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Nogo limits neural plasticity and recovery from injury

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The expression of Nogo-A and the receptor NgR1 limits the recovery of adult mammals from central nervous system injury. Multiple studies have demonstrated efficacy from targeting this pathway for functional recovery and neural repair after spinal cord trauma, ischemic stroke, optic nerve injury and models of multiple sclerosis. Recent molecular studies have added S1PR2 as a receptor for the amino terminal domain of Nogo-A, and have demonstrated shared components for Nogo-A and CSPG signaling as well as novel Nogo antagonists. It has been recognized that neural repair involves plasticity, sprouting and regeneration. A physiologic role for Nogo-A and NgR1 has been documented in the restriction of experience-dependent plasticity with maturity, and the stability of synaptic, dendritic and axonal anatomy.

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Introduction

The longitudinal growth of nerve fibers, the regeneration of injured axons and the structural plasticity of axons and dendrites are confined to very short distances and limited spatial dimensions in the adult mammalian central system (CNS). The successful regeneration of adult CNS axons of multiple origins into peripheral nerve grafts placed into spinal cord, brain or optic nerve over centimeter distances emphasized the key role of factors from the local tissue microenvironment in determining the extent of growth [1]. Twenty years ago, specific neurite growth inhibitory factors, many of which were enriched in myelin, were discovered. Nogo-A, the myelin proteins, MAG and OMgp, several semaphorins and ephrins as well as chondroitin sulphate proteoglycans have been identified [2]. For many of these molecules the detailed expression pattern in the adult CNS, the possible interplay between single factors, as well as their *in vivo* roles in the intact or injured adult CNS are not well characterized yet. More information is available for the membrane protein Nogo-A; some key concepts and the most recent literature on Nogo-A will be summarized in this short review.

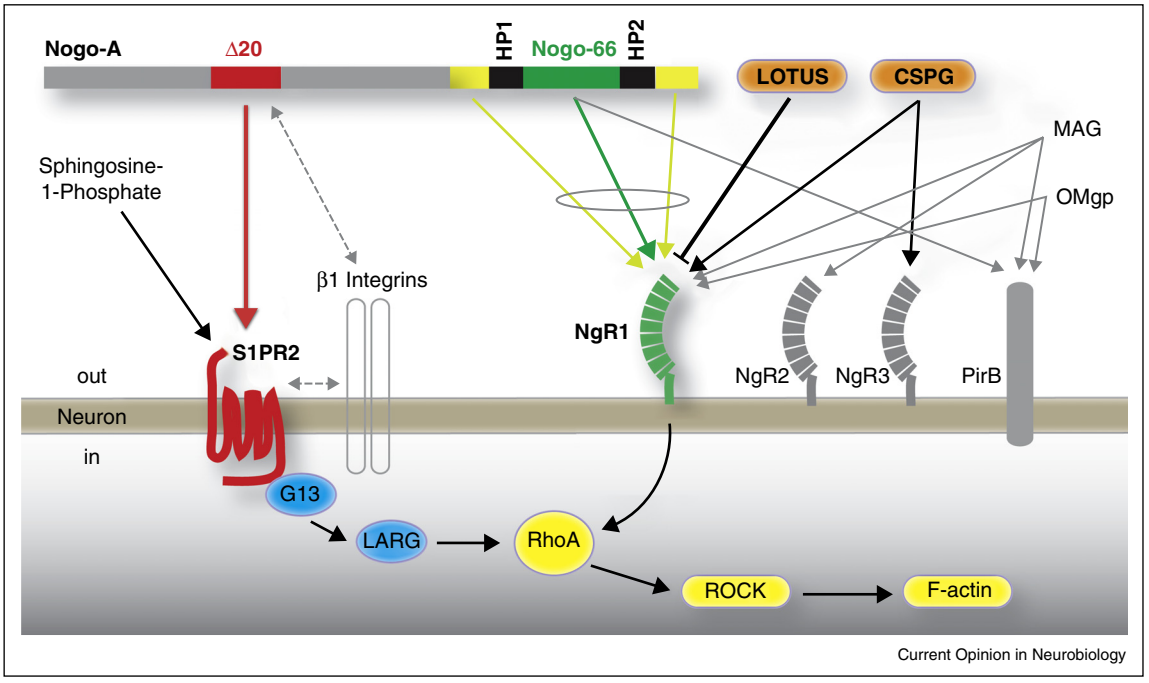
Nogo-A interacts with a multisubunit receptor complex

