Figure 8.

Administration of minocycline reduced the density of microglial inflammation and abolished disease induced changes at paranodal and nodal units. (A- C) The density of CD11b immunoreactivity (mean signal intensity per animal± sem) in transverse spinal cord was greatly reduced in minocycline treated mice in comparison to vehicle dosed MOG immunised animals. (D, G) Minocycline treated animals contained microglia with a resting morphology that lacked TLR4 expression, in contrast to microglia in vehicle MOG immunised mice (E, H). (F) Nfasc155^{+} paranode length was increased in vehicle treated animals in comparison to the minocycline group (Box plot of mean paranodal lengths per case showing minimum, maximum, interquartile and median values), whilst the incidence of nodal abnormalities was greater in the vehicle dosed animals (I). n=5 animals per group; *=$p<0.05$, **=$p<0.01$ Mann-Whitney test. Dpi; days post induction. Scale bars A, B= 200μm, D- H= 30μm.

Supplementary Figure 1.

Microglial inflammation, paranodal disruption and axonal pathology in Parkinson's disease subcortical white matter. (A, B) CD68^{+} microglia were distributed throughout the MS NAWM and PD subcortical WM. (C) Nfasc155^{+} paranode length was significantly increased in areas of subcortical white matter in tissue blocks from six cases of Parkinson's disease in comparison to controls (Mann-Whitney test, ***=$p<0.001$). (D- F) Immunostaining for the paranodal axo-glial protein Caspr1 and βIV spectrin, a component of mature nodes of Ranvier, revealed examples of disrupted and elongated paranodes (arrows). (G, H) Paranodal disruption associated with SMI32^{+} stressed or damaged axons, whilst the aberrant expression of juxtaparanodal $K_v1.2$ channels in Nfasc155^{+} domains is suggestive of breakdown of normal paranodal barrier function (I- K, arrows). NAWM; normal appearing white matter. Scale bars, A, B= 100μm; C- J= 2μm.

Supplementary Table 1. Primary antibodies

Antigen	*Specificity*	*Clone*	*Source*
MOG	Myelin and oligodendrocytes	Z12	S. Piddlesden, University of Cardiff, UK
MBP	Myelin and oligodendrocytes	Polyclonal	R. Reynolds, Imperial College, UK
Neurofilament-H	Primary dendrites and axons	Rt97	Millipore
Smi32	Dephosphorylated neurofilaments	Smi32	Invitrogen Ltd, Paisley, UK
APP	Amyloid precursor protein	22C11	Chemicon International
α-synuclein	α-synuclein 1	42	Beckton-Dickinson, Oxford, UK
iNOS	Inducible nitric oxide synthetase	Polyclonal	Insight Biotechnology, Wembley, UK
HLA-DR	Activated microglia/ macrophages	LN3	Abcam, Cambridge, UK
IBA-1	Microglia/ macrophages	Polyclonal	Wako Pure Chemicals ind, Osaka, Japan,
CD68	Activated microglia/ macrophages	KP1	Dako UK, Ely, UK
TLR-4	Toll-like-receptor-4	hta125	Insight Biotechnology
CD3	T lymphocytes	F7. 2. 38	Abcam, Cambridge, UK
GFAP	Astrocytes	Polyclonal	Dako UK, Ely, UK
Nfasc155	Oligodendrocyte neurofascin	Polyclonal	P. Brophy, University of Edinburgh, UK
Caspr1	Neuronal Caspr1	K65/35	UC Davis/ NINDS/ NIMH NeuroMab Facility
Na_v1	Voltage gated Na^+ channels α1	K58/35	Sigma Aldrich Ltd.
$Na_v1.6$	Voltage gated Na+ channels α1.6	K87A/10	UC Davis/ NINDS/ NIMH NeuroMab Facility

$Na_v1.6$	Voltage gated Na+ channels $\alpha 1.6$	PN4	Sigma Aldrich Ltd.
$K_v1.2$	Voltage gated K^+ type 1.2 channels	K14/16	UC Davis/ NINDS/ NIMH NeuroMab Facility
βIV spectrin	βIV spectrin	Polyclonal	M. Komada, Tokyo Institute of Technology, Japan

Supplementary Table 2: Cause of death had no impact on measured microglial inflammation, axonal pathology or paranodal disruption.

	Density of MHC-II+ microglia (mm^2)	Density of Smi32+ axons (mm^2)	Nfasc155+ paranode length (μm)
Ctrl (sudden)	97.5 (74.8-136)	10.2 (3.5-15.8)	2.3 (1.8-2.7)
Ctrl (infection)	98.6 (81.6-115.6)	9.2 (8.8-9.6)	2.1 (2-2.2)
P value	1.0	0.8	0.58
MS (sudden)	173.3 (115-240)	31.1 (3.5-64.9)	3.1 (2.3-3.6)
MS (Infection)	271.8 (136-612)	33.5 (24.1-46.9)	3.0 (2.2-4.2)
P value	0.1	0.53	0.41

Data range shown in ( )

p value determined by Mann-Whitney test

Supplementary Table 2.

Control and MS cases that died suddenly (myocardial infarct) or with accompanying infection (such as sepsis, respiratory illness) were analysed to determine whether cause of death affected quantifiable measures of micogliosis, axonal pathology or paranode length shown in this report. Cause of death is recorded in Table 1. Two blocks from a single control case that died with an infection, and 4 blocks from 3 MS cases that died suddenly were not significantly different when compared to the respective control and MS values.

Comparing $Nfasc155^+$ structure length between the MS cohort with the shortest post-mortem delay (PMD; less than 15hrs, n=8) and the remaining MS cases (n=10, PMD 15-24hrs) revealed there to be no effect of PMD on Nfasc155 measures (2.90± 0.1 and 2.91± 0.1μm, respectively).

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Supplementary Figure 1