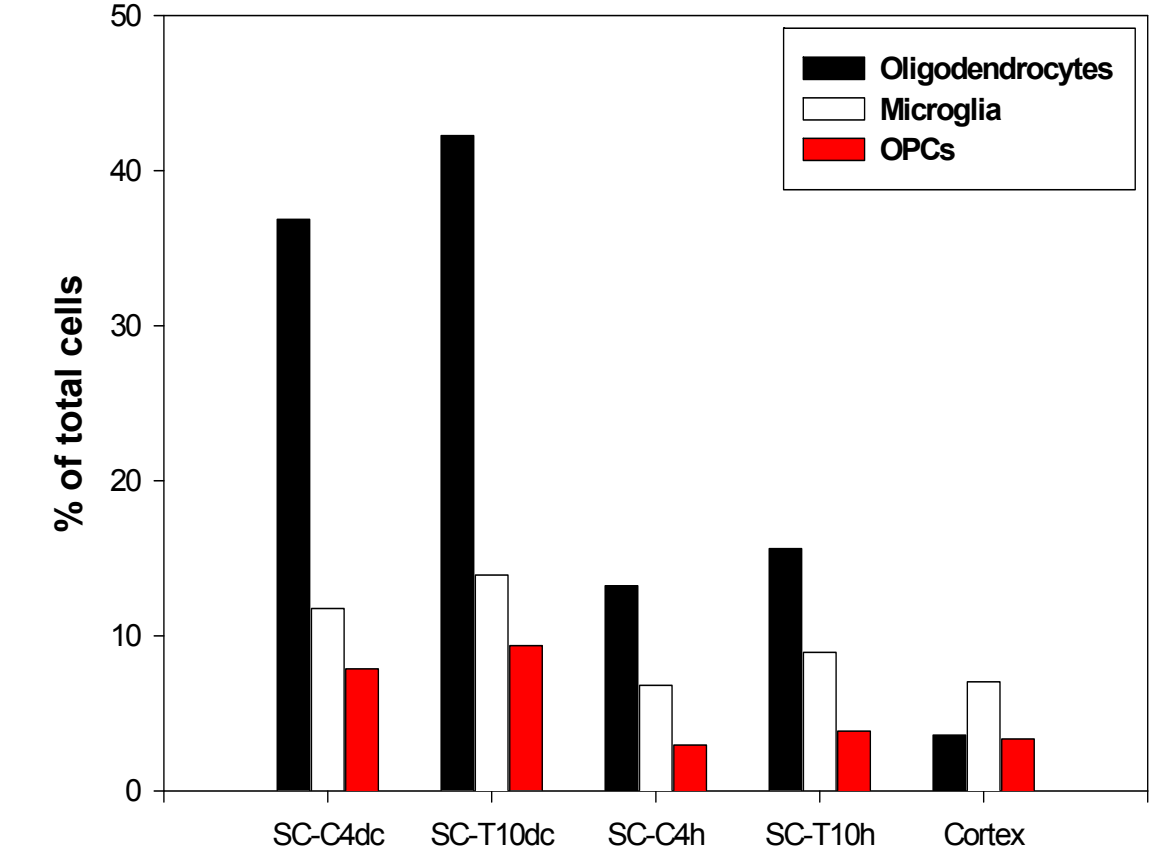


NG2+ progenitors/oligodendrocytes



NG2+ progenitors/microglia

Ratio of oligodendrocytes:microglia:progenitors

