

# Activity-dependent regulation of excitable axonal domains

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**Abstract** Rapid action potential propagation along myelinated axons requires voltage-gated Na<sup>+</sup> channel clustering at the axon initial segments (AISs) and nodes of Ranvier. The AIS is intrinsically defined by cytoskeletal proteins expressed in axons, whereas nodes of Ranvier are formed by interaction between neurons and myelinating glia. These axonal domains have long been considered stable structures, but recent studies revealed that they are plastic and contribute to fine adjustment of neuronal activities and circuit function. The AIS changes its distribution and maintains neural circuit activity at a constant level. Morphological changes in myelinated nerve structures presumably modulate the excitability of nodal regions and regulate the timing of activity, thereby optimizing signal processing in a neural circuit. This review highlights recent findings on the structural plasticity of these excitable axonal domains.

**Keywords** Axon initial segment · Node of Ranvier · Myelin · Neuronal activity · Plasticity · Ion channel

## Introduction

Neurons are highly polarized cells forming specialized structures, which are essential for complicated nervous system functions. Somatodendritic domains of neurons receive input, whereas axonal domains support neuronal output: action potential initiation and propagation to the target. Myelinated axons are divided into functionally and molecularly distinct domains including axon initial segments (AIS), nodes of Ranvier, paranodes, juxtaparanodes, and internodes (reviewed in [1]). The AIS and nodes are the excitable domains of axons characterized by a high accumulation of voltage-gated Na<sup>+</sup> (Nav) channels (Fig. 1a). The AIS is a region approximately 30–60 μm long located at the interface between the neuronal soma and axon that contributes to the generation of action potentials. Nodes of Ranvier are defined as the short gaps (approximately 1 μm) between two adjacent internodal segments located along myelinated nerve fibers. Nodes of Ranvier secure reliable regeneration and conduction of action potentials. At internodes, neurons and myelinating glia interact in areas of the axon wrapped by myelin sheaths. Oligodendrocytes and Schwann cells are the myelinating glia in the central nervous system (CNS) and peripheral nervous system, respectively. Since the internodes are insulated by the myelin sheaths, the action potentials propagate rapidly in a saltatory manner. Reliable generation and conduction of action potentials are important for the function of neural circuits, and these axonal domains play a critical role in the process. The importance of these domains is further underscored by the fact that the disruption of the AIS or nodes plays a key role in the pathophysiology of various nervous system diseases and injuries (reviewed in [2, 3]). Recently, it was revealed that these axonal domains of neurons are not just static

