



**HAL**  
open science

## **Nodes of Ranvier during development and repair in the central nervous system**

Catherine Lubetzki, Nathalie Sol-Foulon, Nathalie Sol Foulon, Anne Desmazières

► **To cite this version:**

Catherine Lubetzki, Nathalie Sol-Foulon, Nathalie Sol Foulon, Anne Desmazières. Nodes of Ranvier during development and repair in the central nervous system. *Nature Reviews Neurology*, 2020, 16 (8), pp.426-439. 10.1038/s41582-020-0375-x. hal-03052020

**HAL Id: hal-03052020**

**<https://hal.science/hal-03052020v1>**

Submitted on 12 Jan 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Nodes of Ranvier during development and repair in the central nervous system

Catherine Lubetzki <sup>1,2</sup>, Nathalie Sol Foulon <sup>1</sup> and Anne Desmazières <sup>1</sup>

1 Sorbonne University, Inserm, CNRS, ICM-Groupe hospitalier Pitié-Salpêtrière, Paris  
France

2 Assistance Publique des Hôpitaux de Paris (APHP), Neurology department, Groupe  
hospitalier Pitié-Salpêtrière, Paris, France

## Abstract

Saltatory conduction of action potentials along myelinated axons depends on small unmyelinated axonal domains, the nodes of Ranvier, where voltage-gated sodium channels are concentrated. Our knowledge on the complex molecular composition of these axonal domains still accumulates, whereas mechanisms of nodal assembly, established in the peripheral nervous system, remain only partially understood in the central nervous system. Apart from their key role in accelerating conduction, novel results have suggested that nodal variations allow to finely tune axonal conduction speed, to meet information processing needs. In addition, through their multiple glial contacts, nodes now appear as key actors for neuro-glial interactions. In pathology, disorganization of axonal domains is involved in the pathophysiology of various neurological diseases. In multiple sclerosis, nodal and perinodal disruption upon demyelination, with subsequent changes of ion channels distribution leads to altered axonal conduction and integrity. Nodal clusters then reform, coincident - but also prior to - remyelination, allowing restoration of axonal conduction. Here, we will review the current knowledge on nodes of Ranvier organization and function in the central nervous system; then discuss their dynamic changes during demyelination and remyelination, highlighting their impact on neuronal physiology in health and diseases and the related therapeutic perspectives.

## 31 **Bullet points**

- 32 • Nodes of Ranvier allow high-speed, saltatory propagation of action potentials along  
33 myelinated fibers
- 34 • Multiple mechanisms contribute to nodal proteins clustering in the central nervous  
35 system
- 36 • Multiple glial contacts at the nodal surface suggest that CNS node might act as an axo-  
37 glial hub, opening new perspectives of neuro-glia communication
- 38 • Variations of nodes of Ranvier length and diameter allow to finely tune axonal  
39 conduction speed, to meet information processing needs at the network scale
- 40 • Nodal domains might represent sites of axonal vulnerability in demyelinating diseases
- 41 • Disruption of node of Ranvier and paranodal junctions in CNS demyelinating diseases  
42 is associated with Nav and Kv channels redistribution, impacting action potential  
43 propagation and axonal integrity.

## 44 45 **Introduction**

46 Nodes of Ranvier, small axonal domains flanked by paranodal junctions, are characteristic of  
47 myelinated fibers<sup>1-3</sup>. In contrast to internodal segments, the axonal membrane at the node is  
48 not wrapped by myelin, but in the peripheral nervous system (PNS) Schwann cells microvilli  
49 cover the nodal domain<sup>4</sup>. Nodes of Ranvier allow fast saltatory propagation of action potentials  
50 (AP), due to nodal clusters of voltage dependent Na<sup>+</sup> channels (Nav) and the insulating property  
51 of myelin. Our knowledge on the complex molecular composition of the nodal domains,  
52 actively explored during the last decade, is still advancing, in particular with the recent  
53 discovery, in mammals, of the presence of specific nodal K<sup>+</sup> channels, independent of voltage  
54 and responsible for rapid axonal membrane repolarisation<sup>5,6</sup>. The multifactorial process leading  
55 to nodal proteins assembly in the central nervous system (CNS) during development is partially  
56 understood, with the recent hypothesis that these mechanisms might differ depending on  
57 neuronal sub-populations. Concerning nodal function, apart from their key role in saltatory  
58 conduction, established long ago, recent data have highlighted other functions for nodes of  
59 Ranvier, in particular a critical impact for regulation/adjustment of AP propagation – a crucial  
60 need for coincident arrival of synaptic inputs from multiple axons<sup>7</sup>. Moreover, the multiple  
61 contacts of nodal domains with the surrounding glial environment has led to the hypothesis that  
62 nodes might act as a functional “hub”, representing a novel way for neuron-glia interactions. In

63 pathology, nodal disruption drastically impact axonal function and integrity<sup>2,8</sup>. Such alterations  
64 are not restricted to demyelinating diseases, but have been observed in different types of  
65 neurological injury. Although some nodal constituents have been identified as the primary  
66 target of the disease process in peripheral demyelinating neuropathies, such demonstration is  
67 still lacking in CNS demyelinating pathology. However, nodal domains, lacking the trophic  
68 support of myelin, might represent a site of axonal vulnerability. In this review, we provide an  
69 update on the molecular organization of CNS nodes of Ranvier and our current understanding  
70 of their physiological function. We review nodal domain alterations in multiple sclerosis (MS)  
71 and related experimental models, and how these changes impact neuronal physiology, paving  
72 the way to therapeutic perspectives for neuroprotection.

### 73 74 **1) *The discovery of nodes of Ranvier***

75 Working at Collège de France in Paris, and studying isolated myelinated fibers, Ranvier showed  
76 that, contrary to the current view that myelin was a continuous and impermeable sheath, there  
77 were specific sites of myelin interruption, that he described as “annular constrictions” (Figure  
78 1A), where the crystalline water soluble dye picocarminate was able to reach the axonal  
79 membrane. Ranvier’s first hypothesis (which might make us smile now) was that these  
80 constrictions had a mechanical role: *“since myelin is essentially a liquid material.... if it was*  
81 *uninterrupted, in the human sciatic nerve for example, which is mostly in the upright position,*  
82 *then the myelin would flow, by gravity, to the more sloping portion of the nerve, ...but this is*  
83 *not the case; the myelin sheath is interrupted by lateral constrictions which retain it”*<sup>9</sup>. Ranvier  
84 also postulated that these constrictions, allowing the diffusion of soluble cues, could be  
85 important for nutrition of the nerve cell. He attributed to the myelin sheath a protective role  
86 against compression. Last but not least, he postulated that myelin, even if not needed for  
87 transmission of sensory and motor “impressions” -because such transmission also takes place  
88 in non myelinated fibers- would result, acting as an electrical insulation, in a “more perfect”  
89 transmission...<sup>9</sup>; a pioneering hypothesis! that would not be confirmed before several decades  
90 <sup>10</sup>.

### 91 **2) *Nodes of Ranvier: structural and molecular organization***

92 This paragraph will provide an update on the current knowledge on nodes of Ranvier  
93 organization, as well as on the mechanisms of nodal assembly, which have been actively

94 explored and debated. Perinodal domains, tightly linked to nodal organization will also be  
95 briefly described.

96 *2-1) Structural organization.* Since the first electron microscope description of nodes of  
97 Ranvier in 1960<sup>11-13</sup>, our knowledge of the nodal ultrastructural organization has extended  
98 (Figure 1B).

99 Although the typical size of the node of Ranvier ranges from 1.0-2.0  $\mu\text{m}$ <sup>14</sup>, it was recently  
100 shown that it can vary (by several fold) between axons, including in an axon-diameter  
101 independent manner<sup>15</sup>. Furthermore, nodal length could partly depend on the neuronal subtype  
102 considered, as they are shorter in cortical GABAergic neurons compared to non-GABAergic  
103 neurons<sup>16,17</sup>. The axonal diameter at the nodes is reduced in size compared to the internode<sup>18</sup>,  
104 suggesting reduced phosphorylation of neurofilaments and/or their higher packing at the nodes  
105<sup>14,19</sup>. Variations of nodal diameter between axons, but also along the same axon, was recently  
106 identified<sup>20</sup>. As we will discuss below, both nodal length and diameter are potential regulators  
107 of conduction speed. Beneath the nodal axolemma, the electron-dense granular cytoplasmic  
108 layer likely corresponds to the cytoskeletal network of scaffold proteins<sup>21</sup>. In the CNS the  
109 density of mitochondria at the node is similar or reduced compared to paranodal and internodal  
110 areas<sup>22,23</sup>, in contrast to PNS. STimulated Emission Depletion (STED) super-resolution  
111 microscopy has opened fascinating new insight into PNS nodal organization, highlighting  
112 preserved periodic cytoskeleton organization at nodes compared to internodal areas<sup>24</sup>, but also  
113 the importance of lateral organization of proteins at nodal gaps<sup>25</sup>. Such nanoscale investigation  
114 is still lacking for CNS nodes, but could clarify some of the mechanisms of protein assembly at  
115 CNS nodes.

116 Contrary to the PNS, where Schwann cells microvilli contact the nodal surface, myelinating  
117 oligodendrocytes in the CNS do not form nodal microvilli. Instead, some nodes are contacted  
118 by perinodal astrocyte and/or oligodendrocyte precursor cell (OPC) processes<sup>26-32</sup>. In addition,  
119 we and others recently identified by light and electron microscopy that microglial processes  
120 also contact the node<sup>33</sup> (Ronzano, Roux et al, in preparation). These multiple glial contacts at  
121 the nodal surface (Figure 2), suggest that CNS node might act as an axo-glial “hub”, opening  
122 new perspective on neuroglial communication. The CNS nodal gap is further embedded in  
123 extracellular matrix (ECM), as described below<sup>34</sup>.

124 Surrounding the nodes are the paranodes, which are formed by the tight interaction between the  
125 distal, uncompacted loops of myelin and the axolemma. Electron microscopy has revealed the

126 existence of transverse bands, corresponding to intercellular periodic densities, between each  
127 paranodal loop and the axon<sup>35,36</sup>. Neighboring the paranodes are the juxtaparanodes, located at  
128 the interface between the internode and the paranode, underneath the myelin sheath (Figure 3).

## 129 2-2) Molecular composition of the nodal area

130 The ability of nodes of Ranvier to regenerate and propagate AP along the axon relies  
131 on their specialized molecular composition associating ion channels, cell adhesion molecules  
132 (CAMs), cytoskeletal scaffold proteins and extra-cellular matrix net (Figure 3). This  
133 molecular organization is partly similar to the axon initial segment (AIS), located at the axon  
134 hillock and where the AP are generated<sup>37</sup>

135 The nodes of Ranvier are highly enriched in Na<sub>v</sub> channels, consisting of a 260kDa pore-forming  
136  $\alpha$ -subunit, enabling inward Na<sup>+</sup> fluxes and depolarization phase of the AP, and one or more  
137 subtypes of 30-40 kDa accessory  $\beta$ -subunits, which modulates  $\alpha$ -subunit membrane targeting  
138 and the opening threshold of the pore<sup>38-43</sup>. Five out of the ten existing  $\alpha$ -subunits isoforms are  
139 found at the CNS nodes in physiological contexts (Nav1.1, 1.2, 1.3, 1.6 and 1.8). Nav1.3 is  
140 restricted to embryonic and developmental stages and mostly absent from adult CNS<sup>44</sup>. Nav1.1  
141 expression depends on the neuronal subtype and is detected in GABAergic neurons in the  
142 hippocampus and cortex<sup>45-49</sup>, as well as in retinal ganglion cells (RGCs) and Purkinje cells  
143<sup>50,51</sup>. Nav1.2 is the major isoform in immature nodes, with a later switch to Nav1.6<sup>52-55</sup>, which  
144 allows for higher discharges frequencies<sup>56,57</sup>. In the adult CNS, a subset of nodes also express  
145 Nav1.2 and Nav1.8<sup>58</sup>.

146 There are four different  $\beta$ Nav isoforms ( $\beta$ 1Nav to  $\beta$ 4Nav), encoded by the *SCNB* genes<sup>42,59</sup>,  
147  $\beta$ 1Nav and  $\beta$ 3 Nav being non-covalently associated to  $\alpha$ -subunit, while  $\beta$ 2 or  $\beta$ 4 subunits are  
148 linked to  $\alpha$ -subunit through disulfure bonds.  $\beta$ 1,  $\beta$ 2 and  $\beta$ 4Nav subunits are found at the nodes  
149 of Ranvier<sup>41,60-63</sup>.  $\beta$ Nav subunits have an immunoglobulin-like domain on the N-terminal  
150 portion, which confers their adhesive properties and allows for direct interaction with other  
151 nodal CAMs, such as contactin or neurofascin 186 (Nfasc186), as well as extracellular matrix  
152 proteins, such as tenascins<sup>62,64,65</sup>.  $\beta$ 1 and  $\beta$ 2Nav subunits can further bind the scaffold protein  
153 ankyrin-G (AnkG) through their intracellular domain, which also plays a role in signaling<sup>66,67</sup>.