Fragment analyses and binding studies showed that the 1200 aa protein Nogo-A contains more than one growth inhibiting domain (Figure 1). The 66 aa extracellular loop between the two C-terminal intramembrane segments binds to a GPI-linked, LRR-containing membrane protein called NgR1. Signaling is induced by a receptor complex containing the membrane proteins p75 and/or Troy and the LRR-protein Lingo1 [3–5]. An alternative Nogo-66 receptor is PirB. PirB is expressed at low or undetectable levels in many parts of the adult CNS, although its expression may be more prominent during neural development or after ischemia [6]. Activation of the signal transducer rho A and ROCK are downstream of the Nogo-66 interaction with NgR1 and mediate growth cone collapse and growth arrest of neurites [3,7]. Binding to NgR1 is enhanced to picomolar affinities by flanking sequences in particular in the Nogo-A specific domain [8].

Recent work has expanded our understanding of NgR1 regulation and function at two levels. Studies of the olfactory system development led to the identification of Cartilage Acidic Protein-1B (LOTUS) as an antagonist of Nogo-66 binding to NgR1 [9•]. The role of this endogenous antagonist for adult injury settings requires further study. Another line of work showed that NgR1 and the related protein NgR3 are not receptors solely for Nogo-A. They also participate in binding and axon growth inhibition by the chondroitin sulfate proteoglycans (CSPGs) known to be enriched in glial scars and perineuronal nets [10•]. Thus, oligodendrocyte-derived Nogo-A may share receptor mechanisms with other, astrocyte-derived inhibitors of nerve fiber growth and repair.

A second active region of Nogo-A is found in the N-terminal extracellular region ('Nogo-A d-20' or 'amino-Nogo') [5]. While its activity was known to depend on integrin engagement [11], its mechanism had been unclear until very recently. The sphingosine-1-phosphate receptor 2 (S1PR2) has now been identified as a specific binding partner and signal transducer for Nogo-A d-20 in neurons and Nogo-A responsive non-neuronal cells by a

Figure 1



Molecular mechanisms related to Nogo-A signaling. The schematic illustrates the two growth inhibiting domains of Nogo-A, the Δ20 region and the Nogo-66 region. The two hydrophobic (HP1, HP2) segments are highlighted, as well as two adjacent regions (light green) that cooperate with Nogo-66 activity. The Δ20 region interacts with S1PR2, and the Nogo-66 region interacts with NgR1, and both can couple to RhoA and Rho-Associated Kinase (ROCK) signaling. Additional recent findings demonstrate that chondroitin sulfate proteoglycans (CSPGs) can inhibit growth via both NgR1 and NgR3, and that the LOTUS protein antagonizes Nogo-66 signaling. Further discussion and references are provided in the text.

yeast 2-hybrid screen, co-immunoprecipitation, binding studies and *in situ* co-localization experiments [12••]. Nogo-A binding to this 7-transmembrane domain, G-protein coupled receptor activated the G-protein G-13, the rhoGEF LARG and rho A; all these steps are required for Nogo-A d-20 induced inhibition of neurite growth or fibroblast spreading. Whether NgR-1 and S1PR2 and their associated co-receptor proteins occur and function always in a complex or also independently, as well as a possible modulatory role of S1P on Nogo-A effects remain to be studied in detail. The role of three other recently identified Nogo-A binding proteins, GPCR-50 [13], olfactomedin [14] and LRP1 [15] is currently unclear. However, since NgR1 binds several other ligands in addition to Nogo-66, a multi-subunit receptor complex for Nogo-A with two (or more) binding subunits represents a highly attractive model for signal transduction with high specificity, analogous to what is known for for example, neurotrophins, semaphorins or Wnt.

