Review TRENDS in Pharmacological Sciences Vol.25 No.11 November 2004

Na^+ channel expression along axons in multiple sclerosis and its models

Stephen G. Waxman, Matthew J. Craner and Joel A. Black

Department of Neurology and Center for Neuroscience and Regeneration Research, Yale School of Medicine, New Haven, CT 06510, and Rehabilitation Research Center, Veterans Affairs Medical Center, West Haven, CT 06516, USA

Following the loss of myelin from axons in multiple sclerosis, some axons recover the ability to conduct impulses despite the absence of an insulating sheath, providing a basis for remission of clinical deficits. By contrast, other axons degenerate and contribute to non-remitting clinical deficits and, thus, disability. Investigations using laboratory models of multiple sclerosis indicate that altered expression of two distinct isoforms of Na^+ channels underlies these two processes, and the study of human tissue reveals similar changes in multiple sclerosis.

Multiple sclerosis (MS), the most common neurological disorder to affect young adults, often displays a relapsing–remitting course, with patients experiencing remissions in which previously lost functions (e.g. vision and gait) are regained. In progressive forms of MS, however, clinical status inexorably worsens and there is acquisition of an increasing burden of non-remitting neurological deficits. Both of these processes – remission and progression – appear to involve voltage-gated Na^+ channels within axons, and the expression of these molecules is therefore crucial in the pathophysiology of MS.

Inflammation, demyelination and axonal degeneration occur within white matter of the brain and spinal cord in MS. In normal myelinated axons within the white matter, Na^+ channels are clustered at a high density in the axon membrane at the nodes of Ranvier, but are present in much lower densities (too low to support secure conduction) in the internodal and paranodal axon under the myelin. This is functionally important because dissipation of current through Na^+ channel-poor portions of the axon membrane after demyelination can cause action potential conduction to fail, producing clinical deficits. Conduction failure in demyelinated axons is not, however, irreparable. Even before the molecular era (i.e. before the cloning of Na^+ channels), it became clear that expression of Na^+ channels along demyelinated (previously Na^+ channel-poor) axon regions can lead to restoration of conduction, which underlies remission of clinical deficits, even in the absence of remyelination (Figure 1a–c) [1].

In addition to this adaptive role, Na^+ channels can play a maladaptive role in disorders such as MS. Axonal degeneration occurs frequently in MS, and produces persistent neurological deficits [2,3]. It became clear early in the molecular era that injury to CNS axons can be triggered by Na^+ influx through persistently activated Na^+ channels, which drives reverse Na^+–Ca^{2+} exchange that imports damaging levels of Ca^{2+} into axons [4,5]. Consistent with a role for Na^+ channels in axonal injury in MS, pharmacological block of Na^+ channels prevents axonal degeneration within CNS white matter after a variety of insults [6–8], including injury produced by nitric oxide (NO) [9,10], which is present at increased concentrations within MS lesions [11]. Furthermore, recent studies have shown that the Na^+ channel blockers phenytoin [12] and flecainide [13] have a protective effect in experimental autoimmune encephalomyelitis (EAE), an experimental model of MS, where they prevent degeneration of CNS axons, maintain axonal conduction and improve clinical outcome.

As a result of molecular analysis it is now clear that at least nine different genes encode distinct voltage-gated Na^+ channels ($Na_v1.1$–$Na_v1.9$), all sharing a common overall motif but with different amino acid sequences, voltage-dependencies and kinetics. $Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ channels are expressed widely within the nervous system, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ channels are expressed selectively within dorsal root ganglion and trigeminal ganglion neurons, and $Na_v1.4$ and $Na_v1.5$ channels are expressed within somatic and cardiac muscle, respectively [14].

Early cytochemical [15] and immunocytochemical [16,17] studies using pan-specific Na^+ channel antibodies demonstrated higher than normal numbers of Na^+ channels in chronically demyelinated axons in experimental model systems, and electrophysiological studies [18] indicated that these axons can support impulse conduction; however, these early studies did not distinguish between channel isoforms and thus did not reveal the molecular identity of the new axonal channels that restore conduction. A fourfold increase in binding sites for the Na^+ channel blocker saxitoxin within demyelinated white matter from patients with MS, compared with controls, also suggested the expression of new Na^+ channels [19] but provided no details about the channel isoform(s) that are expressed. Moreover, the identity of the Na^+ channel isoforms in injured axons in MS has been enigmatic.

Recent studies have begun to identify the Na^+ channel isoforms that are present along demyelinated axons in animal models of MS, and in human MS, and suggest that specific Na^+ channel isoforms are associated

Corresponding author: Stephen G. Waxman (stephen.waxman@yale.edu).
Available online 17 September 2004

