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Synaptic pathology in multiple sclerosis: a role for Nogo-A signaling in astrocytes?

Sheila Espírito-Santo*, Vinícius Gabriel Coutinho, Flávia Carvalho Alcantara Gomes

Multiple sclerosis (MS) is characterized as an inflammatory demyelinating disease that affects the central nervous system (CNS), leading to sensory, motor and cognitive impairments. Ultimately, axonal denudation culminates in axonal lesions and neurodegeneration. Inflammatory demyelinating lesions in MS are associated with infiltration of immune cells combined with activation of the resident CNS inflammatory cells, astro- and microglia. Recently, synaptopathy has been associated with MS pathophysiology, though, intriguingly, it can occur independently of demyelination (Jürgens et al., 2016). Although inflammation also seems to corroborate with synaptic abnormalities, associated or not with demyelinating lesions, the underlying mechanisms are not fully understood (Mandolesi et al., 2015). In the last decades, the myelin inhibitory protein neurite outgrowth inhibitor-A (Nogo-A) has emerged as a potential mediator of axonal and synaptic dysfunctions in MS and a promising target to be neutralized (Ineichen et al., 2017). Based on our recent findings demonstrating that Nogo-A signaling regulates astrocyte-driven synaptogenesis (Espírito-Santo et al., 2021), and considering the critical role of astrocytes in regulating synaptic plasticity and function (Allen and Eroglu, 2017), we propose that modulation of Nogo-A pathway in these cells is a new mechanism driving circuitry alterations of MS.

Synaptopathy in MS: Synaptic alterations are hallmarks of MS expressed even in the early stage of the disease both in patients and in animal models of MS, such as experimental autoimmune encephalomyelitis (EAE) and cuprizone-induced demyelination, being directly associated with neurological disabilities. This profile of synaptopathy has been characterized by perturbations of molecular machinery from Glutamatergic and GABAergic (γ-aminobutyrate, GABA) neurotransmission, implicating in excitatory/inhibitory imbalance, with predominant excitability. Sustained activation of glutamate-activated Ca+2 permeable receptors along with reduced glutamate uptake in the synaptic cleft deregulates Calcium homeostasis, leading to synaptic excitotoxicity. Activated micro- and astroglia release inflammatory molecules, particularly tumor necrosis factor-alpha (TNF-α) and interleukin- 1 beta (IL-1β), that are implicated with neurotransmission perturbation in experimental MS and patients. Contributing to excitation, TNF-α induces α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPAR) insertion on the neuronal surface, promoting an increase in AMPAR-dependent excitatory postsynaptic currents. IL-1β signaling, along with its downstream mediator miR-142-3p, promotes reduction in astrocytic glutamate-aspartate transporter expression and activity, impairing glutamate reuptake and leading to the enhancement in the duration of excitatory postsynaptic currents. Other studies still report a reduction in GABAergic transmission by the action of IL-1β, also favoring excitation. The resulting hyperexcitability, which has been related to a higher probability of epilepsy development in MS patients, seems to be adaptive plasticity that aims to restore the impaired neuronal activity due to demyelination (Mandolesi et al., 2015). Despite its importance to reinforce synaptic inputs into target neurons, it may chronically result in excitotoxicity and synaptic loss. Thus, astrocyte and microglia chronic activation is a critical phenomenon to synaptopathy development and maintenance at the MS course and therapeutic approaches targeting its regulation are required.

Along this line, we recently demonstrated that cuprizone-induced acute demyelination increases the density of excitatory synapses in the visual cortex (Espírito-Santo et al., 2021). These results led to the hypothesis that excitatory synaptogenesis could represent a mechanism that precedes and contributes to hyperexcitability in MS (**Figure 1**). Indeed, evaluation of the pre-synaptic protein synapsin 2 in the brain during different phases of EAE revealed enhanced levels in the pre-symptomatic phase with progressive decreased levels in posterior phases (Raphael et al., 2017). Aligned with this idea, other studies have reported excitatory contacts loss associated with MS synaptopathy and/or grey matter atrophy as a result of long-term injury, especially during later and progressive phases of MS (Mandolesi et al., 2015). Conversely, at the onset or established phase of the disease from EAE model, pre-synaptic proteins and/or spine density are enhanced in the somatosensory cortex (Potter et al., 2016) Therefore, the confirmation if new excitatory contacts is an early step of MS course which results in hyperexcitability, excitotoxicity and synaptic loss in MS, should benefit from further studies investigating different phases of the disease, and distinct regions of different animal models and MS patients. Considering the actual lack of treatments for the later phase of the disease, unveiling the events underlying synaptic pathology will pave an essential way to the search for novel targets to prevent neurological deterioration during MS course.