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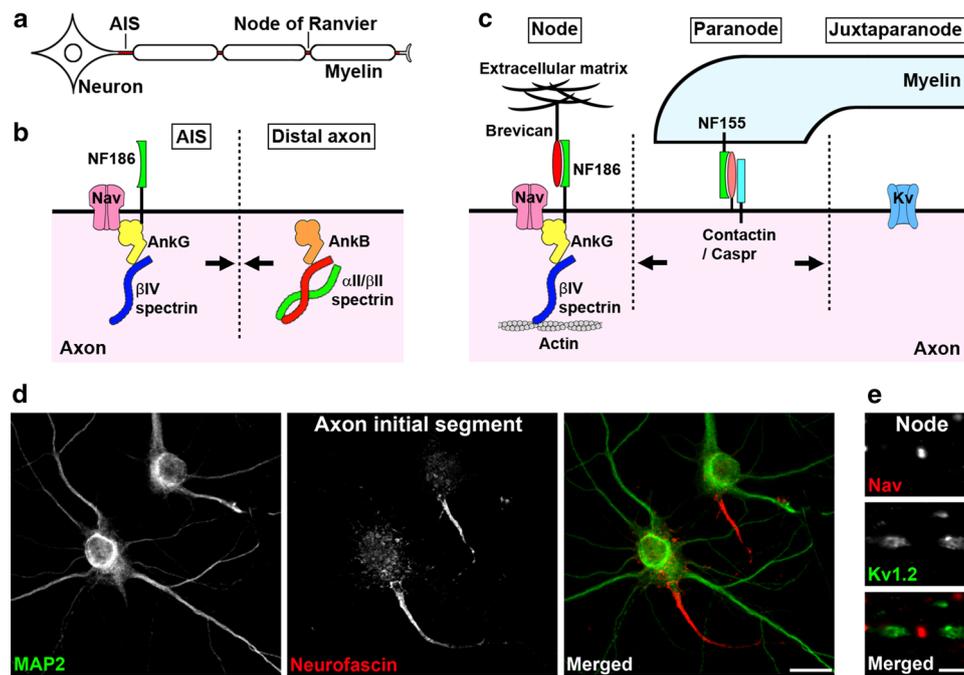
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**Fig. 1** Excitable domains along the myelinated nerve fiber. **a** Cartoon illustrating the structures of the myelinated nerve fiber and excitable axonal domains: axon initial segment (AIS) and nodes of Ranvier. **b** Schematic presentation showing molecular mechanisms of AIS formation. Intra-axonal boundary between the AIS and distal axon is formed by submembranous cytoskeletal complexes, ankyrinG- $\beta$ IV spectrin in the AIS and ankyrinB- $\alpha$ II/ $\beta$ II spectrins in the distal axon. **c** Schematic presentation showing molecular mechanisms of node of Ranvier formation. Paranodal junctions formed by cell adhesion molecules, axonal contactin and contactin-associated protein (Caspr), and glial neurofascin (NF) 155 act as a diffusion barrier to

restrict the mobility of nodal and juxtaparanodal molecules. Nodal protein complex is secured by the interaction between the axonal cell adhesion molecule NF186 and extracellular matrix molecules such as brevican. An ankyrinG- $\beta$ IV spectrin complex links Nav channels to the actin cytoskeleton to further stabilize Nav channel complex. **d** Cultured hippocampal neurons. Antibody to neurofascin shows AIS (red), whereas antibody to MAP2 shows somatodendritic domains (green). Scale bar 20  $\mu$ m. **e** Longitudinal section of mouse optic nerve. Nav channels at nodes (red) and voltage-gated potassium (Kv) 1.2 channels at juxtaparanodes (green). Scale bar 5  $\mu$ m (color figure online)

structures, but are plastic with the ability to undergo neuronal activity-dependent morphological changes. This plasticity is thought to enable fine modulation of neuronal output. This review summarizes current knowledge of axonal domain plasticity and discusses its implications in the nervous system.

### Molecular mechanisms of AIS and node of Ranvier formation

Given their similarity in function, it is not surprising that the AIS and nodes of Ranvier have an almost identical molecular composition. Protein complexes at these domains include voltage-gated ion channels, the scaffolding protein ankyrinG, the cell adhesion molecule neurofascin (NF) 186, and the cytoskeletal protein  $\beta$ IV spectrin (Fig. 1b, c) (reviewed in [1]). However, the assembly of these excitable domains differs. In brief, AIS assembly is intrinsically determined by neurons, whereas node of

Ranvier formation requires interaction between neurons and myelinating glia.

AnkyrinG is the first component that appears at the AIS and is considered to be a master organizer for this region [4]. Loss of ankyrinG blocks clustering of Nav channels, NF186, and  $\beta$ IV spectrin [4]. In developing unmyelinated axons, ankyrinG is restricted to the AIS and is excluded from the distal axon. The submembranous axonal cytoskeleton in distal axons consisting of ankyrinB,  $\alpha$ II spectrin, and  $\beta$ II spectrin defines an intra-axonal boundary limiting ankyrinG and  $\beta$ IV spectrin at the AIS (Fig. 1b; [5]).

