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Nogo-A Represses Anatomical and Synaptic Plasticity in the Central Nervous System

Nogo-A was initially discovered as a myelin-associated growth inhibitory protein limiting axonal regeneration after central nervous system (CNS) injury. This review summarizes current knowledge on how myelin and neuronal Nogo-A and its receptors exert physiological functions ranging from the regulation of growth suppression to synaptic plasticity in the developing and adult intact CNS.

Pioneering work in the late 1980s led to the discovery of specific neurite growth inhibitory factors in CNS myelin and to the identification of two membrane protein fractions that were highly inhibitory for neurite outgrowth in vitro (NI-35 and NI-250) (16). Antibody-mediated neutralization of these fractions was found to largely relieve their growth-inhibitory properties and to increase axonal regeneration and functional recovery in vivo (15, 102, 114). NI-250 was later identified as a new ~1,200-amino acids (aa)-long protein. It was renamed Nogo-A and appeared as a member of the reticulon (RTN) family (18, 41, 96, 115). Ensuing studies using a variety of blockers interfering with Nogo-A, its interaction partners, or associated signaling pathways confirmed the initial results and underlined its role in restricting anatomical plasticity and functional recovery after different types of CNS injuries (147). Surprisingly, an induction of structural plasticity was also observed upon genetic or antibody-mediated inactivation of Nogo-A in the intact CNS, pointing to an important physiological role of Nogo-A. To date, Nogo-A is thought to act as a tonic brake on CNS growth and plasticity, thereby stabilizing neuronal circuits.

This review aims at describing the currently known physiological roles of Nogo-A in the developing and adult CNS with particular emphasis on the underlying cellular mechanisms. The first part focuses on molecular features of Nogo-A and its known receptors, and on the signaling cascades that lead to a destabilization of the cytoskeleton and to a suppression of neurite growth. The second part describes physiological roles of Nogo-A in restricting different forms of plasticity in the adult CNS. The last section summarizes regulatory roles of Nogo-A during development.

Molecular and Cellular Characteristics of Nogo-A

Four mammalian reticulon genes (*rtn1, rtm2, rtm3, rtm4*) give rise to a wide range of different splice variants forming the RTN family of proteins (89). The protein isoforms Nogo-A, Nogo-B, and Nogo-C are encoded by the *rtm4/nogo* gene by alternative splicing or different promoter usage (18, 41, 96) (FIGURE 1A). All RTN members share a COOH-terminal reticulon homology domain (RHD) of 180–200 aa, which consists of two membrane-anchored hydrophobic regions spanning by a 60- to 70-aa-long hydrophilic region, also called Nogo-66 in Nogo-A, and followed by a short COOH terminus (89) (FIGURE 1A). In contrast to the highly conserved RHD, little or no homology can be found between the NH2-terminal regions of RTNs or other proteins (89), suggesting that various RTN isoforms may interact with different proteins and thereby exert a wide range of biological functions.

In the adult CNS, Nogo-A is predominantly expressed in myelin-forming oligodendrocytes but also is found in neurons of highly plastic CNS regions such as the hippocampus or the cortex (50). In the developing CNS, Nogo-A is transiently expressed by different neuronal populations, in particular projection neurons (50). Like other RTN family members, Nogo-A is predominantly localized to the endoplasmatic reticulum (ER) with small (<10%) but functionally significant amounts found at the cell surface of oligodendrocytes, neurons, and some nonneuronal cell types (28, 90). Intracellularly, Nogo-A has a strong preference to localize to the tubular ER where it is required for the formation and maintenance of ER tubules in vitro (128). Unlike other ER proteins or transmembrane proteins delivered to the plasma membrane via the classic ER/Golgi transport route, Nogo-A lacks a signal peptide for its translocation into the ER membrane (90). Despite a COOH-terminal dilysine ER retention motif, several studies have now shown that the transmembrane domains of RTNs are responsible for their proper ER localization (47, 88, 110, 113, 123). These findings, however, need to be confirmed for Nogo-A.

