From the Department of Neuroscience,
Karolinska Institutet,
Stockholm, Sweden

On Nogo Signaling Regulation

Alexandra Trifunovski
M.Sc.

Karolinska Institutet

Stockholm 2006

Cover: Seahorse renditions by Mattias Karlén. The Italian anatomist and surgeon Giulio Cesare Aranzi (1529/30–1589) introduced the Greek word for seahorse, "Hippokampos," to name the hippocampal formation in the brain, because of its visual similarity to a seahorse. In the thesis, the Nogo signaling system has been studied in hippocampus and other brain areas.

Layout: Ida Engqvist
Printed by Larserics Digital Print AB, Bromma, 2006

To my family

Abstract

As neuronal development enters its final stages, axon growth becomes restricted. This lack of regenerative capacity is partly due to the non-permissive environment of growth inhibitory proteins. Three such proteins, Nogo, OMgp and MAG bind to the same receptor, the Nogo receptor (NgR) and induce growth cone collapse and axon growth inhibition. Since NgR is GPI-linked to the cell membrane and lacks a cytoplasmic domain, additional transmembrane molecules are needed for intracellular signaling. The low-affinity NGF receptor p75, Lingo-1 and TROY serve as coreceptors involved in initiating the intracellular signaling cascade. Together, therefore, NgR, p75, Lingo-1, and TROY have important roles controlling regeneration, growth inhibition and neuronal plasticity. We have found that NgR and Lingo-1 are widely expressed in the brain and that these two gene transcripts are efficiently regulated in the CNS by neuronal activity. We hypothesize that the NgR/p75/Lingo-1/TROY system is involved in synaptic plasticity and memory functions. Aims: To examine expression of components of the Nogo receptor complex during development, in the adult and in the aging brain. To analyze the expression of receptor components. To investigate the role of the NgR in mechanisms of learning, memory and hippocampal neurogenesis in transgenic animals which overexpress NgR in forebrain neurons. To test mechanisms underlying regulation of Nogo receptor components and the possible connection between brain function (e.g. memory formation) and Nogo signaling. Methods: Histological analysis of different animal models with regard to the expression of transcripts encoding Nogo receptor components using in situ hybridization. Quantitative mRNA analysis using RT-PCR. Kainic acid administration and intrahippocampal BDNF injections in adult rats. Generation and characterization of novel transgenic mouse lines that overexpress NgR via a tetracycline inducible CamKII promoter. Paper I details the expression of NgR mRNA throughout development and in adult mouse and human tissues. In the adult, NgR is expressed in neurons in specific brain region associated with a high degree of plasticity. Paper II investigates how plasticity is regulated in regions with high NgR expression. We challenged rats with kainic acid and found the NgR was rapidly down-regulated, suggesting activity-dependent regulation. This was confirmed by showing that rats transiently down-regulate NgR during a period of establishing a running behavior, suggesting a role for NgR regulation in learning. Paper III addresses the NgR coreceptor Lingo-1. We found that Lingo-1 mRNA levels are rapidly and strongly increased in hippocampus by treatments thought to increase neuronal activity. We also describe regulation of NgR: Since BDNF upregulation coincides with the downregulation of NgR we tested a possible causal relationship by intracranial injections of BDNF or BSA. NgR was downregulated by both injections, although BDNF caused a bigger effect than BSA in ipsilateral cortex. Lingo-1 was specifically upregulated by BDNF. It is hypothesized that activity-driven structural synaptic plasticity is facilitated by appropriate changes of the levels of Nogo receptor components and trophic factors and that the Nogo system is also involved in stabilizing neuronal networks. Paper IV addresses the role of the NgR complex in a transgenic mouse model (mceph/mceph) characterized by epilepsy and a markedly enlarged brain. Results suggest that NgR down-regulation coincided with an upregulation of growth promoting proteins. We also found that carbamazepine (CBZ), a commonly used antiepileptic drug, counteracted brain overgrowth in mceph/mceph mice and reduced the number and size of neurons. CBZ normalized brain levels of mRNA encoding BDNF and several components of the Nogo signalling system, which were dramatically upregulated in untreated mceph/mceph brains. Paper V uses in situ hybridization to map Lingo-1 gene activity patterns in adult human nervous tissues, as well as inthe developing and adult rat CNS. Lingo-1 mRNA expression was observed in most, but not all neurons of the brain, spinal cord and dorsal root ganglia in developing and adult rats as well as in human adult CNS. We found a good correlation between Lingo-1 mRNA expression and NgR mRNA expression. Paper VI focuses on the Nogo system in the aging rat brain. We examined the levels of mRNA encoding Nogo, OMgp, MAG, as well as the receptor components NgR, Lingo-1 and Troy in cortex and hippocampus. There were no significant changes of receptor components or the ligands OMgp or MAG. However, Nogo mRNA was significantly, albeit modestly, decreased in hippocampal subregions of aged animals. The specific decrease of Nogo mRNA levels in hippocampus during aging may relate to age-dependent decline of brain plasticity. Paper VII is an attempt to determine the significance of activity-dependent NgR downregulation by generating transgenic mice with strong inducible NgR overexpression. The goal was to overexpress NgR in forebrain neurons and test if this may decrease plastic changes in vivo. A tetracycline inducible system was employed. The hypothesis was that NgR over-expressing mice should have impaired learning abilities due to reduced synaptic plasticity. To date, we have generated 3 transgenic mouse lines (CamKIIpTRE/NgR) with inducible (tet-off) and specific overexpression of NgR in forebrain neurons as analyzed by in situ hybridization and western blot. Levels of Nogo, Lingo-1, Troy, p75 and BDNF mRNA are not altered and the endogenous NgR mRNA levels did not change to compensate for the overexpression. CamKIIpTRE/NgR have significantly fewer BrdU labeled cells in the dentate gyrus compared to controls. Behavior of NgR overexpressing mice is currently being investigated using open-field, passive avoidance and swim maze tests. Conclusions: The results contribute to the understanding of the Nogo signaling system in health and disease, focusing on the Nogo receptor components. The dramatic activity-related changes of neuronal levels of NgR and Lingo-1 transcripts suggest fundamental roles in synaptic plasticity. Our transgenic mouse model may become a helpful tool in determining the role of one of the receptor components, NgR, for such plasticity during development and in adulthood. Increased knowledge about components of the Nogo receptor complex may also help the design of more effective means to improve regeneration in CNS.

Keywords: Nogo, Nogo receptor, regulation, neurotrophin, Lingo-1, p75NTR, Troy, synaptic plasticity

Table of Contents

List of Papers

This thesis is based on the following papers, which are referred to by their Roman numerals:

I. Josephson A, **Trifunovski A,** Widmer HR, Widenfalk J, Olson L, Spenger C. Nogo-receptor gene activity: cellular localization and developmental regulation of mRNA in mice and human. Journal of Comparative Neurology, 453:292-304 (2002)

II. Josephson A*, **Trifunovski A*,** Schéele C, Widenfalk J, Wahlestedt C, Brené S, Olson L, Spenger C. Activity-induced and developmental downregulation of the Nogo receptor. Cell and Tissue Research, 311:333-342 (2003)

III. **Trifunovski A,** Josephson A, Ringman A, Brené S, Spenger C, Olson L. Neuronal activity-induced regulation of Lingo-1. NeuroReport 15 (15):2397-2400 (2004)

IV. Lavebratt C*, **Trifunovski A*,** Persson AS, Wang FH, Klasson T, Josephsson A, Olson L, Spenger C, Schalling M. Carbamazepine administration inhibits excessive brain growth in the mceph/mceph mice. Neurobiology of Disease. In press

V. **Trifunovski A,** Josephson A, Erschbamer M, Galter D, Spenger C, Olson L. Lingo-1 mRNA Expression in the Adult Human and Rat CNS. Manuscript

VI. **Trifunovski A,** Josephson A, Bickford P, Olson L, Brené S. Selective decline of Nogo mRNA in the aging brain. NeuroReport 17(9):913-916 (2006)

VII. **Trifunovski A,** Josephson A, Mattsson A, Lundströmer K, Pham T, Griffin EA, Ögren SO, Spenger C, Brené S and Olson L. A role for Nogo signaling for neurogenesis and behavior. Evidence from NgR overexpressing mice. Manuscript

*Equal contributions

Introduction

In contrast to the peripheral nervous system (PNS), where successful axonal regeneration is commonly seen, regeneration in the mammalian central nervous system (CNS) is generally very poor or absent (reviewed in Yiu and He, 2006). As neuronal development enters its final stages in the nervous system, axon growth becomes restricted. Perinatal lesions in CNS can be compensated for by some degree of spontaneous regeneration. However, postnatal and adult lesions are not repaired and axons do not regenerate, resulting in devastating and lasting functional deficits. The absence of axon regeneration in the CNS is not fully understood. Why do CNS and PNS axons differ in their regenerative abilities? Initially, it was thought that the failure of mature axons to regenerate was the result of a loss of inherent regenerative ability of adult CNS neurons. However, observations in 1911 by Tello suggested a possible involvement of the adult CNS environment (see Ramon y Cajal, 1928). After implanting a piece of denervated peripheral nerve into rabbit cortex, silver staining revealed that bundles of axons had entered the peripheral nerve graft and had grown along Schwann cell bands. Later and with novel methods, Aguayo and collaborators (see Richardson et al., 1980; David and Aguayo, 1981; Benfey and Aguayo, 1982) were able to demonstrate that adult CNS neurons could indeed form long projections through peripheral nerve grafts. By grafting segments of peripheral nerve to the injured brain, spinal cord, and optic nerve and applying the tracer horseradish peroxidase, they could demonstrate that axons of CNS neurons can grow long distances through PNS tissue. The fact that the growing CNS neurons stopped soon after reentering the spinal cord with only a few fibers exiting the graft provided additional proof that it is the CNS environment rather than intraneuronal circumstances that curtail regenerative growth of CNS axons. Later work has shown that rerouting CNS axons exiting a peripheral nerve bridge graft to grey rather than white matter (Cheng et al., 1996) or treatment of the graft-CNS interface with chondroitinase (Houle et al., 2006) enable CNS axons in a nerve graft to reenter the CNS environment, leading to a degree of functional improvement in rodent models of spinal cord injury.

CNS versus PNS injuries

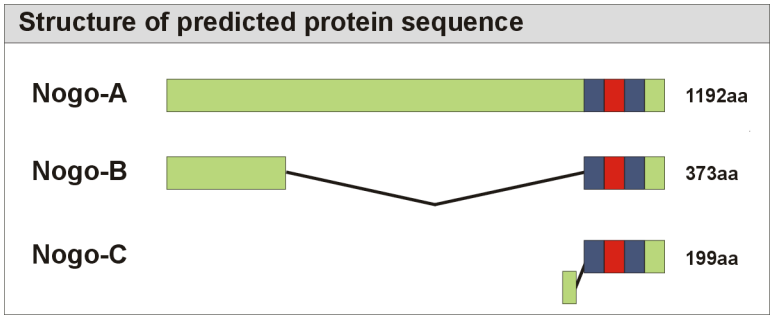
Astrocytes contribute to the formation and maintenance of the blood-brain barrier (Risau and Wolburg, 1990), which is important for protection and ion homeostasis (Bekar and Walz, 1999). Astrocytes are also involved in the production of the extracellular matrix (Liesi and Silver, 1988), neurotransmitter transport and metabolism (Westergaard et al., 1995) and a plethora of additional functions. Microglial cells are the phagocytic cells that remove cellular debris from sites of injury or as the result of normal cell turnover (Banati and Graeber, 1994). In the CNS, myelination is provided by oligodendrocytes (Bunge, 1968) while Schwann cells produce myelin in the PNS. After axonal injury in the PNS, myelin is rapidly cleared by macrophages as the axons degenerate. In contrast, following injury in CNS, myelin is cleared much more slowly, leaving demyelinated axons and the proximal stumps of cut axons exposed to myelin-associated inhibitors present in myelin fragments as well as in the intact surroundings (Filbin, 2003; He and Koprivica, 2004; Yiu and He, 2003). Reactive astrocytes form a glial "scar" at the lesion site and deposit extracellular matrix components, both of which serve as barriers to axon regrowth (Silver and Miller, 2004). The net effect of the cellular and extracellular changes in the immediate environment of severed CNS axons is an environment that does not permit cut axons to regenerate and

reestablish useful functional connections. The following description is an overview focusing on factors in the CNS that have been studied in the thesis.