154 The voltage-gated potassium channels K<sub>v</sub>7.2/3 (*KCNQ2/3*, belonging to the delayed rectifier  
155 K<sub>v</sub>7 family) have also been described at nodes<sup>68-70</sup>. These potassium channels are thought to

156 stabilize the membrane resting potential, thus allowing to maximize AP amplitude <sup>71</sup>. Kv3.1b  
157 channels are also present in a subset of nodes in the CNS, in particular on large myelinated  
158 axons, where they could participate in high-frequency firing <sup>72,73</sup>. New comers at the node are  
159 the very recently described voltage-independent, thermosensitive and mechanosensitive TREK-  
160 1 and TRAAK channels (belonging to the 2-pore domain potassium leak channels (K2P)  
161 family). Rather than conventional voltage-gated K<sup>+</sup> channels, these K2P channels drive AP  
162 regeneration. Moreover, they permit high-speed and high-frequency AP conduction in  
163 mammalian myelinated afferent nerves. Although it remains to be determined whether the role  
164 of K2P channels in rapid AP conduction can be extrapolated to all myelinated nerves, these  
165 results represent a real breakthrough in our understanding of axonal conduction. They may also  
166 have clinical implications in demyelinating pathologies <sup>5,6</sup>.

167 The nodal voltage-gated ion channels are further stabilized by cell-adhesion molecules (CAM)  
168 such as the neuronal neurofascin isoforms, Nfasc186 and Nfasc140 (the latter expressed only  
169 during development) <sup>74,75</sup>, NrCAM <sup>76–78</sup> and the GPI-anchored protein contactin <sup>79</sup>. Through  
170 its IgG domain, Nfasc186 can directly interact with NrCAM,  $\beta$ 1Nav as well as contactin <sup>62,80,81</sup>.

171 This transmembrane complex is further anchored to the actin cytoskeleton through its direct  
172 interaction with the scaffold protein ankyrin-G (AnkG), which binds in turn to  $\beta$ IVspectrin  
173 <sup>37,69,74,82–86</sup>.

174 Various extracellular matrix proteins (ECM) are also enriched at the CNS nodes, namely the  
175 extracellular domain of NrCAM, Bral1, Tenascin-R, as well as the chondroitin proteoglycans  
176 Brevican, neurocan, phosphacan, and Versican V2 <sup>87–95</sup>. The CNS nodal ECM, which is similar  
177 to the perineuronal nets surrounding soma and dendrites of various neuronal populations <sup>96</sup> may  
178 play a role in buffering the nodal environment, through the negative charges of chondroitin  
179 sulfate chains <sup>88,92,97</sup>, and influence neuronal physiology.

180 The paranodal septate-like axoglial junctions, which delimitate the node, comprise the axonal  
181 adhesion proteins Caspr (contactin associated protein or paranodin) and contactin, which  
182 interact with the glial isoform of neurofascin, Nfasc155 <sup>98</sup>. The absence of any of these  
183 constituents leads to a loss of the axoglial junction <sup>98–106</sup>. A specialized cytoskeleton further  
184 stabilizes the septate-like junctions, by the interaction of Caspr with the cytoskeletal adapter  
185 4.1B, which binds to ankyrin-B (AnkB) and  $\alpha$ II/ $\beta$ II spectrin <sup>107,108</sup>.

186 In mammals, the main feature of juxtaparanodes is their high density in delayed rectifier  
187 voltage-gated potassium channels (K<sub>v</sub>1.1, 1.2 and K<sub>v</sub>b2 subunit), which may play a role in  
188 internodal resting potential support, in particular during development and injury<sup>109–111</sup>. K<sub>v</sub>  
189 channels associate with Caspr2, a transmembrane Caspr-related protein, and the GPI-anchored  
190 TAG1 (contactin-2), which is expressed on both the axonal and glial side. The neuronal TAG1  
191 binds in *trans* homophilically to its glial counterpart and to Caspr2 in *cis*<sup>112</sup>.

### 192 *2-3) Mechanisms of nodal assembly and maintenance*

193 Node of Ranvier assembly in the CNS is a complex process, not fully understood to  
194 date. Three main mechanisms, implicating paranodal junction, cytoskeleton and ECM,  
195 participate to node formation, but their respective role may vary depending of the neuronal  
196 subtype considered<sup>3,92,113,114</sup>.

#### 197 The paranodal axo-glial junction

198 Several studies have shown that CNS nodes form after the paranodal junction, leading to the  
199 assumption that CNS node formation relies primarily on the flanking paranodal junctions  
200<sup>92,115,116</sup>. The axoglial junction formed by axonal Caspr and contactin interaction with the glial  
201 Nfasc155 is required to establish an effective paranodal membrane diffusion barrier, with an  
202 essential role of its anchoring to neuronal paranodal cytoskeleton, by interaction with 4.1B,  $\alpha$ II  
203 spectrin and  $\beta$ II spectrin<sup>117,118</sup>. This barrier restricts accumulation of nodal proteins and  
204 participates in nodal clusters localization<sup>107,118,119</sup>.

205 However, paranodal axo-glial junctions might not be essential for nodal clustering, as shown  
206 in mutant models where nodes of Ranvier still assemble in the absence of paranodal junctions  
207<sup>100,101,120–122</sup>. In addition, we and others have shown that early nodal clusters, that we named  
208 prenodes, can form on some neuronal subpopulations prior to paranodal formation and myelin  
209 deposition<sup>63,123–126</sup>. That nodal cytoskeleton and nodal CAMs (Nfasc 186) interaction with  
210 ECM might be sufficient to promote Nav clustering highlights that several mechanisms operate  
211 together for correct node formation in the CNS. It is likely, as shown by knock-out studies, that  
212 compensation takes place whenever one mechanism fails. In addition, different mechanisms  
213 might drive nodal clustering depending on different neuronal subpopulations.

#### 214 The cytoskeletal scaffold proteins ankyrin and $\beta$ IV spectrin

215 Cytoskeletal AnkG has been shown to be a key factor for nodal assembly. Most major nodal  
216 markers, including Nav, K<sub>v</sub>7.2/3 channels, Nfasc 186, NrCAM, and  $\beta$ IV spectrin have AnkG



217 binding domains<sup>69,85,127,128</sup>. It is established that AnkG, through binding to  $\beta$ IV spectrin  
218 provides a scaffold that recruits nodal proteins and anchors them to the  
219 cytoskeleton<sup>86,92,127,129,130</sup>. Although compensatory expression of AnkR in the absence of AnkG  
220 can rescue nodal assembly in the CNS, Nav channels fail to cluster when both AnkG and AnkR  
221 are absent<sup>131</sup>.

222

223

### The nodal ECM

224 In the CNS, nodes of Ranvier are surrounded by a nodal ECM. Some ECM proteins, such as  
225 NrCAM, BraL1, Brevican and Versican, can directly interact with the axonal CAMNfasc186<sup>3</sup>,  
226 which plays a major role in Nav channel clustering. Although ECM-CAMs interactions might  
227 play a role in localizing the nodal clusters, it is now hypothesized that it might be involved in  
228 long-term stabilization rather than the initial assembly of the CNS Nodes<sup>92,132–134</sup>.

229

230 Aside to this tripartite scenario, the role of oligodendroglial secreted cues in early events of  
231 nodes of Ranvier formation, already shown long ago has been revisited by our group, with the  
232 subsequent demonstration that clusters of nodal proteins (prenodes) form in subpopulations of  
233 CNS axons prior to myelin deposition/paranodal junction formation and that oligodendroglial  
234 secreted contactin is a key factor for the aggregation of prenodes constituents.

235

236

### The aggregating oligodendroglial secreted cues

237 Early cytochemical and freeze fracture studies have provided evidence for the differentiation  
238 of the axon membrane prior to formation of compact myelin in spinal nerve roots<sup>135</sup>. In rat  
239 developing optic nerve axons, clusters of intramembranous particles on the axolemma precede  
240 the formation of any compact myelin<sup>136</sup>. In the developing rodent optic nerve, Nav clusters  
241 colocalize with AnkG and Nfasc186 and are transiently detected, either isolated or flanked on  
242 one side by paranodal Caspr (i.e., heminodal structure)<sup>92,115,125</sup>. Immunostainings in retinal  
243 ganglion cell cultures have demonstrated the presence of such Nav channels clusters regularly  
244 spaced along axons, which are induced by protein(s) secreted by oligodendrocytes, in the  
245 absence of glial cell contact<sup>63,123</sup>.

246 These results were reproduced by Freeman et al. in 2015, showing that nodal protein assembly  
247 (prenodes) occurs under the influence of oligodendroglial secreted cues, without the need of  
248 direct oligodendroglial contact on hippocampal parvalbumin and/or somatostatin expressing  
249 GABAergic neurons. In addition, selective expression of Kv1.2 channels has been detected

250 along GABAergic hippocampal neurons with prenodes<sup>137</sup>. Formation of these early nodal  
251 protein clusters along unmyelinated GABAergic axons requires also intrinsic neuronal factors  
252 such as AnkG and  $\beta 2\text{Nav}$ <sup>124,126</sup>. To identify the oligodendroglial cues inducing formation of  
253 prenodes, a mass spectrometry analysis of oligodendrocyte conditioned medium was  
254 performed, resulting in the identification of contactin-1 combined with RPTP/Phosphacan and  
255 Tenascin-R<sup>125</sup>. Contactin expression is detected in neurons and oligodendroglial cells<sup>138</sup>, and  
256 binds both to RPTP/Phosphacan and TenascinR<sup>139–142</sup>, suggesting that the oligodendroglial  
257 aggregating activity might be related to the formation of a multi-molecular complex. Nfasc186  
258 and NrCAM are not critical for contactin-1 induced prenodal clustering, but interactions with  
259 RPTP/phosphacan or Tenascin-R may bridge oligodendrocyte-derived contactin with other  
260 neuronal CAMs (contactin,  $\beta 1$  or  $\beta 2\text{Nav}$  subunits)<sup>64,143</sup>. In addition to the optic nerve and the  
261 hippocampus, prenodes have also been detected in Purkinje cells in the mouse cerebellum (A.  
262 Desmazières, unpublished results), as well as in the *Xenopus* optic nerve (B. Zalc unpublished  
263 results) suggesting that it might be a more general mechanism for nodal clustering. What  
264 distinguishes axons that form prenodes from axons without prenodes is still unclear. Prenode  
265 formation might be related to different modalities of axonal differentiation. Alternatively, it  
266 might be developed to meet the need for early establishment of neuronal connections during  
267 development<sup>124,144–146</sup>. That formation of prenodes is an intrinsic property of subpopulations of  
268 neurons is illustrated by the fact that in the same culture, oligodendroglial conditioned medium  
269 will induce prenodes on GABAergic, but not on pyramidal axons, a specificity that is  
270 reproduced *in vivo* on tissue sections of developing hippocampus<sup>124</sup>.

271 What is the fate of these early nodal clusters has been recently addressed using *in vitro* live  
272 imaging methods, showing that prenodal clusters participate in heminode formation (and thus  
273 ultimately in mature nodes of Ranvier) when myelination proceeds, by fusion with existing  
274 heminodal structures when myelin progresses towards them, or by guiding myelin deposition  
275 initiation at their direct vicinity<sup>126</sup>.

276  
277

### 278 **3) The Node of Ranvier as a regulator of nerve conduction**

279 The nerve impulse propagation results from a transient influx of  $\text{Na}^+$ , responsible for the  
280 rapid depolarization, and an efflux of  $\text{K}^+$  which causes repolarization, as described by the  
281 Hodgkin and Huxley model<sup>147</sup> established from voltage-clamp measurements in the squid giant  
282 axon. Voltage-gated  $\text{Na}^+$  channels are critical drivers for active conduction along the axon, and

283 their clustering at nodes of Ranvier in myelinated fibers allows regeneration of AP and saltatory  
284 conduction <sup>148,149</sup>. Using computational modeling in combination with high-speed voltage-  
285 calibrated optical recordings across the nodal and internodal axolemma of myelinated  
286 neocortical pyramidal axons, as well as electron microscopy analysis, Cohen et al <sup>150</sup> recently  
287 provided evidence for a second longitudinal conducting pathway formed by the periaxonal and  
288 paranodal submyelin spaces, therefore a double cable model for axonal conduction.  
289 Morphological axonal parameters, notably axon diameter, internodal length and myelin  
290 thickness are critical parameters for conduction velocity <sup>151-154</sup> (Figure 4), and might be  
291 regulated by neuronal electrical activity. This adaptive process contributes to strengthening and  
292 synchronization of circuits and improves performances during learning <sup>155,156</sup>. In addition,  
293 nodes of Ranvier length and diameter are potential regulators of conduction speed as they  
294 determine the capacitance of the node and the speed with which nodal Nav channels activate  
295 <sup>15,20</sup>. In this context, variability of node length even at constant axon diameter was recently  
296 shown between different axons in the optic nerve and in the grey matter of the cortex,  
297 contrasting with similar length along the same axon. Using simulation studies, such length  
298 modifications resulted in large variations of conduction speed (increased node length  
299 decreasing conduction speed and decreased node length increasing conduction speed). Taken  
300 together this suggests that axons may be able to adjust their node lengths in order to tune their  
301 conduction speed, to optimize information processing. (Figure 4B). It was recently shown that  
302 perinodal astrocytes, by altering myelin thickness and increasing nodal gap length might also  
303 impact conduction speed <sup>31</sup>. Concerning node of Ranvier diameter, a progressive increase along  
304 the distal segments has been observed in auditory brainstem axons, and simulation studies  
305 suggest that it contributes to temporal processing for sound localization <sup>20</sup>. In addition to these  
306 morphological changes of nodes of Ranvier, clustering of Nav prior to myelin deposition  
307 (prenodes) may constitute an early mechanism to accelerate conduction velocity, <sup>124,157</sup> (Figure  
308 4A). Indeed, single-axon electrophysiological recordings on hippocampal GABAergic neurons  
309 with and without prenodes show that the conduction velocity is higher in neurons with prenodes  
310 compared to same caliber axons without prenodes <sup>124</sup>. These data suggest another level of  
311 influence by oligodendrocytes on the regulation of conduction velocity beyond the production  
312 of a myelin sheath. Prenodes with high-density Nav clusters could serve as acceleration point,  
313 allowing microsaltatory conduction <sup>158,159,160</sup> and could therefore help to overcome axonal  
314 branch point failures and maintain reliable propagation of AP <sup>153,161</sup>.