Membrane Topology and Trafficking of Nogo-A

Given their unusually long hydrophobic segments (~35 aa), RTN proteins have been suggested to
adopt a hairpin topology different from classic transmembrane proteins. In the ER membrane, both the NH\textsubscript{2} and COOH terminus are located on the cytosolic side (FIGURE 1B), reminiscent of the scaffold-assembling proteins caveolin and reggie/flotillin (11, 47, 57, 90, 113, 128). In line with this, RTNs have been proposed to represent a novel class of membrane-shaping proteins, which generate membrane curvature through a local oligomerization of several hairpin loops inserted into one leaflet of the membrane (11, 110). The localization of the Nogo-66 loop might differ between different Nogo isoforms, resulting in a “V” or “W” configuration of the protein, in which each hydrophobic segment fully spans the membrane or adopts a hairpin structure, respectively (90, 128) (FIGURE 1B). Nogo-A is found in a different topology at the plasma membrane, in which the NH\textsubscript{2} terminus and the Nogo-66 loop face the extracellular space and thereby enable a functional engagement with several interaction partners in trans (see below) (41, 90, 105, 142) (FIGURE 1B). Alternate topologies allowing the expression of multiple biological functions in different cellular compartments have been described for a number of other proteins including ER-resident proteins such as the inositol 1,4,5-triphophate receptor (IP\textsubscript{3}R) or the recently characterized Nogo-B receptor (NgBR) (44, 45, 67, 78, 120). Although bioinformatic models have predicted that the NH\textsubscript{2}-terminal sequence of Nogo-A is a strong topological organizer, it is unclear how Nogo-A adopts its different topologies (47). Putative mechanisms may include dimerization, the association with different molecular chaperones known to determine the conformational flexibility of several membrane proteins, as well as the phospholipid composition of the membrane (12, 54, 61, 100). In line with this, Nogo-66 is known to require a phosphocholine lipid surface to fold properly (125).

Along its intriguing topology, it remains elusive how Nogo-A is transported from the ER to the cell surface. Being an unconventional membrane protein, Nogo-A trafficking is likely to occur in a Golgi-independent manner (90, 105). To date, several membrane proteins are known to bypass the Golgi such as the protein tyrosine phosphatase CD45 (9), the paranodal complex of F3/Contactin and Caspr/Paranodin (13), the cystic fibrosis transmembrane conductance regulator (CFTR) (37, 139), Drosophila\textsubscript{a}PS1 integrin (103, 104), and some connexins (Cx) such as Cx26 (74). Plasma membrane targeting might either involve a direct fusion between peripheral components of the ER, in which Nogo-A is strongly expressed, or a vesicle-mediated transport mechanism (128). The latter might be dependent on two recently identified critical components of a Golgi bypass pathway: Golgi reassembly stacking protein (GRASP) 55 and 65 (42). It will be interesting

**FIGURE 1. Structure and membrane topology of RTN4/Nogo proteins**

A: schematic structure of the Nogo protein isoforms Nogo-A, Nogo-B, and Nogo-C. The functional domains Nogo-A/B-Δ2 (purple), Nogo-A-Δ20 (blue), Nogo-A-ext (amino-Nogo), Nogo-66 (red), Nogo-A-24 (green), Nogo-C39 (orange), and Nogo-22 are indicated. Nogo-66 is located between the transmembrane domains (TM) and found in all three isoforms. The Δ20 domain is encoded by parts of exon 3 and is specific to Nogo-A. B: multiple topologies proposed for RTN4 proteins in the ER (left) and plasma membrane (right) of different neuronal and nonneuronal cell types. Nogo-66 is found both inside and outside of the ER lumen, resulting in a V or W configuration of the protein. At the plasma membrane, both Nogo-A-Δ20 and Nogo-66 are facing the extracellular space. Different topologies in different cell types may reflect the functional diversity of RTN4 proteins.
to study whether Nogo-A is trafficked in a GRASP-dependent manner to the cell surface and how GRASP is regulated at the onset of myelination.

**Mechanisms of Nogo-A-Mediated Growth Inhibition**

**Functional Domains**

Two different regions of Nogo-A induce growth-cone collapse and inhibit neurite outgrowth of primary neuronal cultures in vitro: Nogo-A/Δ20 (rat aa544–725) and Nogo-66 (rat aa1026–1091) (41, 90) (FIGURES 1 AND 2; Table 1). Nogo-A-Δ20 additionally inhibits adhesion and cell spreading of various nonneuronal cell types, suggestive of a more ubiquitous function inside and outside the CNS (90). Although Nogo-A-Δ20 is encoded by parts of exon 3 and therefore specific to Nogo-A, the Nogo-66 region is encoded by exons 4 and 5 and found in all RTNs (87). However, the analysis of different RTN-Nogo-66 regions showed that neither RTN1- nor RTN2- nor RTN3-Nogo-66 induce growth cone collapse as opposed to RTN4-Nogo-66 (41). This has been partially linked to a sequence diversity in the COOH-terminal Nogo-66 residues of different RTN family members (62). A third region common to Nogo-A and Nogo-B, Nogo-A/B-Δ2 (rat aa59–172), also inhibits cell spreading but not neurite outgrowth (90) (FIGURE 1; Table 1).