Myelin-derived axon growth inhibitors

Schwab and other researchers have provided experimental evidence for the presence of neurite growth-inhibiting molecules within the CNS (reviewed in Schwab et al., 1993). The investigators performed elegant cell and tissue culture experiments to show how CNS myelin, but not PNS myelin, possesses potent neurite growth inhibitory activity (Schwab and Thoenen, 1985; Carbonetto et al., 1987; Crutcher, 1989; Khan et al., 1990; Sagot et al., 1991; Savio and Schwab, 1989). Furthermore, co-cultures of neurons and glia demonstrated that when growth cones came in contact with oligodendrocytes they collapsed and were retracted, and thus that myelinated areas were strictly avoided by neurons (Caroni and Schwab, 1988; Bandtlow et al., 1990; Moorman, 1996).

Nogo



To further dissect and identify individual oligodendrocyte-derived molecules, Schwab and colleagues fractionated CNS myelin by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). By testing the ability to inhibit axonal extension they identified two gel-extracted proteins with different molecular weights (35kDa and 250kDa) having this dramatic effect on axon growth. These fractions were named NI35 and NI250. Thereafter, an anti-CNS myelin monoclonal antibody to NI250, termed IN-1, was isolated for its ability to neutralize myelin inhibition *in vitro* (Caroni and Schwab, 1988; Schnell and Schwab, 1990). The target of the IN-1 antibody led Schwab's group (Chen et al., 2000) and two other labs (Prinjha et al., 2000; GrandPre et al., 2000) to the discovery of Nogo. Nogo is a member of the reticulon family of membrane proteins, and three major transcripts Nogo-A (1,162 amino acid), -B (373 amino acid), and –C (199 amino acids) originate from this gene by alternative RNA splicing and promoter usage. Two domains of Nogo have been identified as inhibitors of neurite outgrowth, causing growth cone collapse *in vitro*. One is the amino terminal portion of Nogo-A (termed amino-Nogo), not shared by Nogo-B and Nogo-C (Oertle et al., 2003), and the second is a 66 amino acid sequence (Nogo-66) shared by all three isoforms (Chen et al., 2000; Prinjha et al., 2000; GrandPre et al., 2000; Fournier et al., 2001; Oertle et al., 2003). Amino-Nogo and Nogo-66 show independent inhibitory activity (Fournier et al., 2001). While amino-Nogo causes inhibition of axonal growth as well as inhibition of fibroblast spreading *in vitro*, Nogo-66 causes inhibition of axonal extension but does not inhibit fibroblast spreading. Since Nogo-B and Nogo-C expression has not

been detected in oligondendrocytes, most studies have focused on Nogo-A. Nogo-A is highly expressed in CNS oligodendrocytes and also by central and peripheral neurons, while astrocytes and Schwann cells do not express Nogo-A (Josephson et al., 2001; Huber et al., 2002; Wang et al., 2002c). Transgenic mice in which Nogo A or Nogo-C is expressed by Schwann cells show delayed regeneration after sciatic nerve lesions, supporting a general inhibitory role for the Nogo-66 domain that can partly override the nerve growth stimulatory milieu of an injured peripheral nerve (Pot et al., 2002; Kim et al., 2003a).

Myelin-associated glucoprotein (MAG)

The first myelin protein to be identified as an inhibitor of axon outgrowth was MAG. MAG is a transmembrane protein that is expressed in both CNS and PNS (Lai et al., 1987; Salzer et al., 1987). Interestingly, the effect of MAG *in vitro* appears to be bifunctional (DeBellard et al., 1996; Mukhopadhyay et al., 1994). It can either promote or inhibit neurite outgrowth depending on the age of the neurons. Application of MAG to embryonic neurons promotes outgrowth (DeBellard et al., 1996) indicating that the inhibitory activity of MAG *in vitro* is restricted to adult neurons. The *in vivo* inhibitory effect of MAG is less well understood. Since peripheral nerve myelin, where regeneration occurs, expresses MAG, it may not function as an inhibitor *in vivo*. However, analysis of MAG knockout mice has shown that axon regeneration is improved in the PNS (Schafer et al., 1996). One explanation for the axon regeneration seen in PNS even though MAG expression is present might be that peripheral nerve myelin is cleared more efficiently by macrophages (reviewed in Hirata and Kawabuchi, 2002). The basal membrane surrounding peripheral myelinated axons may also act to shield other axons from direct myelin contact in the PNS, while the lack of such a structure in CNS may increase contact between axons and axon growth inhibitory molecules.

Oligodendrocyte myelin glycoprotein (OMgp)

Wang and colleagues identified OMgp as a myelin-associated inhibitor during a search for glycophosphatidylinositol (GPI) -linked inhibitors in CNS myelin. OMgp causes growth cone collapse and inhibits neurite outgrowth *in vitro* (Wang et al., 2002b; Barton et al., 2003b). OMgp contains a leucine-rich repeat (LRR) domain and is a GPI-anchored protein (Mikol et al., 1990) localized to the plasma membrane. Like Nogo, OMgp is also made by neurons (Habib et al., 1998). A study by Huang and colleagues suggests that OMgp acts to prevent collateral sprouting since it is enriched in membranes of oligodendroglia-like processes that encircle nodes of Ranvier (Huang et al., 2005). Effects of OMgp *in vivo* have not yet been analyzed.

Additional inhibitors

In addition to the membrane linked growth inhibitors Nogo-A, MAG and OMgp, a host of other components and circumstances in the immediate surroundings of an injured axon contribute to the failure of meaningful spontaneous axon regeneration in the adult mammalian optic nerve, brain and spinal cord.

Astrocyte-derived axon growth inhibitors - CSPG

In response to injury, microglia, oligodendrocyte precursors, meningeal cells and astrocytes appear at the lesion site. Whereas some astrocytes might support regrowth (Faulkner et al., 2004),

many astrocytes become hypertrophic and adopt a reactive phenotype, depositing inhibitory extracellular matrix molecules including chondroitin sulphate proteoglycans (CSPGs; McKeon et al., 1991), which are rapidly up-regulated after injury, forming an inhibitory gradient that is highest at the center of the lesion. The mechanism by which CSPGs inhibit axon growth is not well understood and may include a mechanical barrier function as well as more specific molecular interactions (Bovolenta and Fernaud-Espinosa, 2000).

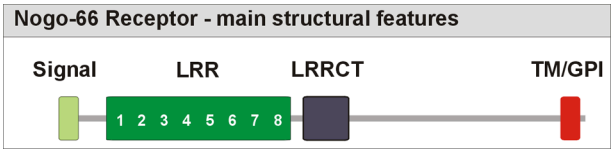
Ephrins and Fibroblast/meningeal cell-derived growth inhibitor – Semaphorin

Repulsive guidance cues found in CNS myelin, with roles in axon pathfinding during development, have also been implicated as inhibitors of axon repair in the adult. For instance, Ephrin B3 functions as a midline repellent during corticospinal tract (CST) formation (Kullander et al., 2001) and continues to be expressed in myelinating oligodendrocytes (Benson et al., 2005). The transmembrane semaphorin Sema4D/CD100 is expressed by mature oligodendrocytes and can be induced by injury resulting in growth cone collapse (Moreau-Fauvarque et al., 2003). It appears that many guidance cues involved in formation of the nervous system, become down-regulated in adulthood, but some continue to be expressed in adulthood and others are reexpressed after injury and may exert axon growth inhibitory activity in CNS.

Axonal receptors for myelin-associated inhibitors

Nogo receptor (NgR)

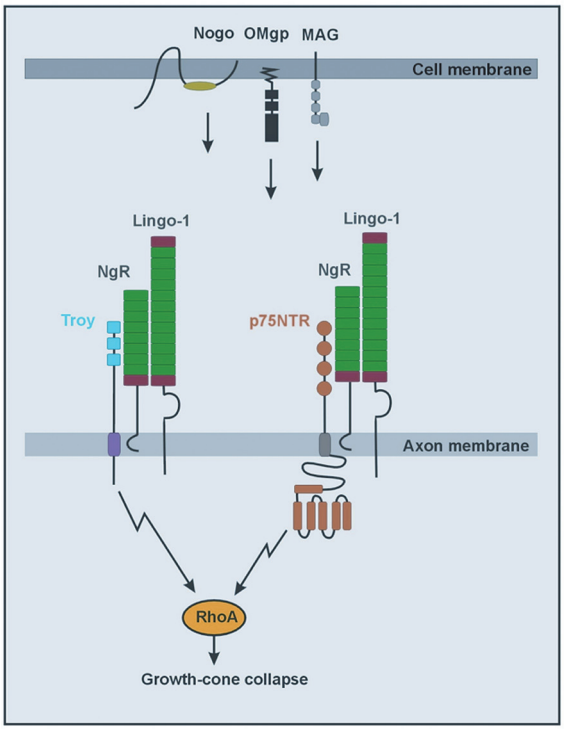
The laboratory of Strittmatter has identified a GPI-linked axonal surface protein (NgR) that binds Nogo-66 with high affinity (Fournier et al., 2001). Although MAG and OMgp are structurally different from Nogo, it was later found that NgR binds all three myelin-associated inhibitors (Domeniconi et al., 2002; Liu et al., 2002; Wang et al., 2002b). NgR is expressed in multiple types of neurons (Josephson et al., 2002, 2003). The receptor is a 473 amino acid protein with a signaling sequence, followed by 8 leucine-rich repeat (LRR) domains, a LRR carboxy-terminal cystein-rich flanking domain, a unique region and a GPI anchorage site (reviewed in Strittmatter, 2002). The GPI linkage serves to anchor the protein to the outer surface of the cell membrane, where it can interact with its ligands. Strittmatter and colleagues analyzed small peptide fragments of Nogo-66 and showed that, although residues 1-31 of Nogo-66 bind with high affinity to NgR, only a peptide containing residues 31-55 inhibits axon outgrowth. These observations led further to development of a competitive antagonist peptide, NEP1-40 (Nogo extracellular peptide, residues 1-40), which specifically blocks Nogo-66, but not the inhibitory activity of amino-Nogo or MAG, showing that Nogo-66 contains discrete binding and inhibitory domains. (GrandPre et al., 2002). Intrathecal (GrandPre et al., 2002) and delayed systemic application (Li and Strittmatter, 2003) of NEP1-40 produced significant axon regrowth and enhanced locomotor recovery following spinal cord injury. In an animal model of stroke, administration of a function-blocking NgR fragment, sNgR310-Fc, promoted both the recovery of motor skills and axonal plasticity without significantly reducing stroke lesion size (Lee et al., 2004). This indicates that reduction of NgR function after stroke allows increased anatomical plasticity and improved motor performance that are not assigned to neuroprotection.