315 In myelinated fibers, K<sub>v</sub>1.1 and K<sub>v</sub>1.2 channels, restricted to the juxtaparanodal compartment,  
316 contribute to internodal resting potential and act to prevent repetitive firing<sup>2</sup>. At nodes, K<sup>+</sup>  
317 conductance mainly relies on leak-type channels belonging to the K2P channel subfamily (see  
318 above), recently identified as TRAAK and/or TREK-1<sup>5,6</sup> and also on slowly opening K<sub>v</sub>7.2/7.3  
319 channels<sup>68</sup>. Their activity largely contributes to repolarizing the nodal membrane during a  
320 spike, and is also important for the stabilization of a negative resting potential and maximum  
321 spike amplitude<sup>5,6,68,71</sup>. The activity of K2P channels can be diversely regulated to control  
322 cellular excitability, notably by force applied directly through the lipid bilayer<sup>162,163</sup> and could  
323 serve a mechano-protective role in node of Ranvier or respond to mechanical component of the  
324 action potential<sup>164</sup>. Other K<sup>+</sup> channels also repolarizes membrane at nodes, such as K<sub>Ca</sub>3.1, a  
325 calcium-activated potassium channel, which is a key determinant for generation of fast  
326 frequency spikes and safe conduction in Purkinje cell axons<sup>165</sup>.

327

#### 328 **4) Nodes of Ranvier alterations in demyelinating pathologies**

329 Whether nodal structures can be the direct target of auto-immune CNS demyelinating  
330 pathologies, as demonstrated for several auto-immune peripheral neuropathies, is not clear to  
331 date. Major changes at and near the node, however, take place as a consequence of myelin loss.  
332 These structural changes are associated with profound modifications of Nav and K<sub>v</sub> channels  
333 expression, and alteration of neuronal physiology.

##### 334 *4-1) Nodal clusters alterations and ion channels changes at and near nodes upon CNS* 335 *demyelination.*

336 In experimental autoimmune encephalomyelitis (EAE), a model of inflammatory CNS  
337 demyelination, disruption of nodes of Ranvier with loss of Nav nodal clusters has been reported  
338 in both optic nerves<sup>166</sup> and spinal cord<sup>167</sup>. Loss of nodal clusters is associated with extended  
339 expression of Nav channels along demyelinated axons. Similar findings with drastic reduction  
340 of Nav nodal clusters were reported in the corpus callosum upon cuprizone-induced  
341 demyelination, a toxic, non-inflammatory model<sup>168,169</sup>. In dysmyelinating mutants, changes of  
342 Nav channels expression appear more heterogeneous : in *myelin-deficient (md)* rats and *jimpy*,  
343 mice, two proteolipid protein (PLP) mutants<sup>58,170</sup>, where myelination is drastically reduced,  
344 Nav channels clusters persist despite lack of myelin and axoglial junctions; similarly, in  
345 *ceramide galactosyltransferase deficient* mice, which undergo spontaneous adult-onset  
346 demyelination, Nav channels clusters persist after myelin sheaths are lost<sup>168</sup>. In contrast, in

347 *shiverer* mice, a myelin basic protein (MBP) mutation resulting in non-compacted myelin, Nav  
348 channel clusters are reduced in number<sup>115</sup> while Nav channel immuno-reactivity is detected  
349 throughout adult axons<sup>55</sup>. Different type of myelin pathology and different life expectancy  
350 between these different mutants might explain this heterogeneity.

351 These alterations of Nav localization upon experimental demyelination are associated with  
352 changes in Nav isoform expression. Reduced expression of Nav1.6 (the mature isoform of Nav,  
353 expressed at nodes in the adult normal CNS, see above) associated with detection of Nav1.2  
354 (normally restricted to immature nodes and unmyelinated fibers) has been reported in different  
355 models<sup>55,166,167,169,171</sup>. That Nav1.2 expression might correspond to *de novo* synthesis was  
356 addressed in the EAE model, where the increased expression of Nav1.2 channels along  
357 demyelinated axons of the optic nerve was accompanied by upregulated Nav1.2 channel mRNA  
358 levels in retinal ganglion cells<sup>172</sup>. In addition to Nav1.2 and 1.6, Nav1.8 is ectopically expressed  
359 by Purkinje neurons in EAE, where it perturbs electrical activity<sup>173,174</sup>.

360 In addition to changes in Nav channels, major modifications of Kv channels are associated with  
361 demyelination. In the dysmyelinating mutants (*md* rats and *jimpy* mice) the localization of Kv  
362 channels, which in mammals normally reside at the juxtaparanodal region beneath the myelin  
363 sheath (e.g., Kv 1.1 and Kv 1.2), is strongly altered and Kv 1.1/ Kv 1.2 channels about the nodal  
364 clusters of Nav<sup>58,72,170</sup>. Similar findings were reported in the spinal cord of EAE mice<sup>175,176</sup>.  
365 Finally, upon cuprizone demyelination Kv 1.1 and Kv 1.2 channels disperse all along the axon  
366<sup>177</sup>.

367 Most of these studies have been focused on white matter axons. Recently, using a murine  
368 cuprizone model, Hamada et al<sup>178</sup> addressed the changes induced by demyelination in the  
369 cortex, by focusing on L5 pyramidal neurons of the somato-sensory cortex. They showed that,  
370 contrary to white matter tracts, Nav1.6 expression was heterogeneously distributed along these  
371 cortical denuded axons, either dissolved or extended into the paranodal domains with increased  
372 nodal area. This was in contrast with the Kv 7.3 channels, which redistribute extensively along  
373 the demyelinated internodes.

374 The few neuropathological studies addressing the fate of nodal structures in MS tissue  
375 demonstrated that nodes of Ranvier on white matter demyelinated axons are profoundly altered  
376 or disrupted, while a diffuse redistribution of Nav channels is detected along denuded axons  
377<sup>172,179</sup>. Other changes such as lengthening of nodes have also been reported<sup>176,179</sup>. Although

378 Nav channels of mature nodes in the normal CNS are Nav1.6, redistributed Nav channels along  
379 demyelinated axons consist both of Nav1.6 and Nav1.2<sup>172</sup>. In addition, in MS, similarly to  
380 EAE, Nav1.8 is ectopically expressed in axons of Purkinje neurons, and it has been suggested  
381 that variants of *SCN10A* gene (encoding Nav1.8) predict the degree of cerebellar dysfunction  
382 in MS<sup>173,180</sup>. Changes of Kv channels also occur, with loss of juxtaparanodal Kv clusters and  
383 extensive expression of Kv channels along the denuded portion of the axon<sup>179</sup>.

384 Whether these nodal alterations occur at the onset of myelin damage or whether they are  
385 secondary to paranodal junction disruption has been extensively studied. In experimental  
386 demyelinating models (EAE, cuprizone)<sup>176,181</sup>, early breakdown of paranodes is detected  
387 contrasting with persisting nodal clusters.<sup>2,58,170</sup> However, nodal changes have been detected  
388 prior to or coincident to disruption of paranodal junctions in an EAE model using coherent anti-  
389 Stokes Raman scattering microscopy (CARS)<sup>182</sup>. In a model of autoimmune optic neuritis both  
390 nodes and paranodes were increased in length during the late induction phase of the disease,  
391 prior to onset of the demyelination and immune cell infiltration<sup>183</sup>. Similarly, in a cuprizone  
392 model with different treatment durations, a small but significant decrease in nodes density was  
393 observed at the onset of demyelination and before significant change in Caspr  
394 immunoreactivity<sup>184</sup>. Analyzing MS normal appearing white matter (NAWM), Howell et al  
395 showed an early elongation and alteration of paranodal structures<sup>176</sup>. Moreover, a displacement  
396 of Kv1.2 channels closer to the nodes is observed indicating that disruption of paranode  
397 precedes alterations at the node itself. Similar findings were reported in the borders of lesions  
398 of patients with chronic progressive MS<sup>176,185</sup>. In line with these results, loose/extended clusters  
399 of nodal proteins are detected on some denuded axons within MS demyelinated lesions<sup>179</sup>,  
400 likely corresponding to remaining nodal structures after paranodal breakdown. Altogether, the  
401 chronology of nodal versus paranodal alteration upon demyelination remains elusive, with the  
402 difficulty to ascertain a temporal pattern from human post-mortem studies. Whether, as  
403 highlighted for nodal formation, the temporal pattern of nodal disaggregation might vary  
404 depending on neuronal subpopulations is an open question.

405 *4-2) The consequences of demyelination-induced nodal disruption on neuronal physiology.*  
406 Disruption of nodal and perinodal domains have a profound impact on nerve conduction and  
407 excitability. Moreover, Nav channels redistributed along the axon might act as a double sword,  
408 participating to an adaptive response to injury on the one hand, to neurodegeneration on the  
409 other hand.

### Change in neuronal conduction and excitability

In the spinal cord, optic nerve, corpus callosum and neocortex myelin loss is known for long to cause a substantial slowing in conduction velocity and often a transitory conduction block<sup>184,186–188</sup>. More recently, optical Voltage-Sensitive Dye Recordings of cortical L5 pyramidal neurons allowed to demonstrate that demyelination is associated with a switch of the modality of AP propagation from saltatory fast conduction to continuous slow conduction<sup>178</sup>, consistent with the observation that even low Nav channel densities at the denuded internode axolemma suffice to ensure propagation of axonal APs<sup>189</sup>. In addition, hyperexcitability, ectopic spikes generation and failure of AP propagation at the presynaptic terminals have been recorded in these demyelinated fibers<sup>178,190</sup> (Figure 4C). The impact of K<sup>+</sup> channels redistribution along demyelinated axons remains unclear. The functional role of Kv7, redistributed into the demyelinated internodes, remains to be further examined. To date no data are reported concerning K2P channels during demyelination. Kv 1.1 and 1.2 channels become exposed from under the myelin, contributing to alteration of AP propagation. Blockade of these channels using 4-amidopyridine (4-AP) is a current treatment in MS and other demyelinating diseases. However, it has been suggested that K<sup>+</sup> channels targeted by 4-AP are also found at synaptic terminals rather than along demyelinated axons<sup>8</sup>.

### Redistribution of Nav channels induces neurodegeneration

Whereas redistribution of Nav channels allow persistent, albeit slow, nerve conduction, it might however be maladaptive, as suggested by the seminal work of Craner et al.<sup>172</sup>, which has demonstrated that the Nav1.6 isoforms, producing persistent Na<sup>+</sup> current might be a key driver of neurodegeneration, with accumulation of intra-axonal sodium and subsequent calcium overload. In contrast, redistribution of the Nav1.2 isoform was considered as less damaging due to less Na<sup>+</sup> persistent current production than the Nav1.6 channels. This hypothesis was only recently tested *in vivo* using gain and loss of function experiments. In mice floxed for *Scn8a*, the gene that encodes the  $\alpha$  subunit of Nav1.6, elimination of Nav1.6 from murine retinal ganglion cells using intra-vitreous injection of an AAV vector harboring the Cre recombinase has resulted, during EAE, in increased neuronal survival. Interpretation of the impact of Nav1.6 suppression on neuroprotection was however complex because loss of Nav1.6 also resulted in reduced inflammation<sup>191</sup> likely related to the expression of Nav1.6 on non neuronal cells, among them microglia and macrophages. Concerning Nav1.2, gain-of-function achieved by inserting a human mutation in the mouse Nav1.2-encoding gene has resulted in increased neuro-

443 axonal degeneration and increased disability during EAE <sup>192</sup> with no impact on the immune  
444 response; such gain of function was not tested for Nav1.6, due to the induction of epileptic  
445 seizures. Altogether these results strengthen the hypothesis that demyelination-induced  
446 redistribution of Nav channels along the axon play a major role in neurodegeneration.