### Table 1. Comparison of the different functional domains of Nogo-A

<table>
<thead>
<tr>
<th>Functional Domain</th>
<th>Function</th>
<th>Receptor/Signal Transducer(s)</th>
<th>Cell Type(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nogo-A/B-Δ2 (aa 59–172)</td>
<td>Cell spreading inhibition</td>
<td>Unknown</td>
<td>3T3</td>
<td>91</td>
</tr>
<tr>
<td>Nogo-A-Δ20 (aa 544–725)</td>
<td>Growth cone collapse</td>
<td>Unknown</td>
<td>E13–15 cDRGs, E19 rHNs</td>
<td>54, 91</td>
</tr>
<tr>
<td></td>
<td>Neurite outgrowth inhibition</td>
<td>Unknown/Integrin-dependent</td>
<td>E7–9 cRGCs, PC12, E9–11 cDRGs, P6–10 rDRGs, P6–8 CGNs</td>
<td>49–50, 54, 91</td>
</tr>
<tr>
<td></td>
<td>Cell spreading inhibition</td>
<td>Unknown/Integrin-dependent</td>
<td>3T3, COS-7, CHO-K1, HUVEC</td>
<td>49–50, 91</td>
</tr>
<tr>
<td>Nogo-A-ext/amo-Nogo (aa 1–979)</td>
<td>Cell spreading inhibition</td>
<td>Unknown/Integrin-dependent</td>
<td>3T3, COS-7</td>
<td>34, 49</td>
</tr>
<tr>
<td></td>
<td>Neurite outgrowth inhibition</td>
<td>E13 cDRGs, P4 msCGNs</td>
<td>34, 49</td>
<td></td>
</tr>
<tr>
<td>Nogo-66 (aa 1,026–1,091)</td>
<td>Growth cone collapse</td>
<td>NgR1, PirB</td>
<td>E12 cDRGs, P10 msDRGs</td>
<td>8, 34, 41</td>
</tr>
<tr>
<td></td>
<td>Neurite outgrowth inhibition</td>
<td>For soluble Nogo-66: NgR1/p75-dependent</td>
<td>E12–13 cDRGs, P4 msCGNs, PC12</td>
<td>19, 34, 41, 132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For substrate-bound Nogo-66: NgR1-independent, PirB-dependent/LINGO-1-, p75-, TROY-dependent</td>
<td>E12–13 cDRGs, P7 msCGNs, P10 or adult msDRGs</td>
<td>8, 19, 34, 41, 78, 93, 109, 132, 146</td>
</tr>
<tr>
<td>Nogo-A-24 (aa 966–989)</td>
<td>Increases the binding affinity of Nogo-66 to NgR 1; not inhibitory per se</td>
<td>NgR1, PirB</td>
<td>E13 cDRGs, COS-7</td>
<td>49, 52</td>
</tr>
<tr>
<td>Nogo-C39 (aa 1126–1163)</td>
<td></td>
<td>NgR1, PirB</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Nogo-22 (aa 966–1163)</td>
<td>Growth cone collapse</td>
<td>NgR1</td>
<td>E13 cDRGs</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Neurite outgrowth inhibition</td>
<td>NgR1, PirB-independent</td>
<td>Adult msDRGs, DIV21 CNs</td>
<td>52</td>
</tr>
</tbody>
</table>

Rat sequences are indicated. CNs, cortical neurons; CGNs, cerebellar granule neurons; DRGs, dorsal root ganglia; HNs, hippocampal neurons; RGCs, retinal ganglion cells; c, chicken; ms, mouse; r, rat; E, embryonic day; P, postnatal day; DIV, days in vitro.

...at Universitaet Zuerich on August 28, 2013...
amino-Nogo) possesses stronger inhibitory properties than Nogo-A-Δ20 (FIGURE 1; Table 1) (90). Together, these results suggest that multivalent interactions of different regions of Nogo-A with a heteromeric receptor complex might be critical for the activation of downstream signaling events.