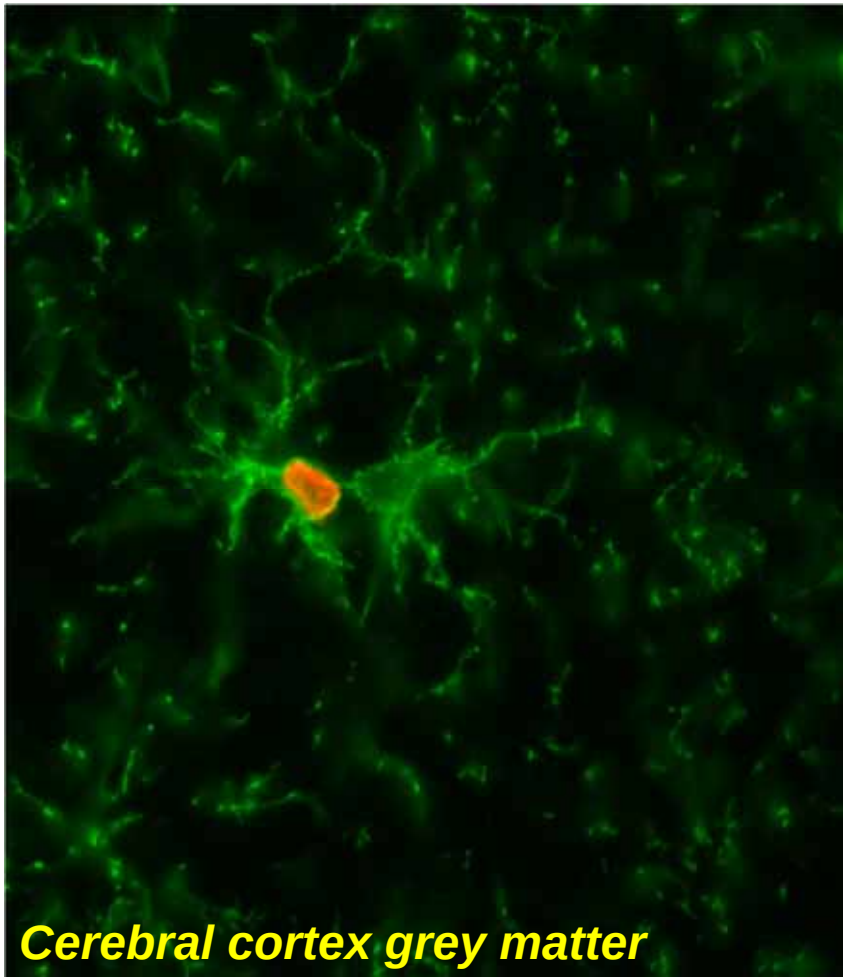


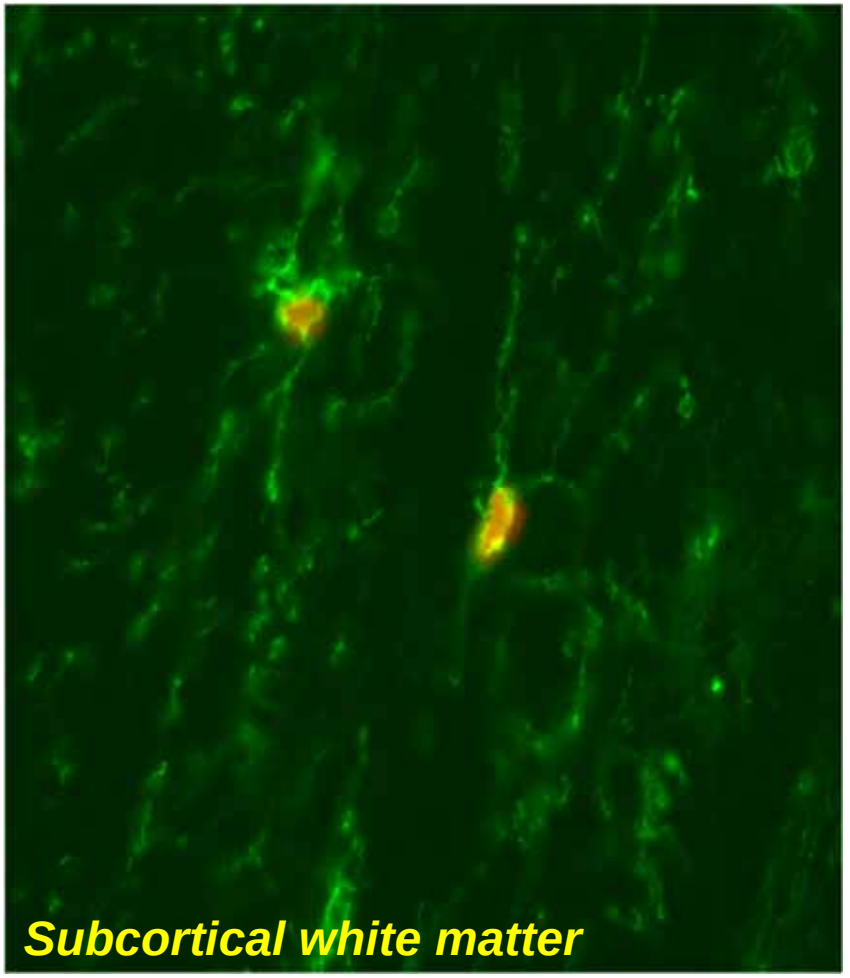
Cell ratios **3:2:1** **4:2:1** **4:2:1** **4:2:1** **1:2:1**

