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

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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.

Bullet points

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

Introduction

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

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

1) The discovery of nodes of Ranvier

Working at Collège de France in Paris, and studying isolated myelinated fibers, Ranvier showed that, contrary to the current view that myelin was a continuous and impermeable sheath, there were specific sites of myelin interruption, that he described as “annular constrictions” (Figure 1A), where the crystalline water soluble dye picrocarminate was able to reach the axonal membrane. Ranvier’s first hypothesis (which might make us smile now) was that these constrictions had a mechanical role: *“since myelin is essentially a liquid material.... if it was uninterrupted, in the human sciatic nerve for example, which is mostly in the upright position, then the myelin would flow, by gravity, to the more sloping portion of the nerve, ...but this is not the case; the myelin sheath is interrupted by lateral constrictions which retain it”*⁹. Ranvier also postulated that these constrictions, allowing the diffusion of soluble cues, could be important for nutrition of the nerve cell. He attributed to the myelin sheath a protective role against compression. Last but not least, he postulated that myelin, even if not needed for transmission of sensory and motor “impressions” -because such transmission also takes place in non myelinated fibers- would result, acting as an electrical insulation, in a “more perfect” transmission...⁹; a pioneering hypothesis! that would not be confirmed before several decades¹⁰.

2) Nodes of Ranvier: structural and molecular organization

This paragraph will provide an update on the current knowledge on nodes of Ranvier 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 ¹¹⁻¹³, 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 μm ¹⁴, it was recently
100 shown that it can vary (by several fold) between axons, including in an axon-diameter
101 independent manner ¹⁵. 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 ^{16,17}. The axonal diameter at the nodes is reduced in size compared to the internode ¹⁸,
104 suggesting reduced phosphorylation of neurofilaments and/or their higher packing at the nodes
105 ^{14,19}. Variations of nodal diameter between axons, but also along the same axon, was recently
106 identified ²⁰. 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 ²¹. In the CNS the
109 density of mitochondria at the node is similar or reduced compared to paranodal and internodal
110 areas ^{22,23}, 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 ²⁴, but also
113 the importance of lateral organization of proteins at nodal gaps ²⁵. 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 ²⁶⁻³². In addition,
119 we and others recently identified by light and electron microscopy that microglial processes
120 also contact the node ³³ (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 ³⁴.

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

existence of transverse bands, corresponding to intercellular periodic densities, between each paranodal loop and the axon^{35,36}. Neighboring the paranodes are the juxtaparanodes, located at the interface between the internode and the paranode, underneath the myelin sheath (Figure 3).

2-2) Molecular composition of the nodal area

The ability of nodes of Ranvier to regenerate and propagate AP along the axon relies on their specialized molecular composition associating ion channels, cell adhesion molecules (CAMs), cytoskeletal scaffold proteins and extra-cellular matrix net (Figure 3). This molecular organization is partly similar to the axon initial segment (AIS), located at the axon hillock and where the AP are generated³⁷

The nodes of Ranvier are highly enriched in Na_v channels, consisting of a 260kDa pore-forming α -subunit, enabling inward Na⁺ fluxes and depolarization phase of the AP, and one or more subtypes of 30-40 kDa accessory β -subunits, which modulates α -subunit membrane targeting and the opening threshold of the pore³⁸⁻⁴³. Five out of the ten existing α -subunits isoforms are found at the CNS nodes in physiological contexts (Nav1.1, 1.2, 1.3, 1.6 and 1.8). Nav1.3 is restricted to embryonic and developmental stages and mostly absent from adult CNS⁴⁴. Nav1.1 expression depends on the neuronal subtype and is detected in GABAergic neurons in the hippocampus and cortex⁴⁵⁻⁴⁹, as well as in retinal ganglion cells (RGCs) and Purkinje cells^{50,51}. Nav1.2 is the major isoform in immature nodes, with a later switch to Nav1.6⁵²⁻⁵⁵, which allows for higher discharges frequencies^{56,57}. In the adult CNS, a subset of nodes also express Nav1.2 and Nav1.8⁵⁸.

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

The voltage-gated potassium channels K_v7.2/3 (*KCNQ2/3*, belonging to the delayed rectifier K_v7 family) have also been described at nodes⁶⁸⁻⁷⁰. These potassium channels are thought to

156 stabilize the membrane resting potential, thus allowing to maximize AP amplitude ⁷¹. 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 ^{72,73}. 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⁺ 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 ^{5,6}.