**Receptors**

Two high-affinity interaction partners/receptors have been characterized for the Nogo-66 domain, whereas the molecular players interacting with Nogo-A-Δ20 are still largely unknown. Nogo-66 interacts with the Nogo-66 receptor 1 (NgR1) (34) and also with the paired immunoglobulin-like receptor B (PirB) (8) (FIGURE 2). NgR1 belongs to a family of three glycosylphosphatidylinositol (GPI)-anchored proteins (NgR1–3) lacking an intracellular signaling domain (126). To transduce intracellular signals, NgR1 interacts with the leucine-rich repeat (LRR) and Ig domain-containing Nogo receptor-interacting protein 1 (LINGO-1) (77) and with the low-affinity neurotrophin receptor p75NTR (131). Because the expression of p75NTR is temporally regulated and restricted in the adult CNS, the related tumor necrosis factor alpha (TNF-α) receptor superfamily member 19 (TROY) functionally substitutes for p75NTR (92, 108) (FIGURE 2). PirB also interacts with p75NTR for signal transduction (36). Surprisingly, two other structurally unrelated myelin proteins with growth inhibitory activity, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp), as well as the inhibitory non-myelin-associated protein B lymphocyte stimulator (BLyS) share the same receptor complex (29, 70, 131, 132, 137, 143). MAG binds the NgR1 homolog NgR2 with higher affinity than NgR1. NgR2, however, does not bind to Nogo-66 or OMgp (126). In addition, NgR1 and its third family member NgR3 bind to chondroitin sulfate proteoglycans (CSPGs) (26). NgR1 also interacts with the secreted proteins leucine-rich glioma inactivated (LG1) and olfactomedin-1, which antagonize

![Diagram](image-url)

Functional evidence for NgR1 and PirB acting as Nogo-66-specific receptors was presented in numerous assays using different neuronal cell types. Acute blockade of NgR1 with a function-blocking antibody, a competitive antagonist of Nogo-66 (NEP1–40), or a soluble NgR1 ectodomain substantially antagonizes neurite outgrowth inhibition and growth cone collapse mediated by Nogo-66, MAG, OMgp, or crude myelin extracts in vitro (29, 33, 40, 70). Conversely, exogenous expression of NgR1 induces growth cone collapse in neurons that would otherwise be insensitive to any of the NgR1 ligands (34, 70, 132). However, studies using neurons derived from NgR1 knockout (KO) mutants have shown that NgR1 is required for acute Nogo-66-mediated growth cone collapse but not for chronic substrate-bound growth inhibition (19, 59, 127, 145). On the other hand, functional deletion of PirB is sufficient to rescue Nogo-66-mediated growth cone collapse and to partially release Nogo-66- and myelin-mediated substrate-bound growth inhibition (8). Together, these results suggest that the molecular mechanisms underlying acute and chronic growth-inhibitory effects can be dissociated and suggest the existence of NgR1-independent mechanisms for long-term growth-inhibitory effects (19).

Several studies have shown that Nogo-A-Δ20-mediated inhibitory effects occur independently of NgR1 (105). Functional receptors for Nogo-A-Δ20 are being studied in several laboratories at present but remain still largely unknown. One study reported that Nogo-A-Δ20 inhibits the activation of the integrin-associated focal adhesion kinase (FAK) and that Nogo-A-Δ20-mediated growth inhibition could be overcome by activating integrins (49, 119). However, a proof of interaction using purified proteins remains elusive, and it is unclear how far the observed effects require intermediate proteins (49). Furthermore, the orphan G-protein-coupled receptor 50 (GPR50) was recently found to interact with Nogo-A-Δ20, albeit intracellularly in cis and mediating opposite effects on neurite outgrowth (43). Thus high-affinity functional receptors mediating Nogo-A-Δ20-induced growth inhibition in trans remain to be discovered.