The role of the NgR as mediator of effects of myelin-associated inhibitors is not fully clarified. By the use of a growth cone collapse assay, Kim and colleagues showed that NgR-deficient neurons had a reduced sensitivity to myelin-associated signals (Kim et al., 2004). However, Zheng and collaborators found that neurons lacking NgR still showed limited neurite outgrowth in the presence of myelin-associated inhibitors (Zheng et al., 2005). Together, these results indicate that there may be other receptor mechanisms operating independently of NgR. In CNS neurons two human homologues of NgR have been identified (NgR2 and NgR3) although neither of them binds to Nogo-66 (Barton et al., 2003a).

Co-receptors of NgR

Since NgR lacks an intracellular domain, it is believed that NgR associates in lipid rafts with other transmembrane signal-transducing polypeptides (Brittis and Flanagan, 2001). Perhaps surprisingly, the low-affinity neurotrophin receptor (P75NTR) was the first co-receptor of NgR to be identified (Wang et al., 2002a; Wong et al., 2002). Thus the p75 receptor has dual roles, being a ligand for either nerve growth factor (NGF) or serving as a co-receptor for Nogo-type inhibitory ligands. So far, evidence suggest two classes of co-receptors to be involved in Nogo signaling, because members of the tumor necrosis factor receptor (TNFR) family, such as p75NTR and Troy (Park et al., 2005; Shao et al., 2005), as well as Lingo-1 (Mi et al., 2004), appear to function as co-receptors for NgR. Interaction of myelin-associated inhibitors with either the p75NTR/NgR/Lingo-1 or the Troy/NgR/Lingo-1 complex is thought to lead to activation of the small GTPase RhoA and eventual growth cone collapse (Yamashita and Tohyama, 2003; Park et al., 2005; Shao et al., 2005).



Intracellular signaling and growth cone collapse

Axon growth inhibitory ligands induce collapse of growth cones via the Rho family of small GTPases (guanosine triphosphatase) signaling pathway (Tang, 2003). RhoA, Cdc42 and Rac1 are the best characterized Rho family members and known as regulators of the actin cytoskeleton and modulators of growth cone motility (Maekawa et al., 1999; Luo, 2000). RhoA acts as a molecular switch being active when bound to guanosine triphosphatase (GTP) and inactive when bound to guanosine diphosphatase (GDP). In the growth cone, the serine-threonine kinase Rho-kinase (ROCK) is probably the most important effector of RhoA (Bito et al., 2000). Yamashita and colleagues have shown that MAG activates RhoA by increasing the proportion of the protein bound to GTP (Yamashita et al., 2002). Data indicate that Nogo-66 and myelin use the same signaling pathway (Fournier et al., 2003; Niederost et al., 2002). Both *in vitro* and *in vivo* experiments suggest that ROCK plays an important role in mediating myelin-induced inhibition via NgR (Niederost et al., 2002; Dergham et al., 2002). Furthermore, in a model of corticospinal tract lesions in the adult rat, inactivation of the Rho pathway can promote spinal cord repair (Dergham et al., 2002) and enhance axonal regeneration (Fournier et al., 2003).

The Nogo system and injury

Several observations have challenged the view that the prime role of Nogo would be to inhibit axon regeneration in the CNS upon injury, especially spinal cord injury. Thus Nogo mRNA is not elevated at the site of a spinal cord injury (Josephson et al., 2001; Huber et al., 2002; Hunt et al., 2003) and NgR mRNA levels are rather low in the adult spinal cord compared to other parts of the brain (Josephson et al., 2003; Hunt et al., 2002). Also, while NgR mRNA levels appear unaffected by spinal cord injury (Josephson et al., 2003; Wang et al., 2002c) we demonstrated that NgR mRNA levels are down-regulated in kainate-induced injury (Josephson et al., 2003). Secondly, three different laboratories have produced Nogo knockout mice with three different regeneration phenotypes (Zheng et al., 2003; Kim et al., 2003b; Simonen et al., 2003). Strittmatter and colleagues analyzed Nogo-A/B mutant mice (Kim et al., 2003b). Nogo-A and –B expression is disrupted, whereas Nogo-C expression remains unaffected in this line. They found marked sprouting of corticospinal axons proximal to a spinal cord dorsal hemisection, although these effects were restricted to young mice. Schwab and collaborators generated a Nogo-A-deficient mouse-line (Simonen et al., 2003) and detected a compensatory increase in Nogo-B. Two weeks after dorsal hemisection, Nogo-A knockout mice had more corticospinal tract fibers growing toward and into the lesion compared to wildtype mice. These data are significant, although more modest than those reported by Strittmatter's group. The more modest regenerative effects may be explained by the up-regulation of Nogo-B, which contains the Nogo-66 inhibitory domain. In addition, Zheng and collaborators (Zheng et al., 2003) analyzed two Nogo knockout strains, one Nogo-A/B mutant, which did not affect Nogo-C, and one Nogo-A/B/C mutant. They report that following dorsal hemisections, tracing of corticospinal tract fibers did not reveal any obvious regeneration or sprouting of these fibers in either strain, suggesting that elimination of Nogo alone is not sufficient to induce axon regeneration. In addition, p75NTR-deficient mice were not better than wildtype mice following spinal cord injury (Song et al., 2004). Functional redundancy of p75NTR and TROY and/or the limited expression of p75 in the CNS might possibly explain the lack of improved regeneration after spinal cord injury in p75NTR knockout mice. Whether TROY-deficient mice will display enhanced regeneration is now an interesting question, given the more abundant expression of Troy. However, since p75NTR is widely up-regulated after injury, p75 might possibly replace TROY in some neuron systems of injured Troy knockout mice.

This issue will no doubt soon be addressed by the generation of TROY/p75 double knockout mice. An important question that still remains is the importance of the Nogo system in tissues outside the general context of spinal cord injury.

The Nogo system and neuropathologic conditions

Nogo has been implicated in neuropathologic conditions that are not due to physical injury. An elevation in Nogo transcript and protein levels was shown in a transgenic amyotrophic lateral sclerosis (ALS) mouse model (Dupuis et al., 2002). The investigators observed that the lumbar spinal cord and gastrocnemius muscle displayed altered expression patterns of Nogo. These findings were confirmed by similar results in postmortem and biopsy samples from diagnosed ALS patients. In patients with temporal lobe epilepsy (TLE), Bandtlow and collaborators (Bandtlow et al., 2004) noted that Nogo mRNA and protein were strikingly up-regulated in most neurons in hippocampus. Moreover, Nogo seems to have a direct role in the disease pathology of autoimmune-mediated demyelination (Karnezis et al., 2004). Using mice with experimental autoimmune encephalomyelitis (EAE) as an animal model of multiple sclerosis (MS), immunization with peptides corresponding to the Nogo-A specific exon 3 region, investigators observed attenuated clinical symptoms, demyelination, and axonal damage. These results are in line with the observations that antibodies to Nogo-A have been detected in serum and cerebrospinal fluid from MS patients (Reindl et al., 2003). Furthermore, Novak and collaborators (Novak et al., 2002) have provided evidence that Nogo levels are elevated in cortex cerebri of schizophrenic patients, while Strittmatter and colleagues have indicated a possible role of the Nogo system in Alzheimer's disease (Strittmatter, 2002; Park et al., 2006). Nie and collaborators (Nie et al., 2003) have also shown that in the paranodal region of axons, Nogo seems to interact with the adhesion molecule Caspr/paranodin (Peles et al., 1997) and indirectly with Caspr-associated potassium (K^+) channels (Bhat et al., 2001).

Structural plasticity and inhibitors

What is the normal physiological role of Nogo and other myelin-associated components? The functions of Nogo, MAG, OMgp and its receptors likely extend beyond axon outgrowth inhibition. Data indicate that MAG and p75NTR regulate aspects of myelination *in vivo* (Li et al., 1994; Montag et al., 1994; Cosgaya et al., 2002). Moreover, Nogo and NgR are expressed during synaptogenesis and NgR is found at synaptic sites (Wang et al., 2002c). There are also indications that these proteins may function to restrict axonal plasticity and prevent excessive sprouting and formation of aberrant connections in the intact adult CNS. If so, antagonism of the Nogo system should lead to increased axonal sprouting, growth and branching in the uninjured nervous system. This notion has been supported by experiments with IN-1 and with anti-Nogo (Thallmair et al., 1998; Buffo et al., 2000).

During a postnatal critical period, monocular deprivation leads to a shift in ocular dominance in the visual cortex, which does not occur in adults (Wiesel and Hubel, 1963a, b). Strikingly, both genetic deletion of NgR (McGee et al., 2005) and infusion of Chondroitinase-ABC (ChABC) into the visual cortex (Pizzorusso et al., 2002) provide evidence that ocular dominance shift plasticity can occur in the adult visual cortex by removal of growth inhibitors in the neuropil. These results suggest that a greater degree of local remodeling in the adult CNS would be generally allowed upon removal of components such as CSPGs and/or myelin inhibitors. This idea is sup-

ported by the enhanced short-range plasticity and stroke recovery noted in the absence of Nogo or NgR expression (Lee et al., 2004). In addition, Huang and colleagues (Huang et al., 2005) demonstrated increased sprouting at nodes of Ranvier in OMgp-deficient mice. Furthermore, data indicate improved collateral sprouting following ChABC treatment after denervation of the superior colliculus (Tropea et al., 2003) or spinal cord injury (Massey et al., 2006). In summary, in higher vertebrates different astrocyte- and oligodendrocyte-derived inhibitors seem to promote stabilization of the mature neural circuitry.

The Nogo system and hippocampal formation

A challenging question raised by our laboratory (Josephson et al., 2002, 2003) is why messenger RNA (mRNA) for both the Nogo receptor and one of its ligands, Nogo, are highly expressed in the brain and spinal cord during development and in regions with high plasticity such as the hippocampus in the adult. The hippocampal formation is of key importance in memory. It is therefore tempting to speculate that the Nogo system might be important for memory and learning. One possibility is that the Nogo system is involved in the formation of nerve fiber connections. Selective perturbation of the Nogo or the NgR gene will provide more insight into further possible roles of the Nogo protein. Understanding the function of Nogo could possibly lead to the development of tools for improving the regeneration of nerve fibers over long distances in the central nervous system. Clearly, the widespread distribution of Nogo and the specific and developmentally regulated patterns of NgR presence suggest that the full functional repertoire of the Nogo system remains to be discovered.

Dynamic change of the actin cytoskeleton (Matus et al., 2000) is required for life-long synaptic plasticity and reorganization. It is a central hypothesis of this thesis that the Nogo signaling system, and in particular the NgR, plays a key role in controlling when and where structural remodeling is allowed in the neuropil, and when it is not. We suggest activated neurons down-regulate cell adhesion molecules such as neural cell adhesion molecule (NCAM), to allow axon terminals to become detached, down-regulate NgR to become temporarily insensitive to Nogo and that this allows a degree of synaptic reorganisation in response to increased local gradients of attractive and repulsive molecules. Thus the Nogo signaling system may be involved in synaptic plasticity, learning and memory. The Nogo system may serve to restrict structural plasticity and suppress synapse remodeling in the CNS under basic conditions. Simultaneous presence of Nogo and its receptor in neurons may confer a locked state to hippocampal and cortical microcircuitry. If so, one or both must be effectively and temporally down-regulated to permit structural changes necessary for the formation of very long-term memory.