- these ratios have significant implications for myelin repair
- in spinal cord progenitors will need to undergo at least 2 cell divisions to replace oligodendrocytes
- in cerebral cortex progenitors will only need to undergo 1 round of cell division

Dawson et al, 2003

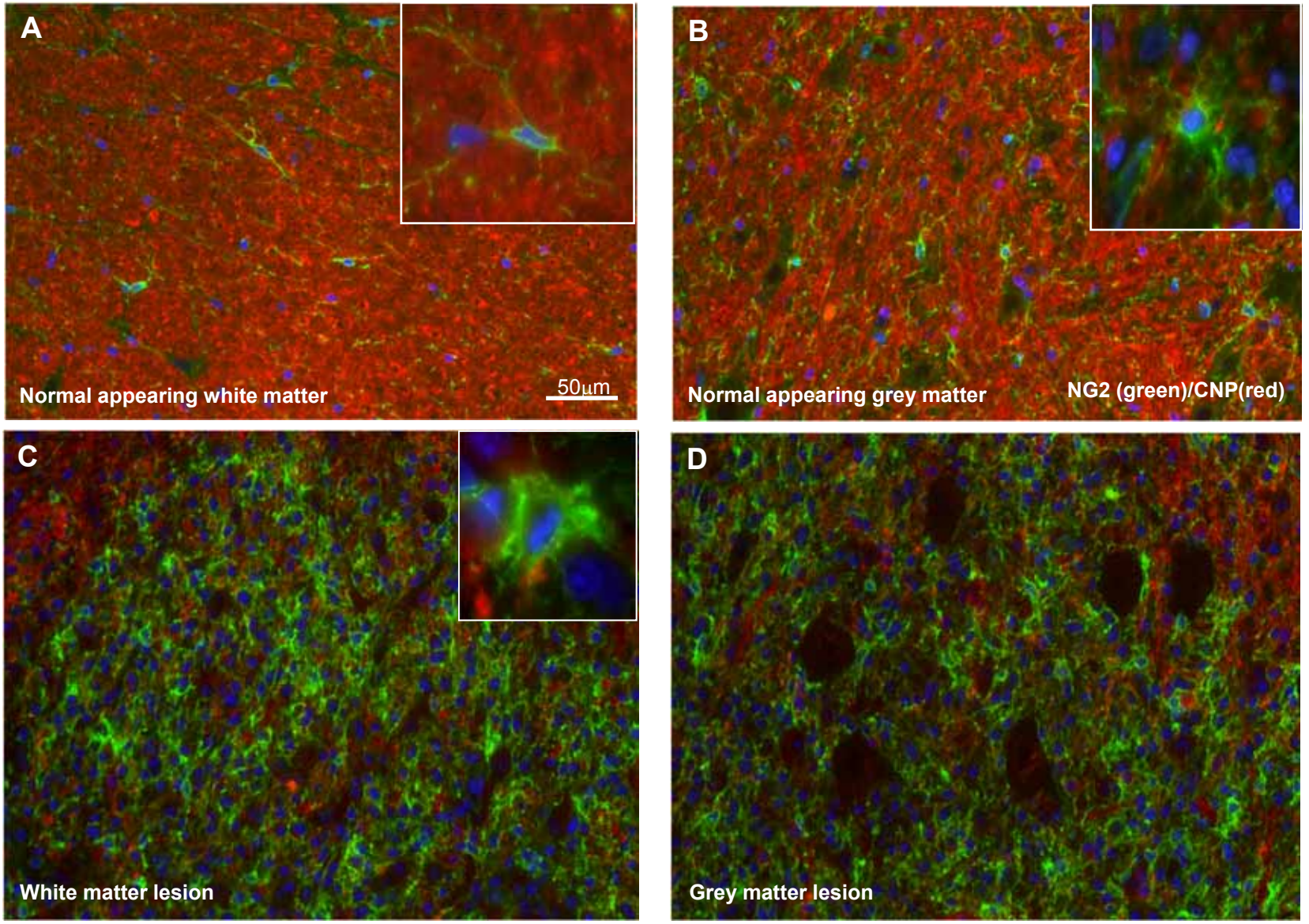
NG2+ glial progenitors are the major cycling population of the mature CNS





