Published in final edited form as:
Trends Neurosci. 2013 June ; 36(6): 363–373. doi:10.1016/j.tins.2013.04.003.

Where No Synapses Go: Gatekeepers of Circuit Remodeling and Synaptic Strength

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Abstract

Growth inhibitory molecules in the adult mammalian CNS have been implicated in blocking axonal sprouting and regeneration following injury. Prominent CNS regeneration inhibitors include Nogo-A, OMgp and CSPGs, and a key question concerns their physiological role in the naïve CNS. Emerging evidence suggests novel functions in dendrites and at synapses of glutamatergic neurons. CNS regeneration inhibitors target the neuronal actin cytoskeleton to regulate dendritic spine maturation, long-term synapse stability, and Hebbian forms of synaptic plasticity. This is accomplished in part by antagonizing plasticity-promoting signaling pathways activated by neurotrophic factors. Altered function of CNS regeneration inhibitors is associated with mental illness and loss of long-lasting memory, suggesting unexpected and novel physiological roles for these molecules in brain health.

Keywords

Nogo receptor; Nogo-A; OMgp; proteoglycan; synaptic structure; dendritic spine; synapse stability

Introduction

Proper nervous system function critically depends on the precise assembly and maintenance of an intricate synaptic network. Once the initial scaffold of neuronal connections has been laid down, refinement processes continue to sculpt and transform microcircuits into a mature brain and spinal cord. In many regions of the early postnatal CNS, neuronal activity drives network refinement. A classic example is the experience-driven formation of ocular

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The authors declare no conflicts of interest

dominance (OD) columns in the primary visual cortex binocular zone. In the mature brain, neuronal architecture is more stable but it remains subject to changes as part of an adaptive response to learning, aging, injury or disease [1–3]. In adulthood, neuronal structural alterations are largely confined to synapses and typically are the result of prolonged changes in activity at these (or nearby) synapses. Remarkably, many human brain disorders, including schizophrenia, autism, and various forms of mental disability, are correlated with changes in synaptic shape or density and are believed to be caused by an imbalance between neuronal excitation and inhibition. Detailed knowledge of the molecular programs that regulate the strength and number of synapses is important for understanding brain function, and ultimately this will provide insights into how these processes are dysregulated in neurological disorders.

Here we discuss recent findings on the physiological function of CNS regeneration inhibitors and their receptors in the naïve brain. Our primary focus is on the function of Nogo-A, oligodendrocyte myelin glycoprotein (OMgp), and chondroitin sulfate proteoglycans (CSPGs) at the synapse. Emerging evidence suggests that these proteins stabilize synaptic structure and also regulate activity-dependent neurotransmission.

A large and structurally diverse group of neuronal growth inhibitors

The adult mammalian CNS is a rich source of molecularly diverse growth inhibitory cues, including proteinaceous components, carbohydrates and lipids (Figure 1). Many repulsive axon guidance molecules, including semaphorins, ephrins, slits, and netrins, continue to be expressed in the CNS long after the initial scaffold of axonal connections has been established [4]. The extracellular matrix (ECM) components CSPGs and keratan sulfate proteoglycans (KSPGs) function as prominent inhibitors of neuronal growth [5–7]. Sulfatide, a major CNS myelin lipid, strongly inhibits retinal ganglion cell axon outgrowth [8]. Additional important players include the prototypic myelin-associated inhibitors (MAIs) Nogo-A, OMgp, and myelin-associated glycoprotein (MAG) [4, 9]. Nogo-A is the largest splice form of the reticulon 4 gene and comprised of at least two distinct growth inhibitory domains, called NogoΔ20 and Nogo66 (Figure 1). Because of their profound growth inhibitory effects on developing neurites *in vitro*, collectively these inhibitory cues are thought to contribute to the regenerative failure of injured CNS axons *in vivo*. Indeed, acute blockage of MAIs or CSPGs in spinal cord injured (SCI) animals has met with some success [5, 6, 10, 11]. However, germline ablation of one or several MAIs in mice results in inconsistent SCI regeneration phenotypes [12, 13]. Here we focus on the physiological role of CNS regeneration inhibitors in the naïve brain. For a more in-depth discussion of these molecules in the injured CNS we refer to recent reviews [4–6, 9, 11, 14].

Mechanistic studies have identified a large number of neuronal surface receptors for CNS regeneration inhibitors (Figure 1), some of which operate in a functionally redundant manner. Moreover, depending on the neuronal cell type, the same inhibitory cue may use different receptors [4]. The Nogo66 receptor 1 (NgR1) is the founding member of a small subfamily of lipid-anchored, leucine-rich repeat (LRR) proteins that also includes NgR2 and NgR3. NgR1 supports binding of the Nogo inhibitory peptide Nogo66, OMgp, and MAG. Similar to NgR1, the type-1 transmembrane protein paired Ig-like receptor B (PirB) can form a complex with Nogo66, MAG, or OMgp [4, 9]. Primary neurons deficient for *NgR1* or *PirB* are largely resistant to growth cone collapse induced by acutely applied inhibitors. However, when plated on substrate-bound growth inhibitors, neurons deficient for *PirB*, but not *NgR1*, exhibit enhanced neurite outgrowth [15–18]. Sulfated proteoglycans, including HSPGs, KSPGs and CSPGs, are comprised of a protein core with covalently attached glycosaminoglycan (GAG) side chain(s) (Table 1). The chemical composition of the CS-GAG chain greatly influences the inhibitory nature of CSPGs and their binding affinity to

the neuronal surface receptors leukocyte common antigen related protein (LAR), its homolog RPTPσ [19–21], or NgR1 and NgR3 [22]. Of particular interest is the di-sulfated GAG CS-E, since it exerts strong inhibitory activity toward primary neurons and binds directly to RPTPσ, NgR1 and NgR3 [21–23]. Additional receptors for CNS regeneration inhibitors have been identified; MAG not only interacts with NgR1 and PirB, but also associates with complex brain gangliosides, NgR2, β1-integrin, and LDL receptor related protein-1 (LRP1) (Figure 1). These molecular interactions contribute to various aspects of neuronal growth inhibition *in vitro* [24–27]. The biological significance of many of these ligand-receptor complexes *in vivo*, both in CNS health and injury, is still poorly understood, and remains a major focus of ongoing research efforts.