Aims

The aims of the present thesis were to advance understanding of the Nogo signaling system in health and disease, focusing on gene activity regulation and behavioral sequelae of NgR overexpression. Specifically, the aims were to:

Map the cellular activity patterns of the NgR gene and its co-receptor Lingo-1 in rodents and humans using *in situ* hybridization.

Investigate if the high NgR and Lingo-1 mRNA expression seen in brain areas endowed with a high degree of plasticity were regulated by neuronal activity.

Study the cellular expression of myelin-associated inhibitors such as Nogo, MAG and OMgp and components of the NgR complex NgR, Lingo-1 and Troy in the aging brain.

Study the cellular expression patterns of components of the Nogo signaling system and brain-derived neurotrophic factor (BDNF) and the effects of carbamazepine treatment in a mouse megencephaly model associated with epilepsy.

Develop and characterize mice with inducible forebrain-directed NgR overexpression using *in situ* hybridization and behavior tests.

Examine the possible role of NgR regulation in the formation of very long-term memories, by using characterized mice with inducible NgR overexpression in different behavior tests.

Materials and Methods

The materials and methods used are summarized below. For more detailed descriptions, see the methods sections of the individual papers and manuscripts (Papers I-VII)

Animals

In paper I, C57B6 mice were used; tissues from fetal, early postnatal and adult mice were included. Female Sprague-Dawley rats (B&K Universal, Sweden) were used in Papers II, III and V. Tissues from early postnatal and adult rats were included. In paper II, male spontaneously hypertensive rats (SHR) were also used. In paper IV, mutated BALB/cByJ-Kv1.1$^{mceph/mceph}$ and BALB/cByJ-Kv1.1$^{+/+}$ mice (originally purchased from The Jackson Laboratory, Bar Harbor, ME) were used. In paper VI, male Fisher 344 rats were used. In paper VII, we used the CamKII-tTAtransgenic mice (The Jackson Laboratory). We also cloned a PCR fragment of the mouse Nogo receptor with flanking HindII-XBA1 sites into the multiple cloning site of the pTRE2 vector (BD Biosciences Clontech) and microinjected into pronuclei of fertilized mouse eggs from C57BL6 (MouseCamp, Stockholm, Sweden) were performed to obtain CamKII-tTA /pTRE-NgR mice with a tissue-specific inducible overexpression of NgR. Animals were kept under standardized light, temperature and humidity and given food and water *ad libitum*. Experiments had been approved by the Ethical Committee of Northern Stockholm (Stockholms Norra Försökdjursetiska Kommitté).

Antiepileptic drug (AED) treatment (Paper V)

Carbamazepine (Sigma-Aldrich, St Louis, MO) was incorporated in pellets (3,5 g/kg) and given orally to BALB/cByJ-Kv1.1$^{mceph/mceph}$ and BALB/cByJ-Kv1.1$^{+/+}$ mice from postnatal week 5 until 12 weeks of age.

Doxycycline treatment (Paper VII)

Doxycycline (Sigma-Aldrich, St Louis, MO) was given in water (100 µg/ml) to turn off transgene expression in mice carrying both the CamKII-tTA and the pTRE-NgR transgenes as well as in monotransgenic controls and normal animals.

Human material

Procurement of human fetal tissues (Paper I)

Fetal tissues were collected following routine abortions and with written consent from the women seeking abortion and the approval of the Ethics Committee of the University of Bern as described earlier (Josephson et al., 2001). Tissues were collected from three fetuses aged 9 weeks post-conception. The tissues were prepared at room temperature in Gey's balanced salt solution (Gibco) under microscopic guidance. Tissue blocks were frozen in isopentane at –70°C and stored at –80°C until further processing.

Procurement of human adult tissue (I and V)

Human brain tissue was provided by the Harvard Brain Tissue Resource Center.

Spinal cord tissue was obtained at the Karolinska Hospital in Stockholm in connection with organ donations as described by Josephson and collaborators (Josephson et al., 2001). The tissue was collected with the informed consent of the relatives of organ donors and the approval of the Ethics Committee of the Karolinska Hospital, Stockholm. After collection of internal organs for transplantation purposes, the 10th, 11th and 12th thoracic vertebral bodies were removed using a ventral approach and the dorsal root ganglia Th11, Th12 and L1 exposed. The identification of the level of the vertebral body was based on its relation to the 12th rib. Following the respective nerve roots, a spinal cord segment between Th11 and L1 was excised with the dorsal root ganglia Th11 and Th12 attached. Specimens were obtained from two adult female (age 19 and 54 years old) human organ donors. The spinal cord pieces were then transversely sectioned into slices, immediately frozen on dry ice and stored at -80°C.

Surgery

Weight-drop injury of the spinal cord (Papers II and V)

One of the most clinically relevant experimental models of spinal cord injury in rodents is the standardized contusion injury caused by a weight-drop injury device, the NYU impactor (Gruner, 1992). This device reproducibly causes graded lesions, typically with a peripheral lateroventral rim of spared white matter.

Kainic acid administration (Papers II and III)

To investigate the hypothesis that neurons regulate Nogo receptor expression, we administered the non-NMDA (N-methyl-D-aspartate) receptor agonist kainic acid. This drug induces seizures as the result of enhanced synaptic transmission, leading also to neuronal death and axonal sprouting accompanied by permanent functional alterations in hippocampal circuits (Sperk et al., 1985). Kainic acid (10 mg/kg) was administered i.p. and animals sacrificed 2, 4 and 24 hours and 3 and 7 days later by decapitation. All animals were observed for development of seizures; only those kainic acid-injected animals that developed grade V seizures, as defined by repeated incidences of rising on the hindlimbs and falling over (Sperk et al., 1985), were included in the study. Control animals had no such symptoms. Brains and spinal cords were frozen on dry ice and stored at –80° until further processing.

Intracranial injections (Paper III)

Since BDNF is up-regulated by increased neuronal activity in specific brain areas where NgR is down-regulated, we speculated that NgR could be down-regulated by local increase of BDNF. Rats were placed in a stereotaxic frame (Kopf) and implanted with bilateral cannulae directed at the dorsal hippocampus. Each animal was then given intrahippocampal BDNF or bovine serum albumin (BSA) injections and sacrificed after 4 and 24 hours.

Behavioral experiments

Wheel running (Papers II and III)

In order to study if the Nogo signaling system is regulated during learning of a motor task, we allowed SHR rats to establish a running behavior known to up-regulate BDNF levels in hippocampus. The SHR strain was used because these rats develop a robust running behavior (Shyu et al., 1984). Rats were placed in individual cages with either unlimited access or no access to running wheels. A computer recorded the running behavior of each animal and animals were sacrificed after 24 h, 3 days, 7 days, and 21 days.

Locomotor activity (Paper VII)

To study locomotor activity and explorative behavior we use a computerized multicage system that uses infrared motion detection. Prior to testing, animals were allowed to habituate to the room. After 30-45 minutes each animal was placed individually in a locomotor cage where horizontal (locomotion and motility) movements are detected by photosensors placed in the floor of the locomotion box. Locomotion and motility counts are summed every five minutes and spontaneous and drug-induced (e.g. amphetamine) locomotor activity is recorded. We carried out these experiments to determine if NgR overexpression during development or in adulthood influences such motor activities.

Step-through passive avoidance (Paper VII)

Passive avoidance (PA) is defined as an emotional memory task, which is hippocampus- and amygdala-dependent (LeDoux, 1993; Stiedl et al., 2000). It is used as a one-trial learning task and the test procedure makes use of the natural tendency of rodents to explore new environments and to avoid bright areas. During training on day 1, entrance to a dark compartment is associated with an electrical current (0,2 ampere), which works as the unconditioned stimulus. On the second day, 24 hours later, memory is investigated in a retention test where the animal is again placed in the light compartment and the latency, "retention latency", to enter the dark compartment is recorded. We also tested retention latency after longer times, up to 4 weeks, to test very long-term memory. We conducted these experiments to determine if very long-term, possibly lifelong memory was impaired in mice unable to down-regulate the NgR.

Magnetic Resonance Imaging

For the MRI experiments, mice were anesthetized with 1.5-2.0% isoflurane via a face mask. The mouse was kept in supine position on a customized rig and the head was fixed using a tooth bar and adhesive tape. The body temperature was maintained between 36 and 37°C using a controlled warm air system. MRI was performed using a 4.7-T magnet with a 40-mm horizontal bore (Bruker Biospec Avance 47/40, Bruker, Karlsruhe, Germany). The magnet was equipped with a 12-cm inner diameter self-shielded gradient system (max. gradient strength 200 mT/m). A birdcage volume coil (Bruker) with an inner diameter of 24 mm was used for excitation and signal detection. An inversion recovery (IR) spin echo sequence with rapid acquisition with relaxation enhancement imaging (RARE; Hennig et al., 1986) was employed to obtain 2D and 3D images of the mouse brain. The following parameters were used for image acquisition: Repetition time (TR) 2566.8 ms, echo time (TE) 35.6 ms, RARE-factor 8, inversion time 450 ms, matrix size

64 x 64 x 128, field of view (FOV) 0.90 x 1.20 x 1.80 cm, 2 averages. The 3D-MR images were analyzed using Amira 3.0 software (Mercury Computer Systems, Berlin, Germany). To ensure accuracy, the borders of the brain and hippocampus were drawn and checked in three orthogonal planes. The total brain volume was reconstructed and included the olfactory bulb as well as parts of the medulla. The border between medulla and spinal cord was defined as a line at the most caudal point of the cerebellum. Measurements of ventral cortex area were performed in coronal sections centered approximately 2.2 mm posterior to Bregma as outlined by Diez and collaborators (Diez et al., 2003) using Paravision 3.0.2 (Bruker). Briefly, The rhinal fissure of the coronal section was defined as dorsal boundary of the ventral cortex and the dark fimbria as medial boundary.

Statistical analysis

Size measurements of brain structures using 3D-MRI were compared using two-tailed non-paired t-tests and Bonferroni correction (STATA version 8.2, Stata Corporation, College Station, TX). Inter-rater reliability was assessed by calculating r between measurements of two investigators as described previously (Diez et al., 2003). For analysis of mRNA expression levels and areas of CA3 and neurons within CA3, statistical differences among groups were determined using analyses of variance (ANOVA) and Tukey's multiple comparison test (GraphPad Prisma version 4.0, GraphPad Software Inc, San Diego, CA). When only two groups were compared in BrdU-, weight- and PA-experiments, a two-tailed t-test (i.e. Mann-Whitney) was used. A probability level of $p<0.05$ was considered statistically significant.

Histological techniques

Tissue preparation

Animals were decapitated and brains and spinal cords removed and rapidly frozen on dry ice. For immunohistochemistry, animals were perfused via the ascending aorta with Tyrode containing heparin, followed by 4% paraformaldehyde with 0.14% picric acid. After dissection, brains were left for postfixation for one hour, and thereafter rinsed in 10% sucrose. Brains were frozen and stored at –80˚C.