**Intracellular Signaling Pathways**

Both Nogo-66 and Nogo-A-Δ20 trigger the activation of the small GTPase RhoA and of its effector Rho-associated, coiled-coil containing protein kinase (ROCK), which results in a destabilization of the growth machinery (84, 85, 105) (FIGURE 2). Rho GTPases, including RhoA, Rac1, and Cdc42, integrate upstream directional cues and trigger downstream cytoskeletal rearrangements, e.g., actin polymerization for growth and protrusion of lamelli- and filopodia on growth cones and ruffling membranes (Rac1, Cdc42), or depolymerization and actomyosin contraction for retraction (RhoA) (72, 101). In line with this, Nogo-66 and Nogo-A-Δ20 not only activate RhoA but also decrease the activity of Rac1 (24, 85). Pharmacological blockade or dominant-negative forms of RhoA and ROCK substantially prevent Nogo-A- and myelin-mediated growth inhibition in vitro (4, 25, 35, 85). In vivo, application of the ROCK blocker Y-27632 stimulates fiber sprouting and regeneration after spinal cord or optic nerve injury but also accelerates functional motor recovery in different models of spinal cord injury (17, 25, 35, 98). Enzymatic inactivation of RhoA via C3 transferase leads to similar but more variable results, most probably due to an inconsistent cell penetration (25, 35, 117). More recently, genetic deletion of the brain-specific ROCK isoform ROCKII confirmed the functional importance of this signaling pathway (31). Given the convergence of multiple inhibitory cues other than Nogo-A onto RhoA and ROCK, e.g., CSPGs or members of the Ephrin and Semaphorin families of axon guidance molecules (38), it is very likely that several inhibitors may be affected by targeting this pathway in vivo.

Downstream cytoskeletal effectors of the RhoA/ROCK pathway include myosin light chain (MLC) II and cofilin, which induce growth cone retraction by either promoting myosin II contractile activity or F-actin depolymerization, respectively (4, 48, 72, 84) (FIGURE 2). More precisely, Nogo-66 modulates the phosphorylation levels of cofilin via a ROCK-dependent sequential activation of LIM (Lin-11, Isl-1, and Mec-3) kinase (LIMK) and Slingshot (SSH) phosphatase (48). Accordingly, deactivation of the ROCK/LIMK/cofilin pathway has been associated with increased growth cone dynamics in Nogo-A KO mice (82). Besides affecting the actin cytoskeleton, Nogo-66 also destabilizes microtubule assembly through phosphorylation of the collapsin response mediator protein 2 (CRMP2) (79) (FIGURE 2). Upstream of Rho GTPases and their associated cytoskeletal effectors, CRMP4 and the Rho-guanine dissociation inhibitor (Rho-GDI) have been proposed to link Nogo-66 to RhoA activation (84). Nogo-66 is found to facilitate RhoA activation by strengthening the interaction of Rho-GDI with p75NTR and thereby releasing RhoA for conversion into its active form (138). In addition, Nogo-66 stimulation results in glycogen synthase kinase 3β (GSK3β) inactivation, thereby allowing an increased complex formation between CRMP4L and RhoA (5, 6) (FIGURE 2). Disruption of the CRMP4L-RhoA complex formation or knockdown of CRMP4L results in decreased growth inhibition on a myelin substrate (5, 6).
Other signaling components of Nogo-A include protein kinase C (PKC) and epidermal growth factor receptor (EGFR), whereby pharmacological blockade of PKCα/β and EGFR was shown to decrease Nogo-66 and myelin-mediated growth inhibition (46, 60, 112). In vivo, application of EGFR antagonists improved motor and sensory function after spinal cord injury (32), increased retinal ganglion cell (RGC) axon regeneration after optic nerve crush (60), and prevented RGC death in a glaucoma model (69). Intriguingly, the latter studies assigned phosphorylated EGFR expression in the adult CNS mainly to glial cells and not to neurons, suggesting that these effects might be glial and indirect (1, 30, 69). However, ensuing studies suggested that growth promotion induced by EGFR blockade might rely on off-target Trk activation by an increased secretion of neurotrophic factors rather than on EGFR inactivation (1, 2, 30). Thus these studies question a direct involvement of intraxonal EGFR in the Nogo-A signaling axis.

**Nogo-A Restricts Axonal Regeneration and Structural Plasticity After CNS Injury**

Acute treatment with Nogo-A neutralizing antibodies was repeatedly shown to increase regenerative sprouting and growth of lesioned as well as spared axons in different models of stroke and spinal cord injury (for detailed reviews, see Refs. 39, 147). Comparable anatomical results as well as improved recovery of lost functions are also obtained by blocking NgRI (33, 40, 133, 135) and the NgRI-associated protein LINGO-1 (52). However, differences were found with regard to the degree of axonal regeneration after spinal cord injury in independently generated Nogo-A, Nogo-A/B, and Nogo-A/B/C targeted or gene trap mutant mice (58, 64, 68, 106, 111, 146). Although two Nogo-A (111) and Nogo-A/B (58) mutant lines displayed increased axonal regeneration following a spinal cord dorsal hemisection, variable results with no significant effects were observed in three other Nogo-A/B and Nogo-A/B/C mutant lines (64, 146). Discrepancies were potentially due to strain background-dependent effects (27) and compensation by other Nogo isoforms or functionally related genes (66, 106).