Putting the brakes on neuronal plasticity

In the postnatal and juvenile brain, the structure of many neurons is refined in an experience-dependent manner in order to optimize internal processing of external inputs. Refinement occurs during the critical period (CP), a time-window during which specific inputs result in heightened network plasticity. After the CP, networks are mature and structurally much more stable ensuring optimal information flow and processing. CPs are of fundamental importance in molding microcircuits in various brain regions associated with sensory perception, motor learning, and language. Pharmacological and genetic manipulations that perturb the timing of CPs have begun to shed light on the molecular basis for network stability and how it can be modulated (Figure 2). In the visual system, enhanced maturation of GABAergic interneurons, or local infusion of benzodiazepines, increases intracortical inhibition and expedites the onset and closure of the CP. Conversely, reducing GABA function by genetic ablation of *GAD67*, or dark-rearing of animals, delays CP onset [28]. Seemingly unrelated manipulations, such as antagonizing inhibitors of neuronal growth, can have a profound impact on CP closure (Figure 2). Extracellular cues that put the brakes on neuronal remodeling after experience-dependent refinement is complete include *Nogo-A/B* [29] and a small subset of classical *MHC1* molecules [30]. Similarly, *NgR1*- and *PirB*-deficient mice fail to stabilize neuronal connectivity in the primary visual cortex (V1), resulting in an expansion of the CP into adulthood [29, 31]. Infusion of the CS-GAG-digesting enzyme chondroitinase-ABC (ChaseABC) into the visual cortex of adult rats is sufficient to increase visual experience-driven neuronal plasticity [32]; remarkably, this can also promote recovery from amblyopia inflicted by reverse suture of one eye during the CP [33]. Toward the end of the CP, the extracellular matrix in V1 undergoes substantial remodeling. CSPG levels increase and condense into ternary structures known as perineuronal nets (PNNs). Interestingly, the ratio of sulfated CS-4 to CS-6, and not the overall CSPG expression, is crucial for the closure of the CP [34]. Several independent lines of evidence show that genetic approaches that perturb PNNs lead to an expansion of the CP into adulthood [34–37]. The recent identification of NgR1, and its close homologue NgR3, as receptors for CSPGs suggest that Nogo-A/B and CSPGs share overlapping receptor components and perhaps signal through related receptor complexes [22]. Moreover, tying Nogo-A, CSPGs, NgR1, and possibly PirB and MHC1, to the same receptor complex may explain why individual manipulation of each of these molecules results in increased OD plasticity beyond the CP. As discussed below, Nogo-A, OMgp, and NgR1 negatively regulate neurotransmission, and similar to what is observed following alteration of GABA signaling, they also influence the balance between excitatory and inhibitory transmission, thereby controlling the onset and closure of the CP. Collectively, these studies show that Nogo-A, CSPGs and their receptors restrict neuronal growth at the end of the CP and thereby help to stabilize and maintain the structure of mature microcircuits. Although beneficial for network stability in the naïve CNS, Nogo-A and CSPGs directly contribute to the growth inhibitory nature of adult CNS tissue, and as such negatively influence network repair flowing injury. The close overlap of molecules that consolidate structure of neuronal

circuits at the end of the CP and limit axonal growth and sprouting following injury is remarkable, and suggests that one important physiological role of CNS regeneration inhibitors is to ensure long-term network stability.

Nogo-A and CSPGs promote dendritic spine maturation and stability

Is there any evidence that CNS regeneration inhibitors regulate neuronal structure in a cell-autonomous manner? Although many growth inhibitors in the adult CNS are expressed by glia, neuronal expression has clearly been demonstrated (Figure 3). Nogo-A and OMgp, for example, are expressed by excitatory and inhibitory neurons and are found in pre- and postsynaptic density fractions [38, 39]. Several CSPGs are expressed by glia and neurons and are enriched in PNNs and the ECM near synapses [40]. Similarly, synaptic localization of their cognate receptors has been reported, including NgR1 [41, 42], PirB [31], LAR, RPTPσ [43–46], LRP1 [47] and integrins [48]. In hippocampal pyramidal neurons, NgR1 and Nogo-A regulate the complexity of apical and basal dendrites and loss of *NgR1* does not alter dendritic spine density but leads to more immature spines in CA1 neurons [42, 49]. Acute knockdown of neuronal *Nogo-A* in layer V pyramidal neurons of the rat neocortex increases the number of filopodia-like dendritic protrusions and decreases spine density *in vivo* [50]. This suggests that neuronal Nogo-A can regulate dendritic spine density and morphology in a cell-autonomous manner. *In vivo*, the combined loss of all three Nogo receptors (*NgR1,2,3*$^{-/-}$) results in an increase in dendritic arborization and more dendritic spines in CA1 pyramidal neurons of juvenile mice [51]. Collectively, these studies suggest that the actin cytoskeleton of dendritic spines is a major target for Nogo-A and NgRs to stabilize neuronal architecture in the juvenile and adult CNS.

In mouse hippocampal slice cultures, local digestion of perisynaptic CSPGs in the stratum radiatum preserves PNNs and leads to an increase in CA1 dendritic spine motility and formation of spine head protrusions [52]. Perisynaptic CSPGs may be of neuronal or astrocytic origin. Mechanistic studies revealed that perisynaptic CSPGs restrict spine structural plasticity in a β1-integrin/focal adhesion kinase (FAK-pY397)-dependent manner [52]. β-integrin activation in hippocampal neurons regulates synapse density and spine stability in a RhoA and Ca^{2+}-calmodulin/CamKII-dependent manner [53]. Similar mechanisms operate in the axonal compartment, as Nogo-A and CSPGs block β1- and β3-integrin activation, decrease FAK-pY397 and increase RhoA activity to inhibit axonal growth [54–56]. We still have yet to learn how neuronal Nogo-A and CSPGs influence neuronal structure and synaptic function in the hippocampus and visual cortex (Box 3). Furthermore, it will be important to define the extent to which different types of CNS regeneration inhibitors influence synaptic function, which of their effects are mediated by RPTPσ, LAR, NgR1 or NgR3, and how these interactions regulate integrin function in nervous system health and disease (Box 3).

Inhibition of synaptogenesis

Over the past several years, much progress has been made in the identification of neuronal cell-surface molecules and astrocyte-derived secreted factors that promote synaptogenesis [40, 57, 58]. Much less is known about the mechanisms that prevent the formation of supernumerary synapses. Members of the semaphorin family have been found to positively or negatively influence the formation and maturation of both excitatory and inhibitory synapses [59, 60]. Moreover, in acute hippocampal slices, bath-applied Sema3F greatly increases the frequency and amplitude of mEPSCs in dentate granule neurons [61], and it has been shown that the ectodomain of soluble Sema5B triggers rapid synapse elimination in primary hippocampal neurons [62].