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) ^{74,75}, NrCAM ^{76–78} and the GPI-anchored protein contactin ⁷⁹. Through
170 its IgG domain, Nfasc186 can directly interact with NrCAM, β 1Nav as well as contactin ^{62,80,81}.

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 β IVspectrin
173 ^{37,69,74,82–86}.

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 ^{87–95}. The CNS nodal ECM, which is similar
177 to the perineuronal nets surrounding soma and dendrites of various neuronal populations ⁹⁶ may
178 play a role in buffering the nodal environment, through the negative charges of chondroitin
179 sulfate chains ^{88,92,97}, 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 ⁹⁸. The absence of any of these
183 constituents leads to a loss of the axoglial junction ^{98–106}. 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 α II/ β II spectrin ^{107,108}.

In mammals, the main feature of juxtaparanodes is their high density in delayed rectifier voltage-gated potassium channels (K_v1.1, 1.2 and K_vb2 subunit), which may play a role in internodal resting potential support, in particular during development and injury^{109–111}. K_v channels associate with Caspr2, a transmembrane Caspr-related protein, and the GPI-anchored TAG1 (contactin-2), which is expressed on both the axonal and glial side. The neuronal TAG1 binds in *trans* homophilically to its glial counterpart and to Caspr2 in *cis*¹¹².

2-3) Mechanisms of nodal assembly and maintenance

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

The paranodal axo-glial junction

Several studies have shown that CNS nodes form after the paranodal junction, leading to the assumption that CNS node formation relies primarily on the flanking paranodal junctions^{92,115,116}. The axoglial junction formed by axonal Caspr and contactin interaction with the glial Nfasc155 is required to establish an effective paranodal membrane diffusion barrier, with an essential role of its anchoring to neuronal paranodal cytoskeleton, by interaction with 4.1B, α II spectrin and β II spectrin^{117,118}. This barrier restricts accumulation of nodal proteins and participates in nodal clusters localization^{107,118,119}.

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

The cytoskeletal scaffold proteins ankyrin and β IV spectrin

Cytoskeletal AnkG has been shown to be a key factor for nodal assembly. Most major nodal markers, including Nav, K_v7.2/3 channels, Nfasc 186, NrCAM, and β IV spectrin have AnkG

binding domains^{69,85,127,128}. It is established that AnkG, through binding to β IV spectrin provides a scaffold that recruits nodal proteins and anchors them to the cytoskeleton^{86,92,127,129,130}. Although compensatory expression of AnkR in the absence of AnkG can rescue nodal assembly in the CNS, Nav channels fail to cluster when both AnkG and AnkR are absent¹³¹.

The nodal ECM

In the CNS, nodes of Ranvier are surrounded by a nodal ECM. Some ECM proteins, such as NrCAM, BraL1, Brevican and Versican, can directly interact with the axonal CAMNfasc186³, which plays a major role in Nav channel clustering. Although ECM-CAMs interactions might play a role in localizing the nodal clusters, it is now hypothesized that it might be involved in long-term stabilization rather than the initial assembly of the CNS Nodes^{92,132–134}.

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

The aggregating oligodendroglial secreted cues

Early cytochemical and freeze fracture studies have provided evidence for the differentiation of the axon membrane prior to formation of compact myelin in spinal nerve roots¹³⁵. In rat developing optic nerve axons, clusters of intramembranous particles on the axolemma precede the formation of any compact myelin¹³⁶. In the developing rodent optic nerve, Nav clusters colocalize with AnkG and Nfasc186 and are transiently detected, either isolated or flanked on one side by paranodal Caspr (i.e., heminodal structure)^{92,115,125}. Immunostainings in retinal ganglion cell cultures have demonstrated the presence of such Nav channels clusters regularly spaced along axons, which are induced by protein(s) secreted by oligodendrocytes, in the absence of glial cell contact^{63,123}.