Besides regenerative plasticity, acute neutralization as well as genetic deletion of Nogo-A have consistently proven effective in eliciting compensatory sprouting of spared fibers, i.e., non-regenerative plasticity, at different anatomical levels after injury. For example, after transection or elimination (by stroke) of the corticospinal tract (CST) that projects from the sensorimotor cortex to the spinal cord, a twofold increase in compensatory sprouting of unlesioned CST fibers was found in anti-Nogo-A vs. control antibody-treated groups (39, 65, 91, 121, 147). Non-regenerative plasticity of spared fibers is thought to allow the formation of “detour” connections from cortical neurons to spinal motor neurons by either sprouting into denervated areas and thereby re-innervating the spinal cord deprived of its major cortical input or by contacting other nonlesioned axonal tracts. Rearrangement of the intact circuitry may provide an important substrate for plasticity and functional recovery in the adult CNS. Thus increasing the low degree of spontaneously occurring non-regenerative plasticity by inactivating the Nogo-A signaling axis may significantly improve the treatment of spinal cord injury and stroke.

**Nogo-A Restricts Neuronal Plasticity in the Intact Adult CNS**

**Structural Plasticity and Fiber Growth in the Adult CNS**

The observation that Nogo-A neutralization results in a marked increase of compensatory, non-regenerative sprouting after CNS injury raised the question as to whether Nogo-A also limits plasticity in the intact CNS. Initial studies showed that acute neutralization of Nogo-A by antibody injection resulted in marked sprouting of uninjured axons in the cerebellum and spinal cord of adult rats (10, 14) (FIGURE 3, A–D). Inactivation of Nogo-A in mature organotypic hippocampal slice cultures also led to an increase in the growth and complexity of pyramidal axons and dendrites (22, 141) (FIGURE 3, E AND F). Similar results were observed in NgR1/2/3 triple knockout mice (136). Using Nogo-A KO and Nogo-A overexpressing transgenic mice, Nogo-A was also recently found to negatively regulate the dendritic growth and complexity of Purkinje cells in the cerebellum (95). At the molecular level, these structural changes are accompanied by a concomitant upregulation of growth-associated markers and transcription factors, which suggests that Nogo-A may actively suppress anatomical plasticity in the adult CNS by a tonic downregulation of growth-associated gene expression (10, 14, 22, 140) (FIGURE 2). Indeed, transcriptional profiling of hippocampal or cerebellar slices treated with Nogo-A-neutralizing antibodies as well as proteomic profiling of the CNS of adult Nogo-A KO mice pointed to a marked regulation of the growth cone cytoskeleton machinery and of growth-associated transcription factors toward increased growth (22, 82, 140). Tonic growth inhibition might result from retrogradely transported inhibitory signals from the axons to the cell bodies. Inactivation of Nogo-A around mature...
myelinated axons would thereby result in a disinhibition of the suppressed growth program, whereby the detailed mechanisms of action remain to be established (105). Interestingly, a recent study has shown that recombinant Nogo-AΔ20 is internalized and subsequently retrogradely transported in signaling endosomes to neuronal cell bodies where it downregulates cAMP response element binding protein (CREB) phosphorylation, i.e., CREB activation (53). Given that CREB is known to be retrogradely activated by the neurotrophic nerve growth factor (NGF) (21), it is tempting to speculate that Nogo-A uses similar signaling platforms to counteract the effects of growth factor signaling and to repress the growth program at a transcriptional level.

**FIGURE 3. Examples of restriction of axonal plasticity by Nogo-A in the intact adult CNS**

A: schematic representation of the corticospinal tract (CST; green) projecting from the sensorimotor cortex to the spinal cord. Anti-Nogo-A antibody induces sprouting of CST fibers ipsilaterally and, to a lesser extent, contralaterally across the midline in the noninjured spinal cord (red). B: magnification of the reorganization of CST projections occurring in the cervical enlargement in anti-Nogo-A- vs. control antibody-treated rats. C: schematic representation of the cerebellum. Purkinje cells extend axons (green) across the granule cell layer into the folium white matter. D: anti-Nogo-A antibody induces sprouting (red) of new collaterals from Purkinje cell axons within the granule cell layer. E: schematic representation of the hippocampus including two major synaptic pathways. Mossy fibers project from the granule cells to CA3 pyramidal neurons and Schaffer collateral fibers from CA3 to CA1 pyramidal neurons. F: anti-Nogo-A antibody induces sprouting of CA3 axons. Scale bar = 100 μm. F is reproduced from Ref. 141 with permission from the Society for Neuroscience.
**Experience-Dependent Plasticity**