Recent work highlights a central role for LRR domain-containing proteins in promoting synaptogenesis [63]. However, NgR1, NgR2, and NgR3 appear to be exceptions, as these LRR proteins inhibit formation of excitatory synapses [51]. In dissociated hippocampal pyramidal neurons, acute RNAi knock-down of individual Nogo receptor family members greatly increases dendritic spine density, whereas their overexpression leads to a significant reduction in spines. Time-lapse studies in hippocampal slice cultures further reveal that *NgRs* attenuate *de novo* formation of dendritic spines in CA1 pyramidal neurons but do not alter the rate of dendritic spine elimination [51]. In wild-type mice there is an age-dependent increase in synaptic stability as animals transition from adolescence to adulthood [64]. Conditional ablation of *NgR1* in 1-year-old mice is sufficient to significantly increase synaptic turnover and reverse it to adolescent levels [65]. Loss of *NgR1* does not increase synapse density in hippocampal CA1 pyramidal neurons; however, juvenile mice null for all three Nogo receptors (*NgR1,2,3*$^{-/-}$) show a significant increase in synapse density *in vivo* [42, 51]. The identity of the "anti-synaptogenic" NgR ligand(s) remains largely elusive (Box 3). Biochemical studies revealed that OMgp and Nogo-A, but not Nogo-B, are present in synaptic density fractions and may function as "anti-synaptogenic" NgR1 ligands [38]. Several additional NgR1 ligands have been identified (Table 2), some of which have been found to antagonize Nogo66 function in the axonal compartment, and it will be interesting to explore their role at synapses. MAG, a ligand for NgR2, is selectively expressed by myelinating glia [24] and as such is an unlikely candidate to influence synaptogenesis. Of interest is the interaction of NgR1 and NgR3 with the GAG portion of neural CSPGs and HSPGs [22]. Numerous studies in invertebrates and vertebrates have established that proteoglycans regulate synaptogenesis [44, 57, 66], synaptic structure [52, 67], and activity-dependent neurotransmission [68, 69]. Some of these functions are exerted by integrins and LAR protein family members. Notably, the function of several repulsive axon guidance molecules can be regulated in a proteoglycan-dependent manner [70–73]. In the presence of HSPGs, Sema5A and RPTPσ promote neurite outgrowth, whereas in the presence of CSPGs, they inhibit growth [70, 71]. Enzymatic digestion of the CSPGs brevican and neurocan promotes the formation of synaptic puncta in neuron-glia co-cultures [74]. Additional studies are needed to establish whether this is caused by a shift in the HSPG to CSPG ratio, and if this leads to altered activation of RPTPσ, NgR1 or NgR3.

CNS regeneration inhibitors regulate Hebbian forms of synaptic plasticity

The observation that NgR1 attenuates fibroblast growth factor 2 (FGF2) function in primary neurons [42], coupled with the known role of FGF2 at excitatory synapses [75], prompted investigations of NgR1 at synapses. In acute hippocampal slices, local application of FGF2 to Schaffer-collateral/CA1 synapses enhances LTP induced by theta-burst stimulation (TBS) in *NgR1*$^{-/-}$, but not *NgR1*$^{+/+}$ slices [42]. Moreover, acute antibody blockade of Nogo-A or NgR1 leads to increased LTP in CA1 neurons [76]. Conversely, acute application of soluble Nogo66 or OMgp to Schaffer-collateral/CA1 synapses decreases LTP in wildtype, but not *NgR1*$^{-/-}$, hippocampal slices [38]. Additional studies revealed that p75, a co-receptor in the NgR1 receptor complex for certain inhibitory functions, is dispensable, and *PirB* plays only a minor role in OMgp-mediated inhibition of LTP [38]. Collectively, these studies provide strong evidence that Nogo-A, OMgp, NgR1 and PirB regulate activity-dependent synaptic strength. The Nogo-A inhibitory peptide NogoΔ20 does not bind to NgR1 or PirB and when applied to Schaffer-collateral/CA1 synapses 5 min before TBS, decreases post-tetanic potentiation and, counter-intuitively, is followed by a rapid and prolonged increase in LTP [76]. Although the underlying mechanism(s) of this enhanced synaptic response remains to be understood, it is known that acute application of NogoΔ20 triggers rapid, EHD4/pincher-dependent endocytosis of surface-bound NogoΔ20 [77]. Rapid internalization of receptor-bound NogoΔ20 could desensitize neurons toward Nogo-A [76].

Interestingly, manipulations that acutely affect Nogo-A/NgR1 signaling have the most pronounced effects on synaptic strength. Germline ablation of *NgR1, PirB* or *Nogo-A* in mice does not significantly alter basal synaptic transmission or LTP at Schaffer collateral/CA1 synapses, however, LTD in juvenile *NgR1−/−* hippocampal slices is absent [38, 42, 76]. The disparity between acute blockage of Nogo-A and NgR1 on LTP and gene ablation through the germline suggests the existence of powerful compensatory mechanisms. Biochemical analysis of primary hippocampal neurons following RNAi knock-down of *Nogo-A* and *NgR1* have begun to provide insight into how these molecules may regulate synaptic strength. Two days following acute knock-down of *Nogo-A* or *NgR1*, a global increase in AMPA (GluA1 and GluA2) and NMDA (GluN1, GluN2a and GluN2b) receptor subunit expression is observed [78]. CSPGs are known to influence activity-dependent synaptic strength. Perturbation studies with ChaseABC, hyaluronidase or germline ablation of *brevican, neurocan*, or *RPTPσ*, demonstrate a decrease in early or late LTP [40, 79, 80]. One mechanism by which CSPGs may influence synaptic transmission is by functioning as a barrier for lateral mobility of AMPA type glutamate receptors in dendritic spines [81]. Although additional studies are needed to determine the full spectrum of Nogo-A-, OMgp-, and CSPG-mediated regulation of activity-dependent synaptic strength, several independent lines of evidence clearly show that CNS regeneration inhibitors and their receptors not only regulate neuronal structure, but also influence Hebbian forms of synaptic plasticity (Figure 3).

Crosstalk between pro- and anti-plasticity signaling cascades

Finding the right degree of synaptic structural stability and functional plasticity is a life-long challenge for many neurons and of critical importance for proper nervous system function. Mounting evidence suggests that there is extensive crosstalk between “pro-” and “anti-plasticity” signaling pathways (Figure 4). Pretreatment of primary neurons with brain-derived neurotrophic factor (BDNF) attenuates myelin inhibition in a p-CREB-dependent manner [82, 83]. Moreover, NogoΔ20 decreases CREB activation and leads to down-regulation of neuronal growth programs [77]. Conversely, pretreatment of primary neurons with crude myelin or Nogo66 attenuates BDNF-elicited activation of pAKT (Ser473) and p70S6K (Thr389) [38], and NogoΔ20 leads to a decrease in pAKT (Ser473) [84]. In a similar vein, CSPG binding to RPTPσ attenuates TrkB activity [85]. In synaptic density fractions of *NgR1−/−* hippocampus, activation of Erk1/2 is increased [38] and *NgR1−/−* neurons are more sensitive to FGF2/FGFR signaling [42]. Collectively, these studies suggest an antagonistic relationship between CNS regeneration inhibitors and neurotrophic factor signaling pathways (Figure 4). Crosstalk between these pathways at the molecular level is only now beginning to be defined. In primary hippocampal neurons, RNAi knock-down of *NgR1* or *Nogo-A* leads to an increase in PSD95, AMPA, and NMDA receptor subunit expression that is blocked in the presence of rapamycin [78]. This suggests that NgR1/Nogo-A signaling inhibits mTOR complex 1 (TORC1)-mediated synthesis of synaptic proteins. Consistent with this idea, Nogo66 attenuates p70S6K (Thr389), a downstream target of TORC1, in primary cortical neurons [38]. mTOR is found at excitatory synapses, where it regulates cap-dependent translation of local mRNAs [86], including BDNF-induced translation and surface expression of GluA1 [87]. Thus, one mechanism for the observed crosstalk between neurotrophic factor and growth inhibitors in neurons is their opposing regulatory influences on mTOR-dependent protein translation. Additional crosstalk may occur at the transcriptional level by opposing regulation of p-CREB. A similar antagonistic interaction between BDNF and CSPGs has recently been reported [85]. The inverse regulation of signaling pathways triggered by CNS regeneration inhibitors and growth factors provides an economical means for neurons to adjust synaptic structure and strength to meet specific demands dictated by network activity.