447 In direct line with these pathophysiological concepts, therapeutic strategies aimed at blocking  
448 Na<sup>+</sup> channels to promote neuroprotection were developed: the lamotrigin trial evaluating a  
449 partial Na<sup>+</sup> channels blocker in secondary progressive MS <sup>193</sup>, with brain volume as a primary  
450 outcome measure was negative; the phase 2 trial of phenytoin in patients with optic neuritis <sup>194</sup>,  
451 showed a 30% reduction of retinal thickness loss (quantified using optical coherence  
452 tomography) with phenytoin compared with placebo. The lack of specificity for Nav1.2 and/or  
453 Nav1.6 of the therapeutic compounds tested in these trials, the lack of robust surrogate markers  
454 for neuroprotection in MS (except for the optic nerve) might have prevented further  
455 development. These strategies might however be re-actualized with the development of more  
456 specific Na<sup>+</sup> channels blockers.

457 In addition, novel perspectives might emerge based on K<sup>+</sup> channels blockade. Blocking K<sup>+</sup>  
458 channels on denuded axons (see above), 4-aminopyridine (4-AP), have some minor  
459 symptomatic efficacy, likely related to reduced axonal excitability, but it lacks specificity.  
460 Some relatively specific drugs now available might represent a promising approach. Finally  
461 visualizing K<sup>+</sup> channels expression might open to novel imaging perspectives. A recent PET-  
462 scan study using [18F]3-F-4-AP ligand in dysmyelinating *Shiverer* mice lacking compact  
463 myelin has suggested that it might be a valuable PET tracer for detecting CNS demyelination<sup>195</sup>.

464

#### 465 *4-3) Nodes of Ranvier: a primary target of the inflammatory attack?*

466 Whether node of Ranvier might represent sites of axonal vulnerability has been suggested. In  
467 this context, live imaging of spinal cord in an EAE model, has shown that initial disruption of  
468 the axon often occurs at putative nodes of Ranvier <sup>196</sup>. This might reflect the vulnerability of  
469 the nodal domains in an inflammatory environment, due to the lack of myelin protection at the  
470 nodal axonal surface. Interestingly, using a spinal cord *in vivo* multiphoton imaging approach  
471 in combination with calcium reporter mouse lines in mice with EAE, Witte et al have shown  
472 that calcium permeable membrane nano-ruptures might influence axonal fate, and that axons  
473 with maintained elevated calcium levels are prone to degenerate <sup>197</sup>. Importantly, demyelination  
474 increased the probability of calcium permeable membrane nano-ruptures, probably because loss  
475 of the myelin sheaths facilitates access to the axonal membrane along the internode, and, in this



476 context, it can be hypothesized that nodes of Ranvier might represent a preferential site for  
477 calcium induced axonal membrane injury

478 In addition, microglia infiltration was shown to be correlated with nodal and paranodal  
479 alterations in MS patients and in EAE <sup>176</sup>, whereas the inhibition of microglia activation  
480 minimized the nodal/paranodal alterations. This indicates that inflammation can participate in  
481 MS etiology by affecting node organization prior to demyelination.

482 Alternatively to this hypothesis of nodal vulnerability, the possibility that nodal antigens might  
483 be the target of the immune response has been actively studied. The first report <sup>198</sup> that  
484 autoantibodies recognizing the extracellular domain of both Nfasc155 and Nfasc186 were  
485 detected in some MS patients, more frequently in chronic progressive than relapsing remitting  
486 MS, has led to the hypothesis that recognition of Nfasc186 at the node of Ranvier by these  
487 antibodies might exacerbate axonal injury and accelerate disease progression. Another report,  
488 however, described no difference in the prevalence of anti-Nfasc seropositivity between  
489 patients with primary progressive MS (4.8%) or relapsing-remitting MS (0.6%) and healthy  
490 controls (2.0%) <sup>199</sup>, and three later studies indicate that Nfasc is not the dominant target of  
491 antibodies in MS <sup>200–202</sup>. Contactin-2, expressed at juxta-paranodes, was identified as an  
492 autoantigen targeted by T cells and autoantibodies in MS, with the hypothesis that a contactin-  
493 2-specific T-cell response contributes to the development of gray matter pathology <sup>203</sup>, but this  
494 has not been confirmed to date.

495 Taken together, and even if both technical issues to detect seropositivity and underestimation  
496 due to the lack of systematic screening might account for this low prevalence of anti-  
497 nodal/perinodal antibodies in MS, there is no clear evidence for an immune response targeting  
498 nodal antigens in MS.

499 This is in contrast with peripheral nervous system (PNS) inflammatory diseases, where  
500 antibodies targeting Nfasc and contactin-1 have been detected in patients with chronic  
501 inflammatory demyelinating polyneuropathy (CIDP). These antibodies were in most cases  
502 directed against Nfasc155, the glial isoform of neurofascin expressed at paranodes <sup>204,205</sup> but in  
503 few cases against Nfasc186 <sup>206–208</sup> or contactin-1 <sup>206,209,210</sup>. In patients with both CNS and PNS  
504 demyelinating lesions (a rare, not yet well defined condition named combined central and  
505 peripheral demyelination, CCPD), anti-Nfasc155 antibody were detected, but their prevalence

506 strikingly varies between studies <sup>211,212</sup>. These discrepancies likely reflect technical issues  
507 related to the detection of the seropositivity.

#### 508 *4-4) Nodes of Ranvier reform during remyelination. Can it impact myelin repair?*

509 The fact that remyelination is associated with reclustered nodes of Ranvier has been shown  
510 in different experimental models <sup>169,172</sup>. Remyelinated axons, characterized by short internodal  
511 lengths, indicating that new nodes are formed, have restored conduction <sup>213</sup>. In MS, Coman et  
512 al <sup>179</sup> showed that, within shadow plaques, nodal clusters, flanked by paranodes, and  
513 undistinguishable from nodes of Ranvier present in the periplaque, in the NAWM and in control  
514 tissue (Figure 5A), were a hallmark of these remyelinated areas. The chronology of this re-  
515 aggregation is still unknown. In addition, Coman et al <sup>179</sup> reported that, although remyelinated  
516 internodes were always flanked by heminodal clusters, aggregates of Nav channels were often  
517 detected on denuded axons in the vicinity of remyelinated axons. These isolated clusters might  
518 represent nodes of Ranvier persisting after demyelination. Alternatively, they might represent  
519 early clusters of nodal proteins (prenodes), clustered prior to remyelination (as they occur prior  
520 to myelination). Further reinforcing this hypothesis is the detection of prenodes prior to  
521 remyelination, in an *ex-vivo* model of murine cerebellar slices, where demyelination, induced  
522 by lysophosphatidylcholine, is followed by axoglial junction formation and remyelination (our  
523 unpublished results, figure 5B). The role of these prenodes during the remyelination process  
524 remains to be determined. It can be envisaged that they impact conduction speed, allowing  
525 increased velocity prior to/in the absence of remyelination. In agreement with this hypothesis  
526 are the seminal results by Smith et al. <sup>214</sup> in a murine model of spinal cord demyelination, where  
527 conduction velocity increased prior to myelin wrapping. It was suggested by the authors that  
528 this increased conduction was related to “precursors of nodes of Ranvier also named phi-nodes”  
529 distributed along myelinated nerve fibers, and that it “may indicate aggregates of sodium  
530 channels which form along the demyelinated axolemma prior to remyelination”.

531 Alternatively, prenodes might influence the position of the new myelin sheaths. Indeed, our live  
532 imaging approach, showing that myelin wrapping often begins at, or near the site of contact  
533 between an oligodendroglial process and a prenode <sup>126</sup> suggests that prenodes might guide the  
534 myelin wrapping onset. In line with the long-term imaging of myelin remodeling after myelin  
535 ablation in zebrafish, which has shown that new sheaths are positioned almost exactly where  
536 the ablated ones were, it can therefore be hypothesized that prenodes could serve as landmarks  
537 for myelin positioning of regenerating sheaths <sup>126,215</sup>.

538

539 ***Conclusion /perspectives***

540 The granularity of our knowledge on the molecular composition of the nodal and perinodal  
541 axonal domains, as well as our understanding of the impact of nodes of Ranvier in neuronal  
542 physiology, has moved forward recently.

543 Deciphering the structural and molecular alterations of the nodal components, in particular of  
544 the Na<sup>+</sup> and K<sup>+</sup> ion channels upon demyelination and remyelination, together with the  
545 development of more specific channels blockers, might pave the way for novel therapeutic  
546 perspectives favoring neuroprotection and restoration of function to prevent disability  
547 progression in MS. In addition, even if this is a more general mechanism of axonal damage,  
548 novel perspectives of neuroprotection might emerge from the recent results of calcium induced  
549 nano-ruptures <sup>197</sup>.

550 There is also growing evidence that nodal abnormalities can occur in other, non  
551 de/dysmyelinating diseases (traumatic injury, cerebral hypoperfusion, hyperoxic model of  
552 premature brain injury) in which demyelination is secondary to the initial insult <sup>8,216-219</sup>. In  
553 addition alterations of ion channels have been identified in the pathophysiology of several  
554 disorders of neuronal excitability such as epilepsy, linked to mutations in Nav or Kv channels  
555 or alterations in channel trafficking [reviewed in <sup>220,221</sup>], but also migraine and psychiatric  
556 diseases. It is not clear, however, whether ion channel dysfunctions specifically at the nodes  
557 alone underlie the development of these diseases, since there are also high densities of these ion  
558 channels at the AIS. Loss of function of the TREK-1 and TRAAK (K2P) channels required for  
559 high speed conduction, detected at node of Ranvier, and not at the AIS (see above) is associated  
560 *in vivo* with altered sensory behavioral responses. Mutations of *TRAAK* causes a syndrome  
561 associating facial dysmorphism, hypertrichosis, epilepsy, intellectual disability <sup>222</sup>. The  
562 consequences of demyelination on K2P channels is unknown to date, but changes of these  
563 recently identified thermo- and mechano-sensitive channels at the nodes might impact  
564 conduction and function.

565 Finally, it can be envisaged that favoring nodal clustering might be a promising therapeutic  
566 perspective of neuroprotection in demyelinating pathologies. Clustering Nav channels prior to  
567 remyelination might be beneficial, as it might accelerate conduction velocity prior to  
568 remyelination, -similarly to developmental myelination-, therefore allowing to maintain  
569 physiological functions. In addition, preventing diffuse expression of ion channels along  
570 demyelinated axons and the related damaging cascade, by promoting early clusters of nodal

571 proteins might also have a neuroprotective effect. In this context, and with the caveat that *in*  
572 *vivo* evidence is still lacking, the recent discovery of oligodendroglial cues inducing clustering  
573 might open to promising perspectives.

574

575 Acknowledgements: We thank ARSEP foundation, FRM (Fondation pour la Recherche  
576 Médicale), Bouvet-Labruyere Family, INSERM-DHOS, APHP (Assistance Publique des  
577 Hôpitaux de Paris), “Investissements d’avenir” ANR and NRJ foundation for their precious  
578 support related to basic and clinical research on remyelination. We thank Jean Michel Vallat  
579 for the EM picture of the node of Ranvier, Rémi Ronzano, Thomas Roux and Elisa Mazuir for  
580 the figures. We thank Bernard Zalc for fruitful discussions and careful reading of the  
581 manuscript.

582

583

584

585

586

587

## 588 **Figure Legends**

589 **Figure 1** (A) Original drawings of sciatic nerves stained with silver nitrate. Louis-Antoine  
590 Ranvier, in “Leçons sur l’Histoire du Système Nerveux”, 1878. (B) Longitudinal view of a node  
591 of Ranvier in human brain tissue by electron microscopy. The boxed area shows paranodal  
592 loops. Scale Bar : 1  $\mu$ m. Gift of Prof J. M. Vallat, CHU de Limoges, France.

593

594 **Figure 2** Schematic representation of neuroglial interactions at the node of Ranvier in the  
595 central nervous system. OPC : oligodendrocyte precursor cell.

596

597 **Figure 3** Schematic representation of nodal and perinodal molecular organization

598

599 **Figure 4** Overview of the impact of prenodes and nodes of Ranvier on action potential (AP)  
600 conduction velocity during development (A); possible physiological modifications of myelin  
601 and nodes increasing AP conduction velocity (B); in demyelinating pathologies, alterations of  
602 the nodal-paranodal region and ion channels diffusion, perturbing axonal physiology and  
603 conduction velocity (C).

604

605 **Figure 5** (A) Myelinated fiber organization in human cerebellar post-mortem tissue: the nodes  
606 of Ranvier (Nav, red, zoomed in the boxed area) are surrounded by the paranodes (Caspr,  
607 green). Myelin is detected using MBP (blue). Scale Bar : 5 $\mu$ m (B) Myelinating, myelinated and  
608 remyelinating organotypic cultures of mouse cerebellar slices stained for myelin (PLP, green),  
609 nodal structures (Nav, red) and paranodes (Caspr, white). Mature nodes of Ranvier are  
610 presented in the boxed area of the myelinated condition. Prenodal structures (in red) are  
611 indicated by arrowheads in the boxed area of myelinating and remyelinating conditions. Scale  
612 bar : 20  $\mu$ m; box : 5  $\mu$ m.