In contrast to the AIS, myelinating glial cells are required for node of Ranvier formation (Fig. 1c; [6]). At paranodes flanking each side of a node, myelinating glia form junction complexes with axons. These complexes include axonal proteins (contactin and contactin-associated protein) and the glial molecule NF155, and they function as a diffusion barrier to restrict the mobility of nodal proteins. In the CNS, node assembly starts when paranodal axoglial

junctions form, providing the primary mechanism of nodal Nav channel clustering. CNS node formation is also aided by glia-derived extracellular matrix molecules such as brevican that directly interact with axonal NF186 and promote its clustering. Moreover, Nav channel and ankyrinG complexes at the node are stabilized through a  $\beta$ IV spectrin-based submembranous cytoskeleton. The molecular and structural organization of these proteins critically determines the characteristics of plasticity at individual axonal domains.

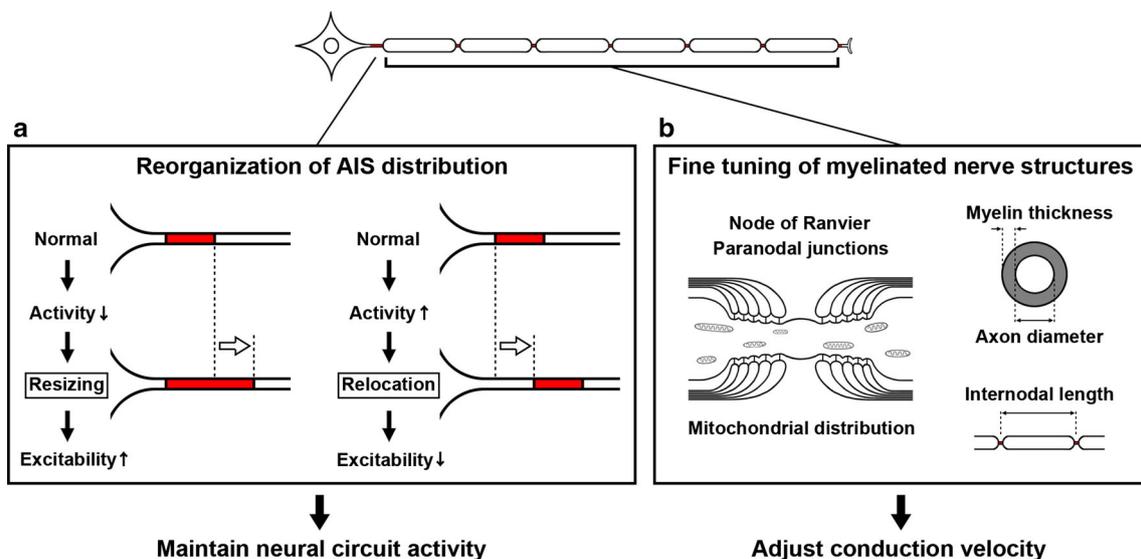
### Structural plasticity of the AIS

The distribution of the AIS changes with neuronal activity. These structural changes work as homeostatic plasticity and contribute to maintaining activity in a circuit at a constant level. There are two forms of structural AIS plasticity: (1) changes in length (resizing) and (2) location (relocation) (Fig. 2a). Resizing of the AIS was originally found in neurons of the avian cochlear nucleus [7]. When afferent inputs are deprived, the AIS of these neurons increases in length. This elongation increases the number of Nav channels at the AIS, augments membrane excitability, and causes spontaneous action potentials in the neurons, suggesting that the elongation compensates for the loss of afferent activity. On the other hand, relocation of the AIS was first revealed in hippocampal pyramidal

neurons in dissociated culture, where chronic activation of neurons moves the entire AIS distally, leading to a decrease in the membrane excitability [8].