In situ hybridization (Papers I, II, III, IV, V, VI and VII)

In situ hybridization was used to detect mRNA in cells. For cryostat sectioning, frozen tissue blocks were embedded (Tissue-Tek, Sakura Finetek USA Inc., Torrance, CA) and 14-µm sections thawed onto slides (ProbeOn, Fisher Biotech, Pittsburgh, USA). High stringency *in situ* hybridization was performed (Dagerlind et al., 1992; Josephson et al., 2001). *In situ* hybridization protocols were designed for use with complementary radiolabeled DNA oligoprobes optimized for the detection of mRNAs in fetal and adult rodent and human tissues.

Autoradiography (Papers I, II, III, IV, V, VI and VII)

Optical density values from *in situ* hybridizations were quantified using appropriate software (NIH-image analysis program, version 1.62 and ImageJ 1.32). To determine amounts of ^{33}S-labeled mRNA, a ^{14}C step standard (Amersham) was included to calibrate optical density readings on the film and convert these values to the corresponding nCi/g values. Measurements of

optical density on film autoradiograms were performed in hippocampus (CA1, CA3 and dentate gyrus), cortex and amygdala.

CA3 size measurements (Paper IV)

The cross-sectional area of neurons within CA3 and the area of CA3 itself were measured in digital images (Zeiss AxioCam and AxioVision software) of three months old *mceph/mceph* and wild-type mice without and after CBZ treatment. Measurements of 150 cells per slide and animal were taken from both right and left hemispheres in CA3 region. Likewise, measurements of CA3 area were taken from each hemisphere and the average area for each mouse was used.

BrdU labeling (Paper VII)

The thymidine analog 5-bromo-2-deoxyuridine (BrdU), which is incorporated during the S-phase of DNA synthesis, was administered to detect effects of NgR overexpression on survival of newly formed cells. In these experiments mice received BrdU (100 mg/kg, i.p., n=10/group) three times during a period of 48 hours. The animals were sacrificed 14 d after the first BrdU injection. Serial coronal 30-µm sections through the entire hippocampus were cut on a cryostat. Every tenth section was processed for BrdU immunohistochemistry. Sections were post-fixed in 4% formaldehyde in PBS for 10 min, rinsed in PBS followed by incubation in 2 M HCl for 30 min at 37˚C to increase exposure of BrdU epitopes. After washing in PBS, sections were blocked in a blocking solution (10% goat serum in 0.1% Tween in PBS) for 60 min at room temperature. Sections were then incubated overnight at 4°C with the primary antibody against BrdU (rat anti-BrdU, Harlan Seralab, Leics, UK, 1:100) diluted in the blocking solution, followed by 3 x 15 min 0.1% Tween PBS rinses. Sections were next incubated with biotinylated goat anti-rat biotin (Vector Laboratories, Burlingame, CA, USA, 1:200) in the blocking solution for 60 min at room temperature. After another series of rinses (0.1% Tween PBS, 45 minutes), avidin-biotin (Vector Laboratories) was applied for 40 min followed by rinses in PBS alone for 1 h, and visualization using 3,3k-diaminobenzidine (DAB) (Sigma). Sections were counterstained with hematoxylin (Vector Laboratories) and mounted.

Stereology (Paper VII)

The 'optical fractionator' was used to count BrdU immunoreactive cells in the dentate gyrus (West and Gundersen, 1990). Briefly, every tenth section was systematically sampled [section sampling fraction (s.s.f.)=1/10] after randomly selecting the first section within the first interval. An unbiased counting frame with known area was superimposed on the field of view by appropriate software (Stereologer™, SPA Inc., VA, USA). Counting frames are then systematically distributed with known x and y steps throughout the marked region from a random starting point. The area of the counting frame relative to the area associated with the x and y steps gives the second fraction [area sampling fraction (a.s.f.)]. The height of the optical dissector relative to the thickness of the section results in the third fraction [height (h)/thickness (h)]. The total number of neurons is given by the optical fractionator formula: Ntotal= $\sum Q$ - • t/h • 1/a.s.f. • 1/s.s.f., where $\sum Q$- is the number of neurons counted in the dissectors. Optical fractionator estimates are free of assumptions about cellular shape and size and are unaffected by tissue shrinkage. The dentate gyrus including an area exceeding the subgranular zone by two cell diameters and an area exceeding the molecular layer by one cell diameter were manually outlined using a 10x lens.

Cell counts were performed with a 60x lens. Values are means from counts in both hemispheres (n=10 animals).

Western blot (Paper VII)

Adult mouse tissues were dissected and sonicated in 200-500 µl of 1% sodium dodecylsulfate. Aliquots (100 µl) of the homogenate were used for protein content determination with BCA (bicinchoninic acid, Pierce Europe). Equal amounts of protein (60 µg) from each sample were loaded onto 10% polyacrylamide gels, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidine difluoride membranes (Amersham Pharmacia Biothech, Uppsala, Sweden). The membranes were immunoblotted using an affinity-purified polyclonal antibody that selectively detects goat anti-mouse NgR (R&D Systems, UK). Antibody binding was revealed by incubation with affinity-purified anti-goat IgG diluted 1:10 000 (Rockland, Gilbertsville, PA).

Real-time PCR (Paper II)

For determining the presence or absence of a specific sequence a real-time polymerase chain reaction (PCR) was performed (TaqMan). We measured the relative expression of NgR mRNA in rat cortex, spinal cord and DRG in P1 and P7 animals. Three samples of cortex and seven samples of spinal cord from P1 and P7 animals each were investigated. For the analysis of DRG, 15 dorsal root ganglia from different animals were pooled in each sample. One sample of DRG at P1 and two samples of DRG at P7 were investigated. In every sample measurements were done in triplicate and sample averages used for statistical evaluation. For the assay, ß-Actin was chosen as the most appropriate endogenous control. The target gene and the endogenous control must have the same amplification efficiency to be able to calculate the relative mRNA levels with the ΔCt-method (Applied Biosystems, User Bulletin #2). This was confirmed by subtracting the Ct values from the dilution series of the target gene.

Results and Discussion

The results of Papers I–VI are summarized below. For detailed descriptions, see appended publications and manuscripts.

Expression of NgR in mice and humans (Paper I)

The identification of Nogo and a Nogo receptor (NgR) (Chen et al., 2000; Prinjha et al., 2000; GrandPre et al., 2000; Fournier et al., 2001) represents important advances in understanding axon inhibition by CNS myelin components. To further the understanding of the physiological roles of the Nogo system, we have mapped the expression of the NgR gene using *in situ* hybridization in human and rodent tissues.

We found that the NgR is expressed in nerve cells of the developing nervous system in both humans and mice. Expression is prominent in brain regions such as hippocampus, endowed with a high degree of plasticity in the adult mouse and human. Specifically, NgR mRNA expression was detected in developing neurons in the forebrain, brainstem, trigeminal ganglion, spinal cord, and DRG at all investigated prenatal stages of mouse development (E14–E18). In the adult mouse, NgR mRNA was found in high amounts in nerve cells in several brain areas including cerebral cortex, hippocampus, thalamus, piriform cortex, amygdala, the mitral cell layer of the olfactory bulb and the granular cell layer of cerebellum. Fournier et al. (2001) reported NgR signals in neurons in cortex, hippocampus, pons and in Purkinje cells in cerebellum. While we confirmed the presence of NgR in cortex, hippocampus and pons, we did not find Purkinje neurons to be positive. This difference might be due to the use of different probes or other methodological differences, but a safe conclusion appears to be that Purkinje neurons have less (if any) NgR mRNA than many other neuron types. NgR was not found in the adult mouse striatum, white matter of the brain, the medial septal nucleus, the spinal cord or in investigated peripheral organs.

We also analyzed fetal and adult human tissue obtained from organ donors. Fetal human tissue revealed prominent NgR expression in the ventral half of the spinal cord and a lower expression in the dorsal spinal cord. Dorsal root ganglion cells also expressed NgR mRNA. In adult human tissue, we found that cortical layers III and V were positive for NgR mRNA. In addition, NgR mRNA was detected in human dentate hilar cells, the granular layer of the dentate gyrus and in the amygdaloid nuclear complex. Furthermore, we observed that NgR mRNA was absent in both white and gray matter of the adult human spinal cord. This was therefore in contrast to the widespread occurrence of NgR mRNA in the developing human spinal cord. This could indicate that during development, the Nogo-NgR system may act to control and direct nerve fibre outgrowth.

In human DRG we found that only a subset of the ganglion cells (20-25%) consisting of large and medium-sized neurons expressed the receptor. Small DRG neurons did not express NgR mRNA. The observation that a large majority of adult sensory nerve cells do not express NgR may explain why they, when transplanted to the white matter of the brain or spinal cord, can extend long nerve fibers. Without NgR, they would not be inhibited by the presence of Nogo in white matter. Pyramidal nerve cells of the cerebral cortex, on the other hand, express the receptor, and therefore their nerve fibers, many of which run down into spinal cord to control voluntary movement, would not be able to regenerate in white matter after spinal cord injury.

Possible ways in which Nogo/MAG/OMgp inhibition of nerve growth might be neutralized

In the present work we have demonstrated widespread expression of NgR mRNA in neurons and shown how rapid down-regulation of NgR mRNA occurs in neurons in situations associated with plasticity and the presumed formation of new memories. For this to affect plasticity one has to assume that the down-regulation of mRNA is coupled to a decrease of NgR protein. However, this is only one of many ways in which nerve-growth inhibitory signaling could be temporarily overcome, several other regulatory possibilities must be considered:

(1) *Membrane trafficking*: It is conceivable that the NgR (and associated molecules in the lipid raft) could be brought to and from the axon membrane by mechanisms similar to those proposed for e.g. neurotransmitter receptors (reviewed in Huang and Reichardt, 2003).

(2) *Receptor shedding*. Secretases acting at different levels could lead to detachment of NgR and/or other receptor components (Ahmed et al., 2006) acting as decoy binding sites for Nogo-type ligands.

(3) *Receptor-receptor interactions*. Emerging evidence in the neurotransmitter field suggests that interactions between adjacent receptor complexes, and topical rearrangements may determine receptor availability and sensitivity (reviewed in Agnati et al., 1995).

(4) *Downstream regulatory events.* The mechanisms by which Nogo-66 binding to NgR leads to growth cone collapse involves RhoA and several additional steps that finally influence actin filament organization and function. While these steps offer several additional ways in which a neuron may regulate its sensitivity to Nogo, such regulation would be less specific, if other receptor-mediated effects (e.g. from the epidermal growth factor (EGF)-receptor) converge or partially converge on the same intracellular pathway.

(5) *Modulation of ligand availability*. We have found the NgR gene to be much more prone to down-regulation by neuronal activity than its ligand Nogo (and, although less studied, possibly MAG and OMgp). Nevertheless, Nogo, particularly Nogo expressed by neurons (rather than Nogo expressed by glial cells) might be regulated in terms of synthesis, availability in the cell membrane and by various protein degrading enzymes, much like as suggested above for NgR.

Regulation of Nogo receptor expression (Paper II)

To further examine developmental regulation of NgR we used detailed *in situ* hybridization and real-time PCR (TaqMan) to locate and quantify NgR mRNA. We found the NgR gene to be developmentally regulated. NgR mRNA expression in the rat spinal cord and DRG was down-regulated after/during the first postnatal week.