Nogo-A destabilizes the cytoskeleton and suppresses the cellular growth program

Growth cones collapse and neurite elongation stops upon contact with Nogo-A in a rho/ROCK dependent way. Interestingly, internalization of Nogo-A was shown to be required for these effects in growth cones of hippocampal neurons [16]. Integrin function is also affected by Nogo-A [11], and activation of integrins can overcome the Nogo-A mediated growth inhibition [17•]. The actin cytoskeleton-based lamellipodia and filopodia of growth cones are affected at an early stage of the collapse. Subsequently, internalized Nogo-A d-20 is transported retrogradely to neuronal cell bodies in signaling endosomes, a process leading to increased rho A and decreased phospho-CREB levels in cultured sensory neurons [16]. In hippocampal neurons Nogo-A was shown to decrease the activation of the key growth regulator mTOR [18].

Long-term effects of Nogo-A KO or neutralization showed sprouting of hippocampal axons and dendrites as well as upregulation of a number of growth associated genes in the adult brain and spinal cord [19–21]. Together with the findings on the role of Nogo-A as a negative controller of CNS plasticity summarized below, these results suggest a physiological role of Nogo-A as a factor controlling growth and plasticity in the adult CNS.

Suppression of Nogo-A/Nogo receptor signaling enhances repair after CNS injury

Spinal cord injuries affecting up to half of the spinal cord diameter or strokes destroying only part of the motor cortex often have a good prognosis with substantial

spontaneous functional recovery in animals and humans. Compensatory sprouting of intact fiber systems and formation of new circuits including indirect 'detour' pathways are currently emerging as important mechanisms underlying these functional recovery processes [22–24]. The factors which trigger and guide growing fibers, select and stabilize functionally meaningful connections and prune circuits leading to malfunction are almost entirely unknown, however. Neurite growth and regeneration can be stimulated by upregulating the neuronal growth machinery, in particular via stimulation of the mTOR and the Stat3 pathways as shown mostly by anatomical data in rodents [25]. Overstimulation of sprouting by deletion of the important mTOR regulator PTEN can also lead to epilepsies and tumors, however [26–28]. In injury models, the functional consequences of unregulated stimulation of the neuronal growth program remain to be shown.

Many years of animal studies in a variety of models and species have shown that down-regulation or functional blockade of Nogo-A signaling leads to enhanced regenerative sprouting and fiber elongation and to enhanced compensatory fiber growth over distances exceeding those occurring spontaneously [2,28,29]. Interestingly, these anatomical changes were consistently associated with functional improvements in the absence of malfunctions, in different lesion models, species and using different ways to suppress Nogo function. These results suggest that temporarily restricted suppression of Nogo/Nogo receptor actions enhance growth within a CNS microenvironment that still allows for some growth control and target selection.

The *methods used to suppress Nogo-A/Nogo receptor functions* in the adult, injured CNS include the intrathecal application of function blocking anti-Nogo-A antibodies, soluble NgR1-Fc fusion proteins, peptides blocking NgR1, immunization against Nogo or NgR1, or pharmacological blockers of rho or ROCK [28]. Interestingly, these acute interventions gave consistently stronger results than mice KO for Nogo-A, double KO for Nogo-A and MAG, or triple KO for Nogo-A, MAG and OMgp [5,30,31]. This suggests an important role of the compensatory up-regulation of other inhibitory factors as seen in the Nogo-A KO for ephrinA3, EphA4, Sema 4D and 3F and plexin B2 [32]. While compensatory and regenerative sprouting was consistently enhanced in different KO lines from different laboratories, the extent of growth and regeneration differed among the lines. The higher endogenous growth capacity of Sv129 strain neurons compared to C57Bl6 neurons in these mixed background lines may be an additional confounding factor [33].