Regulation of Nogo receptor function

The prominent role of Nogo-A, OMgp and NgR1 in neuronal plasticity suggests that the NgR1 signaling pathway is not constitutively active but subject to strict regulation. Table 2 lists a growing number of NgR1 binding partners, including molecules with antagonistic action toward Nogo66. Of these, leucine-rich glioma inactivated (LGI1) is of particular interest because it enhances AMPA receptor-mediated synaptic transmission in hippocampal slices [88]. NgR1 and ADAM22 collaborate to form a functional LGI1-binding complex [89]. Both NgR1 and ADAM22 are enriched postsynaptically, and it will be interesting to explore whether LGI1 sequesters NgR1 away from ligands that attenuate activity-dependent synaptic strength. Unlike antibody blocking of NgR1, however, acute application of LGI1 to hippocampal slices does not increase LTP in CA1 neurons, suggesting a more complex mechanism for LGI1 function than simply blocking synaptic NgR1 [88]. ADAM22 shares homology with a large family of transmembrane ADAM metalloproteases but is catalytically inactive. Nevertheless, NgR1 function is regulated by shedding of its ectodomain from the neuronal surface. In cortical neurons, basal shedding of endogenous NgR1 is mediated by membrane-type matrix metalloproteinases-3 (MT3-MMP). In the presence of tissue inhibitor of metalloproteinase-2 (TIMP-2) and TIMP-3, NgR1 shedding is largely absent [90, 91]. The MT3-MMP processing site on NgR1 is located distal to the LRR cluster at position Ala358. Therefore, the released NgR1 ectodomain fragment retains Nogo, MAG, and OMgp (but not GAG) binding activity and may exert a dominant-negative function [91]. Physiological MMP activity is required for dendritic remodeling, synaptogenesis and LTP, and it appears likely that this is accomplished, at least in part, through regulation of Nogo receptor shedding. The discovery of endogenous regulatory mechanisms for these inhibitors and their receptors is of interest from a biological point of view, and may be exploited for therapeutic purposes following CNS injury or disease.

Coordination of structural and functional synaptic plasticity

Prolonged changes in synaptic activity alter neuronal structure. Since growth inhibitors in the adult CNS have the potential to regulate synaptic structure and influence synaptic efficacy, these cues are well suited for linking changes in electrical activity to neuronal structural alterations. In the adult neocortex and hippocampus, neuronal activity regulates the *NgR1* promoter [92]. Increased voluntary exercise or administration of kainic acid leads to a reduction of *NgR1* and myelin inhibitor expression *in vivo* [92, 93]. There appears to be an inverse relationship between activity-dependent down-regulation of *NgR1* and activity-dependent upregulation of neurotrophic factors such as FGF2 and BDNF [92, 94, 95]. Coordination of activity-induced upregulation of molecules that promote synaptic plasticity with simultaneous downregulation of synaptic plasticity inhibitors may prime neurons for adaptive structural changes. When coupled with more local regulatory mechanisms, such as activity-dependent release of BDNF at synaptic sites or local shedding of NgR1, strengthening of individual synapses and changes in morphology may be achieved rapidly and with high temporal and spatial specificity. Because of the apparent antagonistic effects between growth factors and myelin inhibitors on synaptic structure and function, and their opposite regulation by prolonged neural activity, it is tempting to speculate that similar to BDNF, Nogo-A/NgR1 participate in synaptic scaling and homeostasis (Box 3).

Are some things not meant to be undone?

Recent work indicates that a significant fraction of synapses in the mature brain is structurally stable over a very long time scale [1, 64], thus, it seems quite likely that stable synaptic contacts are required for long-term memory storage. Perturbation studies suggest that CSPGs and Nogo-A play important roles in the stabilization and maintenance of axonal,

dendritic and synaptic structure. The diversity and functional redundancy of known CNS growth inhibitors begs the question of why the CNS of higher vertebrates has gone to enormous trouble to evolve mechanisms of turning off adult neuronal growth and regeneration. Although the growth inhibitory nature of adult CNS tissue likely contributes to the regenerative failure of injured neurons, the emerging physiological role of CNS regeneration inhibitors as a stabilizing force that limits exuberant growth in the healthy (uninjured) mature brain may be critical for proper nervous system function and formation of long-lasting memories. Lifting the stabilizing constraints in the adult CNS to promote neuronal plasticity for therapeutic purposes may need to be carefully controlled, both temporally and spatially. Similar to developmental CPs, a more plastic adult CNS may need to be subjected to task-specific rehabilitative training as a means to refine newly formed circuits in an activity-dependent manner and to maximize behavioral outcomes following stroke or other forms of CNS injury [96]. Caution must be exercised, as some forms of neuronal plasticity may be maladaptive, and too much growth and sprouting has been associated with severe complications including allodynia, seizures and epilepsy. Recent evidence suggests that interfering with growth inhibitory signaling cascades may have detrimental consequences for intellectual abilities by impairing key neurologic functions such as long-term memory storage and mental health. Loss of *NgR1* does not impair hippocampal learning in the Morris Water Maze (MWM), and forebrain-specific overexpression of *NgR1* does not alter short-term memory [97, 98]; however, it does impair the formation of long-lasting memory in the MWM task [98]. In the amygdala, ChaseABC digestion of CS-GAGs renders acquired fear memory susceptible to erasure, supporting the notion that stable synapses are important for long-term memory storage [99]. Key open questions concern the extent to which the plethora of known CNS regeneration inhibitors (Figure 1) exert similar functions, protecting certain types of memory from erasure, and whether these molecules can be targeted for therapeutic purposes following injury or disease without impairing vital neurologic functions (Box 3).

Concluding remarks

Recent work examining the physiological role of CNS regeneration inhibitors in the naïve brain of juvenile and adult rodents reveals novel functions for Nogo-A, OMgp, CSPGs and their receptors in the dendritic compartment of different types of CNS projection neurons. From a biological point of view it will be important to understand how these growth inhibitory molecules cross-talk with growth-promoting mechanisms to strike a delicate balance that keeps a neuron in the “goldilocks zone” of synaptic plasticity (Box 3). Balancing the degree of excitation vs inhibition and finding the right degree of synaptic stability is absolutely critical for proper nervous system function. Too much dendritic remodeling and synaptic turnover may erase memories, and conversely, too much stability in microcircuits may compromise experience-dependent network refinement, formation of new synapses and acquisition of specific types of new memory. As we learn more about CNS regeneration inhibitors, they may in many ways be viewed as the counter players of neurotropic factors. In addition to their role in controlling neuronal architecture, regeneration inhibitors regulate activity-dependent synaptic strength, possibly through blocking mTOR-dependent protein synthesis at synapses. Because altered mTOR activity in the brain has been linked to cognitive and social dysfunction, and mutations in *NgR1* and *Nogo-A* have been associated with schizophrenia [100, 101], a detailed understanding of the physiological role of CNS regeneration inhibitors in the developing and adult brain will be of great interest both from a biological and clinical point of view.