An interesting finding was that in the adult CNS, Nogo-A and NgR mRNA expressions are confined to neurons in brain areas with high plasticity such as hippocampus. The formation of long-term memories presumably involves synaptic rearrangements as dictated by neuronal activity in hippocampus and cortex cerebri (Bliss and Collingridge, 1993; Engert and Bonhoeffer, 1999). We speculated that the Nogo system could be involved in regulating such structural change and asked if neural activity regulated the transcription of Nogo-A or NgR. To test this we subjected rats to kainic acid (KA), an excitoxin and non-NMDA receptor agonist that induces strong neuronal activity resulting in seizures and also axonal sprouting in hippocampus. We found that KA transiently down-regulated NgR mRNA levels in the dentate gyrus, hippocampus and neocor-

tex. We did not detect any regulation of Nogo mRNA. Up-regulation of BDNF mRNA in the brain in response to epileptogenic treatments is known (Schmidt-Kastner et al., 1996; Zafra et al., 1990). Therefore we also compared the regulation of NgR mRNA with that of BDNF mRNA and observed a strong negative temporal correlation between levels of BDNF and NgR mRNA in measured brain regions. The brain region in which the first and most dramatic NgR down-regulation and BDNF up-regulation was noted was the dentate gyrus. While KA administration can lead to death of hippocampal pyramidal cells, granule cells of the dentate gyrus are spared (Ben-Ari, 1985). Instead, the dentate granule cells undergo sprouting and synaptic reorganization (Tauck and Nadler, 1985; Cronin and Dudek, 1988). Our findings are thus in line with the idea that the Nogo signaling system might have a role in structural rearrangements at the synaptic level in the neuropil.

To analyze possible effects of changes of neuronal activity on NgR mRNA levels under physiological conditions, we allowed male rats of the spontaneously hypertensive rat (SHR) strain to establish a running behavior known to upregulate BDNF levels in hippocampus (Widenfalk et al., 1999). The SHR strain was chosen because it is known to develop a robust running behavior (Shyu et al., 1984). Strikingly, we were able to detect that establishment of a running behavior involves a period of NgR mRNA down-regulation in hippocampus and cortex. NgR mRNA was down-regulated after 3 and 7 days of running, whereas at 21 days levels were back to normal despite the continued use of the running wheels. We hypothesize that a 3–7 day interval, a period when rats intensify running, is also when they establish robust learning of this motor behavior and become habituated to daily running.

To study regulation of NgR mRNA in response to injury, we induced spinal cord trauma in adult rats and analyzed the expression of NgR mRNA at different time points using *in situ* hybridization. We failed to detect any changes in the expression levels in the spinal cord itself following weight-drop injury. This does not exclude that regulation of NgR mRNA levels might occur in different areas of the brain in response to spinal cord injury.

In conclusion, both chemically induced increase of neuronal activity (administration of KA) and behaviorally induced neuronal activity under physiological conditions (establishment of a running behavior) involves a period of NgR mRNA down-regulation in cortex and hippocampus. At the same time and in the same brain regions we confirmed BDNF up-regulation. This suggests that the full functional reportoire of the Nogo system remains to be discovered and that the Nogo signaling system might function beyond axon growth inhibition in white matter.

We suggest one such role for the Nogo system to be to regulate plasticity and learning. Perhaps the activity-induced NgR mRNA changes occur in synchrony with the well-known changes of BDNF during these events and also in synchrony with changes of other neurotrophic factors and receptors. We hypothesized that high levels of Nogo and its receptor in neurons would lock hippocampal and cortical networks and that Nogo and/or its receptor must be temporally down-regulated to permit plastic structural changes as needed for the formation of long-term memory. Down-regulation of cell adhesion molecules such as NCAM in situations of plastic changes have been shown (reviewed in Martin and Kandel, 1996). Hence a bouton might become detached and become temporarily insensitive to Nogo to allow plastic responses, as directed by local BDNF increases, after which synapses would become locked in their new configurations by normalization of NCAM, NgR and BDNF levels.

Lingo-1 mRNA expression in rat and human CNS tissue and regulation in rat following weight drop injury, KA and BDNF injections (Papers III and V)

When NgR was identified (Fournier et al., 2001) it was realized that the receptor for Nogo lacks an intracellular signaling domain, suggesting that additional components were required. A member of the tumor necrosis factor receptor (TNFR) family, p75NTR was the first component to be identified as a NgR co-receptor (Wang et al., 2002a; Wong et al., 2002). However, NgR in combination with p75NTR is not sufficient for transduction leading to RhoA activation. This led to the identification of another molecule, Lingo-1, that was shown to be required for intracellular transduction (Mi et al., 2004). The data also indicate that Lingo-1 may function primarily as a modulator that requires additional molecules for signal transduction. The mismatch between the scarcity of p75-expressing neurons in the brain and the richness of NgR-expressing neurons suggested additional co-receptor components. Troy, another member of the TNFR family, was shown to interact with NgR and Lingo-1 and able to replace p75NTR in a functional NgR complex consisting of NgR, Lingo-1 and Troy (Park et al., 2005; Shao et al., 2005). Troy is indeed more widely expressed in the brain than p75.

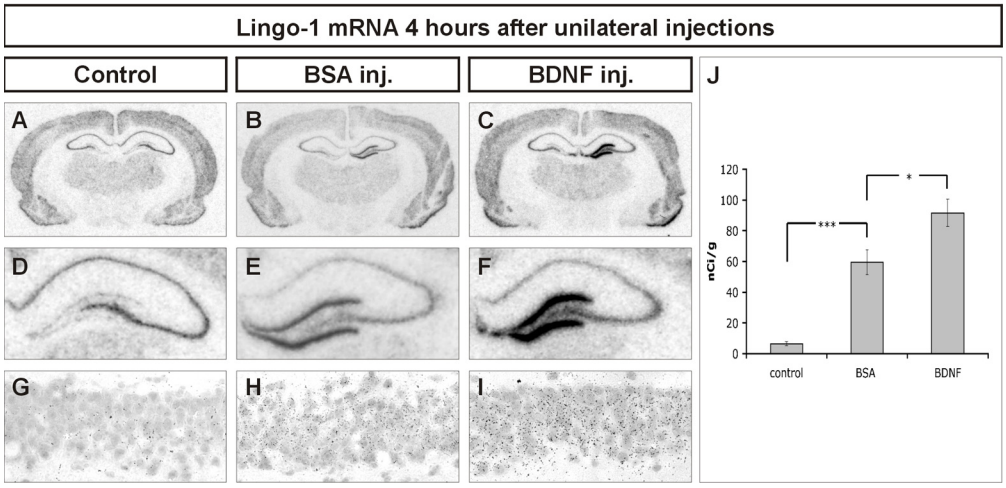
In Paper V, we compared the expression of Lingo-1 to the other components in the NgR complex by *in situ* hybridization in adult human brain and spinal cord and in the nervous system of developing and adult rat CNS. We also analyzed Lingo-1 gene transcription responses to weight drop injury of the adult rat spinal cord (Paper V), kainic acid administration and intrahippocampal injections of BDNF (Paper III). We found neuron-specific expression of Lingo-1 in adult human and rat CNS and DRG. We saw strong similarities between the patterns of Lingo-1 mRNA expression in the rat and human CNS. Lingo-1 mRNA is expressed in the adult hippocampus, neocortex and thalamus and the signal is slightly down-regulated in the adult rat compared to postnatal rat brain. During postnatal development, Lingo-1 mRNA was detected in motor neurons in the spinal cord and in dorsal root ganglia. While the expression of Lingo-1 weakens in the adult spinal cord, the strong expression in sensory neurons remains in adulthood. Moreover, all three layers of the P1 cerebellum displayed a positive signal for Lingo-1 mRNA although, from P7 onwards, only the granular layer of cerebellum showed Lingo-1 mRNA expression. Further investigations are needed to understand the functional implications of these expression patterns.

One week following weight drop injury, we observed a strong induction of Lingo-1 mRNA expression in cranial and caudal gray matter of the spinal cord and the up-regulation was longlasting. In contrast, Lingo-1 expression was markedly down-regulated at the site of injury. Erschbamer and colleagues (Erschbamer et al., 2005) have previously reported a clear increase of RhoA expression in the injury zone 1-4 weeks following injury in the same animals. The induction of RhoA was astrocyte and oligodendrocyte specific. These observations may possibly suggest that neurons in the damaged area become temporarily less sensitive to Nogo/MAG/OMgp inhibition allowing neurite extensions, while neurons in other areas of the damaged spinal cord become less prone to neurite sprouting.

In paper III we assessed Lingo-1 levels after KA administration and after a running period, conditions where neuronal activity is induced and where we previously detected a NgR mRNA down-regulation. Since local transient increases of BDNF are seen in response to neuronal activity, we also performed unilateral intrahippocampal BDNF injections and measured Lingo-1 and NgR gene regulation. We could not detect any Lingo-1 mRNA changes in cortex, CA1, CA3 or the dentate gyrus of animals that were exposed to running wheels. However, we found that Lingo-

1 mRNA was up-regulated in the dentate gyrus in response to either KA or BDNF injections. Even control injection of bovine serum albumin (BSA) into hippocampus induced an increase of Lingo-1 mRNA. After 24 hours Lingo-1 levels were back to control levels in the dentate gyrus. In line with our previous reports of NgR down-regulation by neuronal activity, we found a similar down-regulation of NgR mRNA after intrahippocampal injection of either BDNF or BSA.

Throughout life a degree of morphological plasticity is retained in the hippocampal formation. One trophic factor that is closely regulated by activity both under physiological and pathologi-



cal circumstances is BDNF. BDNF is also involved in plasticity of synapses during development and in adulthood (Lu and Figurov, 1997). We found that NgR is down-regulated in response to increased neuronal activity whereas BDNF was instead up-regulated. Therefore, we attempted to determine if there was a causal relationship, by using intrahippocampal BDNF injections. While BDNF delivery was indeed associated with a decrease of NgR mRNA levels, so was delivery of a control protein (BSA). Hence we were not able to demonstrate a cause-relationship between BDNF and NgR levels. Instead we found that the insult of the needle and injection procedure *per se* had effects. This is thus another example of the notion that it is impossible to physically probe the brain without causing significant local changes of cellular functions. Another example is implantation of a microdialysis probe, which *per se* causes marked increases of basic FGF mRNA in surrounding brain tissue (Humpel et al 1994).

Our experiments suggest that NgR may play a unique role in brain plasticity, by being the only known receptor component to be down-regulated in response to increased neuronal activity. Lingo-1 mRNA levels are instead increased in situations when NgR mRNA levels are decreased.

Decline of Nogo-A message in the aging brain (Paper VI)

Impairments of learning and memory are associated with aging and aspects of these impairments are similar to those caused by hippocampal damage (reviewed in Rosenzweig and Barnes, 2003). One reason for declining memory function in the aging cortex cerebri and hippocampus might be

decreased plasticity, impairing the ability to form new long-lasting memories. The Nogo system has been implicated in the pathophysiology of aging in Alzheimer's disease (Park et al., 2006).

To better understand age-related deficits we examined if the Nogo system components change in the aging brain. Sexual maturity (2–3 months in rodents) is used to define the start of adulthood. This definition varies depending on species, genetic background, and experimental conditions. We used Fisher 344 rats to model aging and measured the mRNA expression levels of Nogo, MAG, OMgp, NgR, Lingo-1 and Troy, in the hippocampal formation of adult (up to 10 months), middle-aged (10-20 months) and aged (from 20 months) rats. In this study, we found a small but significant decrease of Nogo mRNA in the neuronal layers of CA1, CA3, CA4 and the dentate gyrus when comparing 16- and 24-month-old animals. Neither the receptor components NgR, Lingo-1 and Troy, nor the ligands MAG and OMgp were changed in the aging cortex and hippocampus.