Spinal cord injury

In rat, mouse and monkey models of large spinal cord injuries, suppression of Nogo-A/Nogo receptor signaling by many of the methods mentioned above resulted in enhanced anatomical plasticity and regrowth of functionally important fiber systems such as the corticospinal tract or descending serotonin and dopamine fibers. Significant recovery of locomotion, balance, skilled stepping over irregular ladders and fine forepaw (rats, mice) and finger movements (monkeys) appeared within 2–4 weeks of the treatment [28,34]. Starting the anti-Nogo therapy within a few days after the lesion was more efficient than delayed treatments [35], although efficacy has been demonstrated with soluble NgR1–Fc fusion proteins in the chronic phase of spinal cord contusion, months after the trauma [36]. Serotonin fibers showed layer specific reinnervation patterns, and the decrease of post-lesion spastic contractions after anti-Nogo-A antibodies in spinal cord injured rats suggest formation of functionally correct connections [37,38].

Stroke models

A high degree of functional restoration of skilled forelimb reaching in rats with large unilateral strokes or pyramidal tract lesions was observed with many interventions blocking Nogo-A or its receptor. Anatomically, corticospinal fibers from the intact side sprout across the midline, for example, of the spinal cord, retract their original arbor and functionally innervate the denervated spinal cord [23,39]. Interestingly, the functional and anatomical effects of intrathecal anti-Nogo-A antibodies were also observed in aged rats as well as after antibody application delayed up to 2 months [40,41]. Recovery of hand function and cortical map shifts were also observed in macaques following Nogo-A suppression [42]. Combined suppression of Nogo-A (by antibodies) and CSPGs (by chondroitinase) resulted in a higher degree of recovery of food pellet reaching in rats with large unilateral strokes [43].

Optic nerve lesions

In optic nerve lesions, NgR1 knock down or KO, Nogo-A neutralization or rho/ROCK blockade leads to enhanced regenerative sprouting, but rarely to elongation over more than 1–2 mm [44]. In this system, however, the rapid death of retinal ganglion cells and the very low intrinsic growth potential of these neurons complicate the situation. High levels of CNTF, Stat 3 activation or PTEN deletion or combinations of these interventions led to a higher level of optic nerve axon regrowth, but also to many misdirected axons, including about half of the axons returning toward the retina [45•,46•]. Only limited evidence exists up to now for functional benefits with innervation beyond the chiasm [47]. Of note, NgR1 decoy receptor therapy rescues retinal ganglion cell survival in a glaucoma model [48].

Multiple sclerosis

In the multiple sclerosis animal model of EAE, antibody mediated Nogo-A neutralization, systemic siRNA against Nogo-A, Nogo-A/B KO, or NgR1 deletion showed a beneficial effect on the clinical outcome [49–52].

Whether enhanced fiber growth, circuit plasticity and functional compensation and/or effects of myelin repair [53•] or immunological reactions are responsible for these functional improvements remains to be analysed.

Clinical studies

Early phase clinical trials are currently in progress for spinal cord injury, MS and amyotrophic lateral sclerosis (ALS) using intrathecal or systemic administration of function blocking anti-Nogo-A antibodies (see www.clinicaltrials.gov) [54]. Parallel studies with anti-MAG antibodies in human stroke recovery gave indications for improved walking speeds with therapy [55]. A trial using a function blocking NgR1-Fc protein is in preparation.