 0165-6147/$ - see front matter doi:10.1016/j.tips.2004.09.001

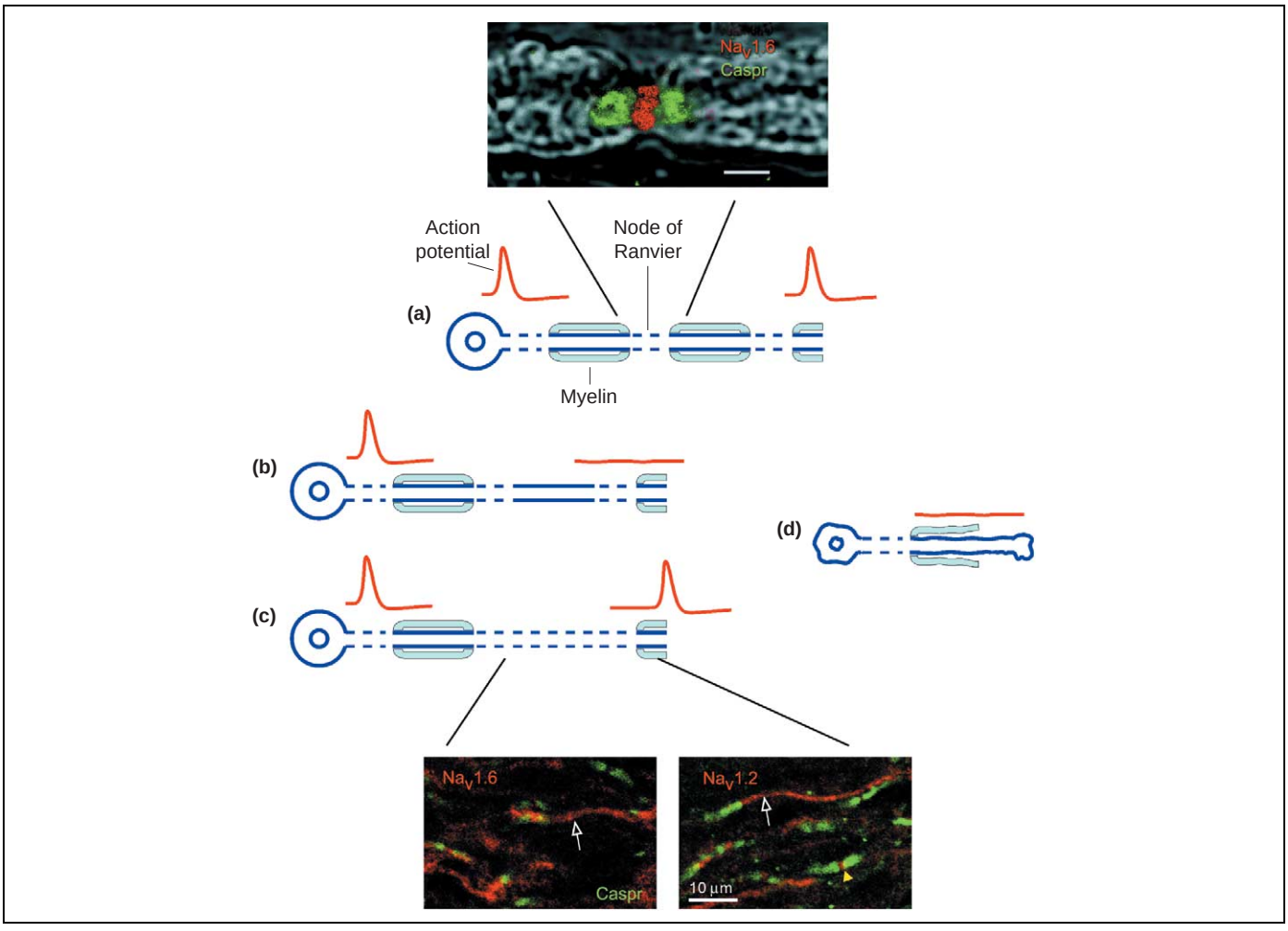

Figure 1. (a) Voltage-gated Na^+ channels, now known to be of the $Na_v1.6$ channel subtype, are clustered at a high density within the normal axon membrane at nodes of Ranvier but are sparse in the paranodal and internodal axon membrane under the myelin. Aggregation of $Na_v1.6$ channels (red) is shown at a node of Ranvier, bounded by Caspr (a constituent of the paranodal apparatus) in paranodal regions (green), in a normal myelinated axon. The fluorescence image of the node was merged with differential contrast image, which shows the myelin sheath. Scale bar = 5 μm. Image reproduced, with permission, from [52]. **(b)** Demyelination in multiple sclerosis (MS) initially exposes axon membrane with low Na^+ channel density that cannot support secure action potential conduction, and conduction block ensues. **(c)** Some demyelinated axons acquire higher than normal densities of Na^+ channels in demyelinated (formerly paranodal and/or internodal) regions, supporting the restoration of conduction that underlies clinical remissions. Changes in $Na_v1.6$ and $Na_v1.2$ channel expression are shown in optic nerve axons in experimental autoimmune encephalomyelitis (EAE). There is a loss of $Na_v1.6$ channel immunostaining (left) and an increase in $Na_v1.2$ channel immunostaining (right) at nodes (arrowhead). Demyelinated axonal profiles display diffuse $Na_v1.6$ channel (left) and $Na_v1.2$ channel (right) immunostaining (white arrows), which can extend for tens of microns along the fiber axis. Scale bar = 10 μm. Images reproduced, with permission, from Oxford University Press [28]. **(d)** Degeneration of axons also occurs in MS, and produces non-remitting, permanent loss of function. The steps that lead to axonal degeneration are only partially understood.

with restoration of conduction and axonal degeneration; these observations might have important therapeutic implications. In this article, recent progress in this rapidly evolving area will be reviewed.

$Na_v1.2$ and $Na_v1.6$ channels in normal and dysmyelinated axons

Within the normal nervous system at early stages before glial ensheathment, Na^+ channels are present at a low density along the entire length of pre-myelinated axons [20]. It is now known that $Na_v1.2$ channels are distributed diffusely along non-myelinated axons [21–23], and support action potential conduction that is known to occur in pre-myelinated axons [24,25]. By contrast, $Na_v1.6$ channels cluster at the nodes at Ranvier in myelinated axons (Figure 1) [26]. Isoform-specific studies have shown that after myelination, there is a loss of $Na_v1.2$ channels and an aggregation of $Na_v1.6$ channels at mature nodes of Ranvier in normal axons [23,27]; $Na_v1.6$ channels are not detectable in the paranodal and internodal axon membrane under the myelin.

Studies using isoform-specific antibodies in dysmyelinated mutants, in which myelin fails to form as a result of an abnormality within glial cells, have also provided information about the expression of Na^+ channels along axons where myelin is absent. These studies showed that $Na_v1.2$ channels continue to be expressed [21,23] whereas $Na_v1.6$ channels are not expressed [23] along dysmyelinated axons within *Shiverer* mutant tracts that have failed to form compact myelin. Together with the studies on developing axons, the observations from studies of dysmyelinated axons suggest that a relationship exists between myelin formation and the sequential expression of the $Na_v1.2$ and $Na_v1.6$ channel isoforms; however, these studies do not provide information about Na^+ channel expression in demyelinated axons.

$Na_v1.2$ channels in demyelinated axons: EAE

The Na^+ channel isoforms expressed along demyelinated axons have been identified recently in studies that used subtype-specific immunocytochemical methods and *in situ* hybridization to examine white matter axons in mice with EAE [28,29]. $Na_v1.1$ and $Na_v1.3$ channels were not detectable along axons in control or EAE animals; this is notable because $Na_v1.3$ channels are upregulated and expressed within dorsal root ganglion cells and their axons [30], and in higher-order nociceptive neurons within the spinal cord dorsal horn, after peripheral nerve injury [31] and spinal cord injury [32]. $Na_v1.8$ channels are not detectable along CNS axons in control or EAE animals, although they are aberrantly expressed within cerebellar Purkinje neurons [33] and retinal ganglion cells (M.J. Craner *et al.*, unpublished) in EAE and MS, and appear to distort the firing pattern of these cells [34].