NG2 - green, BrdU - red

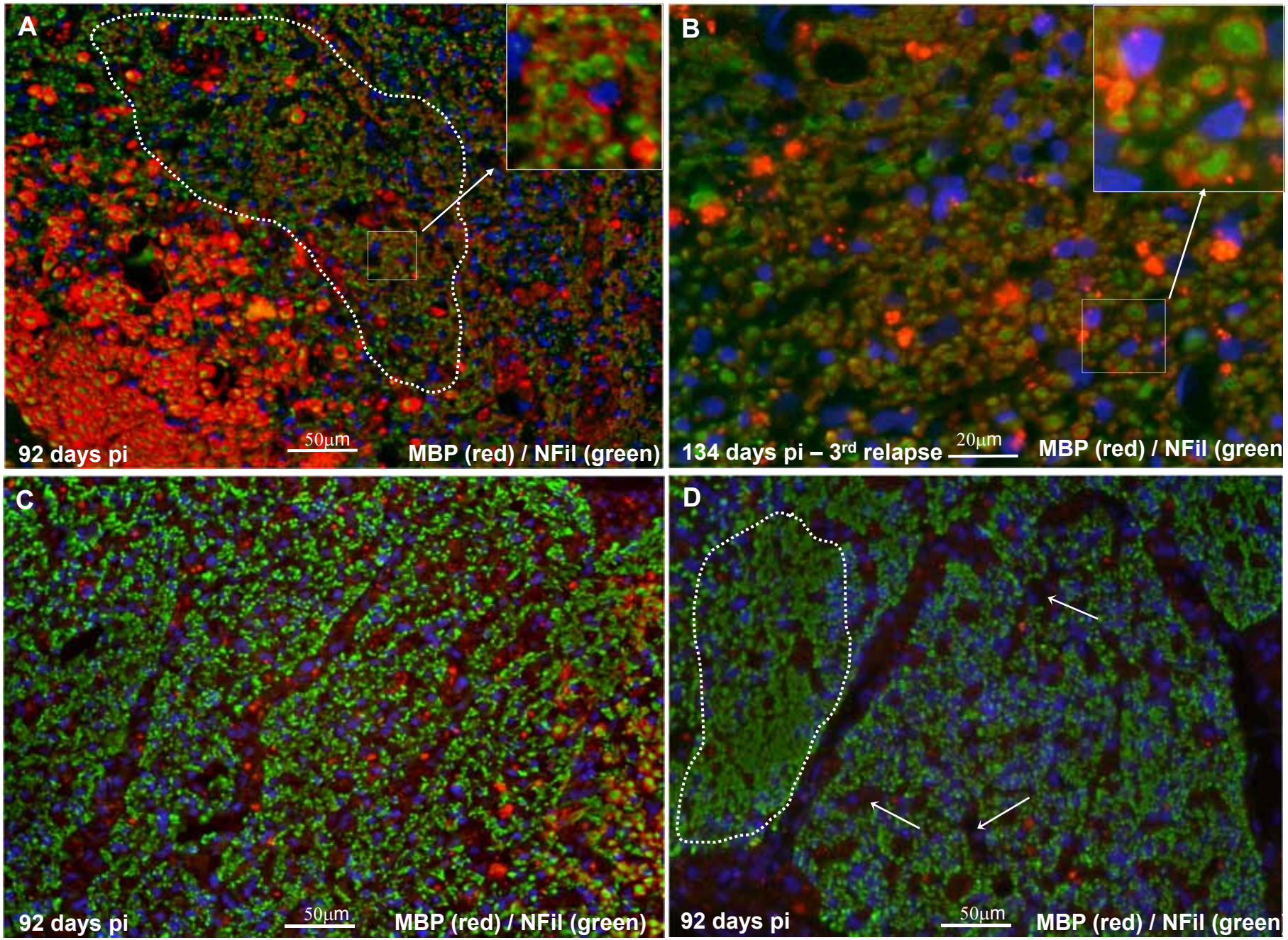
Response of NG2^{+} OPCs to demyelination in EAE