Nogo-A and NgR1 signaling titrates experience-dependent plasticity

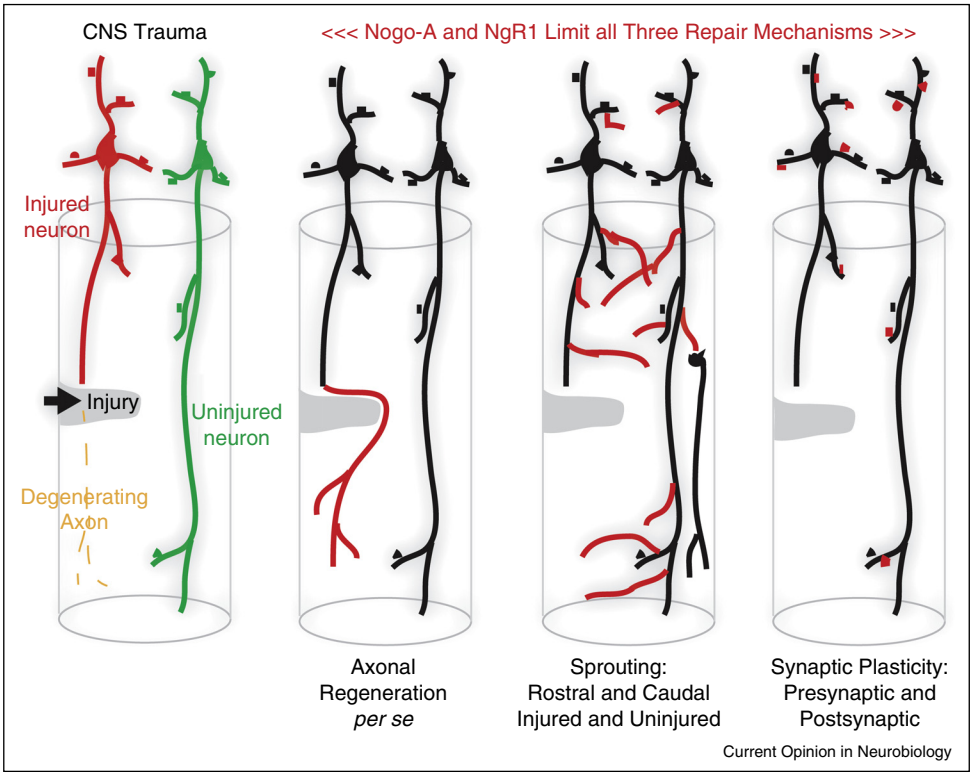
It is abundantly clear that interruption of the Nogo-A to Nogo-66 receptor pathway increases functional recovery after injury by a combination of neuronal plasticity, short range sprouting and axonal regeneration. The observation of anatomical rearrangements far from the injury site and by uninjured pathways raises the question of the natural role of this Nogo pathway for anatomical stability in the adult nervous system (Figure 2).

It is well appreciated that experience-dependent neural plasticity decreases during the transition from adolescence to adulthood, and a classic manifestation of this phenomenon is the closing of a critical period for robust ocular dominance plasticity in binocular cerebral cortex. Indeed, it has been shown that mice lacking either NgR1 or Nogo-A maintain adolescent critical period plasticity of the visual system into adulthood [56]. Since a similar phenotype of enhanced adult plasticity is also observed when CSPGs are digested [57], each of the major molecular categories limiting recovery from injury appears to play a natural role in stabilizing the adult central nervous system. A recent description of CSPGs as ligands for NgR1 and the related cell surface protein, NgR3, suggests that functional similarities may derive from mechanistic similarities in signal transduction [10•].

Acute electrophysiological plasticity

The role of Nogo-A and NgR1 in limiting experience-dependent plasticity in adulthood might occur by either

Figure 2



Cellular mechanisms of neural repair limited by Nogo-A and NgR1. Injuries to the central nervous system of traumatic, ischemic or other etiology typically damage certain axonal pathways but spare parallel pathways. Recovery and repair can be supported by a range of cellular events, here grouped into three main mechanisms. The cut axon can grow back over long distances by regeneration, or uninjured and injured fibers that are rostral and caudal to the injury can sprout to form new connections, or plastic changes of presynaptic and postsynaptic connections throughout the nervous system. Data show that Nogo-A and NgR1 limit all three of these mechanisms, and blockade of their action supports repair and recovery. Further discussion and references are provided in the text.

acute electrophysiological alterations and/or more chronic changes in anatomical stability. While the latter morphologic mechanism appears to parallel the role of Nogo-A and NgR1 in the recovery from neurological trauma, there is also evidence for more acute regulation of synaptic transmission by these molecules. Studies of hippocampal slice long-term potentiation (LTP) demonstrated that administration of soluble Nogo-66 or OMgp altered synaptic plasticity, suppressing LTP and enhancing LTD [58,59]. These effects were dependent on the presence of NgR1 as a receptor. In separate studies, acute neutralization of endogenous Nogo-A or NgR1, or *in vivo* knock down of Nogo-A increased LTP [60,61]. Thus, acute modulation of synaptic plasticity may contribute to the ability of Nogo-A and NgR1 blockade to enhance recovery from CNS injury.