Both $Na_v1.2$ and $Na_v1.6$ channels are expressed along CNS axons in EAE where there is an increased frequency of $Na_v1.2$ channel-positive nodes and a reduction in the frequency of $Na_v1.6$ channel-positive nodes within white matter (Figure 1). An increase in the overall number of nodes in EAE suggests that some new myelin sheaths are formed, but it is not clear whether the $Na_v1.2$ channel-expressing nodes are formed along remyelinated (but possibly not yet fully mature) axons or by replacement of $Na_v1.6$ channels with $Na_v1.2$ channels at preexisting nodes.

There is a large number of demyelinated axons in EAE. Diffuse Na^+ channel immunostaining for $Na_v1.2$ and $Na_v1.6$ channels extends for tens of microns along demyelinated axons in EAE optic nerve (Figure 1); by contrast, diffuse Na^+ channel immunostaining is very rare in control optic nerves (which do not contain unmyelinated axons) [28]. Similar changes are observed in the demyelinated spinal cord in EAE [29]. The increased expression of $Na_v1.2$ channels along demyelinated axons in EAE is accompanied by upregulated $Na_v1.2$ channel mRNA levels within retinal ganglion cells, which give rise to demyelinated optic nerve axons [28]. Thus, the emergence of increased numbers of nodes expressing $Na_v1.2$ channels, and of large regions of $Na_v1.2$ channel expression along extended regions of demyelinated fibers, appears to be associated with upregulated transcription of the gene encoding $Na_v1.2$ channels.

$Na_v1.6$ channels in injured axons: EAE

The activity of Na^+ channels can trigger Ca^{2+}-mediated injury of white matter axons, by providing a sustained Na^+ influx that drives reverse (Ca^{2+}-importing) activity of the Na^+–Ca^{2+} exchanger [4]. The available evidence indicates that a persistent (non-inactivating) Na^+

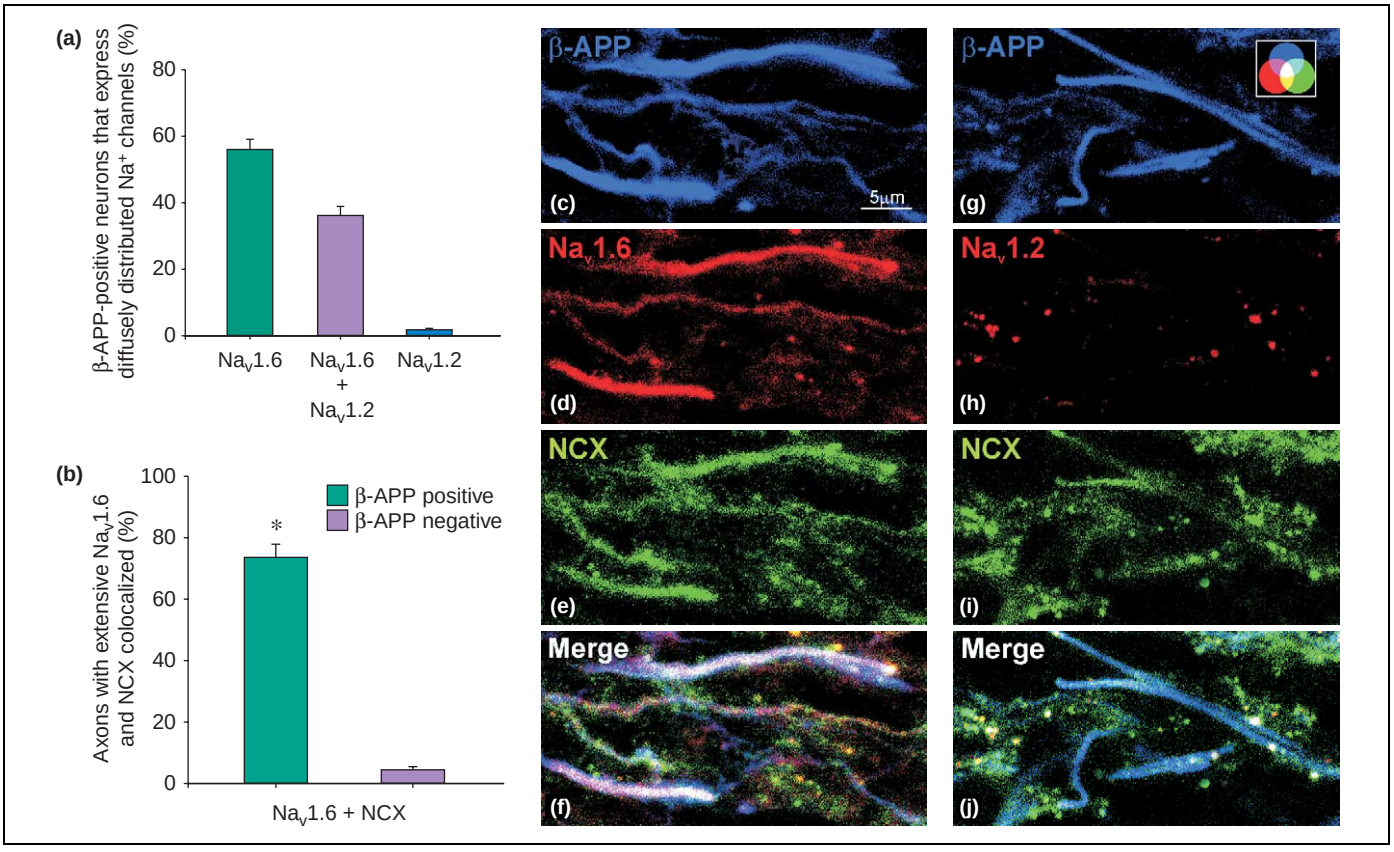

Figure 2. Coexpression of $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger in degenerating spinal cord axons in experimental autoimmune encephalomyelitis (EAE). **(a)** The percentage of β-amyloid precursor protein (β-APP)-positive axons in EAE that express immunoreactivity for $Na_v1.6$ channels alone (green), both $Na_v1.2$ and $Na_v1.6$ channels (purple), and $Na_v1.2$ channels alone (blue) is shown. More than 90% of β-APP-positive axons express $Na_v1.6$ channels, either alone or together with $Na_v1.2$ channels. **(b)** Coexpression of the Na^+–Ca^{2+} exchanger (NCX) and $Na_v1.6$ channels in β-APP-positive axons in EAE, determined by triple immunolabeling. The proportion of β-APP-positive axons that coexpress NCX and $Na_v1.6$ channels over extensive regions is significantly higher (green) than for β-APP-negative axons (purple). *$P<0.001$. Images: spinal cord axons in EAE immunostained for **(c,g)** β-APP (blue), **(d)** $Na_v1.6$ channels (red), **(h)** $Na_v1.2$ channels (red) and **(e,i)** the NCX (green) are shown. Panels **(f)** and **(j)** show merged images (white). Note the coexpression of $Na_v1.6$ channels, NCX and β-APP (a marker of axonal injury) **(c–f)**. Modified, with permission, from Oxford University Press [29].