The fact that other components of the Nogo system were not changed by aging, argues against the Nogo mRNA decline being part of a general decrease of mRNA species in cells of the aging brain. Instead, the decrease might possibly reflect a compensatory mechanism of the aging brain. Lately, Nogo has also been implicated in neuronal pathologies that are not induced by physical injury. Patients with temporal lobe epilepsy have elevated Nogo-A levels in hippocampal neurons (Bandtlow et al., 2004). Patients with amyotrophic lateral sclerosis (ALS) have been reported to have elevated Nogo-A levels (Dupuis et al., 2002), whereas levels of Nogo-C protein are decreased in postmortem muscular samples of ALS patients. In a transgenic mouse model of ALS, similar changes have been observed. Also, autoantibodies recognizing Nogo-A have been detected in serum and cerebrospinal fluid (CSF) from patients with multiple sclerosis (MS) but not in controls (Reindl et al., 2003). Thus clinical observations to date have focused on Nogo itself. Investigations of the other ligands and the receptor components should help clarify the possible roles of the Nogo signaling system in injury and diseases of the nervous system.

*Normalization of BDNF, Lingo-1, Nogo and Troy after carbamazepine administration in the **mceph/mceph** mice (Paper IV)*

Megalencephaly in human neurological diseases is associated with symptoms ranging from mild to severe. Features often associated with megencephaly include epileptic seizures, learning disabilities and impaired intelligence, as well as motor dysfunction and morphological abnormalities. A mouse model for megencephaly was recently found to be caused by a spontaneous mutation in the Shaker-related, voltage-gated potassium channel gene 1, KCNA1, causing brain enlargement and seizure-like behavior (Petersson et al., 2003). Previously, Diez and colleagues (Diez et al., 2003) have reported that homozygous such mice (*mceph/mceph*) have increased total brain volume, ventral cortex, hippocampal formation and cerebral cortex from eight weeks onwards. Additionally, the investigators proposed a possible role of neurothrophic factors such as BDNF, for increase in size since a disturbance in BDNF expression was detected.

The *mceph/mceph* mouse offered an opportunity to study to which extent the Nogo signaling system might be involved in megencephaly pathophysiology, particularly because it had already been shown that BDNF levels are increased very early and it has been suggested that developmental mechanisms continue to be active in these mice (Diez et al., 2003). The effects of carbamazepine (CBZ) on neural growth in these mice were also studied. We speculated that the seizure-induced neural activity might mimic the findings from KA administration. Mice fed

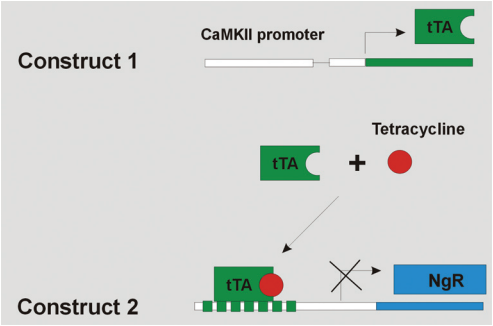
with CBZ were analyzed for seizure behavior, brain structure size and expression of markers for neuronal plasticity and rescue in the brain. We found the megencephalic brain to be characterized by a pattern of changes in the Nogo system that was clearly different from that caused by increased neuronal activity alone. Thus Nogo mRNA levels were increased in several areas, and NgR mRNA increased (rather than decreased) in the dentate gyrus and not markedly changed in other investigated areas. Lingo-1 mRNA levels were dramatically increased in e.g. the dentate gyrus, while Troy mRNA levels were decreased. OMgp mRNA levels were also increased. Strikingly, our results show that CBZ normalized the brain growth and counteracted the increased size of CA3 pyramidal neurons in hippocampus. CBZ also counteracted the abnormal BDNF, Gap-43, Nogo, OMgp, NgR, Lingo-1 and Troy mRNA levels in the *mceph/mceph* brain. Notably, these effects occurred at CBZ doses that did not fully suppress seizures. These observations suggest that certain forms of postnatal abnormal brain overgrowth are associated not only with abnormal expression of BDNF, but also with pathological changes in genes involved in the Nogo signaling system, and that these abnormalities can be prevented by treatment with known antiepileptic drugs such as CBZ.

Overexpression of Nogo receptor in the forebrain (Paper VII)

Based on our observations of specific down-regulation of NgR mRNA in situations of increased neuronal activity, we formulated the hypothesis that such NgR down-regulation could be a necessary event in neural plasticity underlying synaptic reorganization and long-term memory formation (Josephson et al., 2003), and conversely, that the steady-state high levels of Nogo and its receptor in neuropil serve to stabilize neuronal networks.

To directly test our hypothesis, we set out to generate mice with cell-specific overexpression of NgR. We reasoned that overexpression of NgR in neurons during development might disturb development and lead to adult brain dysfunction, and that overexpression in adulthood only might impair learning and long-term memory. To direct expression to forebrain neurons we used the Ca^{2+} calmodulin-dependent protein kinase II (Cam KII) promoter. To be able to turn transgene expression on and off we used the Tet-off system.

In paper VII, we describe the generation of NgR overexpressing mice and provide a preliminary characterization of some of their phenotypic traits. We have obtained data from three viable transgenic mouse lines ($CamKII^{pTRE/NgR}$) with inducible (removal of doxycycline) and specific overexpression of NgR in forebrain neurons. All three lines were found to overexpress NgR message in forebrain neurons as expected. As typical with pronuclear injections, there were certain differ-



ences in the precise patterns of overexpression between the lines, which we may use to our advantage to determine which levels and/or regions of NgR overexpression may be most important.

For line 1 (L1) we have determined that the overexpression of NgR transcript is also paralleled by overexpression of NgR protein (Fig. A). The overexpression is striking in the olfactory bulb, cortex cerebri, striatum and hippocampus. Overexpression is not seen in thalamus, cerebellum (Fig. B) or spinal cord (not shown). We have also determined that the expression is dependent on the absence of tetracycline. As long as mice receive tetracycline (doxycycline) in the drinking water, the transgene is not expressed. Together, these findings are evidence that the transgenic mice express the transgene as planned. We therefore used the L1 strain for our initial experiments. When doxycycline is not present, these mice will begin overexpression in forebrain neurons about 14 days after birth, at which time the CamKII gene is normally activated. Since the experimental animals are bitransgenic, monotransgenic animals can serve as controls.

Body weight measurements of mice with NgR overexpression from two weeks of age show that such animals will begin to lag behind littermate controls in adulthood, starting at about 3.5 months of age. Since one aspect of hippocampal plasticity is neurogenesis, we performed BrdU labeling to test if NgR-overexpressing mice have disturbed formation or survival of newly formed cells. Counting BrdU-labeled cells in the subgranular zone and the granular cell layer of the dentate gyrus, we noted fewer BrdU-labeled cells in the L1 strain compared to control animals. This

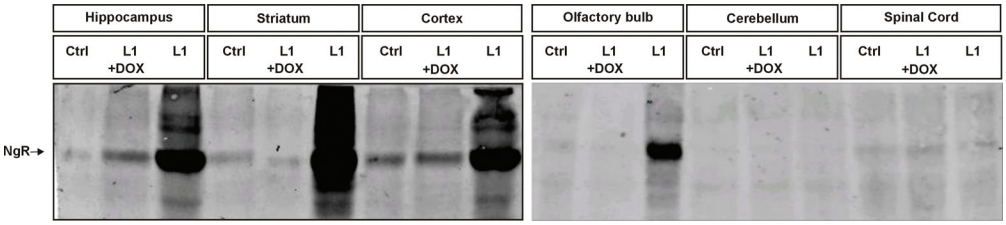


Fig. A. Tissue-specific expression of NgR in NgR overexpressing mice (L1) shown by Western blotting.

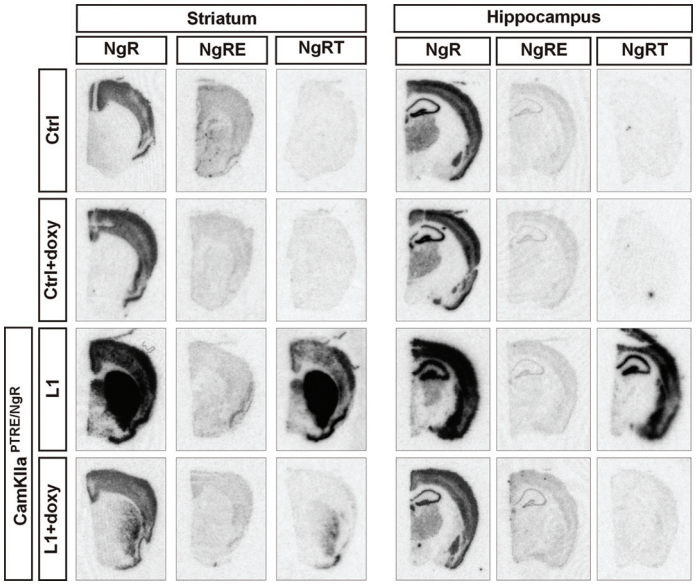


Fig. B. Tissue-specific overexpression of NgR mRNA in L1 mice.

argues for an impaired proliferation and/or survival of new cells in the L1 strain.
We next measured locomotor activity in L1 mice that had not been treated with doxycycline. Preliminary data suggest that spontaneous activity did not differ from controls, while the locomotor response to amphetamine was attenuated, particularly in response to a second amphetamine injection. These data need confirmation, but may indicate that NgR-overexpressing L1 mice either have developmental disturbances, e.g. of the reward system, or that they may be less prone to become sensitized to amphetamine.

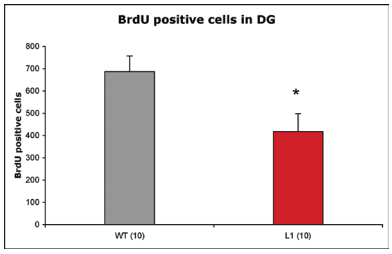


Fig. C. Counts of BrdU-positive cells in the dentate gyrus.

Passive avoidance, a hippocampus- and amygdala-dependent emotional memory task (LeDoux et al., 1993; Stiedl et al., 2000), was tested to determine if very long-term memory was affected in NgR-overexpressing mice. Commonly, this test is used to measure if drugs influence the tendency to avoid entering a dark compartment about 24 hours after animals have had an unpleasant experience of entering. We did not see any difference between L1 NgR-overexpressing mice and controls 1 day after conditioning. One week after conditioning there was still no difference. However, 4 weeks after the training day, 3 of 6 animals with overexpression of NgR in the forebrain crossed over. In contrast, none of the control animals entered the dark compartment during 5 minutes of testing. These preliminary observations suggest that overexpression of NgR may impair the establishment of long-term memory. However, other explanations such as a developmental disturbance caused by the continuous overexpression of NgR from the third week of life may have also influenced the behavior of the overexpressing mice.

To further test our hypothesis, we have initiated studies of mice, which receive doxycycline in the drinking water until adulthood and from which doxycycline is then withdrawn to allow transgene expression. Tests similar to those described above, as well as open field tests and tests in Morris swim maze are underway. These studies require four groups to be analyzed (controls and overexpressing mice with and without doxycyline removal in adulthood) in order to sort out effects of doxycycline removal versus turning on NgR overexpression. If and when a robust behavior effect of adult NgR overexpression is identified, the definitive test would be to correlate such effect to the on or off state of the transgene in the same individual animals.