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

along GABAergic hippocampal neurons with prenodes¹³⁷. Formation of these early nodal protein clusters along unmyelinated GABAergic axons requires also intrinsic neuronal factors such as AnkG and $\beta 2\text{Nav}$ ^{124,126}. To identify the oligodendroglial cues inducing formation of prenodes, a mass spectrometry analysis of oligodendrocyte conditioned medium was performed, resulting in the identification of contactin-1 combined with RPTP/Phosphacan and Tenascin-R¹²⁵. Contactin expression is detected in neurons and oligodendroglial cells¹³⁸, and binds both to RPTP/Phosphacan and TenascinR^{139–142}, suggesting that the oligodendroglial aggregating activity might be related to the formation of a multi-molecular complex. Nfasc186 and NrCAM are not critical for contactin-1 induced prenodal clustering, but interactions with RPTP/phosphacan or Tenascin-R may bridge oligodendrocyte-derived contactin with other neuronal CAMs (contactin, $\beta 1$ or $\beta 2\text{Nav}$ subunits)^{64,143}. In addition to the optic nerve and the hippocampus, prenodes have also been detected in Purkinje cells in the mouse cerebellum (A. Desmazières, unpublished results), as well as in the *Xenopus* optic nerve (B. Zalc unpublished results) suggesting that it might be a more general mechanism for nodal clustering. What distinguishes axons that form prenodes from axons without prenodes is still unclear. Prenode formation might be related to different modalities of axonal differentiation. Alternatively, it might be developed to meet the need for early establishment of neuronal connections during development^{124,144–146}. That formation of prenodes is an intrinsic property of subpopulations of neurons is illustrated by the fact that in the same culture, oligodendroglial conditioned medium will induce prenodes on GABAergic, but not on pyramidal axons, a specificity that is reproduced *in vivo* on tissue sections of developing hippocampus¹²⁴.

What is the fate of these early nodal clusters has been recently addressed using *in vitro* live imaging methods, showing that prenodal clusters participate in heminode formation (and thus ultimately in mature nodes of Ranvier) when myelination proceeds, by fusion with existing heminodal structures when myelin progresses towards them, or by guiding myelin deposition initiation at their direct vicinity¹²⁶.

3) The Node of Ranvier as a regulator of nerve conduction

The nerve impulse propagation results from a transient influx of Na^+ , responsible for the rapid depolarization, and an efflux of K^+ which causes repolarization, as described by the Hodgkin and Huxley model¹⁴⁷ established from voltage-clamp measurements in the squid giant axon. Voltage-gated Na^+ channels are critical drivers for active conduction along the axon, and

their clustering at nodes of Ranvier in myelinated fibers allows regeneration of AP and saltatory conduction^{148,149}. Using computational modeling in combination with high-speed voltage-calibrated optical recordings across the nodal and internodal axolemma of myelinated neocortical pyramidal axons, as well as electron microscopy analysis, Cohen et al¹⁵⁰ recently provided evidence for a second longitudinal conducting pathway formed by the periaxonal and paranodal submyelin spaces, therefore a double cable model for axonal conduction. Morphological axonal parameters, notably axon diameter, internodal length and myelin thickness are critical parameters for conduction velocity^{151–154} (Figure 4), and might be regulated by neuronal electrical activity. This adaptive process contributes to strengthening and synchronization of circuits and improves performances during learning^{155,156}. In addition, nodes of Ranvier length and diameter are potential regulators of conduction speed as they determine the capacitance of the node and the speed with which nodal Nav channels activate^{15,20}. In this context, variability of node length even at constant axon diameter was recently shown between different axons in the optic nerve and in the grey matter of the cortex, contrasting with similar length along the same axon. Using simulation studies, such length modifications resulted in large variations of conduction speed (increased node length decreasing conduction speed and decreased node length increasing conduction speed). Taken together this suggests that axons may be able to adjust their node lengths in order to tune their conduction speed, to optimize information processing. (Figure 4B). It was recently shown that perinodal astrocytes, by altering myelin thickness and increasing nodal gap length might also impact conduction speed³¹. Concerning node of Ranvier diameter, a progressive increase along the distal segments has been observed in auditory brainstem axons, and simulation studies suggest that it contributes to temporal processing for sound localization²⁰. In addition to these morphological changes of nodes of Ranvier, clustering of Nav prior to myelin deposition (prenodes) may constitute an early mechanism to accelerate conduction velocity,^{124,157} (Figure 4A). Indeed, single-axon electrophysiological recordings on hippocampal GABAergic neurons with and without prenodes show that the conduction velocity is higher in neurons with prenodes compared to same caliber axons without prenodes¹²⁴. These data suggest another level of influence by oligodendrocytes on the regulation of conduction velocity beyond the production of a myelin sheath. Prenodes with high-density Nav clusters could serve as acceleration point, allowing microsaltatory conduction^{158,159,160} and could therefore help to overcome axonal branch point failures and maintain reliable propagation of AP^{153,161}.