conductance is involved [5]. $Na_v1.6$ channels are known to produce a persistent current in many cell types and this current is larger than the persistent current produced by $Na_v1.2$ channels [35–37]. Coexpression of $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger within demyelinated axons might predispose these axons to injury and so Craner *et al.* [29] attempted to determine whether there is a correlation between the expression of $Na_v1.6$ channels, the Na^+–Ca^{2+} exchanger and β-amyloid precursor protein (β-APP), a marker of axonal injury [2], in spinal cord axons from mice with EAE. Double-label immunocytochemistry demonstrated that 92% of β-APP-positive axons in EAE are $Na_v1.6$ channel immunopositive [either expressing $Na_v1.6$ channels alone (56%) or coexpressing $Na_v1.6$ and $Na_v1.2$ channels (36%)], whereas only 1.8% of β-APP-positive axons express only $Na_v1.2$ channels. Because these findings suggested that $Na_v1.6$ channels are preferentially associated with axonal injury, triple-labeling fluorescence immunohistochemistry was used to determine whether $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger are colocalized in β-APP-positive axons in EAE [29]. Axon counts showed that 74% of β-APP-positive axons displayed extensive regions of coexpression of $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger, whereas only 4% of β-APP-negative axons exhibited such coexpression (Figure 2). Thus, these data provide evidence for colocalization of $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger within injured axons in a model of MS.

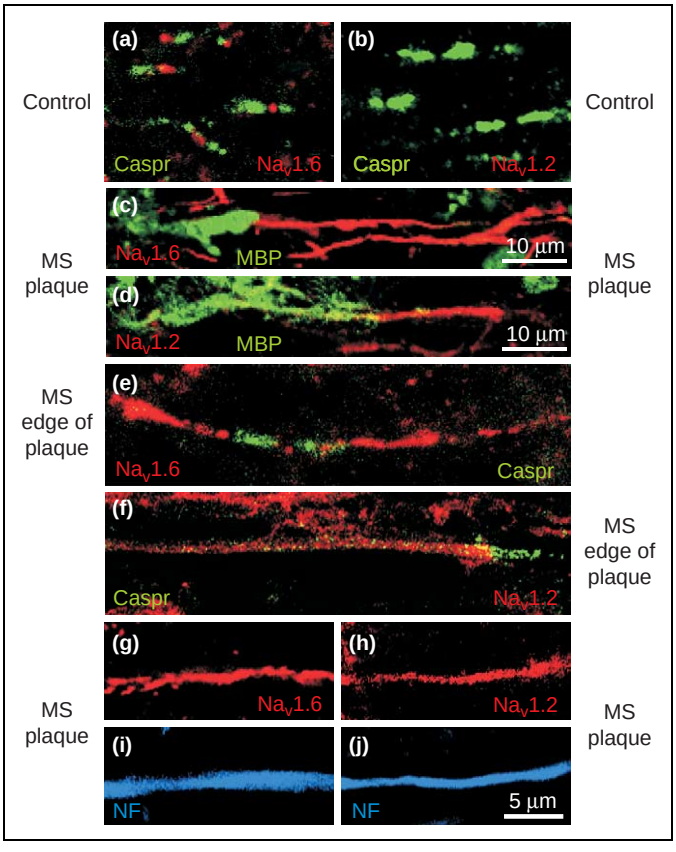

Figure 3. Changes in $Na_v1.6$ and $Na_v1.2$ channel expression along demyelinated axons within active lesions from patients with disabling secondary progressive multiple sclerosis (MS). Note the expression of **(a)** $Na_v1.6$ channels (red), bounded by Caspr (a constituent of the paranodal apparatus) (green), at nodes of Ranvier, whereas **(b)** $Na_v1.2$ channels (red) are not detectable at nodes within spinal cord from controls without neurological disease. Panels **(c)** and **(d)** show active MS lesions within spinal cord, where axons display residual damaged myelin (green) next to extensive regions of diffuse expression of $Na_v1.6$ channels (c) (red) and $Na_v1.2$ channels (d) (red), which establishes the identity of these profiles as axons. The presence of neurofilaments (NFs) **(i,j)** (blue) further confirms the diffuse distribution of $Na_v1.6$ channels **(g)** and $Na_v1.2$ channels **(h)** along axon regions that lack myelin. In some cases extensive regions of **(e)** $Na_v1.6$ channels (red) or **(f)** $Na_v1.2$ channels (red) are bounded by Caspr (green), without overlap, consistent with the expression of $Na_v1.6$ and $Na_v1.2$ channels within the demyelinated axon membrane. Abbreviation: MBP, myelin basic protein. Modified, with permission, from [38]. ©2004 National Academy of Sciences USA.

$Na_v1.2$ and $Na_v1.6$ channels in demyelinated axons: MS

Although EAE is the most commonly studied animal model of MS, there is no model that perfectly mimics all of the features of the human disorder, and the question 'which Na^+ channels are expressed along demyelinated axons in MS?' therefore requires the examination of human tissue. Such a study [38] has been carried out recently on post-mortem spinal cord and optic nerve tissue from patients with disabling secondary progressive MS and from controls, acquired via a rapid autopsy protocol [39]; the study revealed a pattern of Na^+ channel expression within acute MS lesions that is similar to the pattern observed in EAE. Control white matter, from patients with no neurological disease, displayed abundant myelin basic protein (MBP) and the expected pattern of focal expression of $Na_v1.6$ channels that was confined to nodes of Ranvier. Within acute MS plaques (which could be identified on the basis of attenuated MBP immunostaining and evidence of inflammation and recent phagocytosis of myelin), $Na_v1.6$ and $Na_v1.2$ channels were expressed along extensive regions running tens of microns along demyelinated axons (Figure 3). In some cases the region of $Na_v1.6$ or $Na_v1.2$ channel immunostaining was bounded by damaged myelin (Figure 3c,d) or Caspr (Figure 3e,f), a constituent of the paranodal apparatus [40,41], further confirming the identity of these profiles as previously myelinated axons.