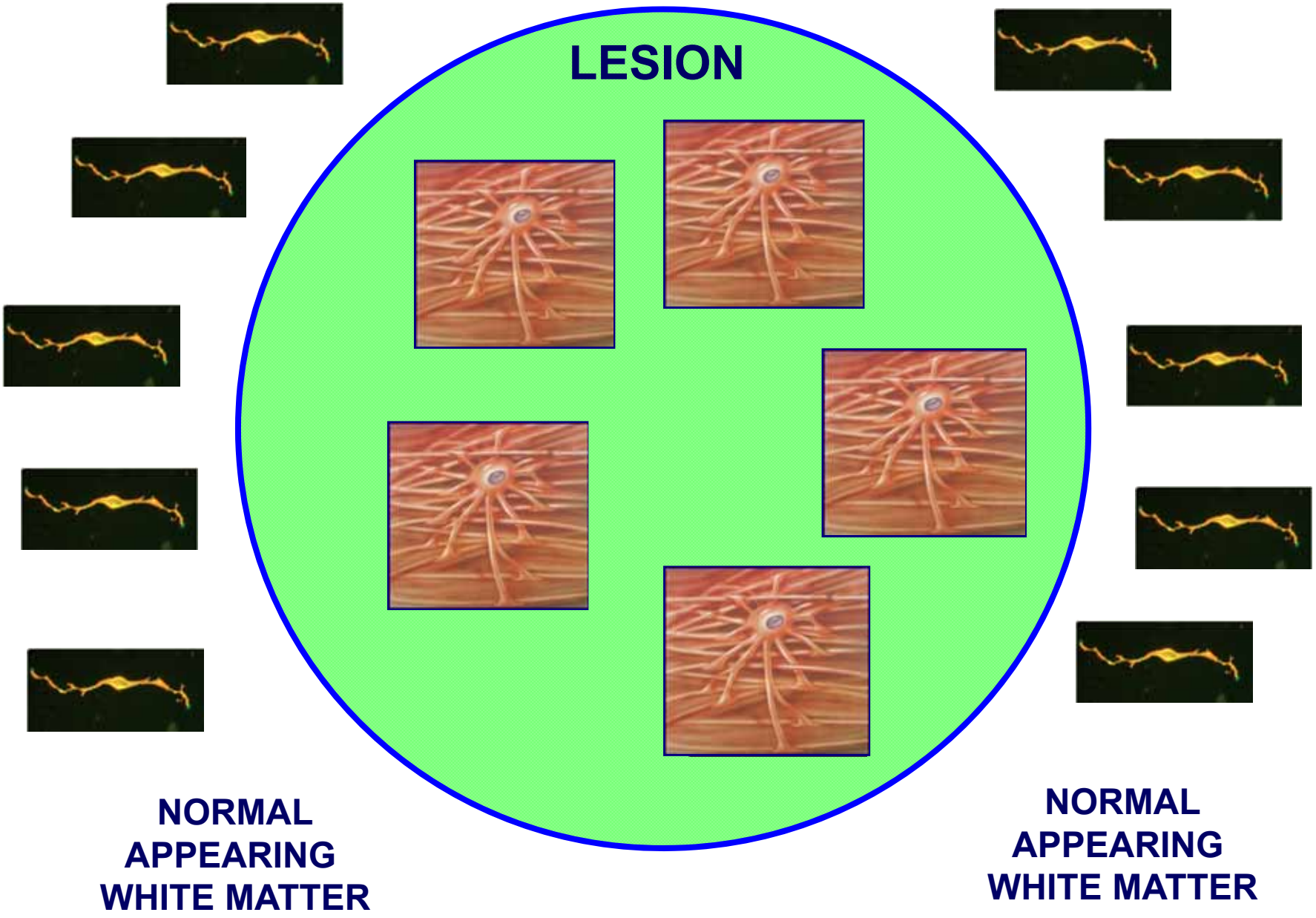
NG2^{+} OPCs respond to demyelination with an increase in cell number, approximately 3 fold, irrespective of whether the demyelination occurred in the white matter or grey matter (C and D)