Nogo-A in MS: The mechanisms underlying synaptic dysfunction in the MS are still not fully understood, but the Nogo-A protein, a potent neuroplastic inhibitor of adult CNS, has emerged as a candidate in this context. Nogo-A is a component of the myelin sheath that exists as a transmembrane protein expressed by oligodendrocytes, and in a lower degree by neurons. In general, it binds to Nogo Receptor 1 (NgR1), which, through TROY/p75 and LINGO-1 coreceptors, activates the RhoA pathway, modulating actin-based morphology (Ineichen et al., 2017). Particularly, Nogo-A inhibits synapse formation, through ROCK-cofilin signaling, downstream to RhoA activation, which leads to the destabilization of the actin cytoskeleton and decrease of dendritic spine density (Kellner et al., 2016).

In the context of MS, studies in animal models have reported that suppression of Nogo-A promotes: clinical improvement, slowed disease progression, reduced demyelination and axonal damage. Despite the lack of consensus of its physiopathological role in MS, both Nogo-A and its receptor neutralization have been employed in different phase I and II clinical trials, with inconclusive results so far (Ineichen et al., 2017). Besides, the last years also experienced an influx of studies investigating Nogo-A and its receptor as biomarkers for MS and other demyelinating inflammatory diseases of the CNS, both in liquor and blood serum. In fact, both in MS and animal models, the levels of Nogo-A and its receptor are regulated in the course of the disease, by mechanisms still unknown: whereas Nogo-A is reduced in the acute phase of MS, it increases in the chronic phase, mainly in surviving oligodendrocytes and myelin sheath. Interestingly, this MS stage-dependent variation