To delineate the relationship between axonal injury and $Na_v1.6$ and $Na_v1.2$ channel expression within these MS lesions, colocalization of these channel isoforms with β-APP was examined. A large number of β-APP-positive axons were present within these MS plaques, suggesting the presence of ~7500 injured axons per mm^3 of tissue within these acute lesions, similar to the value (11 000 per mm^3) reported in earlier studies [2]. Almost all β-APP-immunopositive axons in these MS lesions expressed $Na_v1.6$ channels over extensive regions, whereas few β-APP-immunopositive axons expressed $Na_v1.2$ channel immunostaining [38]. $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger tended to be colocalized within β-APP-positive axons within acute MS lesions. A majority of β-APP-positive axons displayed extensive regions where both $Na_v1.6$ channels and the Na^+–Ca^{2+} exchanger were expressed (Figure 4). By contrast, $Na_v1.2$ channels and the Na^+–Ca^{2+} exchanger tended to be expressed in β-APP-negative axons. Thus, similar to EAE, the majority of injured axons in acute MS lesions display extensive regions where both $Na_v1.6$ channels and Na^+–Ca^{2+} exchanger are present, in contrast to apparently uninjured axons, which tend to express $Na_v1.2$ channels.

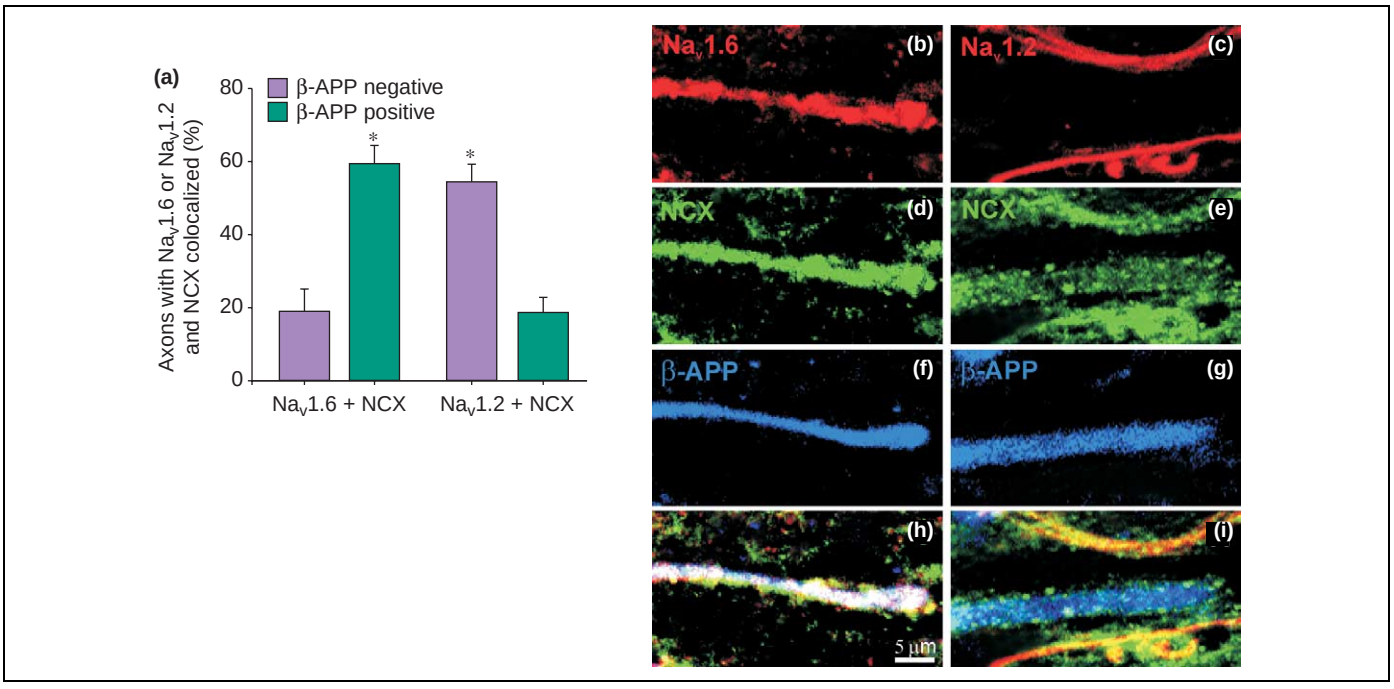

Figure 4. The Na^+–Ca^{2+} exchanger and $Na_v1.6$ channels are coexpressed in β-amyloid precursor protein (β-APP)-positive axons in multiple sclerosis (MS). **(a)** The proportions of β-APP-positive axons (green) and β-APP-negative axons (purple) that coexpress the Na^+–Ca^{2+} exchanger (NCX) and $Na_v1.6$ channels or the NCX and $Na_v1.2$ channels over extensive regions are shown. Coexpression of $Na_v1.6$ channels and the NCX is observed in a significantly higher proportion of β-APP-positive axons than in β-APP-negative axons. *$P<0.005$. The images show representative axons in MS spinal cord white matter immunostained for **(b)** $Na_v1.6$ channels (red) or **(c)** $Na_v1.2$ channels (red), **(d,e)** the NCX (green) and **(f,g)** β-APP (blue). Panels **(h)** and **(i)** show merged images (white). Panels **(b,d,f,h)** show coexpression of $Na_v1.6$ channels and the NCX within axons displaying β-APP. By contrast, panels **(c,e,g,i)** demonstrate NCX staining but an absence of $Na_v1.2$ channels within β-APP-positive axons, and coexpression of the NCX and $Na_v1.2$ channels within β-APP-negative axons. Modified, with permission, from [38]. ©2004 National Academy of Sciences USA.

What do $Na_v1.2$ channels do in demyelinated axons?

The widespread distribution of $Na_v1.2$ channels, extending for tens of microns along demyelinated but apparently uninjured axons in EAE and MS, is similar to the diffuse distribution of $Na_v1.2$ channels along pre-myelinated axons [23], in which action potential conduction is known to occur [24,25]. $Na_v1.2$ channels are also present along non-myelinated axons within the CNS [22,42,43] where they appear to support action potential conduction.