613

614

615

616

617

618

619

620 **References**

- 621 1. Poliak, S. & Peles, E. The local differentiation of myelinated axons at nodes of Ranvier. *Nat.*  
622 *Rev. Neurosci.* **4**, 968–980 (2003).
- 623 2. Faivre-Sarrailh, C. & Devaux, J. J. Neuro-glial interactions at the nodes of Ranvier: implication  
624 in health and diseases. *Front. Cell. Neurosci.* **7**, 196 (2013).
- 625 3. Rasband, M. N. & Peles, E. The Nodes of Ranvier: Molecular Assembly and Maintenance. *Cold*  
626 *Spring Harb. Perspect. Biol.* **8**, a020495 (2015).
- 627 4. Salzer, J. L., Brophy, P. J. & Peles, E. Molecular domains of myelinated axons in the peripheral  
628 nervous system. *Glia* **56**, 1532–1540 (2008).
- 629 5. Brohawn, S. G. *et al.* The mechanosensitive ion channel TRAAK is localized to the mammalian  
630 node of Ranvier. *eLife* **8**, (2019).
- 631 6. Kanda, H. *et al.* TREK-1 and TRAAK Are Principal K(+) Channels at the Nodes of Ranvier for  
632 Rapid Action Potential Conduction on Mammalian Myelinated Afferent Nerves. *Neuron* **104**, 960-  
633 971.e7 (2019).
- 634 7. Seidl, A. H. Regulation of conduction time along axons. *Neuroscience* **276**, 126–134 (2014).
- 635 8. Arancibia-Carcamo, I. L. & Attwell, D. The node of Ranvier in CNS pathology. *Acta*  
636 *Neuropathol. (Berl.)* **128**, 161–175 (2014).
- 637 9. Ranvier, Louis-Antoine. *Leçons sur l'histologie du système nerveux, par M. L. Ranvier,*  
638 *recueillies par M. Ed. Weber. Paris.* (1878).
- 639 10. Lillie, R. S. Factors affecting transmission and recovery in the passive iron nerve model. *J. Gen.*  
640 *Physiol.* **7**, 473–507 (1925).
- 641 11. Peters, A. The formation and structure of myelin sheaths in the central nervous system. *J.*  
642 *Biophys. Biochem. Cytol.* **8**, 431–446 (1960).
- 643 12. Uzman, B. G. & Villegas, G. M. A comparison of nodes of Ranvier in sciatic nerves with node-  
644 like structures in optic nerves of the mouse. *J. Biophys. Biochem. Cytol.* **7**, 761–762 (1960).
- 645 13. Metzuzals J. Ultrastructure of myelinated nerve fibers and nodes of Ranvier in the central nervous  
646 system of the frog. *Proceedings of the European Regional Conference on Electron Microscopy* (1960).
- 647 14. Salzer, J. L. Polarized domains of myelinated axons. *Neuron* **40**, 297–318 (2003).
- 648 15. Arancibia-Carcamo, I. L. *et al.* Node of Ranvier length as a potential regulator of myelinated  
649 axon conduction speed. *eLife* **6**, (2017).
- 650 16. Micheva, K. D. *et al.* A large fraction of neocortical myelin ensheathes axons of local inhibitory  
651 neurons. *eLife* **5**, (2016).
- 652 17. Micheva, K. D. *et al.* Distinctive Structural and Molecular Features of Myelinated Inhibitory  
653 Axons in Human Neocortex. *eNeuro* **5**, (2018).
- 654 18. Landon, D. N. & Williams, P. L. Ultrastructure of the node of Ranvier. *Nature* **199**, 575–577  
655 (1963).
- 656 19. Pannese, E. *Neurocytology: Fine Structure of Neurons, Nerve Processes, and Neuroglial Cells.*  
657 (2015).
- 658 20. Ford, M. C. *et al.* Tuning of Ranvier node and internode properties in myelinated axons to adjust

- 659 action potential timing. *Nat. Commun.* **6**, 8073–8073 (2015).
- 660 21. Rosenbluth, J. Electrophysiology and morphology of myelinated nerve fibers. V.  
661 Intramembranous particle distribution in nerve fiber membranes. *Experientia* **39**, 953–963 (1983).
- 662 22. Edgar, J. M., McCulloch, M. C., Thomson, C. E. & Griffiths, I. R. Distribution of mitochondria  
663 along small-diameter myelinated central nervous system axons. *J. Neurosci. Res.* **86**, 2250–2257 (2008).
- 664 23. Ohno, N. *et al.* Myelination and axonal electrical activity modulate the distribution and motility  
665 of mitochondria at CNS nodes of Ranvier. *J. Neurosci. Off. J. Soc. Neurosci.* **31**, 7249–7258 (2011).
- 666 24. D’Este, E. *et al.* Subcortical cytoskeleton periodicity throughout the nervous system. *Sci. Rep.*  
667 **6**, 22741 (2016).
- 668 25. D’Este, E., Kamin, D., Balzarotti, F. & Hell, S. W. Ultrastructural anatomy of nodes of Ranvier  
669 in the peripheral nervous system as revealed by STED microscopy. *Proc. Natl. Acad. Sci. U. S. A.* **114**,  
670 E191–E199 (2017).
- 671 26. Black, J. A. & Waxman, S. G. The perinodal astrocyte. *Glia* **1**, 169–183 (1988).
- 672 27. Butt, A. M., Duncan, A. & Berry, M. Astrocyte associations with nodes of Ranvier:  
673 ultrastructural analysis of HRP-filled astrocytes in the mouse optic nerve. *J. Neurocytol.* **23**, 486–499  
674 (1994).
- 675 28. Butt, A. M. *et al.* Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white  
676 matter. *Glia* **26**, 84–91 (1999).
- 677 29. Serwanski, D. R., Jukkola, P. & Nishiyama, A. Heterogeneity of astrocyte and NG2 cell  
678 insertion at the node of ranvier. *J. Comp. Neurol.* **525**, 535–552 (2017).
- 679 30. Ffrench-Constant, C., Miller, R. H., Kruse, J., Schachner, M. & Raff, M. C. Molecular  
680 specialization of astrocyte processes at nodes of Ranvier in rat optic nerve. *J. Cell Biol.* **102**, 844–852  
681 (1986).
- 682 31. Dutta, D. J. *et al.* Regulation of myelin structure and conduction velocity by perinodal  
683 astrocytes. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 11832–11837 (2018).
- 684 32. Hildebrand, C. & Waxman, S. G. Postnatal differentiation of rat optic nerve fibers: Electron  
685 microscopic observations on the development of nodes of Ranvier and axoglial relations. *J. Comp.*  
686 *Neurol.* **224**, 25–37 (1984).
- 687 33. Zhang, J., Yang, X., Zhou, Y., Fox, H. & Xiong, H. Direct contacts of microglia on myelin  
688 sheath and Ranvier’s node in the corpus callosum in rats. *J. Biomed. Res.* **33**, 192–200 (2019).
- 689 34. Oohashi, T., Edamatsu, M., Bekku, Y. & Carulli, D. The hyaluronan and proteoglycan link  
690 proteins: Organizers of the brain extracellular matrix and key molecules for neuronal function and  
691 plasticity. *Exp. Neurol.* **274**, 134–144 (2015).
- 692 35. Dermietzel, R. Junctions in the central nervous system of the cat. II. A contribution to the tertiary  
693 structure of the axonal-glial junctions in the paranodal region of the node of Ranvier. *Cell Tissue Res.*  
694 **148**, 577–586 (1974).
- 695 36. Rosenbluth, J., Petzold, C. & Peles, E. Dependence of paranodal junctional gap width on  
696 transverse bands. *J. Comp. Neurol.* **520**, 2774–2784 (2012).
- 697 37. Waxman, S. G. & Ritchie, J. M. Molecular dissection of the myelinated axon. *Ann. Neurol.* **33**,  
698 121–136 (1993).

- 699 38. Catterall, W. A. From ionic currents to molecular mechanisms: the structure and function of  
700 voltage-gated sodium channels. *Neuron* **26**, 13–25 (2000).
- 701 39. Patton, D. E., Isom, L. L., Catterall, W. A. & Goldin, A. L. The adult rat brain beta 1 subunit  
702 modifies activation and inactivation gating of multiple sodium channel alpha subunits. *J. Biol. Chem.*  
703 **269**, 17649–17655 (1994).
- 704 40. Isom, L. L. *et al.* Structure and function of the beta 2 subunit of brain sodium channels, a  
705 transmembrane glycoprotein with a CAM motif. *Cell* **83**, 433–442 (1995).
- 706 41. Chen, C. *et al.* Reduced sodium channel density, altered voltage dependence of inactivation, and  
707 increased susceptibility to seizures in mice lacking sodium channel  $\beta$ 2-subunits. *Proc. Natl. Acad. Sci.*  
708 **99**, 17072 (2002).
- 709 42. Namadurai, S. *et al.* A new look at sodium channel  $\beta$  subunits. *Open Biol.* **5**, 140192–140192  
710 (2015).
- 711 43. Lopez-Santiago, L. F. *et al.* Sodium channel beta2 subunits regulate tetrodotoxin-sensitive  
712 sodium channels in small dorsal root ganglion neurons and modulate the response to pain. *J. Neurosci.*  
713 *Off. J. Soc. Neurosci.* **26**, 7984–7994 (2006).
- 714 44. Thimmapaya, R. *et al.* Distribution and functional characterization of human Nav1.3 splice  
715 variants. *Eur. J. Neurosci.* **22**, 1–9 (2005).
- 716 45. Duflocq, A., Le Bras, B., Bullier, E., Couraud, F. & Davenne, M. Nav1.1 is predominantly  
717 expressed in nodes of Ranvier and axon initial segments. *Mol. Cell. Neurosci.* **39**, 180–192 (2008).
- 718 46. Van Wart, A. & Matthews, G. Impaired firing and cell-specific compensation in neurons lacking  
719 nav1.6 sodium channels. *J. Neurosci. Off. J. Soc. Neurosci.* **26**, 7172–7180 (2006).
- 720 47. Ogiwara, I. *et al.* Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a  
721 circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J. Neurosci. Off. J. Soc.*  
722 *Neurosci.* **27**, 5903–5914 (2007).
- 723 48. Lorincz, A. & Nusser, Z. Cell-type-dependent molecular composition of the axon initial  
724 segment. *J. Neurosci. Off. J. Soc. Neurosci.* **28**, 14329–14340 (2008).
- 725 49. Tian, C., Wang, K., Ke, W., Guo, H. & Shu, Y. Molecular identity of axonal sodium channels  
726 in human cortical pyramidal cells. *Front. Cell. Neurosci.* **8**, 297–297 (2014).
- 727 50. Van Wart, A., Trimmer, J. S. & Matthews, G. Polarized distribution of ion channels within  
728 microdomains of the axon initial segment. *J. Comp. Neurol.* **500**, 339–352 (2007).
- 729 51. Schaller, K. L. & Caldwell, J. H. Expression and distribution of voltage-gated sodium channels  
730 in the cerebellum. *Cerebellum Lond. Engl.* **2**, 2–9 (2003).
- 731 52. Caldwell, J. H., Schaller, K. L., Lasher, R. S., Peles, E. & Levinson, S. R. Sodium channel  
732 Na(v)1.6 is localized at nodes of ranvier, dendrites, and synapses. *Proc. Natl. Acad. Sci. U. S. A.* **97**,  
733 5616–5620 (2000).
- 734 53. Tzoumaka, E. *et al.* Differential distribution of the tetrodotoxin-sensitive rPN4/NaCh6/Scn8a  
735 sodium channel in the nervous system. *J. Neurosci. Res.* **60**, 37–44 (2000).
- 736 54. Schafer, D. P., Custer, A. W., Shrager, P. & Rasband, M. N. Early events in node of Ranvier  
737 formation during myelination and remyelination in the PNS. *Neuron Glia Biol.* **2**, 69–79 (2006).
- 738 55. Boiko, T. *et al.* Compact myelin dictates the differential targeting of two sodium channel  
739 isoforms in the same axon. *Neuron* **30**, 91–104 (2001).