Based on their role in limiting growth, Nogo-A and NgR1 were hypothesized to stabilize activity-dependent anatomical rearrangements and neuronal circuits during development, in particular at the end of the so-called “critical periods,” which are characterized by a highly plastic fine-tuning of synaptic connections (55, 105). In the visual cortex, monocular deprivation of one eye results in an expansion of the ocular dominance regions of the non-deprived contralateral eye within a defined postnatal time window. This can be measured by electrophysiological recordings or optical imaging. Interestingly, the onset of myelination and Nogo-A expression in oligodendrocytes of the visual cortex tightly correlates with the termination of the critical period (3, 76). Genetic deletion of Nogo-A/-B or its receptors NgR1 or PirB induces an increase in adult optical dominance plasticity, suggesting that these proteins are involved in the closure of highly plastic developmental periods (76, 118). However, relatively little is known about the precise underlying mechanisms and the extent to which changes in structural connections and/or synaptic strengthening are involved.

**Synaptic Plasticity, Long-Term Potentiation**

A number of developmental axon guidance cues including repulsive molecules of the Ephrin/Eph and Semaphorin/Plexin families are found at synapses and have been shown to influence synaptic plasticity, in particular long-term potentiation (LTP) and long-term depression (LTD) (109). Nogo-A and NgR1 are also found pre- and postsynaptically in different brain regions including the hippocampus (7, 63, 71, 134). Antibody-mediated neutralization of Nogo-A or NgR1 in acute hippocampal slices induces an increase in LTP that is not due to a change in basal synaptic transmission or short-term plasticity (23) (FIGURE 4A). Accordingly, application of Nogo-66 or OMgp attenuates LTP in a NgR1-dependent manner (97). However, the genetic deletion of Nogo-A,

![Graph A](image1.png)

**FIGURE 4.** Nogo-A restricts synaptic plasticity in the hippocampus

A: LTP was induced in acute hippocampal slices at Schaffer collateral-CA1 synapses by theta burst stimulation (TBS; arrow). Nogo-A or NgR1 neutralization significantly increases LTP. *Significant difference (P < 0.05). A is reproduced from Ref. 63 with permission from the National Academy of Sciences.

B: changes in dendritic spine morphology of CA1 pyramidal neurons. Genetic deletion of NgR1 induces morphological changes toward a more stubby- and less thin- or mushroom-shaped phenotype. Spine density remains unchanged. Significant difference: *P < 0.05; **P < 0.001. Scale bar = 5 μm. B is reproduced from Ref. 23 with permission from the Society for Neuroscience.
Neuronal Nogo-A levels then many types of neurons during their axonal growth system, in particular in postmitotic neurons and in During CNS Development.

Nogo-A Perturbs Cell Migration, Neurite Growth and Myelination During CNS Development

Nogo-A is expressed in the developing nervous system, in particular in postmitotic neurons and in many types of neurons during their axonal growth phase (50, 81, 94). Neuronal Nogo-A levels then decline progressively except for some types of neurons in highly plastic regions, and oligodendrocytes become the main site of Nogo-A synthesis during postnatal development and in adulthood (105). This pattern suggests functions of Nogo-A distinct from its role as a myelin-associated growth inhibitor (50). Anecdotally, an antibody originally designed to recognize growing axons in the developing olfactory bulb was found to specifically bind to Nogo-A (124). In vitro, genetic deletion or functional blockade of cell surface Nogo-A leads to an increase in neurite outgrowth, growth cone area, and motility in dissociated DRG neurons as well as to an increase in neurite fasciculation in DRG explants (82, 94). Similar results were also obtained by blocking NgR1 or downstream signaling molecules (82, 94). This suggests that axonal surface Nogo-A may have growth-, migration-, and fascicle-related functions during development, possibly by acting as a repulsive or growth-restricting cue.