Reynolds et al (2002) J Neurocytol 31:523-536

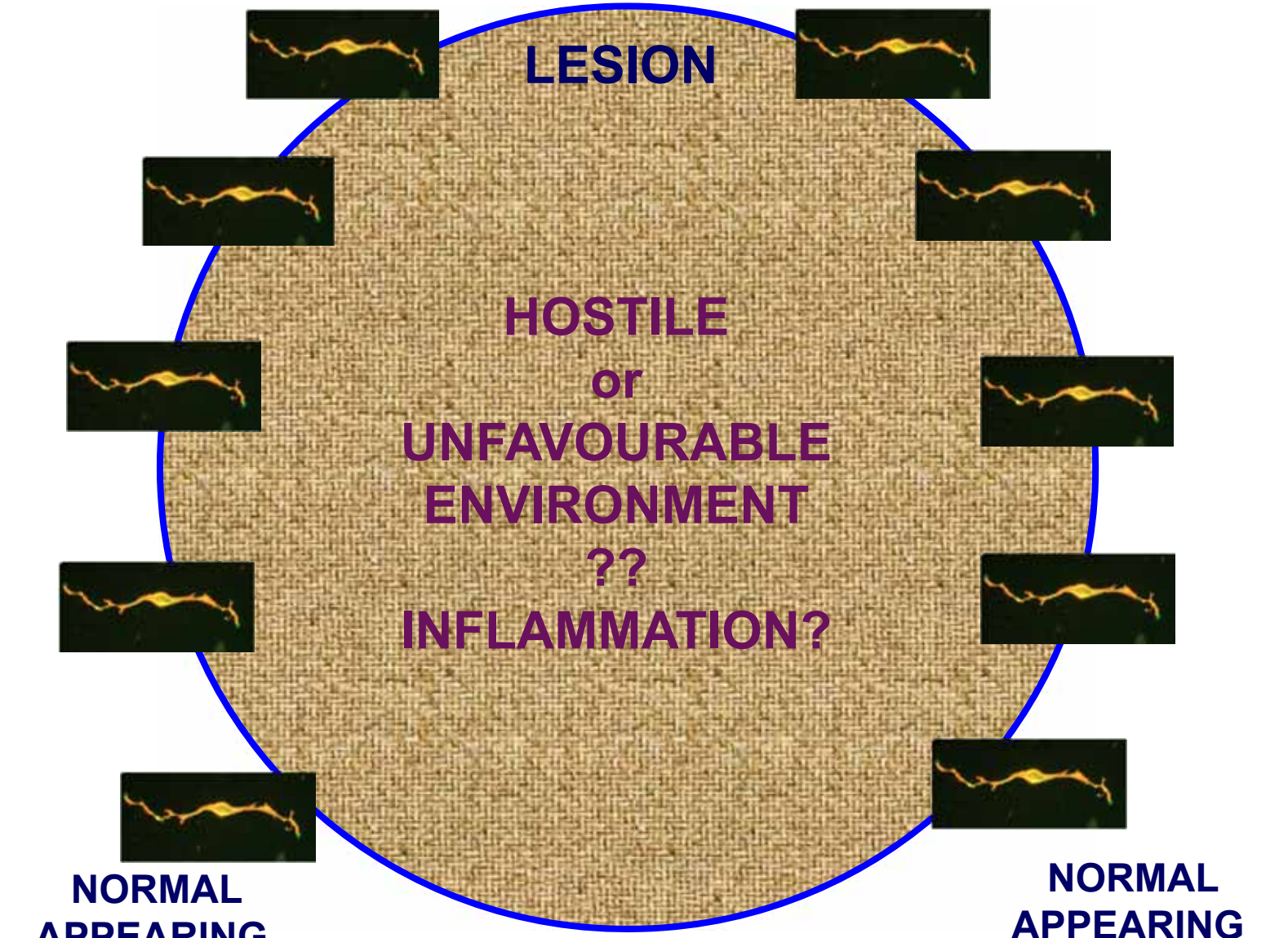
Remyelination of MOG-induced EAE lesions