Acknowledgments

We thank Alex Kolodkin and members of the Giger lab for critical reading of the manuscript. This work was supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (AMRF), the NIH Cellular and Molecular Biology Training Grant T32-GM007315 and R01-NS081281.

Abbreviations

LOT	lateral olfactory tract
MAG	myelin-associated glycoprotein
OMgp	oligodendrocyte myelin glycoprotein, p75
Troy/Taj	members of the TNF receptor superfamily
GT1b	complex ganglioside
APP	amyloid precursor protein
Aβ	neurotoxic fragment of APP
FGF	fibroblast growth factor
LGI1	leucine-rich glioma inactivated
BLyS/CD253	TNF family member B lymphocyte stimulator
MT3-MMP	membrane-type matrix metalloproteinases-3
ADAM22	a disintegrin and metalloproteinase domain-containing protein 22
Crtac1B/ LOTUS	cartilage acidic protein-1B/ lateral olfactory tract usher substance

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- Insights into the physiology of CNS regeneration inhibitors in the naïve brain
- CNS regeneration inhibitors serve as negative regulators of synaptic plasticity
- Nogo-A restricts synaptic plasticity by antagonizing neurotrophic factor signaling

Box 1: GAGs have evolved as major regulators of neuronal function

Glycosaminoglycans (GAGs) are comprised of repeating (up to ~20–200) disaccharide units and form long, unbranched polymers. The chemical composition of the disaccharide unit and its sulfation pattern can vary, giving rise to different types of GAGs, including chondroitin sulfate (CS), keratan sulfate (KS), heparan sulfate (HS), hyaluronic acid (HA) and heparin. In proteoglycans, CS-, HS-, and KS-GAGs are covalently linked to a protein core. HA and heparin GAGs are not covalently linked to proteins. CS-GAGs are composed of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) repeats. During biosynthesis, GalNAc is sulfated at position C4 by chondroitin-4 sulfotransferase 1 (C4ST-1) and C6 by chondroitin-6 sulfotransferase-1 (C6ST-1). Depending on the sulfation pattern and epimerization of GlcA at position C5 into iduronic acid (IdoA), CS-GAGs are further classified into CS-A, CS-B, CS–C, CS-D, and CS-E. Of importance for regulation of neuronal structure is the observation that different CS-GAG subclasses contribute to various degrees to the growth inhibitory nature of CSPGs. The receptor binding specificity and biological activity of CS-GAGs is regulated by sulfation [21, 22] and the ratio of CS-4-sulfation/CS-6-sulfation is a critical regulator of OD plasticity in the mouse visual cortex [34]. The CS-GAG binding patterns of RPTPσ, NgR1 and NgR3 are largely overlapping. These receptors strongly interact with CS-B and CS-E but fail to support binding of CS-A and CS-C [21, 22]. Few proteoglycans (e.g. aggrecan) can carry two different types of GAG chains (e.g. KS-GAGs and CS-GAGs). A recent study found that KS-GAGs exert growth inhibitory activity toward growing axons *in vitro* and regenerating fibers following spinal cord injury *in vivo* [7]. Heparan sulfate proteoglycans form a large class of HS-GAG proteins, with prominent members including transmembrane syndecans and the lipid-anchored glypicans. HSPGs are often an integral part of large surface receptor complexes with diverse functions. In the nervous system, HSPGs have been shown to participate in axonal growth and guidance, synapse formation and maturation, and synaptic transmission. Hyaluronan (HA) is a major component of the extracellular matrix. In the brain, HA associates with tenascins and CSPGs and is an integral part of perineuronal nets.

Box 2: Candidate ligands that regulate Nogo receptor dependent synaptic function

A large number of NgR1 binding partners has been identified, including co-receptors and agonistic/antagonistic ligands. Thus far, most studies have focused on the role of these interactions in the axonal compartment. Of interest, many NgR binding partners are found at synapses and have been shown to influence synapse structure, maturation, or efficacy of transmission. Proteins with known synaptic function include Nogo-A, OMgp, FGF2, APP, LGI1, HSPGs and CSPGs. Thus, it will be important to examine which synaptic functions by any of these ligands are exerted through association with Nogo receptor family members. The synaptic function of the NgR1 antagonists LGI1, Crtac1B/LOTUS and olfactomedin-1 is poorly understood, but may offer new approaches to modulate NgR1 function. All three NgR family members interact with and have been implicated in APP processing. Loss of endogenous *NgR1* in an Alzheimer disease (AD) mouse model increases Aβ deposition [102]. In the same AD model, the interaction of NgR2 with APP favors processing of APP by BACE1 and loss of endogenous *NgR2* reduces Aβ production and formation of amyloid plaques [103].

Box 3: Outstanding questions

- The receptor mechanisms that govern dendritic and axonal functions of Nogo-A, OMgp and CSPGs are still incompletely understood. What is the molecular identity of the neuronal NogoΔ20 receptor? What are the transmembrane-spanning co-receptors that complex with NgR1, NgR2 and NgR3 in the dendritic compartment, and what are the “anti-synaptogenic” ligands of NgR1, NgR2 and NgR3?
- What is the functional significance of the interaction between NgR1 and NgR3 with CSPGs and HSPGs in the axonal and dendritic compartments of naïve (uninjured) CNS neurons?
- How do CNS regeneration inhibitors regulate protein levels of AMPA and NMDA type glutamate receptors?
- Nogo-A/NgR1 and CPSGs consolidate neuronal structure at the end of the CP in the visual system. Do these molecules also regulate duration of the CP in the auditory cortex, somatosensory cortex, and perhaps in brain regions associated with higher-level processing such as language acquisition?
- Does manipulation of CNS inhibitors offer therapeutic opportunities following brain or spinal injury, mental illness, or help to erase certain types of memories in the adult brain associated with excessive fears, anxiety, or addiction?