- 740 56. Zhou, W. & Goldin, A. L. Use-dependent potentiation of the Nav1.6 sodium channel. *Biophys.*  
741 *J.* **87**, 3862–3872 (2004).
- 742 57. Herzog, R. I., Liu, C., Waxman, S. G. & Cummins, T. R. Calmodulin binds to the C terminus  
743 of sodium channels Nav1.4 and Nav1.6 and differentially modulates their functional properties. *J.*  
744 *Neurosci. Off. J. Soc. Neurosci.* **23**, 8261–8270 (2003).
- 745 58. Arroyo, E. J. *et al.* Genetic dysmyelination alters the molecular architecture of the nodal region.  
746 *J. Neurosci. Off. J. Soc. Neurosci.* **22**, 1726–1737 (2002).
- 747 59. Kazen-Gillespie, K. A. *et al.* Cloning, localization, and functional expression of sodium channel  
748 beta1A subunits. *J. Biol. Chem.* **275**, 1079–1088 (2000).
- 749 60. Buffington, S. A. & Rasband, M. N. Na<sup>+</sup> channel-dependent recruitment of Navβ4 to axon  
750 initial segments and nodes of Ranvier. *J. Neurosci. Off. J. Soc. Neurosci.* **33**, 6191–6202 (2013).
- 751 61. Chen, C. *et al.* Mice lacking sodium channel beta1 subunits display defects in neuronal  
752 excitability, sodium channel expression, and nodal architecture. *J. Neurosci. Off. J. Soc. Neurosci.* **24**,  
753 4030–4042 (2004).
- 754 62. Ratcliffe, C. F., Westenbroek, R. E., Curtis, R. & Catterall, W. A. Sodium channel beta1 and  
755 beta3 subunits associate with neurofascin through their extracellular immunoglobulin-like domain. *J.*  
756 *Cell Biol.* **154**, 427–434 (2001).
- 757 63. Kaplan, M. R. *et al.* Differential control of clustering of the sodium channels Na(v)1.2 and  
758 Na(v)1.6 at developing CNS nodes of Ranvier. *Neuron* **30**, 105–119 (2001).
- 759 64. Srinivasan, J., Schachner, M. & Catterall, W. A. Interaction of voltage-gated sodium channels  
760 with the extracellular matrix molecules tenascin-C and tenascin-R. *Proc. Natl. Acad. Sci.* **95**, 15753  
761 (1998).
- 762 65. Patino, G. A. & Isom, L. L. Electrophysiology and beyond: multiple roles of Na<sup>+</sup> channel β  
763 subunits in development and disease. *Neurosci. Lett.* **486**, 53–59 (2010).
- 764 66. Malhotra, J. D. *et al.* Structural requirements for interaction of sodium channel beta 1 subunits  
765 with ankyrin. *J. Biol. Chem.* **277**, 26681–26688 (2002).
- 766 67. Kim, D. Y. *et al.* BACE1 regulates voltage-gated sodium channels and neuronal activity. *Nat.*  
767 *Cell Biol.* **9**, 755–764 (2007).
- 768 68. Devaux, J. J., Kleopa, K. A., Cooper, E. C. & Scherer, S. S. KCNQ2 is a nodal K<sup>+</sup> channel. *J.*  
769 *Neurosci. Off. J. Soc. Neurosci.* **24**, 1236–1244 (2004).
- 770 69. Pan, Z. *et al.* A common ankyrin-G-based mechanism retains KCNQ and NaV channels at  
771 electrically active domains of the axon. *J. Neurosci. Off. J. Soc. Neurosci.* **26**, 2599–2613 (2006).
- 772 70. Schwarz, J. R. *et al.* KCNQ channels mediate IKs, a slow K<sup>+</sup> current regulating excitability in  
773 the rat node of Ranvier. *J. Physiol.* **573**, 17–34 (2006).
- 774 71. Battefeld, A., Tran, B. T., Gavrilis, J., Cooper, E. C. & Kole, M. H. P. Heteromeric Kv7.2/7.3  
775 channels differentially regulate action potential initiation and conduction in neocortical myelinated  
776 axons. *J. Neurosci. Off. J. Soc. Neurosci.* **34**, 3719–3732 (2014).
- 777 72. Devaux, J. *et al.* Kv3.1b is a novel component of CNS nodes. *J. Neurosci. Off. J. Soc. Neurosci.*  
778 **23**, 4509–4518 (2003).
- 779 73. Lien, C.-C. & Jonas, P. Kv3 potassium conductance is necessary and kinetically optimized for  
780 high-frequency action potential generation in hippocampal interneurons. *J. Neurosci. Off. J. Soc.*

- 781 *Neurosci.* **23**, 2058–2068 (2003).
- 782 74. Davis, J. Q., Lambert, S. & Bennett, V. Molecular composition of the node of Ranvier:  
783 identification of ankyrin-binding cell adhesion molecules neurofascin (mucin+/third FNIII domain-) and  
784 NrCAM at nodal axon segments. *J. Cell Biol.* **135**, 1355–1367 (1996).
- 785 75. Zhang, A. *et al.* Neurofascin 140 is an embryonic neuronal neurofascin isoform that promotes  
786 the assembly of the node of Ranvier. *J. Neurosci. Off. J. Soc. Neurosci.* **35**, 2246–2254 (2015).
- 787 76. Grumet, M., Mauro, V., Burgoon, M. P., Edelman, G. M. & Cunningham, B. A. Structure of a  
788 new nervous system glycoprotein, Nr-CAM, and its relationship to subgroups of neural cell adhesion  
789 molecules. *J. Cell Biol.* **113**, 1399–1412 (1991).
- 790 77. Custer, A. W. *et al.* The role of the ankyrin-binding protein NrCAM in node of Ranvier  
791 formation. *J. Neurosci. Off. J. Soc. Neurosci.* **23**, 10032–10039 (2003).
- 792 78. Feinberg, K. *et al.* A glial signal consisting of gliomedin and NrCAM clusters axonal Na<sup>+</sup>  
793 channels during the formation of nodes of Ranvier. *Neuron* **65**, 490–502 (2010).
- 794 79. Rios, J. C. *et al.* Contactin-associated protein (Caspr) and contactin form a complex that is  
795 targeted to the paranodal junctions during myelination. *J. Neurosci. Off. J. Soc. Neurosci.* **20**, 8354–  
796 8364 (2000).
- 797 80. Lustig, M. *et al.* Nr-CAM and neurofascin interactions regulate ankyrin G and sodium channel  
798 clustering at the node of Ranvier. *Curr. Biol. CB* **11**, 1864–1869 (2001).
- 799 81. Labasque, M. & Faivre-Sarrailh, C. GPI-anchored proteins at the node of Ranvier. *FEBS Lett.*  
800 **584**, 1787–1792 (2010).
- 801 82. Leterrier, C., Brachet, A., Dargent, B. & Vacher, H. Determinants of voltage-gated sodium  
802 channel clustering in neurons. *Semin. Cell Dev. Biol.* **22**, 171–177 (2011).
- 803 83. Berghs, S. *et al.* betaIV spectrin, a new spectrin localized at axon initial segments and nodes of  
804 ranvier in the central and peripheral nervous system. *J. Cell Biol.* **151**, 985–1002 (2000).
- 805 84. Jenkins, S. M. & Bennett, V. Developing nodes of Ranvier are defined by ankyrin-G clustering  
806 and are independent of paranodal axoglial adhesion. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 2303–2308  
807 (2002).
- 808 85. Garrido, J. J. *et al.* A targeting motif involved in sodium channel clustering at the axonal initial  
809 segment. *Science* **300**, 2091–2094 (2003).
- 810 86. Gasser, A. *et al.* An ankyrinG-binding motif is necessary and sufficient for targeting Nav1.6  
811 sodium channels to axon initial segments and nodes of Ranvier. *J. Neurosci. Off. J. Soc. Neurosci.* **32**,  
812 7232–7243 (2012).
- 813 87. Bekku, Y. & Oohashi, T. Neurocan contributes to the molecular heterogeneity of the perinodal  
814 ECM. *Arch. Histol. Cytol.* **73**, 95–102 (2010).
- 815 88. Bekku, Y. *et al.* Bral1: its role in diffusion barrier formation and conduction velocity in the  
816 CNS. *J. Neurosci. Off. J. Soc. Neurosci.* **30**, 3113–3123 (2010).
- 817 89. Bekku, Y., Rauch, U., Ninomiya, Y. & Oohashi, T. Brevican distinctively assembles  
818 extracellular components at the large diameter nodes of Ranvier in the CNS. *J. Neurochem.* **108**, 1266–  
819 1276 (2009).
- 820 90. Dours-Zimmermann, M. T. *et al.* Versican V2 assembles the extracellular matrix surrounding  
821 the nodes of ranvier in the CNS. *J. Neurosci. Off. J. Soc. Neurosci.* **29**, 7731–7742 (2009).

- 822 91. Hedstrom, K. L. *et al.* Neurofascin assembles a specialized extracellular matrix at the axon  
823 initial segment. *J. Cell Biol.* **178**, 875–886 (2007).
- 824 92. Susuki, K. *et al.* Three mechanisms assemble central nervous system nodes of Ranvier. *Neuron*  
825 **78**, 469–482 (2013).
- 826 93. Zimmermann, D. R. & Dours-Zimmermann, M. T. Extracellular matrix of the central nervous  
827 system: from neglect to challenge. *Histochem. Cell Biol.* **130**, 635–653 (2008).
- 828 94. Melendez-Vasquez, C. *et al.* Differential expression of proteoglycans at central and peripheral  
829 nodes of Ranvier. *Glia* **52**, 301–308 (2005).
- 830 95. Weber, P. *et al.* Mice deficient for tenascin-R display alterations of the extracellular matrix and  
831 decreased axonal conduction velocities in the CNS. *J. Neurosci. Off. J. Soc. Neurosci.* **19**, 4245–4262  
832 (1999).
- 833 96. Fawcett, J. W., Oohashi, T. & Pizzorusso, T. The roles of perineuronal nets and the perinodal  
834 extracellular matrix in neuronal function. *Nat. Rev. Neurosci.* **20**, 451–465 (2019).
- 835 97. Susuki, K. & Rasband, M. N. Molecular mechanisms of node of Ranvier formation. *Curr. Opin.*  
836 *Cell Biol.* **20**, 616–623 (2008).
- 837 98. Charles, P. *et al.* Neurofascin is a glial receptor for the paranodin/Caspr-contactin axonal  
838 complex at the axoglial junction. *Curr. Biol. CB* **12**, 217–220 (2002).
- 839 99. Girault, J.-A. & Peles, E. Development of nodes of Ranvier. *Curr. Opin. Neurobiol.* **12**, 476–  
840 485 (2002).
- 841 100. Bhat, M. A. *et al.* Axon-glia interactions and the domain organization of myelinated axons  
842 requires neurexin IV/Caspr/Paranodin. *Neuron* **30**, 369–383 (2001).
- 843 101. Boyle, M. E. *et al.* Contactin orchestrates assembly of the septate-like junctions at the paranode  
844 in myelinated peripheral nerve. *Neuron* **30**, 385–397 (2001).
- 845 102. Collinson, J. M., Marshall, D., Gillespie, C. S. & Brophy, P. J. Transient expression of  
846 neurofascin by oligodendrocytes at the onset of myelinogenesis: implications for mechanisms of axon-  
847 glial interaction. *Glia* **23**, 11–23 (1998).
- 848 103. Einheber, S. *et al.* The axonal membrane protein Caspr, a homologue of neurexin IV, is a  
849 component of the septate-like paranodal junctions that assemble during myelination. *J. Cell Biol.* **139**,  
850 1495–1506 (1997).
- 851 104. Menegoz, M. *et al.* Paranodin, a glycoprotein of neuronal paranodal membranes. *Neuron* **19**,  
852 319–331 (1997).
- 853 105. Sherman, D. L. *et al.* Neurofascins are required to establish axonal domains for saltatory  
854 conduction. *Neuron* **48**, 737–742 (2005).
- 855 106. Tait, S. *et al.* An oligodendrocyte cell adhesion molecule at the site of assembly of the paranodal  
856 axo-glial junction. *J. Cell Biol.* **150**, 657–666 (2000).
- 857 107. Ogawa, Y. *et al.* Spectrins and ankyrinB constitute a specialized paranodal cytoskeleton. *J.*  
858 *Neurosci. Off. J. Soc. Neurosci.* **26**, 5230–5239 (2006).
- 859 108. Chang, K.-J. *et al.* Glial ankyrins facilitate paranodal axoglial junction assembly. *Nat. Neurosci.*  
860 **17**, 1673–1681 (2014).
- 861 109. Rasband, M. N. Clustered K<sup>+</sup> channel complexes in axons. *Neurosci. Lett.* **486**, 101–106 (2010).