$Na_v1.6$ channels appear particularly well suited to support conduction at nodes, which can conduct high-frequency trains of impulses. $Na_v1.2$ and $Na_v1.6$ channels both produce rapidly activating and inactivating currents that can support action potential electrogenesis, but the rapid repriming kinetics of $Na_v1.6$ channels [37] can support sustained high-frequency opening whereas $Na_v1.2$ channels have slower repriming kinetics, and are more likely to generate activity in response to slow depolarizations [37,44]. The two isoforms also differ in terms of their ability to produce persistent currents, with $Na_v1.2$ channels producing significantly less persistent current than $Na_v1.6$ channels [35], a factor that might (see later) permit $Na_v1.2$ channels to be expressed along demyelinated axons without triggering reverse Na^+–Ca^{2+} exchange. Thus, the deployment of $Na_v1.2$ channels along demyelinated axons might serve an adaptive function, supporting the conduction of action potentials. Evidence for a non-injurious role of diffusely distributed $Na_v1.2$ channels is provided by observations in *Shiverer* mice, which lack compact myelin and express $Na_v1.2$ channels along dysmyelinated CNS axons [21,23] but do not display evidence of ongoing axonal degeneration [45]. It should be noted, nonetheless, that expression of $Na_v1.2$ channels along demyelinated axons might not fully restore the function of these axons because the ability of $Na_v1.2$ channels to sustain high-frequency conduction might be limited and the sensitivity of $Na_v1.2$ channels to slow depolarizations might contribute to ectopic firing or unstable patterns of firing after demyelination.

What do $Na_v1.6$ channels do in demyelinated axons?

Rapidly inactivating Na^+ current would not be expected to produce the sustained influx of Na^+ that is needed to drive reverse Na^+–Ca^{2+} exchange and, as described earlier, a persistent Na^+ conductance has been shown to have a prominent role in the triggering of reverse Na^+–Ca^{2+} exchange and resultant Ca^{2+} entry that results in injury of myelinated axons [4,5]. $Na_v1.6$ channels produce a persistent Na^+ current that becomes larger with depolarization [35,36]. Patch clamp studies on neurons expressing $Na_v1.6$ channels (using recombinant channels that were resistant to tetrodotoxin, permitting unequivocal identification of $Na_v1.6$ channel current) detected persistent $Na_v1.6$ channel currents in all cells that were studied [37]. Although $Na_v1.2$ channels might produce a persistent current when coexpressed with G-protein βγ-subunits [46], $Na_v1.6$ channels produce a much larger persistent current than $Na_v1.2$ channels [35], and in many cells types $Na_v1.6$ channels are responsible for the majority of persistent current [47]. Moreover, there is evidence that traumatic injury of axons can trigger Ca^{2+}-induced proteolytic cleavage of $Na_v1.6$ channels at the III–IV linker that damages their inactivation mechanism, suggesting a feed-forward process that might further

increase $Na_v1.6$ channel current amplitude [48]. Calmodulin also increases the $Na_v1.6$ channel current amplitude in a Ca^{2+}-dependent manner [49]. These observations raise the possibility that persistent current through $Na_v1.6$ channels might be further increased by secondary changes in channel function triggered by an initial rise in intra-axonal Ca^{2+} levels early in the course of injury. Irrespective of this, the physiological data together with the immunocytochemical results are consistent with the proposal that $Na_v1.6$ channels, when coexpressed with the Na^+–Ca^{2+} exchanger along demyelinated axons, can contribute to axonal injury.

Mapping the Na^+ channels in MS

The molecular identities and distribution of the Na^+ channels that are deployed in demyelinated and degenerating CNS axons are now being delineated both in animal models of MS and in MS itself. Interestingly, the two Na^+ channel isoforms that have been detected thus far, $Na_v1.2$ and $Na_v1.6$, show different patterns of distribution: $Na_v1.2$ channels tend to be present in axons that are β-APP-negative (i.e. in axons that do not display signs of injury), whereas $Na_v1.6$ channels tend to be expressed in axons that are β-APP-positive and thus appear to be injured and, in these axons, this Na^+ channel isoform is often coexpressed with the Na^+–Ca^{2+} exchanger. This pattern of channel distribution suggests the model illustrated in Figure 5, in which the presence of $Na_v1.6$ channels, but not $Na_v1.2$ channels, might predispose axons to injury. Consistent with this proposal, dysmyelinated CNS axons, which express $Na_v1.2$ channels rather than $Na_v1.6$ channels [21,23], are substantially less sensitive than myelinated axons to injury triggered by activity of Na^+ channels [50]. Also consistent with this proposal is the observation that demyelinated axons are less sensitive than myelinated axons to injury triggered by activity of Na^+ channels [51], an observation that might be explained by the expression of $Na_v1.2$ channels along demyelinated axons that have not degenerated.

If, as proposed in this article, expression of $Na_v1.2$ channels along demyelinated axons serves to support conduction, strategies that induce expression of this channel might promote restoration of conduction, thereby encouraging remission in MS. It also might also be predicted that subtype-specific blockade of $Na_v1.6$ channels (or, preferably, of the persistent component of the current produced by $Na_v1.6$ channels) might have a