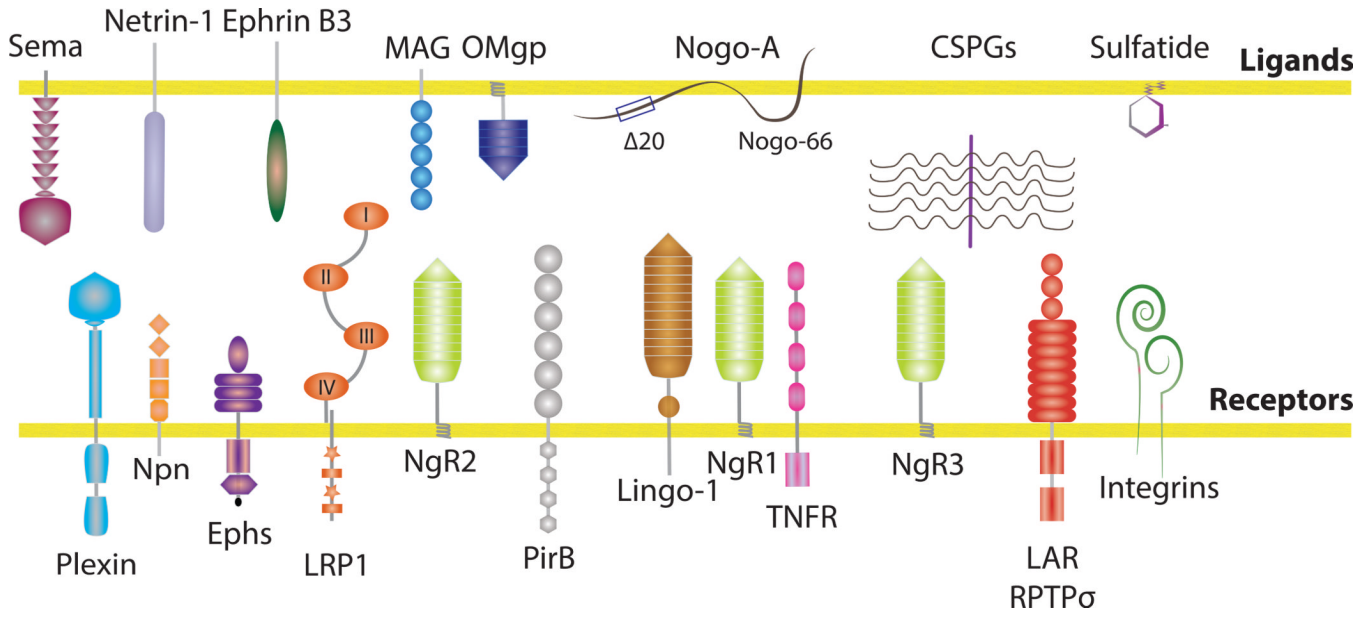


Figure 1. Prominent CNS inhibitors and their receptors

CNS inhibitors abundantly found in the postnatal and adult brain and spinal cord include canonical axon guidance molecules such as semaphorins, ephrinB3, netrin-1 and the glial inhibitors Nogo-A, OMgp, MAG, CSPGs and sulfatide [4, 9]. Nogo, a member of the reticulon family is strongly expressed by neurons and oligodendrocytes. The splice variant Nogo-A harbors two distinct neurite outgrowth inhibitory regions: NogoΔ20 and Nogo-66. MAG is a sialic-acid binding Ig-lectin expressed by myelinating glia that inhibits neurite outgrowth in a developmental stage-dependent manner. OMgp is a heavily glycosylated lipid-anchored LRR family member expressed by neurons and oligodendrocytes. Inhibitory CSPGs in the extracellular matrix include lecticans (versican, neurocan, brevican, aggrecan), phosphacans and NG2. Sulfatide is a sulfated galactosylceramide that is abundant in CNS myelin. Neuronal surface receptors for CNS inhibitors have been identified. Plexins, neuropilins, and Ephs function as principal receptors for semaphorins and ephrins [4]. NgR1 and PirB support direct binding of Nogo66, MAG and OMgp. NgR1 is a lipid-anchored protein and in some cells is part of a tripartite receptor complex that also includes Lingo-1 and the TNFR family member p75 or Troy/Taj [9]. MAG binds directly to NgR2, β1-integrin and the low-density lipoprotein related receptor LRP1. Receptors for CSPGs include RPTPσ, LAR, NgR1, and NgR3 [19, 20, 22]. Indirect and integrin-dependent mechanisms of growth inhibition have been identified for NogoΔ20 and CSPGs [54, 55]. The neuronal receptors for sulfatide and NogoΔ20 are unknown.

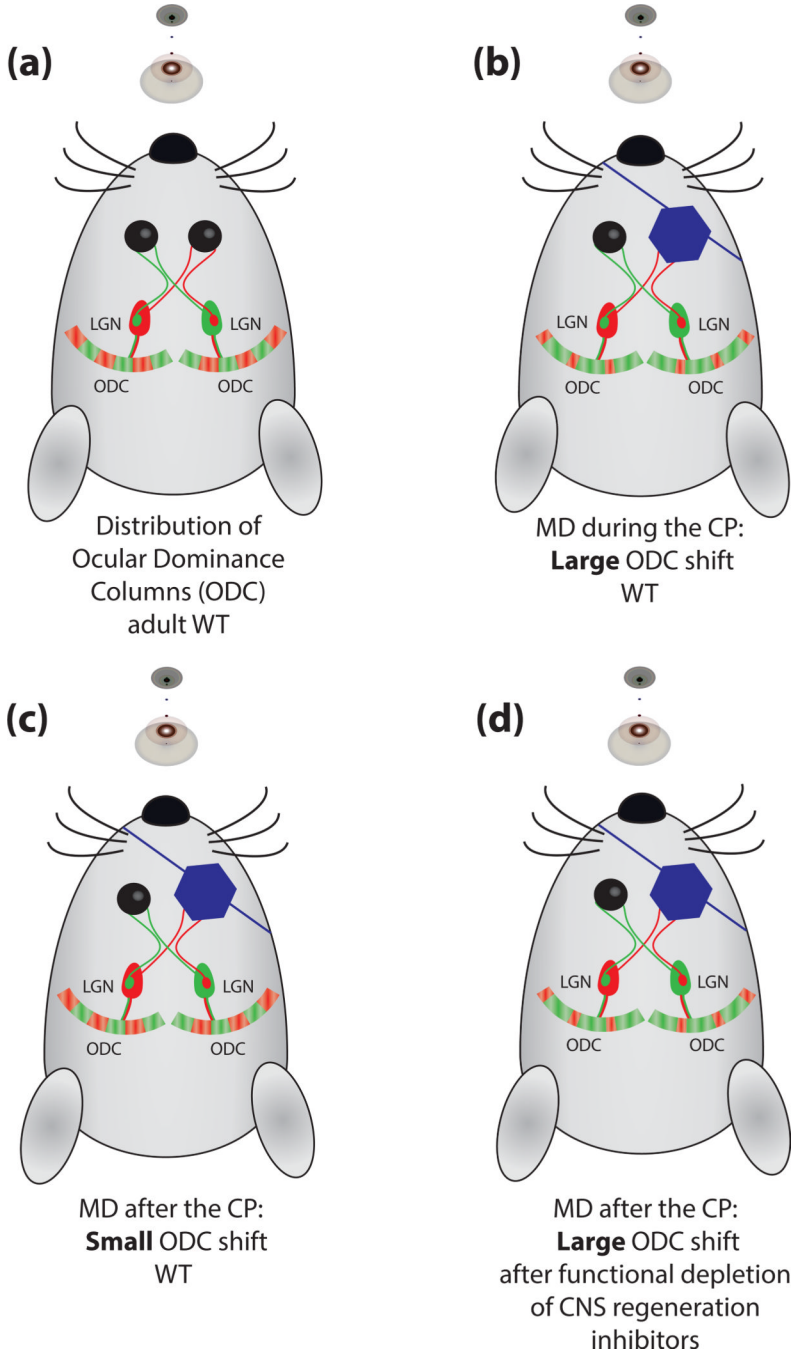


Figure 2. CNS inhibitors consolidate neuronal structure in the visual cortex

A) In the developing visual system, inputs from both eyes first overlap in the primary visual cortex (V1). Over time, visual experience-driven competition segregates eye specific (red and green) inputs into specific cortical regions, also called ocular dominance columns (ODC), in which one eye will dominate both functionally and anatomically. B) Nearly 50 years ago, David Hubel and Torsten Wiesel observed that following monocular deprivation (MD) during the CP, the non-deprived (green) eye shifts and expands its representations in the binocular zone of V1 at the expense of the deprived (red) eye. C) This shift in ODC occurs in layer IV of V1 and is most robust during the CP, as MD in adulthood results in a comparatively small shift in ODC. D) Toward the end of the CP, the extracellular matrix

forms aggregates around fast-spiking parvalbumin-positive interneurons that are called perineuronal nets (PNNs). PNNs are comprised of CSPGs in association with hyaluronic acid, and tenascins. Perturbation of PNNs with pharmacological or genetic manipulations leads to a more pronounced shift in ODC following MD [32, 34–37]. In mice, functional ablation of *Nogo-A/B, NgR1*, or *PirB* through the germline results in robust ODC shift following MD throughout adulthood [29, 31].