AIS plasticity is cell-type specific and includes various patterns. For example, in dissociated olfactory bulb cultures, the AIS in dopaminergic inhibitory interneurons relocated proximally and lengthened in response to elevated neuronal activity; however, excitatory neuron AIS changes occurred in the opposite direction [9]. These complementary changes in AIS structures are suggested to counterbalance the excess activity in the circuit, supporting the idea that AIS plasticity provides a negative-feedback mechanism to maintain homeostasis of the neural circuit. Indeed, AIS plasticity is hypothesized to be critical in adjusting the activity and maintaining homeostasis of neural circuits in various pathological conditions [10–13]. Notably, in the cuprizone-induced demyelination model, cortical pyramidal neurons with demyelinated axons showed a proximal shift of their AIS [13]. These neurons were intrinsically more excitable, whereas the proximal shift of the AIS slightly reduced the efficacy of the action potential generation in the neurons, confirming the compensatory role of AIS plasticity.

In addition to maintaining neuronal activity, AIS plasticity contributes to refinement of neural circuits during development [14–16]. The avian cochlear nucleus and its target nucleus are involved in processing of timing information of auditory signals. In these nuclei, AIS length varies greatly among cells, with a longer AIS in neurons



**Fig. 2** Activity-dependent regulation of the axonal domain structures. **a** Reorganization of axon initial segment (AIS) distribution is expressed as its resizing and/or relocation. *Left panel* illustrates an example of AIS resizing in the avian cochlear nucleus after deprivation of afferent inputs [7]. *Right panel* illustrates an example of AIS relocation in hippocampal pyramidal neurons in culture after chronic depolarization or photostimulation [8]. These structural

reorganizations of the AIS contribute to the maintenance of neural circuit activity at a constant level. **b** The structures and organelles along the myelinated nerve fibers are modulated by both neurons and myelinating glial cells. Reorganization of these structures contributes to adjustment of conduction velocity, thereby regulating signal processing in neural circuits

tuned to lower-frequency sound. This is critical for the reliable and precise signal processing of these neurons [17, 18]. Depriving afferent inputs during development diminishes this variation, suggesting the involvement of activity-dependent mechanisms in AIS differentiation [16].

What are the molecular mechanisms underlying AIS plasticity? Some studies demonstrate that  $\text{Ca}^{2+}$  is the key to inducing AIS plasticity. AIS relocation is triggered by activation of L-type  $\text{Ca}^{2+}$  channels. This then activates  $\text{Ca}^{2+}$ - and calmodulin-dependent protein phosphatase (calcineurin) in hippocampal pyramidal neurons [19], while it activates cyclin-dependent kinase 5 (cdk5) in olfactory bulb dopaminergic interneurons [9]. Notably, cdk5/p35 also regulates the distribution of the AIS-like structure in mushroom body neurons of *Drosophila* by moving the position of its distal boundary [20]. The mechanism by which ankyrin-spectrin complexes at the distal AIS boundary (see above; Fig. 1b; [5]) are remodeled during AIS plasticity remains unknown, although post-translational modifications of these proteins such as phosphorylation or palmitoylation are expected to be involved in the process (reviewed in [21]).

### Activity-dependent regulation of myelinated nerve fibers

In contrast to AIS plasticity, which is regulated intrinsically by neurons, the plasticity of myelinated nerve fibers occurs via interaction between neurons and glial cells. Indeed, cumulative evidence demonstrates that myelinating glia have the ability to modulate myelination in response to neuronal activities. During development, the electrical activity in axons provides signals to oligodendrocytes to control the initiation of myelination [22–24]. Even after maturation, myelin sheaths can be remodeled. For example, protracted social isolation of adult wild-type mice induced behavioral, transcriptional, and ultrastructural changes in oligodendrocytes of the prefrontal cortex and impaired adult myelination, presumably adapting the myelin thickness to axonal firing rate [25]. This neuronal-activity- and social-experience-dependent myelination may be involved in the pathophysiology of psychiatric disorders (reviewed in [23]).