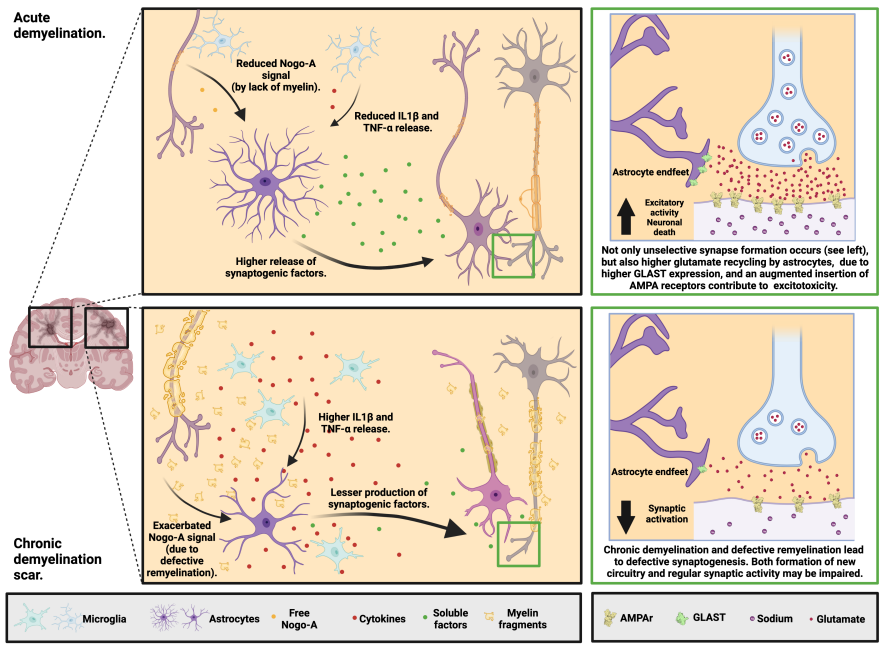

Figure 1 | **Synaptic dysfunctions mediated by Nogo-A in the MS.**
(Upper Panel) In the acute phase of MS, myelin loss results in reduced levels of the Nogo-A protein and consequently leads to a decrease in Nogo-A signaling on neurons and glial cells. In the short term, reduced Nogo-A signaling, directly on neurons or mediated by astrocytes, provides an increase in synaptic plasticity, with an enhance in excitatory synaptogenesis and insertion of AMPAR, which favors the recovery of neuronal activity. Exacerbated and continued excitability might result in excitotoxicity and synaptic loss. (Lower panel) In the chronic phase of MS, increased levels of Nogo-A and increased signaling in neurons and astrocytes result in restriction of synaptic plasticity and synaptogenesis, respectively, culminating in the reduction of AMPA receptors, neuronal activity and synaptic density, compromising the recovery of neural networks. In both phases, Nogo-A signaling in microglia and modulation of secreted inflammatory factors, such as TNF-α and IL-1β, may contribute to synaptic change. Created with BioRender com. AMPAr: Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GLAST: glutamate-aspartate transporter; IL-1β: interleukin-1 beta; MS: multiple sclerosis; Nogo-A: neurite outgrowth inhibitor-A; TNF-α: tumor necrosis factor-alpha.

in Nogo-A expression inversely correlates with expression of growth-associated protein-43, a protein relevant to synapse formation and remodeling (Theotokis et al., 2016). Thus, the acute phase of MS seems to provide a favorable environment for synaptic plasticity, as opposed to the chronic phase, and Nogo-A expression seems to contribute to this scenario (**Figure 1**). Although the content of NgR1 does not vary in the MS brain throughout acute phase, it enhances in the chronic phase exhibiting a cellular redistribution, with higher expression in microglia and astrocytes compared to neurons (Theotokis et al., 2016). The role of Nogo-A signaling in glial cells has only recently begun to be unraveled *in vitro*. It has been shown that Nogo-A signaling stimulates the release of pro-inflammatory cytokines, such as TNF-α, by microglia (Fang et al., 2015). It is worth mentioning that an extensive amount of evidence has correlated neuroinflammation with hyperexcitability and synaptic alterations, particularly with TNF-α and IL-1β released from activated microglia and astrocytes (Mandolesi et al., 2015). If Nogo-A also contributes to the inflammatory state in the MS remains to be investigated. Additionally, it would be important to investigate Nogo-A effect on microglial phagocytosis, since it has been demonstrated in the hippocampus from MS patients that activated microglia promotes synaptic pruning through C1q and C3 complement proteins-mediated synapse opsonization (Michailidou et al., 2015). Therefore, manipulation of the Nogo-A signaling (and NgR1) in glial cells may represent a new avenue for therapeutic intervention in MS. Besides, since astrocytes regulate synapse formation and function, it is expected Nogo-A signaling on astrocytes may indirectly contribute to synaptic dysfunction in MS.

Nogo-A signaling in astrocytes and synaptopathy in MS: Astrocytes are regulators of synapse formation and plasticity in CNS. They can control glutamatergic excitatory synapses mainly through released soluble factors that have been described by our group and others: transforming growth factor-beta 1 and Hevin favor formation of silent excitatory synapses; secreted protein acidic and rich in cysteine (SPARC), the antagonist of Hevin activity, prevents the synaptogenesis and promotes the removal of AMPAR from active synapses; glypican-4 and -6 contribute to the maturation of newly formed synaptic sites through insertion of AMPAR; TNF-α increases AMPAR insertion mainly in pre-existing synapses and reduces GABA receptor levels (Allen and Eroglu, 2017). Therefore, dysregulation of astrocytic homeostatic functions may contribute to the synaptic pathology seen in MS.

Although astrocytes have considerable relevance for the physiopathology of MS, their relation with synaptopathy has not been much explored. Reactive astrocytes containing myelin debris within phagosomes are found on lesioned areas and, intriguingly, are also observed in the pre-injury areas in the early MS, even before the infiltration of peripheral immune cells. By using the cuprizone-induced MS model, we observed reactive astrocytes associated with massive loss of myelin and increased density of excitatory synapses in the visual cortex (Espírito-Santo et al., 2021). Particularly relevant for synapse density and function, our group and others have found altered levels of Hevin and SPARC in the grey matter of cuprizone and EAE models of MS (Blakely et al., 2015; Espírito-Santo et al., 2021). Interestingly, we showed increased expression of Hevin specifically in astrocytes, indicating regulation of its synaptogenic activity (Espírito-Santo et al., 2021). However, if the altered synaptogenic factors expressed by astrocytes are responsible for the synaptic abnormalities in MS remains to be confirmed.