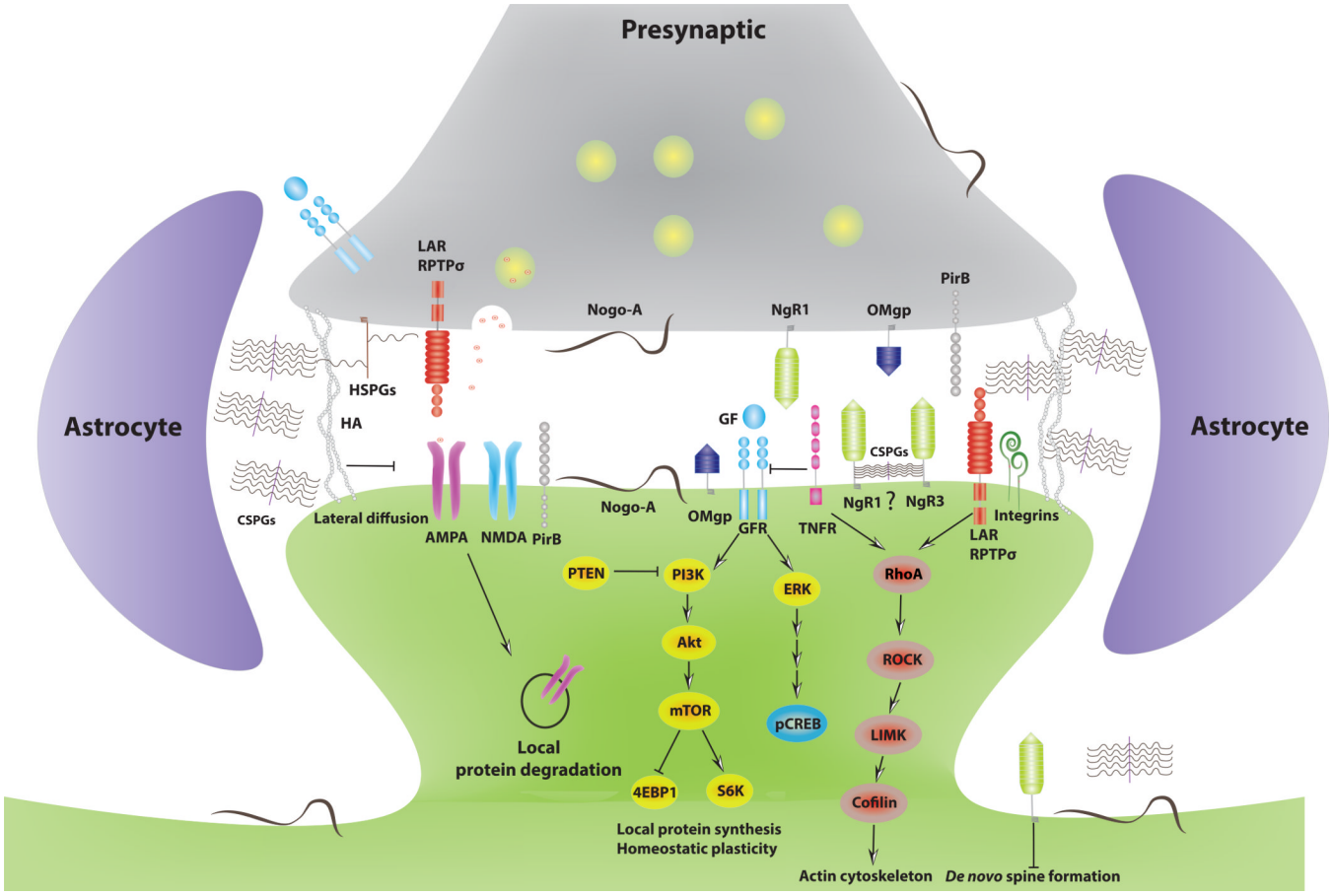
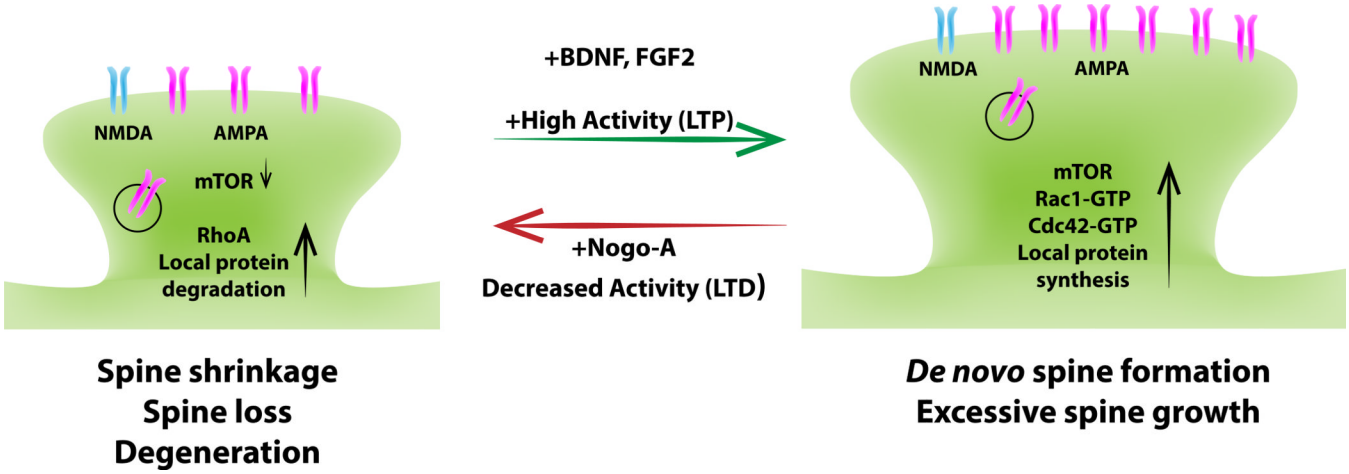