- 862 110. Wang, H., Kunkel, D. D., Martin, T. M., Schwartzkroin, P. A. & Tempel, B. L.  
863 Heteromultimeric K<sup>+</sup> channels in terminal and juxtaparanodal regions of neurons. *Nature* **365**, 75–79  
864 (1993).
- 865 111. Vabnick, I. & Shrager, P. Ion channel redistribution and function during development of the  
866 myelinated axon. *J. Neurobiol.* **37**, 80–96 (1998).
- 867 112. Traka, M. *et al.* Association of TAG-1 with Caspr2 is essential for the molecular organization  
868 of juxtaparanodal regions of myelinated fibers. *J. Cell Biol.* **162**, 1161–1172 (2003).
- 869 113. Eshed-Eisenbach, Y. & Peles, E. The clustering of voltage-gated sodium channels in various  
870 excitable membranes. *Dev. Neurobiol.* 10.1002/dneu.22728 (2019) doi:10.1002/dneu.22728.
- 871 114. Ghosh, A., Sherman, D. L. & Brophy, P. J. The Axonal Cytoskeleton and the Assembly of  
872 Nodes of Ranvier. *Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry* **24**, 104–110 (2018).
- 873 115. Rasband, M. N. *et al.* Dependence of nodal sodium channel clustering on paranodal axoglial  
874 contact in the developing CNS. *J. Neurosci. Off. J. Soc. Neurosci.* **19**, 7516–7528 (1999).
- 875 116. Eshed-Eisenbach, Y. & Peles, E. The making of a node: a co-production of neurons and glia.  
876 *Curr. Opin. Neurobiol.* **23**, 1049–1056 (2013).
- 877 117. Horresh, I., Bar, V., Kissil, J. L. & Peles, E. Organization of myelinated axons by Caspr and  
878 Caspr2 requires the cytoskeletal adapter protein 4.1B. *J. Neurosci. Off. J. Soc. Neurosci.* **30**, 2480–2489  
879 (2010).
- 880 118. Zhang, C., Susuki, K., Zollinger, D. R., Dupree, J. L. & Rasband, M. N. Membrane domain  
881 organization of myelinated axons requires  $\beta$ II spectrin. *J. Cell Biol.* **203**, 437–443 (2013).
- 882 119. Brivio, V., Faivre-Sarrailh, C., Peles, E., Sherman, D. L. & Brophy, P. J. Assembly of CNS  
883 Nodes of Ranvier in Myelinated Nerves Is Promoted by the Axon Cytoskeleton. *Curr. Biol. CB* **27**,  
884 1068–1073 (2017).
- 885 120. Zonta, B. *et al.* Glial and neuronal isoforms of Neurofascin have distinct roles in the assembly  
886 of nodes of Ranvier in the central nervous system. *J. Cell Biol.* **181**, 1169–1177 (2008).
- 887 121. Pillai, A. M. *et al.* Spatiotemporal ablation of myelinating glia-specific neurofascin (Nfasc  
888 NF155) in mice reveals gradual loss of paranodal axoglial junctions and concomitant disorganization of  
889 axonal domains. *J. Neurosci. Res.* **87**, 1773–1793 (2009).
- 890 122. Rios, J. C. *et al.* Paranodal interactions regulate expression of sodium channel subtypes and  
891 provide a diffusion barrier for the node of Ranvier. *J. Neurosci. Off. J. Soc. Neurosci.* **23**, 7001–7011  
892 (2003).
- 893 123. Kaplan, M. R. *et al.* Induction of sodium channel clustering by oligodendrocytes. *Nature* **386**,  
894 724–728 (1997).
- 895 124. Freeman, S. A. *et al.* Acceleration of conduction velocity linked to clustering of nodal  
896 components precedes myelination. *Proc. Natl. Acad. Sci. U. S. A.* **112**, E321–328 (2015).
- 897 125. Dubessy, A.-L. *et al.* Role of a Contactin multi-molecular complex secreted by oligodendrocytes  
898 in nodal protein clustering in the CNS. *Glia* **67**, 2248–2263 (2019).
- 899 126. Thetiot, M. *et al.* An alternative mechanism of early nodal clustering and myelination onset in  
900 GABAergic neurons of the central nervous system. *Glia* 10.1002/glia.23812 (2020)  
901 doi:10.1002/glia.23812.
- 902 127. Yang, Y., Ogawa, Y., Hedstrom, K. L. & Rasband, M. N. betaIV spectrin is recruited to axon

- 903 initial segments and nodes of Ranvier by ankyrinG. *J. Cell Biol.* **176**, 509–519 (2007).
- 904 128. Hill, A. S. *et al.* Ion channel clustering at the axon initial segment and node of Ranvier evolved  
905 sequentially in early chordates. *PLoS Genet.* **4**, e1000317–e1000317 (2008).
- 906 129. Yang, Y., Lacas-Gervais, S., Morest, D. K., Solimena, M. & Rasband, M. N. BetaIV spectrins  
907 are essential for membrane stability and the molecular organization of nodes of Ranvier. *J. Neurosci.*  
908 *Off. J. Soc. Neurosci.* **24**, 7230–7240 (2004).
- 909 130. Dzhashiashvili, Y. *et al.* Nodes of Ranvier and axon initial segments are ankyrin G-dependent  
910 domains that assemble by distinct mechanisms. *J. Cell Biol.* **177**, 857–870 (2007).
- 911 131. Ho, T. S.-Y. *et al.* A hierarchy of ankyrin-spectrin complexes clusters sodium channels at nodes  
912 of Ranvier. *Nat. Neurosci.* **17**, 1664–1672 (2014).
- 913 132. Chang, K.-J. & Rasband, M. N. Excitable domains of myelinated nerves: axon initial segments  
914 and nodes of Ranvier. *Curr. Top. Membr.* **72**, 159–192 (2013).
- 915 133. Desmazieres, A. *et al.* Differential stability of PNS and CNS nodal complexes when neuronal  
916 neurofascin is lost. *J. Neurosci. Off. J. Soc. Neurosci.* **34**, 5083–5088 (2014).
- 917 134. Taylor, A. M., Saifetiarova, J. & Bhat, M. A. Postnatal Loss of Neuronal and Glial Neurofascins  
918 Differentially Affects Node of Ranvier Maintenance and Myelinated Axon Function. *Front. Cell.*  
919 *Neurosci.* **11**, 11–11 (2017).
- 920 135. Waxman, S. G. & Foster, R. E. Development of the axon membrane during differentiation of  
921 myelinated fibres in spinal nerve roots. *Proc. R. Soc. Lond. B Biol. Sci.* **209**, 441–446 (1980).
- 922 136. Waxman, S. G., Black, J. A. & Foster, R. E. Freeze-fracture heterogeneity of the axolemma of  
923 premyelinated fibers in the CNS. *Neurology* **32**, 418–421 (1982).
- 924 137. Bonetto, G. *et al.* Selective Axonal Expression of the Kv1 Channel Complex in Pre-myelinated  
925 GABAergic Hippocampal Neurons. *Front. Cell. Neurosci.* **13**, 222 (2019).
- 926 138. Çolakoğlu, G., Bergstrom-Tyrberg, U., Berglund, E. O. & Ranscht, B. Contactin-1 regulates  
927 myelination and nodal/paranodal domain organization in the central nervous system. *Proc. Natl. Acad.*  
928 *Sci. U. S. A.* **111**, E394–E403 (2014).
- 929 139. Brümmendorf, T. *et al.* The axonal recognition molecule F11 is a multifunctional protein:  
930 specific domains mediate interactions with Ng-CAM and restrictin. *Neuron* **10**, 711–727 (1993).
- 931 140. Peles, E. *et al.* The carbonic anhydrase domain of receptor tyrosine phosphatase beta is a  
932 functional ligand for the axonal cell recognition molecule contactin. *Cell* **82**, 251–260 (1995).
- 933 141. Rathjen, F. G., Wolff, J. M. & Chiquet-Ehrismann, R. Restrictin: a chick neural extracellular  
934 matrix protein involved in cell attachment co-purifies with the cell recognition molecule F11. *Dev.*  
935 *Camb. Engl.* **113**, 151–164 (1991).
- 936 142. Zacharias, U., Nörenberg, U. & Rathjen, F. G. Functional interactions of the immunoglobulin  
937 superfamily member F11 are differentially regulated by the extracellular matrix proteins tenascin-R and  
938 tenascin-C. *J. Biol. Chem.* **274**, 24357–24365 (1999).
- 939 143. Xiao, Z. C. *et al.* Tenascin-R is a functional modulator of sodium channel beta subunits. *J. Biol.*  
940 *Chem.* **274**, 26511–26517 (1999).
- 941 144. Black, J. A., Foster, R. E. & Waxman, S. G. Rat optic nerve: freeze-fracture studies during  
942 development of myelinated axons. *Brain Res.* **250**, 1–20 (1982).

- 943 145. Jinno, S. *et al.* Neuronal diversity in GABAergic long-range projections from the hippocampus.  
944 *J. Neurosci. Off. J. Soc. Neurosci.* **27**, 8790–8804 (2007).
- 945 146. Bonifazi, P. *et al.* GABAergic hub neurons orchestrate synchrony in developing hippocampal  
946 networks. *Science* **326**, 1419–1424 (2009).
- 947 147. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its  
948 application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544 (1952).
- 949 148. Huxley, A. F. & Stämpfli, R. Evidence for saltatory conduction in peripheral myelinated nerve  
950 fibres. *J. Physiol.* **108**, 315–339 (1949).
- 951 149. Tasaki, I. New measurements of the capacity and the resistance of the myelin sheath and the  
952 nodal membrane of the isolated frog nerve fiber. *Am. J. Physiol.* **181**, 639–650 (1955).
- 953 150. Cohen, C. C. H. *et al.* Saltatory Conduction along Myelinated Axons Involves a Periaxonal  
954 Nanocircuit. *Cell* **180**, 311–322.e15 (2020).
- 955 151. Rushton, W. A. H. A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**,  
956 101–122 (1951).
- 957 152. Waxman, S. G. & Bennett, M. V. Relative conduction velocities of small myelinated and non-  
958 myelinated fibres in the central nervous system. *Nature. New Biol.* **238**, 217–219 (1972).
- 959 153. Debanne, D., Campanac, E., Bialowas, A., Carlier, E. & Alcaraz, G. Axon physiology. *Physiol.*  
960 *Rev.* **91**, 555–602 (2011).
- 961 154. Freeman, S. A., Desmazières, A., Fricker, D., Lubetzki, C. & Sol-Foulon, N. Mechanisms of  
962 sodium channel clustering and its influence on axonal impulse conduction. *Cell. Mol. Life Sci. CMLS*  
963 **73**, 723–735 (2016).
- 964 155. Snaidero, N. & Simons, M. The logistics of myelin biogenesis in the central nervous system.  
965 *Glia* **65**, 1021–1031 (2017).
- 966 156. Monje, M. Myelin Plasticity and Nervous System Function. *Annu. Rev. Neurosci.* **41**, 61–76  
967 (2018).
- 968 157. Waxman, S. G., Black, J. A., Kocsis, J. D. & Ritchie, J. M. Low density of sodium channels  
969 supports action potential conduction in axons of neonatal rat optic nerve. *Proc. Natl. Acad. Sci. U. S. A.*  
970 **86**, 1406–1410 (1989).
- 971 158. Johnston, W. L., Dyer, J. R., Castellucci, V. F. & Dunn, R. J. Clustered voltage-gated Na<sup>+</sup>  
972 channels in Aplysia axons. *J. Neurosci. Off. J. Soc. Neurosci.* **16**, 1730–1739 (1996).
- 973 159. Zeng, S. & Jung, P. Simulation analysis of intermodal sodium channel function. *Phys. Rev. E*  
974 *Stat. Nonlin. Soft Matter Phys.* **78**, 061916–061916 (2008).
- 975 160. Neishabouri, A. & Faisal, A. A. Saltatory conduction in unmyelinated axons: clustering of Na<sup>+</sup>  
976 channels on lipid rafts enables micro-saltatory conduction in C-fibers. *Front. Neuroanat.* **8**, 109–109  
977 (2014).
- 978 161. Rama, S., Zbili, M. & Debanne, D. Signal propagation along the axon. *Curr. Opin. Neurobiol.*  
979 **51**, 37–44 (2018).
- 980 162. Brohawn, S. G., Su, Z. & MacKinnon, R. Mechanosensitivity is mediated directly by the lipid  
981 membrane in TRAAK and TREK1 K<sup>+</sup> channels. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 3614–3619 (2014).
- 982 163. Renigunta, V., Schlichthörl, G. & Daut, J. Much more than a leak: structure and function of