In myelinated fibers, K_v1.1 and K_v1.2 channels, restricted to the juxtaparanodal compartment, contribute to internodal resting potential and act to prevent repetitive firing². At nodes, K⁺ conductance mainly relies on leak-type channels belonging to the K2P channel subfamily (see above), recently identified as TRAAK and/or TREK-1^{5,6} and also on slowly opening K_v7.2/7.3 channels⁶⁸. Their activity largely contributes to repolarizing the nodal membrane during a spike, and is also important for the stabilization of a negative resting potential and maximum spike amplitude^{5,6,68,71}. The activity of K2P channels can be diversely regulated to control cellular excitability, notably by force applied directly through the lipid bilayer^{162,163} and could serve a mechano-protective role in node of Ranvier or respond to mechanical component of the action potential¹⁶⁴. Other K⁺ channels also repolarizes membrane at nodes, such as K_{Ca}3.1, a calcium-activated potassium channel, which is a key determinant for generation of fast frequency spikes and safe conduction in Purkinje cell axons¹⁶⁵.

4) Nodes of Ranvier alterations in demyelinating pathologies

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

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

In experimental autoimmune encephalomyelitis (EAE), a model of inflammatory CNS demyelination, disruption of nodes of Ranvier with loss of Nav nodal clusters has been reported in both optic nerves¹⁶⁶ and spinal cord¹⁶⁷. Loss of nodal clusters is associated with extended expression of Nav channels along demyelinated axons. Similar findings with drastic reduction of Nav nodal clusters were reported in the corpus callosum upon cuprizone-induced demyelination, a toxic, non-inflammatory model^{168,169}. In dysmyelinating mutants, changes of Nav channels expression appear more heterogeneous : in *myelin-deficient (md)* rats and *jimpy*, mice, two proteolipid protein (PLP) mutants^{58,170}, where myelination is drastically reduced, Nav channels clusters persist despite lack of myelin and axoglial junctions; similarly, in *ceramide galactosyltransferase deficient* mice, which undergo spontaneous adult-onset demyelination, Nav channels clusters persist after myelin sheaths are lost¹⁶⁸. In contrast, in

347 *shiverer* mice, a myelin basic protein (MBP) mutation resulting in non-compacted myelin, Nav
348 channel clusters are reduced in number¹¹⁵ while Nav channel immuno-reactivity is detected
349 throughout adult axons⁵⁵. 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^{55,166,167,169,171}. 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 cells¹⁷². In addition to Nav1.2 and 1.6, Nav1.8 is ectopically expressed
359 by Purkinje neurons in EAE, where it perturbs electrical activity^{173,174}.

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^{58,72,170}. Similar findings were reported in the spinal cord of EAE mice^{175,176}.
365 Finally, upon cuprizone demyelination Kv 1.1 and Kv 1.2 channels disperse all along the axon
366 ¹⁷⁷.

367 Most of these studies have been focused on white matter axons. Recently, using a murine
368 cuprizone model, Hamada et al¹⁷⁸ 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 ^{172,179}. Other changes such as lengthening of nodes have also been reported^{176,179}. Although

Nav channels of mature nodes in the normal CNS are Nav1.6, redistributed Nav channels along demyelinated axons consist both of Nav1.6 and Nav1.2¹⁷². In addition, in MS, similarly to EAE, Nav1.8 is ectopically expressed in axons of Purkinje neurons, and it has been suggested that variants of *SCN10A* gene (encoding Nav1.8) predict the degree of cerebellar dysfunction in MS^{173,180}. Changes of Kv channels also occur, with loss of juxtaparanodal Kv clusters and extensive expression of Kv channels along the denuded portion of the axon¹⁷⁹.

Whether these nodal alterations occur at the onset of myelin damage or whether they are secondary to paranodal junction disruption has been extensively studied. In experimental demyelinating models (EAE, cuprizone)^{176,181}, early breakdown of paranodes is detected contrasting with persisting nodal clusters.^{2,58,170} However, nodal changes have been detected prior to or coincident to disruption of paranodal junctions in an EAE model using coherent anti-Stokes Raman scattering microscopy (CARS)¹⁸². In a model of autoimmune optic neuritis both nodes and paranodes were increased in length during the late induction phase of the disease, prior to onset of the demyelination and immune cell infiltration¹⁸³. Similarly, in a cuprizone model with different treatment durations, a small but significant decrease in nodes density was observed at the onset of demyelination and before significant change in Caspr immunoreactivity¹⁸⁴. Analyzing MS normal appearing white matter (NAWM), Howell et al showed an early elongation and alteration of paranodal structures¹⁷⁶. Moreover, a displacement of Kv1.2 channels closer to the nodes is observed indicating that disruption of paranode precedes alterations at the node itself. Similar findings were reported in the borders of lesions of patients with chronic progressive MS^{176,185}. In line with these results, loose/extended clusters of nodal proteins are detected on some denuded axons within MS demyelinated lesions¹⁷⁹, likely corresponding to remaining nodal structures after paranodal breakdown. Altogether, the chronology of nodal versus paranodal alteration upon demyelination remains elusive, with the difficulty to ascertain a temporal pattern from human post-mortem studies. Whether, as highlighted for nodal formation, the temporal pattern of nodal disaggregation might vary depending on neuronal subpopulations is an open question.