Stability of adult synaptic anatomy is dependent on Nogo-A and NgR1 signaling

A broad range of studies have implicated Nogo-A and NgR1 in determining the formation and stability of synaptic morphology over longer time scales. In hippocampal slices, post-synaptic dendritic spine architecture, as well as axonal length, was found to depend on Nogo-A and NgR1 signaling [20•]. Interruption of Nogo-A or NgR1 expression produced more immature appearing dendritic spines. Genetic deletion of NgR1, 2 and 3 demonstrated a key role for these proteins as a brake on synaptogenesis both *in vitro* and *in vivo* during the initial stages of circuit formation [62•]. Interactions amongst the NgR family suggest partial redundancy in function, and this emphasizes the overlapping roles of CSPGs and myelin derived inhibitors in both limitation of neural plasticity and neural repair. Nogo-A regulation of synaptic development is not restricted to hippocampus. A recent study demonstrated that cerebellar Purkinje cell expression of Nogo-A limits both dendritic arborisation and synaptic inputs [63].

More recently, studies of Nogo-A and NgR1 regulation of synaptic stability have been extended via chronic time lapse *in vivo* imaging of anatomy [64••]. In mice lacking these molecules, synaptic structures were monitored for weeks in the living mouse. The dynamics of dendritic spines and axonal varicosities in the superficial layers of cerebral cortex was enhanced in mice lacking either NgR1 or Nogo-A. In fact, the stabilization of synaptic anatomy which normally occurs in late adolescence was not observed. In addition, the ability of altered sensory input to enhance anatomical turnover was shifted such that lesser degrees of vibrissal activity yielded greater degrees of anatomical change.

Behavioral consequences of Nogo-A and NgR1 signaling

Without Nogo-A or NgR1 signaling, adolescent critical periods for experience dependent plasticity remain open and the recovery from CNS injury is enhanced. Are these data associated with altered behavior? One clue may come from the ability of high levels of neuronal excitation to suppress NgR1 levels [62•,65], perhaps allowing anatomical plasticity to follow periods of hyperexcitation. In a study of mice expressing elevated levels of NgR1 [65], learning was intact but lasting memory formation was impaired. It was suggested that NgR1 downregulation is required to allow the anatomical rearrangement of synaptic connections underlying long term recall. The extinction of conditioned fear memories has been associated with the formation of new dendritic spines in prefrontal cortex [66]. Correspondingly, the enhanced cortical spine turnover of NgR1 null mice is associated with enhanced fear extinction [64••], suggesting the possibility of therapeutic blockade of Nogo-A or NgR1 to reduce posttraumatic stress disorder. It has been suggested that excess unregulated plasticity related to Nogo-A, myelin or NgR1 genetic variability could contribute to neuropsychiatric conditions such as schizophrenia [61,67–69].

Conclusions

The Nogo-A and NgR1 signaling cascade have been studied extensively with regard to neurological trauma. Blockade of these pathways leads to greater functional recovery through a combination of neural plasticity, sprouting and axonal regeneration. The benefits of interrupting Nogo-A or NgR1 are observed in a broad spectrum of preclinical models and are beginning clinical evaluation. Recent molecular studies have demonstrated a signaling pathway for a second domain of Nogo-A that involves the S1PR2 receptor. In addition, substantial overlaps between Nogo-A and CSPGS have been revealed for both signaling molecules and cellular consequences. The physiologic role of Nogo-A and NgR1 signaling has attracted much attention. This pathway limits adult brain plasticity at the level of acute electrophysiological modulation, shown most dramatically in the anatomical stability of synaptic morphology. Thus, releasing the brakes on anatomical plasticity, which are applied during the transition from adolescence to adulthood, provides an attractive pathway for neural repair therapeutics.

Conflict of interest

SMS is a co-founder of Axerion Therapeutics, seeking to develop NgR-based and PrP-based therapeutics.

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