- 983 K<sub>2</sub>p-channels. *Pflugers Arch.* **467**, 867–894 (2015).
- 984 164. Holland, L., de Regt, H. W. & Drukarch, B. Thinking About the Nerve Impulse: The Prospects  
985 for the Development of a Comprehensive Account of Nerve Impulse Propagation. *Front. Cell. Neurosci.*  
986 **13**, 208–208 (2019).
- 987 165. Gründemann, J. & Clark, B. A. Calcium-Activated Potassium Channels at Nodes of Ranvier  
988 Secure Axonal Spike Propagation. *Cell Rep.* **12**, 1715–1722 (2015).
- 989 166. Craner, M. J., Lo, A. C., Black, J. A. & Waxman, S. G. Abnormal sodium channel distribution  
990 in optic nerve axons in a model of inflammatory demyelination. *Brain J. Neurol.* **126**, 1552–1561  
991 (2003).
- 992 167. Craner, M. J., Hains, B. C., Lo, A. C., Black, J. A. & Waxman, S. G. Co-localization of sodium  
993 channel Nav1.6 and the sodium-calcium exchanger at sites of axonal injury in the spinal cord in EAE.  
994 *Brain J. Neurol.* **127**, 294–303 (2004).
- 995 168. Dupree, J. L. *et al.* Oligodendrocytes assist in the maintenance of sodium channel clusters  
996 independent of the myelin sheath. *Neuron Glia Biol.* **1**, 179–192 (2004).
- 997 169. Pfeiffer, F., Frommer-Kaestle, G. & Fallier-Becker, P. Structural adaption of axons during de-  
998 and remyelination in the Cuprizone mouse model. *Brain Pathol. Zurich Switz.* **29**, 675–692 (2019).
- 999 170. Mathis, C., Denisenko-Nehrbass, N., Girault, J. A. & Borrelli, E. Essential role of  
1000 oligodendrocytes in the formation and maintenance of central nervous system nodal regions. *Dev. Camb.*  
1001 *Engl.* **128**, 4881–4890 (2001).
- 1002 171. Rasband, M. N., Kagawa, T., Park, E. W., Ikenaka, K. & Trimmer, J. S. Dysregulation of axonal  
1003 sodium channel isoforms after adult-onset chronic demyelination. *J. Neurosci. Res.* **73**, 465–470 (2003).
- 1004 172. Craner, M. J. *et al.* Molecular changes in neurons in multiple sclerosis: altered axonal expression  
1005 of Nav1.2 and Nav1.6 sodium channels and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. *Proc. Natl. Acad. Sci. U. S. A.* **101**,  
1006 8168–8173 (2004).
- 1007 173. Black, J. A. *et al.* Sensory neuron-specific sodium channel SNS is abnormally expressed in the  
1008 brains of mice with experimental allergic encephalomyelitis and humans with multiple sclerosis. *Proc.*  
1009 *Natl. Acad. Sci. U. S. A.* **97**, 11598–11602 (2000).
- 1010 174. Roostaei, T. *et al.* Channelopathy-related SCN10A gene variants predict cerebellar dysfunction  
1011 in multiple sclerosis. *Neurology* **86**, 410–417 (2016).
- 1012 175. Jukkola, P. I., Lovett-Racke, A. E., Zamvil, S. S. & Gu, C. K<sup>+</sup> channel alterations in the  
1013 progression of experimental autoimmune encephalomyelitis. *Neurobiol. Dis.* **47**, 280–293 (2012).
- 1014 176. Howell, O. W. *et al.* Activated microglia mediate axoglial disruption that contributes to axonal  
1015 injury in multiple sclerosis. *J. Neuropathol. Exp. Neurol.* **69**, 1017–1033 (2010).
- 1016 177. Bagchi, B. *et al.* Disruption of myelin leads to ectopic expression of K(V)1.1 channels with  
1017 abnormal conductivity of optic nerve axons in a cuprizone-induced model of demyelination. *PLoS One*  
1018 **9**, e87736 (2014).
- 1019 178. Hamada, M. S. & Kole, M. H. P. Myelin loss and axonal ion channel adaptations associated  
1020 with gray matter neuronal hyperexcitability. *J. Neurosci. Off. J. Soc. Neurosci.* **35**, 7272–7286 (2015).
- 1021 179. Coman, I. *et al.* Nodal, paranodal and juxtaparanodal axonal proteins during demyelination and  
1022 remyelination in multiple sclerosis. *Brain J. Neurol.* **129**, 3186–3195 (2006).
- 1023 180. Han, C., Huang, J. & Waxman, S. G. Sodium channel Nav1.8: Emerging links to human disease.

- 1024 *Neurology* **86**, 473–483 (2016).
- 1025 181. Zoupi, L., Markoullis, K., Kleopa, K. A. & Karageorgos, D. Alterations of juxtaparanodal  
1026 domains in two rodent models of CNS demyelination. *Glia* **61**, 1236–1249 (2013).
- 1027 182. Fu, Y. *et al.* Paranodal myelin retraction in relapsing experimental autoimmune  
1028 encephalomyelitis visualized by coherent anti-Stokes Raman scattering microscopy. *J. Biomed. Opt.* **16**,  
1029 106006 (2011).
- 1030 183. Stojic, A., Bojceviski, J., Williams, S. K., Diem, R. & Fairless, R. Early Nodal and Paranodal  
1031 Disruption in Autoimmune Optic Neuritis. *J. Neuropathol. Exp. Neurol.* **77**, 361–373 (2018).
- 1032 184. Crawford, D. K., Mangiardi, M., Xia, X., López-Valdés, H. E. & Tiwari-Woodruff, S. K.  
1033 Functional recovery of callosal axons following demyelination: a critical window. *Neuroscience* **164**,  
1034 1407–1421 (2009).
- 1035 185. Wolswijk, G. & Balesar, R. Changes in the expression and localization of the paranodal protein  
1036 Caspr on axons in chronic multiple sclerosis. *Brain J. Neurol.* **126**, 1638–1649 (2003).
- 1037 186. McDonald, W. I. & Sears, T. A. Focal experimental demyelination in the central nervous  
1038 system. *Brain J. Neurol.* **93**, 575–582 (1970).
- 1039 187. Bostock, H. & Sears, T. A. The internodal axon membrane: electrical excitability and continuous  
1040 conduction in segmental demyelination. *J. Physiol.* **280**, 273–301 (1978).
- 1041 188. Felts, P. A., Baker, T. A. & Smith, K. J. Conduction in segmentally demyelinated mammalian  
1042 central axons. *J. Neurosci. Off. J. Soc. Neurosci.* **17**, 7267–7277 (1997).
- 1043 189. Shrager, P. Axonal coding of action potentials in demyelinated nerve fibers. *Brain Res.* **619**,  
1044 278–290 (1993).
- 1045 190. Hamada, M. S., Popovic, M. A. & Kole, M. H. P. Loss of Saltation and Presynaptic Action  
1046 Potential Failure in Demyelinated Axons. *Front. Cell. Neurosci.* **11**, 45 (2017).
- 1047 191. Alrashdi, B. *et al.* Nav1.6 promotes inflammation and neuronal degeneration in a mouse model  
1048 of multiple sclerosis. *J. Neuroinflammation* **16**, 215 (2019).
- 1049 192. Schattling, B. *et al.* Activity of NaV1.2 promotes neurodegeneration in an animal model of  
1050 multiple sclerosis. *JCI Insight* **1**, e89810 (2016).
- 1051 193. Kapoor, R. *et al.* Lamotrigine for neuroprotection in secondary progressive multiple sclerosis:  
1052 a randomised, double-blind, placebo-controlled, parallel-group trial. *Lancet Neurol.* **9**, 681–688 (2010).
- 1053 194. Raftopoulos, R. *et al.* Phenytoin for neuroprotection in patients with acute optic neuritis: a  
1054 randomised, placebo-controlled, phase 2 trial. *Lancet Neurol.* **15**, 259–269 (2016).
- 1055 195. Brugarolas, P. *et al.* Development of a PET radioligand for potassium channels to image CNS  
1056 demyelination. *Sci. Rep.* **8**, 607 (2018).
- 1057 196. Nikic, I. *et al.* A reversible form of axon damage in experimental autoimmune encephalomyelitis  
1058 and multiple sclerosis. *Nat. Med.* **17**, 495–499 (2011).
- 1059 197. Witte, M. E. *et al.* Calcium Influx through Plasma-Membrane Nanoruptures Drives Axon  
1060 Degeneration in a Model of Multiple Sclerosis. *Neuron* **101**, 615–624.e5 (2019).
- 1061 198. Mathey, E. K. *et al.* Neurofascin as a novel target for autoantibody-mediated axonal injury. *J.*  
1062 *Exp. Med.* **204**, 2363–2372 (2007).
- 1063 199. Stich, O. *et al.* Prevalence of neurofascin-155 antibodies in patients with multiple sclerosis. *J.*



- 1064 *Neurol. Sci.* **364**, 29–32 (2016).
- 1065 200. Devaux, J. J., Odaka, M. & Yuki, N. Nodal proteins are target antigens in Guillain-Barré  
1066 syndrome. *J. Peripher. Nerv. Syst. JPNS* **17**, 62–71 (2012).
- 1067 201. Elliott, C. *et al.* Functional identification of pathogenic autoantibody responses in patients with  
1068 multiple sclerosis. *Brain J. Neurol.* **135**, 1819–1833 (2012).
- 1069 202. Kawamura, N. *et al.* Anti-neurofascin antibody in patients with combined central and peripheral  
1070 demyelination. *Neurology* **81**, 714–722 (2013).
- 1071 203. Derfuss, T. *et al.* Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis  
1072 patients and mediates gray matter pathology in animals. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 8302–8307  
1073 (2009).
- 1074 204. Ng, J. K. M. *et al.* Neurofascin as a target for autoantibodies in peripheral neuropathies.  
1075 *Neurology* **79**, 2241–2248 (2012).
- 1076 205. Querol, L. *et al.* Neurofascin IgG4 antibodies in CIDP associate with disabling tremor and poor  
1077 response to IVIg. *Neurology* **82**, 879–886 (2014).
- 1078 206. Cortese, A. *et al.* Antibodies to neurofascin, contactin-1, and contactin-associated protein 1 in  
1079 CIDP: Clinical relevance of IgG isotype. *Neurol. Neuroimmunol. Neuroinflammation* **7**, e639 (2019).
- 1080 207. Delmont, E. *et al.* Autoantibodies to nodal isoforms of neurofascin in chronic inflammatory  
1081 demyelinating polyneuropathy. *Brain J. Neurol.* **140**, 1851–1858 (2017).
- 1082 208. Vallat, J.-M. *et al.* Subacute nodopathy with conduction blocks and anti-neurofascin 140/186  
1083 antibodies: an ultrastructural study. *Brain J. Neurol.* **141**, e56–e56 (2018).
- 1084 209. Querol, L. *et al.* Antibodies to contactin-1 in chronic inflammatory demyelinating  
1085 polyneuropathy. *Ann. Neurol.* **73**, 370–380 (2013).
- 1086 210. Cortese, A. *et al.* Antibodies to neurofascin, contactin-1, and contactin-associated protein 1 in  
1087 CIDP: Clinical relevance of IgG isotype. *Neurol. Neuroimmunol. Neuroinflammation* **7**, (2020).
- 1088 211. Ogata, H. *et al.* Characterization of IgG4 anti-neurofascin 155 antibody-positive  
1089 polyneuropathy. *Ann. Clin. Transl. Neurol.* **2**, 960–971 (2015).
- 1090 212. Cortese, A. *et al.* Neurofascin-155 as a putative antigen in combined central and peripheral  
1091 demyelination. *Neurol. Neuroimmunol. Neuroinflammation* **3**, e238 (2016).
- 1092 213. Sasaki, M. *et al.* Molecular reconstruction of nodes of Ranvier after remyelination by  
1093 transplanted olfactory ensheathing cells in the demyelinated spinal cord. *J. Neurosci. Off. J. Soc.*  
1094 *Neurosci.* **26**, 1803–1812 (2006).
- 1095 214. Smith, K. J., Bostock, H. & Hall, S. M. Saltatory conduction precedes remyelination in axons  
1096 demyelinated with lysophosphatidyl choline. *J. Neurol. Sci.* **54**, 13–31 (1982).
- 1097 215. Auer, F., Vagionitis, S. & Czopka, T. Evidence for Myelin Sheath Remodeling in the CNS  
1098 Revealed by In Vivo Imaging. *Curr. Biol. CB* **28**, 549-559.e3 (2018).
- 1099 216. Reimer, M. M. *et al.* Rapid disruption of axon-glia integrity in response to mild cerebral  
1100 hypoperfusion. *J. Neurosci. Off. J. Soc. Neurosci.* **31**, 18185–18194 (2011).
- 1101 217. Ritter, J. *et al.* Neonatal hyperoxia exposure disrupts axon-oligodendrocyte integrity in the  
1102 subcortical white matter. *J. Neurosci. Off. J. Soc. Neurosci.* **33**, 8990–9002 (2013).
- 1103 218. Marin, M. A. *et al.* Reassembly of Excitable Domains after CNS Axon Regeneration. *J.*

- 1104 *Neurosci. Off. J. Soc. Neurosci.* **36**, 9148–9160 (2016).
- 1105 219. Marion, C. M., Radomski, K. L., Cramer, N. P., Galdzicki, Z. & Armstrong, R. C. Experimental  
1106 Traumatic Brain Injury Identifies Distinct Early and Late Phase Axonal Conduction Deficits of White  
1107 Matter Pathophysiology, and Reveals Intervening Recovery. *J. Neurosci. Off. J. Soc. Neurosci.* **38**,  
1108 8723–8736 (2018).
- 1109 220. Eijkelkamp, N. *et al.* Neurological perspectives on voltage-gated sodium channels. *Brain J.*  
1110 *Neurol.* **135**, 2585–2612 (2012).
- 1111 221. Cooper, E. C. Potassium Channels (including KCNQ) and Epilepsy. in *Jasper's Basic*  
1112 *Mechanisms of the Epilepsies* (eds. Noebels, J. L., Avoli, M., Rogawski, M. A., Olsen, R. W. & Delgado-  
1113 Escueta, A. V.) (National Center for Biotechnology Information (US), 2012).
- 1114 222. Bauer, C. K. *et al.* Mutations in KCNK4 that Affect Gating Cause a Recognizable  
1115 Neurodevelopmental Syndrome. *Am. J. Hum. Genet.* **103**, 621–630 (2018).
- 1116