Notably, in addition to myelin remodeling, neuronal activity can cause oligodendrocyte depolarization leading to an increase in both the excitability of the axon and the conduction velocity of action potentials [26, 27]. Myelin remodeling takes days to weeks to occur; however, oligodendrocyte depolarization can occur within minutes. The ability to modulate signaling in shorter time scales provides another layer of functional plasticity for white matter. Since the paranodes are the site of interaction between

oligodendrocytes and axons (see above, Fig. 1c), and are critical in regulating nodal excitability and conduction of action potentials [28, 29], it has been speculated that oligodendrocyte depolarization augments action potential generation by modulating the extent of insulation at paranodes [26, 27].

Changes in myelinated nerve fiber structure may affect neuronal circuit function in multiple ways. Nerve conduction velocity is determined by various factors including axon diameter, myelin thickness, and internodal length [30, 31]. Recent reports suggest that these structural characteristics can be tuned to modulate nerve conduction velocity and optimize neural circuit functions (Fig. 2b; [32–34]). For example, in mammalian brainstem auditory circuits, axons responding best to lower-frequency sounds had a larger diameter and shorter internodal length, thereby accelerating conduction velocity in the lower-frequency fibers [34]. In addition, internodal length decreased and axonal diameter at nodes of Ranvier increased progressively toward axon terminals at distal segments in the fibers. This spacial variation as well as cell-type-specific differences of axon, myelin, and nodal morphology may compensate for the difference in the axonal path length among fibers, presumably adjusting the timing of action potential propagation to their target. Activity-dependent mechanisms might be involved in these morphological variations in the auditory circuit, although detailed mechanisms remain unknown. It has been reported that exposure to loud sound induces sensorineural hearing loss and causes decreased myelin thickness, elongated nodes of Ranvier, and altered paranodes and juxtaparanodes along the auditory nerve [35]. This introduces the possibility that myelin and nodal structures could be altered in response to neuronal activities.

Activity-dependent structural reorganization is also observed in organelles in CNS myelinated axons. At nodal regions, mitochondrial distribution and transport are modulated by neuronal activity, which is dependent on oligodendrocyte-axon interactions at paranodes [36]. This emphasizes the role of neuro-glial interactions in maintaining the energy necessary for reliable action potential regeneration at the nodal region. The changes in both axonal mitochondrial distribution and myelinated nerve morphology described above presumably modulate the excitability at nodal regions. Reliable and precisely timed action potential generation is required for accurate synaptic function including integration and plasticity, representation of information, and activity synchronization. Because the plasticity at the myelinated fibers is an effective way to regulate the timing of activity, it should play a critical role in the computation of neural circuits (Fig. 2b). Nevertheless, further studies are necessary both to determine whether structural refinements indeed occur at mature nodes of

Ranvier and to define the molecular mechanism(s) underlying this plasticity.

## Conclusion

As reviewed here, activity-dependent regulation of excitable axonal domains directly modulates neuronal output. Plasticity of the AIS occurs intrinsically by changing its distribution. This process can regulate action potential generation, providing a negative-feedback mechanism that maintains homeostasis for the circuit. In contrast, the plasticity of myelinated nerve properties is mediated by both neurons and myelinating glia, possibly regulating nodal integrity and internodal length. These mechanisms contribute to modulating the timing of action potential propagation and to tuning the signal processing in the circuit. Following this scheme, it is reasonable that the effects of AIS plasticity are global and imposed on every downstream target, whereas those of myelinated nerve plasticity can be local and spatially restricted to targets of specific branches, which would be preferable for fine modulation of circuit function. Neuronal activity is crucial for viable computation of neural circuits and also for the formation, refinement, and maintenance of the circuits. Because plasticity at axonal domains has a strong impact on neuronal activity, elucidating the mechanism(s) of this plasticity will promote better understanding of the function(s) and diseases of our nervous system.

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**Compliance with ethical standards**

**Conflict of interest** We declare no conflict of interest.

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