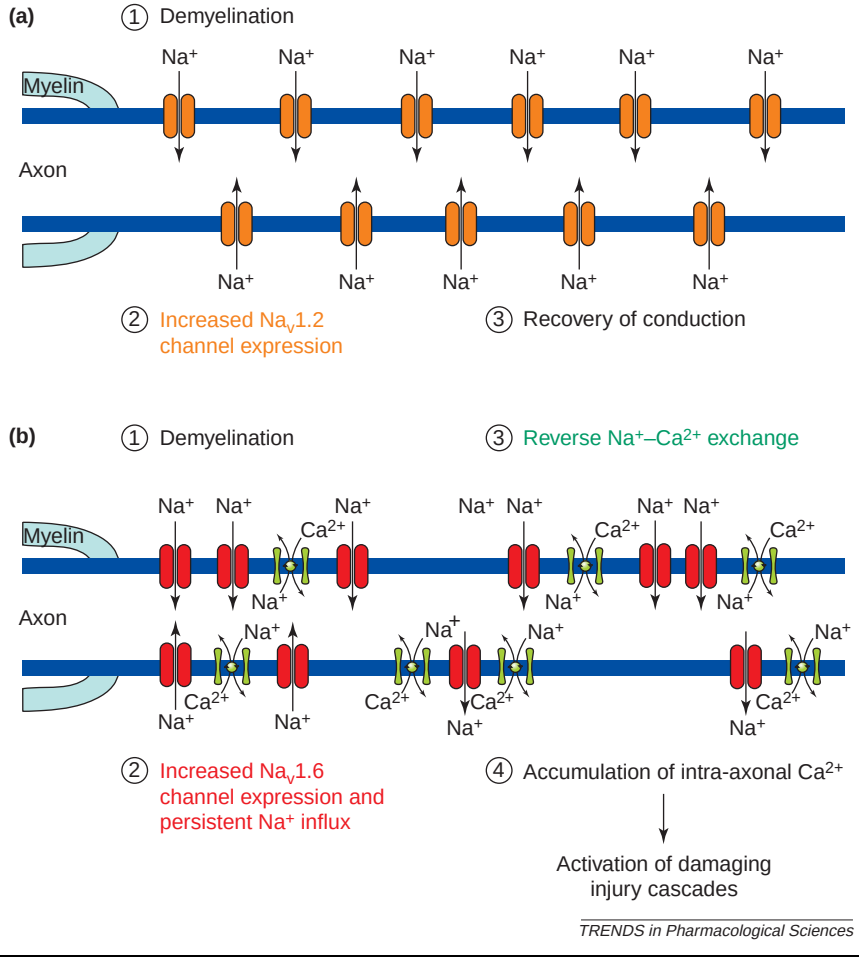

Figure 5. Different patterns of expression of $Na_v1.2$ and $Na_v1.6$ channels along demyelinated axons suggest different functions. **(a)** Following demyelination (1), $Na_v1.2$ channels are expressed diffusely (2) along some axons and support recovery of action potential conduction (3). **(b)** Expression of $Na_v1.6$ channels is upregulated in some axons following demyelination (1). In addition to producing a rapidly activating and inactivating current, these channels also produce a persistent Na^+ current (2) that can drive reverse Na^+–Ca^{2+} exchange (3) and lead to accumulation of intra-axonal Ca^{2+} (4), triggering injurious secondary cascades and axonal injury. Modified, with permission, from [38]. ©2004 National Academy of Sciences USA.

protective effect, preventing axonal degeneration. The clinical efficacy of such an approach might depend, however, on the proportion of $Na_v1.6$ channels along demyelinated axons that require blockade for protection, and on the safety factor (i.e. the fraction of $Na_v1.6$ channels that are required to remain operable) at nodes along normal axons where this channel is required for saltatory conduction. It is encouraging, in this context, that the nonspecific Na^+ channel blockers phenytoin [12] and flecainide [13] are protective in EAE, preventing axonal degeneration, maintaining axonal conduction and improving clinical outcome, without apparent negative side-effects in this animal model. Whether $Na_v1.6$ channel-specific blockade or nonspecific Na^+ channel blockade will be useful clinically as a neuroprotective strategy in MS remains to be determined. The relatively safe side-effect profile of Na^+ channel blockers that are already in clinical use might make it relatively straightforward to begin to answer these questions.

In addition to issues relating to protection of axons, several other questions remain unanswered. It would be interesting to correlate directly the changes in Na^+ channel expression along demyelinated axons with clinical status. Comparison of patterns of Na^+ channel expression within symptomatic versus asymptomatic (subclinical) lesions might, for example, demonstrate differences. There is, in addition, the possibility of lesion-to-lesion (acute versus chronic active versus chronic inactive) variability in Na^+ channel expression.

In addressing these questions, it will be important to juxtapose observations in experimental models with results obtained in human tissue. As illustrated in this article, conclusions from the two are beginning to converge. We will undoubtedly soon understand more about the multiple roles of Na^+ channels, not only in models of MS, but also in MS.

Acknowledgements

Research described in this article was supported, in part, by grants from the National Multiple Sclerosis Society and the Medical Research Service and Rehabilitation Research Service, Department of Veteran Affairs, and by gifts from Destination Cure and the Nancy Davis Foundation. The Neuroscience and Regeneration Research Center is a Collaboration of the Paralyzed Veterans of America and the United Spinal Association with Yale University. M.J.C. thanks Medical Director General, UK for support.