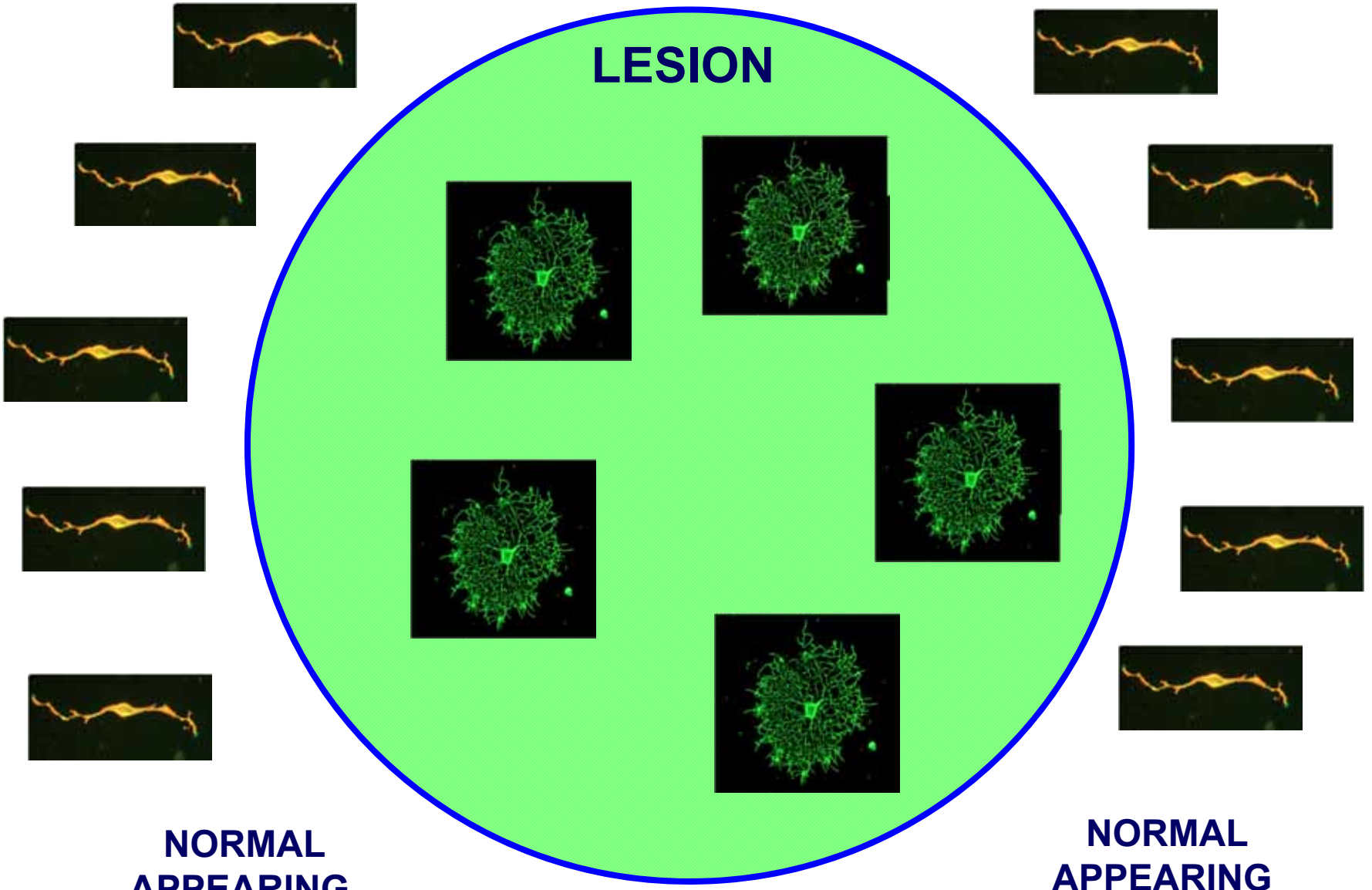
Mechanism for repair



Mechanism for repair



Mechanism for repair



When and why does remyelination fail in MS?

1. Remyelination may not fail early on in MS and can be extensive at later stages

2. The natural history of remyelination awaits good MRI measures

3. Failure of remyelination is determined by the chronicity of individual lesions

4. Reasons for failure of remyelination are many and may vary from patient to patient and from lesion to lesion

5. It remains possible that preserving axon integrity may be sufficient to stimulate remyelination

Kuhlmann et al (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202-2212.
Peterson et al (2001) Transected neurites, apoptotic neurons, and reduced inflammation in cortical MS lesions. Ann Neurol 50:389-400.
Trapp & Stys, Lancet Neurol 2009.
Mathy et al (2007) Neurofascin as a novel target for autoantibody-mediated axonal injury. J Exp Med 204:2363-2372.
Papadopoulos et al (2006) Axon loss is responsible for chronic neurological deficit following inflammatory demyelination in the rat. Exp Neurol 197:373-385.
Magliozzi et al (2010) A gradient of neuronal loss and meingeal inflammation in MS. Ann Neurol 68:477-493.
Patani et al (2007) Remyelination can be extensive in MS despite a long disease course. Neuropath App Neurobiol 33:277-287.
Patrikios et al (2006) Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129:3165-3172
Dawson et al (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24:476-488.
Reynolds et al (2002) The response of NG2-expressing oligodendrocyte progenitors to demyelination in MOG-EAE and MS. J Neurocytol 31:523-536
Franklin (2002) Why does remyelination fail in multiple sclerosis. Nat Rev Neurosci 3:1-10.
Polito & Reynolds (2005) NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult CNS. J Anatomy 207:707-716.