Figure 3. CNS inhibitors regulate synaptic plasticity

Independent lines of evidence show that many CNS inhibitors and their receptors are present at or near synapses. Nogo receptors suppress dendritic spine formation in the juvenile CNS and promote spine maturation and stability in adulthood [42, 49, 51]. Emerging evidence suggests that the Nogo-A/NgR1 complex antagonizes growth factor (FGF2 and BDNF) signaling by suppressing the activity of the mammalian target of rapamycin complex 1 (mTORC1) and MAP kinase signaling from Erk1/2 to nuclear p-CREB pathway. mTORC1 is activated by PI3K–AKT signaling and promotes translation of synaptic mRNAs, including NMDA receptor and AMPA receptor subunits, via phosphorylation of specific downstream effectors such as elF4E binding protein (4EBP1) and p70S6-kinase [38, 78]. One negative regulatory pathway of mTOR includes phosphatase and tensin homolog (PTEN), a synaptic protein associated with autism spectrum disorders. How exactly NgR1 activates downstream signaling pathways at the synapse is largely unknown but may involve RhoA-ROCK-LIMK-cofilin mediated breakdown of the actin cytoskeleton. The extracellular matrix components hyaluronic acid (HA), CSPGs and HSPGs are found near synapses where they form surface compartments that limit lateral diffusion and exchange of AMPA-type glutamate receptors [40, 81]. The CSPG receptors LAR and RPTPσ are present both pre- and post-synaptically, but their CSPG-dependent function at the synapse has not yet been established. Both NgR1 and NgR3 bind CSPGs, but whether this interaction takes place at or near spine synapses is not known. Integrins have been implicated in the regulation of homeostatic synaptic plasticity, and recent evidence suggests an important role for integrin signaling in CSPG-mediated spine morphogenesis [52].



Spine shrinkage
Spine loss
Degeneration

***De novo* spine formation**
Excessive spine growth

Figure 4. Antagonistic action of growth factors and growth inhibitors at the synapse

Dendritic spines are small actin-rich protrusions that form the postsynaptic compartment of the majority of excitatory synapses. Depending on their morphological appearance, spines are categorized into different subclasses: thin, stubby, and mushroom-type. Many human brain disorders are associated with abnormal spine density, shape or volume. Long-term potentiation (LTP) of synaptic transmission is correlated with an increase in spine size or formation of new spines, whereas long-term depression (LTD) is associated with spine shrinkage or loss. On a molecular level, changes in spine density, shape or volume are primarily a reflection of reorganization of the actin cytoskeleton. Typically, activation of the small GTPases Rac1 and cdc42 by synaptic GEFs promotes the formation and growth of dendritic spines, whereas activation of RhoA by synaptic GEFs causes spine shrinkage and synapse loss. Neuronal activity regulates local protein turnover at postsynaptic sites. Local protein synthesis can be increased by BDNF-elicited activation of mTORC1-dependent protein translation and protein degradation. Emerging evidence suggests that CNS inhibitors (particularly Nogo-A) antagonize neurotrophic factor signaling cascades at the synapse. Nogo-A/NgR1 signaling negatively regulates mTORC1 dependent local protein synthesis.

Table 1

GAGs form a class of structurally related but functional diverse glycans

glycosaminoglycans (GAGs)	**adult CNS expression**	**upregulation by CNS injury**	**inhibitory activity toward neurites *in vitro***	**neural binding partners/ receptors**	**function in the nervous system**	**references**
CS-A (CS-4) GlcA-GalNAc4S	most abundant	no	modest	unknown	unknown	[104]
CS-B (dermatan) IdoA-GalNAc4S	modest	yes	no	RPTPσ, NgR1, NgR3	fibrotic scar formation	[21, 22, 105, 106]
CS-C (CS-6) GlcA-GalNAc6S	modest	yes	no	unknown	OD plasticity?	[23, 34, 104]
CS-D (CS-2,6) GlcA2S–GalNAc6S	minor	?	unknown	NgR1, NgR3	OD plasticity?	[22, 34, 36]
CS-E (CS-4,6) GlcA-GalNAc4S6S	minor	yes	very strong	RPTPσ, NgR1, NgR3	OD plasticity? blocks CNS axon regeneration	[21–23, 34]
Keratan sulfate (KS1 and KS2)	brain, spinal cord, highest in cornea	yes	strong	unknown	axon guidance, blocks CNS regeneration	[7]
Heparan sulfate (HS) GlcA-GlcNAc GlcA-GlcNS IdoA-GlcNS IdoA(2S)-GlcNS IodA-GlcNS(6S) IdoA(2S)-GlcNS(6S)	yes, prominent members include glypicans and syndecans	yes	context-dependent growth promotion or growth inhibition	NgR1, NgR3, EphB, CAMs, FGFRs, LARs, semaphorins, slits	axon guidance, synaptogenes is, synaptic structure, neurotransmission	[22, 42, 44, 57, 66, 68, 71, 73]
Hyaluronan GlcA-GlcNAc (non-sulfated)	abundant in perineuronal nets (PNNs)	no	largely inert	Toll-like receptor 2	OD plasticity, myelination	[23, 40]

Table 2

Nogo receptor family interactions

	ligand	ligand localization in the CNS	function of the interaction in the axonal compartment	function of the interaction in the dendritic compartment	references
NgR1	Nogo66	pre- and postsynaptic, myelin,	growth cone collapse	synaptic strength	[15, 18]
NgR1	MAG	myelin	growth cone collapse		[15, 18]
NgR1	OMgp	pre- and postsynaptic, myelin	growth cone collapse	synaptic strength	[15, 18]
NgR1	NgR1, NgR3	neuron pre- and postsynaptic	?	?	[22]
NgR1	Lingo-1	neuron, pre-synaptic	co-receptor	?	[9]
NgR1	p75, Troy/Taj	neurons, astrocytes	co-receptor	?	[9]
NgR1	GT1b	mostly neuronal	co-receptor	?	[107]
NgR1	APP, Aβ	neurons and glia	decreases Aβ production	?	[102]
NgR1	FGF2, FGF1	ECM/proteoglycans	decreased branching	synaptic strength	[42]
NgR1	BLyS	astrocytes, microglia, macrophages	growth inhibition	?	[108]
NgR1	LGI1	neuronal, excitatory synapses	Nogo66 antagonist	?	[89]
NgR1	ADAM22	postnatal neurons	enhances binding of LGI1	?	[89]
NgR1	MT3-MMP	microglia, ECM	NgR1 shedding	NgR1 shedding?	[91]
NgR1	Crtac1B/ LOTUS	neurons, lateral olfactory tract axons	Nogo66 antagonist, LOT fasciculation	?	[109]
NgR1	CSPGs	ECM, perineuronal nets	inhibition together with NgR3	?	[22]
NgR1	HSPGs	neurons and glia	?	?	[22]
NgR1	Olfactomedin-1	DRG and RGC neurons	NgR1 antagonist	?	[110]
NgR2	MAG	myelin	inhibition when overexpressed in vitro		[4]
NgR2	APP	neurons and glia	enhanced APP processing and Aβ production	?	[103]

	ligand	ligand localization in the CNS	function of the interaction in the axonal compartment	function of the interaction in the dendritic compartment	references
NgR3	CSPGs	ECM, perineuronal nets	inhibition together with NgR1	?	[22]
NgR3	HSPGs	ECM	?	?	[22]
NgR3	APP	neurons and glia	APP processing	?	[103]