References

1 Waxman, S.G. (1998) Demyelinating diseases: New pathological insights, new therapeutic targets. *New Engl. J. Med.* 338, 323–325
2 Trapp, B.D. *et al*. (1998) Axonal transection in the lesions of multiple sclerosis. *New Engl. J. Med.* 338, 278–285
3 Kuhlmann, T. *et al*. (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain* 125, 2202–2212
4 Stys, P.K. *et al*. (1992) Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. *J. Neurosci.* 12, 430–439
5 Stys, P.K. *et al*. (1993) Noninactivating, tetrodotoxin-sensitive Na^+ conductance in rat optic nerve axons. *Proc. Natl. Acad. Sci. U. S. A.* 90, 6976–6980
6 Stys, P.K. *et al*. (1992) Tertiary and quaternary local anesthetics protect CNS white matter from anoxic injury at concentrations that do not block excitability. *J. Neurophysiol.* 67, 236–240
7 Imaizumi, T. *et al*. (1997) Anoxic injury in the rat spinal cord: pharmacological evidence for multiple steps in Ca^{2+} -dependent injury of the dorsal columns. *J. Neurotrauma* 14, 299–311
8 Rosenberg, L.J. *et al*. (1999) Effects of the sodium channel blocker tetrodotoxin on acute white matter pathology after experimental contusive spinal cord injury. *J. Neurosci.* 19, 6122–6133
9 Kapoor, R. *et al*. (2003) Blockers of sodium and calcium entry protect axons from nitric oxide-mediated degeneration. *Ann. Neurol.* 53, 174–180
10 Garthwaite, G. *et al*. (2002) Nitric oxide toxicity in CNS white matter: an *in vitro* study using rat optic nerve. *Neuroscience* 109, 145–155
11 Smith, K.J. and Lassmann, H. (2002) The role of NO in multiple sclerosis. *Lancet Neurol.* 1, 232–241
12 Lo, A.C. *et al*. (2003) Phenytoin protects spinal cord axons and preserves axonal conduction and neurological function in a model of neuroinflammation *in vivo*. *J. Neurophysiol.* 90, 3566–3572
13 Bechtold, D.A. *et al*. (2004) Axonal protection using flecainide in experimental autoimmune encephalomyelitis. *Ann. Neurol.* 55, 607–616
14 Catterall, W.A. *et al*., eds (2002). *The IUPHAR Compendium of Voltage-Gated Ion Channels*, IUPHAR Media, Leeds, UK
15 Foster, R.E. *et al*. (1980) Reorganization of the axonal membrane of demyelinated nerve fibers: morphological evidence. *Science* 210, 661–663
16 Novakovic, S.D. *et al*. (1998) Disruption and reorganization of sodium channels in experimental allergic neuritis. *Muscle Nerve* 21, 1019–1032
17 England, J.D. *et al*. (1991) Increased numbers of sodium channels form along demyelinated axons. *Brain Res.* 548, 334–337
18 Bostock, H. and Sears, T.A. (1978) The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. *J. Physiol.* 280, 273–301
19 Moll, C. *et al*. (1991) Increase of sodium channels in demyelinated lesions of multiple sclerosis. *Brain Res.* 556, 3ll–6
20 Waxman, S.G. *et al*. (1989) Low density of sodium channels supports action potential conduction in axons of neonatal rat optic nerve. *Proc. Natl. Acad. Sci. U. S. A.* 86, 1406–1410
21 Westenbroek, R.E. *et al*. (1989) Differential subcellular localization of the RI and RII Na^+ channel subtypes in central neurons. *Neuron* 3, 695–704
22 Gong, B. *et al*. (1999) Type I and type II Na^+ channel alpha-subunit polypeptides exhibit distinct spatial and temporal patterning, and association with auxiliary subunits in rat brain. *J. Comp. Neurol.* 412, 342–352
23 Boiko, T. *et al*. (2001) Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. *Neuron* 30, 91–104
24 Foster, R.E. *et al*. (1982) Rat optic nerve: electrophysiological, pharmacological and anatomical studies during development. *Dev. Brain Res.* 3, 361–376
25 Rasband, M.N. *et al*. (1999) Dependence of nodal sodium channel clustering on paranodal axo-glial contact in the developing CNS. *J. Neurosci.* 19, 7516–7528
26 Caldwell, J.H. *et al*. (2000) Sodium channel Na(v)1.6 is localized at nodes of Ranvier, dendrites, and synapses. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5616–5620
27 Kaplan, M.R. *et al*. (2001) Differential control of clustering of the sodium channels Na(v)1.2 and Na(v)1.6 at developing CNS nodes of Ranvier. *Neuron* 30, 105–119
28 Craner, M.J. *et al*. (2003) Abnormal sodium channel distribution in optic nerve axons in a model of inflammatory demyelination. *Brain* 126, 1552–1562
29 Craner, M.J. *et al*. (2004) Colocalization of sodium channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE. *Brain* 127, 294–303
30 Black, J.A. *et al*. (1999) Upregulation of a silent sodium channel after peripheral, but not central, nerve injury in DRG neurons. *J. Neurophysiol.* 82, 2776–2785
31 Hains, B.C. *et al*. (2004) Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury. *J. Neurosci.* 24, 4832–4840
32 Hains, B.C. *et al*. (2003) Upregulation of sodium channel $Na_v1.3$ and

functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. *J. Neurosci.* 23, 8881–8892
33 Black, J.A. *et al*. (2000) Sensory neuron specific sodium channel SNS is abnormally expressed in the brains of mice with experimental allergic encephalomyelitis and humans with multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11598–11602
34 Renganathan, M. *et al*. (2003) Expression of Na_v 1.8 sodium channels perturbs the firing patterns of cerebellar Purkinje cells. *Brain Res.* 959, 235–243
35 Smith, M.R. *et al*. (1998) Functional analysis of the mouse Scn8a sodium channel. *J. Neurosci.* 18, 6093–6102
36 Raman, I.M. and Bean, B.P. (1997) Resurgent sodium current and action potential formation in dissociated cerebellar purkinje neurons. *J. Neurosci.* 17, 4517–4526
37 Herzog, R.I. *et al*. (2003) Distinct repriming and closed state inactivation kinetics of $Na_v1.6$ and $Na_v1.7$ sodium channels in spinal sensory neurons. *J. Physiol.* 551, 741–751
38 Craner, M.J. *et al*. (2004) Molecular changes in neurons in MS: altered axonal expression of $Na_v1.2$ and $Na_v1.6$ sodium channels and Na^+/Ca^{2+} exchanger. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8168–8173
39 Newcombe, J. and Cuzner, M.L. (1993) Organization and research application of the UK Multiple Sclerosis Society tissue bank. *J. Neural Transm.*Suppl. 39, 155–163
40 Einheber, S. *et al*. (1997) The axonal membrane protein Caspr, a homologue of neurexin IV, is a component of the septate-like paranodal junctions that assemble during myelination. *J. Cell Biol.* 139, 1495–1506
41 Bhat, M.A. *et al*. (2001) Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. *Neuron* 30, 369–383
42 Westenbroek, R.E. *et al*. (1989) Differential subcellular localization of the RI and RII Na^+ channel subtypes in central neurons. *Neuron* 3, 695–704
43 Whitaker, W.R. *et al*. (2000) Distribution of voltage-gated sodium channel alpha-subunit and beta-subunit mRNAs in human hippocampal formation, cortex, and cerebellum. *J. Comp. Neurol.* 422, 123–139
44 Zhou, W. and Goldin, A.L. (2002) Functional differences between the $Na_v1.6$ and $Na_v1.2$ sodium channels. *Soc. Neurosci. Abstr.* 834, 4
45 Griffiths, I. *et al*. (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. *Science* 280, 1610–1613
46 Ma, J.Y. *et al*. (1997) Persistent sodium currents through brain sodium channels induced by G protein betagamma subunits. *Neuron* 19, 443–452
47 Maurice, N. *et al*. (2001) D1/D5 dopamine receptor activation differentially modulates rapidly inactivating and persistent sodium currents in prefrontal cortex pyramidal neurons. *J. Neurosci.* 21, 2268–2277
48 Iwata, A. *et al*. (2004) Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. *J. Neurosci.* 24, 4605–4613
49 Herzog, R.I. *et al*. (2003) Calmodulin binds to the C terminus of sodium channels $Na_v1.4$ and $Na_v1.6$ and differentially modulates their functional properties. *J. Neurosci.* 23, 8261–8270
50 Waxman, S.G. *et al*. (1990) Anoxic injury of mammalian central white matter: Decreased susceptibility in myelin-deficient optic nerve. *Ann. Neurol.* 28, 335–340
51 Imaizumi, T. *et al*. (1998) Resistance to anoxic injury in rat spinal cord following demyelination. *Brain Res.* 779, 292–296
52 Black, J.A. *et al*. (2002) Sodium channel Nav1.6 is expressed along nonmyelinated axons and it contributes to conduction. *Mol. Brain Res.* 105, 19–28