During embryonic corticogenesis, Nogo-A is expressed in migrating and postmigratory neurons as well as in radial glial cells (75, 80). Using Nogo-A and Nogo-A/-B/-C KO mice, two studies have shown that Nogo-A deletion transiently delays tangential and radial migration patterns of neuronal precursor cells in vivo, suggesting a pro-migratory effect of Nogo-A (75, 80). Similarly, in the adult CNS, Nogo-A was recently shown to promote tangential migration of Nogo-A-expressing neuroblasts from the subventricular zone (SVZ) toward the olfactory bulb (OB) by providing Nogo-A-Δ20-specific anti-adhesive signals (99). In contrast, the Nogo-66 domain of Nogo-A inhibits the migration of olfactory ensheathing glial cells, which are themselves essential for the elongation of olfactory receptor axons, in vitro and in vivo (86, 116). Although it is difficult to understand how these results are reconciled, it was suggested that Nogo-A differentially modulates migration depending on the signaling domains and cellular systems (99). Along this line, whereas Nogo-A-Δ20 promotes the migration of neuroblasts toward the OB, Nogo-66 reduces the proliferation of neural stem cells in the SVZ before their migration, thereby also contributing to neurogenesis in the adult SVZ (99).

Because neuronal migration and axon guidance are tightly coordinated during development and often share similar substrates and cues, it was not surprising to find that neutralization of Nogo-A also results in misguidance phenotypes (73). Herein, Nogo-A inactivation led to miscrossed projections of optic nerve axons and spinal cord commissural axons during embryonic development (129, 130). In ovo antibody-mediated neutralization of Nogo-A in chicken embryos resulted in enhanced fascilitation and reduced branching of peripheral nerves leading to aberrant innervation patterns of the
hindlimb (94). Similar results were also observed in Nogo-A KO mouse embryos (94).

On the other hand, glial-derived Nogo-A is specifically involved in oligodendrocyte differentiation, channeling of CST axons down the spinal cord, and myelin formation in vivo (20, 93, 107, 144). Recently, Nogo-A was shown to participate as a repulsive signal in the competition of developing oligodendrocytes for axonal myelination and spatial distribution of myelin segments (internodes) (20). Internodes vary greatly in number and length in similar CNS axon tracts (20). Interestingly, Nogo-A20 but not Nogo-66 was found to inhibit intercellular interactions between oligodendrocytes competing for axon space, resulting in a spatial segregation of myelin internodes. In line with this, genetic deletion of Nogo-A resulted in an increased myelinogenic potential of oligodendrocytes in the developing cerebral cortex without altering global myelination patterns (20). This was accompanied by an increase in the number of oligodendrocyte precursor cells and a decrease of mature oligodendroytes (20). Consistent with a role of Nogo-A on oligodendrocyte differentiation, a transient delay in oligodendrocyte maturation was observed in the optic nerve of Nogo-A KO mice in vivo (93). In vitro, antibody-mediated neutralization of Nogo-A inhibited the differentiation of cortical OPCs (144).

Conclusions and Future Perspectives

Nogo-A was initially identified as a major myelin-associated inhibitor of axonal regeneration after CNS injury. Nogo-A binds to different cell surface receptors and activates signaling cascades that induce a collapse of the cytoskeleton and a down-regulation of the neuronal growth machinery. Although specific receptors for the Nogo-A-A20 domain remain to be identified, there is a general consensus on the existence of a multi-site/multi-subunit ligand/receptor complex consisting of structurally unrelated components. Long-term changes of Nogo-A signaling include a suppression of growth-related gene expression at the transcriptional level, albeit the exact mechanisms remain to be elucidated in details.

In the intact adult CNS, Nogo-A is thought to act as a stabilizer and regulator of neuronal networks by restricting various plastic processes, e.g., structural, activity- and experience-dependent plasticity. Increasing evidence also suggests that Nogo-A modulates migratory, growth, and contact-dependent processes during development. It will be important to understand to which extent these processes are affected by neuron-to-oligodendrocyte, neuron-to-neuron, or oligodendrocyte-to-oligodendrocyte interactions. Given the dual expression pattern and spatio-temporal regulation of Nogo-A, its receptor-binding promiscuity and multiple signaling domains, the cellular mechanisms by which Nogo-A exerts different physiological roles seem to be very complex and are far from being fully understood. Overall, Nogo-A/Nogo-A receptor interactions restrict growth-dependent processes, leading to a stabilization of the existing CNS circuitry. Lifting these brakes allows for the induction of extensive structural and functional rearrangements, e.g., after injury.

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References