4-2) The consequences of demyelination-induced nodal disruption on neuronal physiology. Disruption of nodal and perinodal domains have a profound impact on nerve conduction and excitability. Moreover, Nav channels redistributed along the axon might act as a double sword, participating to an adaptive response to injury on the one hand, to neurodegeneration on the 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^{184,186–188}. 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 conduction¹⁷⁸, consistent with the observation that even low Nav channel densities at the denuded internode axolemma suffice to ensure propagation of axonal APs¹⁸⁹. In addition, hyperexcitability, ectopic spikes generation and failure of AP propagation at the presynaptic terminals have been recorded in these demyelinated fibers^{178,190} (Figure 4C). The impact of K⁺ 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⁺ channels targeted by 4-AP are also found at synaptic terminals rather than along demyelinated axons⁸.

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.¹⁷², which has demonstrated that the Nav1.6 isoforms, producing persistent Na⁺ 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⁺ 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 α 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 inflammation¹⁹¹ 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-

axonal degeneration and increased disability during EAE¹⁹² with no impact on the immune response; such gain of function was not tested for Nav1.6, due to the induction of epileptic seizures. Altogether these results strengthen the hypothesis that demyelination-induced redistribution of Nav channels along the axon play a major role in neurodegeneration.

In direct line with these pathophysiological concepts, therapeutic strategies aimed at blocking Na⁺ channels to promote neuroprotection were developed: the lamotrigine trial evaluating a partial Na⁺ channels blocker in secondary progressive MS¹⁹³, with brain volume as a primary outcome measure was negative; the phase 2 trial of phenytoin in patients with optic neuritis¹⁹⁴, showed a 30% reduction of retinal thickness loss (quantified using optical coherence tomography) with phenytoin compared with placebo. The lack of specificity for Nav1.2 and/or Nav1.6 of the therapeutic compounds tested in these trials, the lack of robust surrogate markers for neuroprotection in MS (except for the optic nerve) might have prevented further development. These strategies might however be re-actualized with the development of more specific Na⁺ channels blockers.

In addition, novel perspectives might emerge based on K⁺ channels blockade. Blocking K⁺ channels on denuded axons (see above), 4-aminopyridine (4-AP), have some minor symptomatic efficacy, likely related to reduced axonal excitability, but it lacks specificity. Some relatively specific drugs now available might represent a promising approach. Finally visualizing K⁺ channels expression might open to novel imaging perspectives. A recent PET-scan study using [18F]3-F-4-AP ligand in dysmyelinating *Shiverer* mice lacking compact myelin has suggested that it might be a valuable PET tracer for detecting CNS demyelination¹⁹⁵.

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

Whether node of Ranvier might represent sites of axonal vulnerability has been suggested. In this context, live imaging of spinal cord in an EAE model, has shown that initial disruption of the axon often occurs at putative nodes of Ranvier¹⁹⁶. This might reflect the vulnerability of the nodal domains in an inflammatory environment, due to the lack of myelin protection at the nodal axonal surface. Interestingly, using a spinal cord *in vivo* multiphoton imaging approach in combination with calcium reporter mouse lines in mice with EAE, Witte et al have shown that calcium permeable membrane nano-ruptures might influence axonal fate, and that axons with maintained elevated calcium levels are prone to degenerate¹⁹⁷. Importantly, demyelination increased the probability of calcium permeable membrane nano-ruptures, probably because loss of the myelin sheaths facilitates access to the axonal membrane along the internode, and, in this

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

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

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

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

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

strikingly varies between studies ^{211,212}. These discrepancies likely reflect technical issues related to the detection of the seropositivity.

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

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

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

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⁺ and K⁺ 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 ¹⁹⁷.

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 ^{8,216–219}. 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 ^{220,221}], 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 ²²². 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

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

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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 μ 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 μ 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 μ m; box : 5 μ m.

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