The mechanisms regulating the synaptogenic potential of astrocytes in MS are still under investigation. Pioneeringly, our recent pieces of evidence strongly support the hypothesis that Nogo-A signaling is a negative regulator of astrocyte-derived synaptogenesis. We demonstrated that Nogo-A signals through RhoA pathway in cultured cortical astrocytes and induces downregulation of the pro-synaptogenic molecules, Hevin, Glypican-4, transforming growth factor beta 1, and brain-derived neurotrophic factor, and upregulation of the anti-synaptogenic factor, SPARC. This regulatory effect is probably dependent on Rho-A activation, since inhibition of its downstream target, ROCK, reversed the effects of Nogo-A on the SPARC content. Consequently, a reduced number of structural and functional synapses is formed in cortical neuronal cultures incubated with conditioned medium from Nogo-A-treated astrocytes when compared to conditioned medium from control astrocytes (Espírito-Santo et al., 2021).

Although *in vivo* direct pieces of evidence of Nogo-A signaling as a regulator of astrocyte-mediated synaptogenesis are still missing, our findings from the cuprizone-model supports our *in vitro* data, that is: demyelination-induced lower Nogo-A expression and increased Hevin expression in astrocytes is associated with increased excitatory synaptic density in the visual cortex. Interestingly, increased NgR1 and decreased Hevin expression in astrocytes are combined with decreased excitatory synaptic density in the lateral geniculate nucleus (Espírito-Santo et al., 2021). So, it also seems to occur *in vivo*, a similar inverse correlation between Nogo-A signaling and astrocyte synaptogenic potential, impacting synapse density. Alternatively, it has been shown that inflammatory cytokines are able to modulate SPARC and Hevin transcripts in astrocytes *in vitro* (Blakely et al., 2015). Thus, both Nogo-A and inflammatory factors could affect astrocyte-derived synaptogenesis in MS (**Figure 1**).

Future directions: For a better understanding of the physiopathological events of MS, the hypothesis that Nogo-A signaling in astrocytes contributes to increased cortical excitability through excitatory synaptogenesis and excitotoxicity needs to be tested. This question can be answered using animals models of MS submitted to astrocytic conditional deletion of proteins belonging to the Nogo-A-activated pathway. The effect on hyperexcitability could be investigated by behavioral analysis and electroencephalogram evaluation of provoked seizures, while cortical neurodegeneration could be evaluated by histological or volumetric imaging

Another remaining question is whether synaptic loss and neurodegeneration during the chronic phase of MS, in which inflammation is less relevant, is associated to increased Nogo-A levels and astrocyte dysfunctions. Also in this case, astrocytic conditional deletion of proteins belonging to the Nogo-A-activated pathway would be helpful. These results can clarify a new field of investigation, that might contribute to the identification of novel targets to fight the synaptic-related symptoms of MS.

Finally, the available treatments for MS are based on immunomodulation, which is effective for the inflammatory, but inefficient for the neurodegenerative phase (Ineichen et al., 2017). Besides, current treatments end up neglecting synaptopathy of MS that can occur dissociated from demyelination foci (Jürgens et al., 2016). Therefore, a treatment combining inflammatory and synaptic modulators, such as inhibitors of Nogo-A pathway should provide a more efficient response.

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Sheila Espírito-Santo*, Vinícius Gabriel Coutinho, Flávia Carvalho Alcantara Gomes
Laboratório de Biologia Celular e Tecidual, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil (Espírito-Santo S)
Laboratório de Neurobiologia Celular, Universidade Federal do Rio de Janeiro, Instituto de Ciências Biomédicas, Rio de Janeiro, RJ, Brazil (Coutinho VG, Gomes FCA)
***Correspondence to:** Sheila Espírito-Santo, PhD, espiritosanto.sheila@gmail.com
https://orcid.org/0000-0001-8359-3435 (Sheila Espírito-Santo)
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