Strong support for our hypothesis that NgR regulation is a key event in brain plasticity was recently provided by McGee and colleagues (McGee et al., 2005), who demonstrated that adult NgR knockout mice, in contrast to wildtype mice, lack maturation-dependent restriction of the ocular dominance shift phenomenon in visual cortex. This remarkable observation suggests that the absence of NgR allows synaptic plasticity to continue into adulthood in the visual cortex, despite the presence of Nogo.

Concluding remarks

The presented work contributes to the understanding of ligand and receptor components of the Nogo signaling system in health and disease. We suggest a role for NgR in the regulation of synaptic plasticity. Our transgenic mouse model with inducible and specific overexpression of NgR should help determine the role of NgR in CNS plasticity. Understanding the regulation of Nogo signaling and its role in synaptic plasticity may aid in the design of novel treatments for CNS injuries and diseases including conditions with failing memory

Acknowledgements

This thesis has been carried out in the Department of Neuroscience, at Karolinska Institutet, Stockholm, Sweden. I would like to take the opportunity to express my genuine appreciation for the help and encouragement that I have received during my thesis work. There are so many people that I feel enormously grateful to, and I do hope I will not forget to mention any of you.

I would like to thank the following people:

Anna Josephson, my supervisor, first for your brilliance and enthusiasm, but most of all for helping and supporting me in a strong and positive way and always being there when I needed you. I wish I had your talent in getting things done and at the same time looking so calm and elegant. No one can resist your charm and humor. I hope you realize what a wonderful supervisor you have been.

Lars Olson, my co-supervisor, for your dedication to science and guiding me to become a scientist. Thank you so much for taking me under your wing, for your patience, for your ability to teach and explain to me and the rest of the group not only about science but everything that is worth knowing. Moreover, I thank you, Lars, for helping me understand the importance of accepting both negative and positive results, for your fantastic sharp mind and eye that have always spotted my mistakes and for your incredible sense about what experiment to do next. I don't think that I will ever again have the privilege and joy to work with a boss such as you. Thank you for being a huge part in making these years the most interesting, most rewarding and stimulating I have ever experienced in my life as a student of science.

Christian Spenger for introducing me to the field of neuroscience and for giving me the opportunity to join the Olson laboratory. For teaching me the practical work such as how to do dissections, cell culture, microscopy, statistics and the basics of writing scientific papers. Thank you for your help, support and generosity.

Stefan Brené for your fantastic knowledge, patience and for being such a creative teacher. And for your endless support, humor, and encouragement when experiments fail. Thank you for always taking time to listen to my questions and for being so positive and enthusiastic about our results.

To all past and present chairmens at the Department of Neuroscience, Sten Grillner, Lars Olson, and Staffan Cullheim. Thank you for accepting me as a graduate student.

The Olson angels: Eva Lindqvist, for your energy and strength, Karin Lundströmer, for your problem-solving skills, no matter how difficult the situation, and Karin Pernold, for your calmness and dependability. Thank you all, not only for your invaluable help, excellent technical teaching and assistance in different laboratory techniques, without which none of this would have been possible, but also for your enthusiasm and always being so kind to me.

Ida Engqvist, thank you for your great help with the thesis layout, for your efficient help with computers, and for everything concerning conferences or travel arrangements. Ida – Your computer knowledge is matched by none!

To everyone on the animal staff, especially Margareta Almström, Niklas Lilja, Tua Finman, Annika Mattsson, Anna Eriksson, Helena Ericsson, Linda Thors and Martina Andersson, for taking so good care of all the animals.

Professor Eva Brittebo and Professor Håkan Aldskogius at BMC in Uppsala for helping me during my first steps in science and for all the support for my master thesis in your laboratories.

Co-authors Dagmar Galter for your kindness and teaching me the true art of human histology, Camilla Schéele for friendship and introducing me to Mr TaqMan, Johan Widenfalk for your clever tricks in designing in situ probes and autoradiography and Therese Pham, Catharina Lavebratt, Ann-Sophie Persson, Sven Ove Ögren, Andreas Ringman, Hans Widmer, Paula Bickford, Eddie Griffin, Fu-Hua Wang, Martin Schalling, and Claes Wahlestedt for all your scientific input.

To past and present members of the Olson group, you are not only colleagues but have become good friends of mine. Thank you for making the everyday laboratory work such a pleasant experience filled with chats, food, cakes, seminars and laughter. Thank you: my closest roommates, the warmest people with the biggest hearts; Anna Mattsson, for putting up with my theories about everything, my messy desk but most of all for your honest input, help and inspiration in life and science. Andrea Carmine Belin, for being a source of support, generous help with dresses at all parties, weddings (dissertation?) and for your creativity and sense of style, but most of all for your friendship during all these years. Mathew Abrams, what can I say…you are "the entertainer" in our lab! Thank you for all the good laughs, for telling us your hilarious stories about your life experience. You have an incredible talent for reading people and I envy your sharp and quick humor. Elin Åberg, I am so happy you started in the lab at the same time as I. In you, I found true friendship, support, strength and most of all reliability. But I will never go boxing with you again…you scared me. Astrid Bjørnebekk, for being a fanstastic person and a "duracellkanin". Although it took half a year to understand your Norweigian, we become friends at once. I am so happy Mattias and I met up with you and Sonny at Perhentian. I am sorry if we couldn't keep up all the time, we did our best. "då juv häv haj her". Saga, for knowing what's important in life: love, friends and good food. Thank you for all the endless hours we spent down at the animal department. Your independence and experience in the lab has meant a lot and you have been a role model for how to plan and perform experiments. Matthias Erschbamer, a living encyclopedia. I am so thankful that you share your knowledge. See it as a chance to enlighten the rest of us ☺. Marie Westerlund, our princess, thank you for always being so happy! I sometimes go and look on your "ordning & reda" desk in the hope that some of your structure and order will rub off on me. I-Hui Lee, I miss you and for your honest and sometimes provocative questions. I always hold my breath when you say: "Alexandra, can I ask you a question?". Martin Werme, you were my first deskmate when I came to the lab and I always look forward to your stories and jokes. Toshiki Endo, for your amazing humor and discipline. Erik Westman, for your charm, Sofia Savage and Anna Anvret for bringing new blood to the lab. Jeff Blakington for sharing my passion for Hawaii, Johan Lilja for your kindness and Christoph Hofstetter for showing me your straightforward approach to science.

Paddock laboratory: Silvia Paddock for your calmness and incredible knowledge about everything and for building up such a great team with Magnus Lekman and Lisette Hörnblad.

Former and present colleagues at the Department of Neuroscience, for stimulating contacts: Olov Andersson, Stefan Plantman, Dan Sjöstrand, Johan Zelano, Niklas Lindgren, Anders Borgkvist, Andries Blokzijl, Lorenzo Cangiano, Julianna Kele, Henrik Jörnvall, Evanthia Nanou, Margarita Diez, Malin Höistad, Christian Broberger, Kerstin Håkansson, Matilda Bäckberg, Malin Sandberg, Fredrik Andersson, Katarina Luhr, Göran Klement, Elin Elvander-Tottie, Johan Sandin, Danny Marcellino, Eva Backström, Jeremy Young, Nina Törnqvist, Elin Nordström, Zoltan

Alessandros Kyriakatos, Haleh Razani, Mikael Nygård, Sebastian Tham, Jeppe Ericsson, Line Lundfald, Ernesto Restrepo, Pablo Brumovsky, Eugenia Kuteeva, Simret Beraki, Nora Kerekes, Petronella Kettunen, Tara Wardi, Alexander Mata, Goncalo Castelo-Branco, and Alison Canty.

I especially want to thank Göran Sandberg for being such a great teaching coordinator at the department and for giving essential guidelines in how to teach histology. Also for making me feel welcome to "the Educational group".

Professors, researchers and administrative staff for creating a good atmosphere at the department: Staffan Cullheim, Ola Hermanson, Gustavo Paratcha, Fernanda Ledda, Gilberto Fisone, Tomas Hökfelt, Peter Wallén, Katarina Eriksson, Liz Strandelin, Ole Kiehn, Lennart Brodin, Peter Löw, Kjell Fuxe, Carlos Ibáñez, Abdel El Manira, Brun Ulfhake, Maja Deckner, Krister Kristensson, Björn Meister, Christina Ingvarsson, Ingrid Olofsson, Karin Lagerman, Elzbieta Holmberg, Eliana Sobarzo, Vassiliki Galandaris and Tommy Nord.

Thank you, past and present members of CMB/Ludwig/CGB/CMM and their partners: Ulrika and Kristian, Madelen and Pelle with little Signe, Lizzy, Camilla S, Anna V and Johan, Joanna and Steve, Fabrice and Virginie, José, Stina, Qiaolin, Johan E and Annika, Alistair, Malin A, Niklas H, Lia and Toni.

To my friends outside KI: Lisa, my best friend thank you for all wonderful memories since we were nine years old. I wish you lived closer, but no matter if you live in London, Dubai, New Dehli, Varna or New York, you always feel close to me. Marie, Tobbe, Mickan, Linda, Sofie and Pelle, Åsa and Kenneth, Virginia, Sara and Micke, Pau, Pär and Gunsan, Oscar, Viktor, Martin and Jenny, Stefan and Sofia, Nina, Elin, David, Danne, Jenny, Tanner, Pekka, Camilla, Heli. Vad skulle jag göra utan er.

To my family:
My relatives in Stockholm: the Trifunovski, Graholski and Janis family. To my new in-laws the Fäldt family. My relatives in Bitola; "baba" i "dedo". My relatives in Split; Jadranka, Dragana and Saso, teta Milka and Sanja. Thank you so much for all the incredible memories you created during my childhood summers. They are safe in my heart!

To my "mother-in-law", Kristina for your trust and inspiration. I admire your strength and your courage!

To my brother, Toni, for bugging me since I was born. You have challenged me and inspired me to become who I am. Thank you for always being their when I need you!

To my parents, Dunja and Hristo, the nicest people in the world! For your endless source of support, love and for trying to teach me how to be good to other people. You are the best!

To the love of my life, Mattias "the owl". It is impossible to put down what I feel in words. vi.

✧

Projects included in this thesis were supported by the Swedish Research Council,
NIDA, NIH (Grant No. 1-HD-1-3138), AMF, Petrus and Augusta Hedlunds stiftelse,
A. and J. Mattsons Minnesstiftelse för sonen Johan, Karolinska Institutets fonder,
USPHS grants, the Swedish Society for Medical Research,
the Torsten and Ragnar Söderberg Foundation, AFA and the Swedish Brain Power Initiative.

References

Agnati, L. F., Ferre, S., Cortelli, P., and Fuxe, K. (1995). A brief appraisal on some aspects of the receptor-receptor interaction. Neurochem Int *27*, 139-146.

Ahmed, Z., Mazibrada, G., Seabright, R. J., Dent, R. G., Berry, M., and Logan, A. (2006). TACE-induced cleavage of NgR and p75NTR in dorsal root ganglion cultures disinhibits outgrowth and promotes branching of neurites in the presence of inhibitory CNS myelin. FASEB J. 2006 Jul 18; [Epub ahead of print].

Banati, R. B., and Graeber, M. B. (1994). Surveillance, intervention and cytotoxicity: is there a protective role of microglia? Dev Neurosci *16*, 114-127.

Bandtlow, C., Zachleder, T., and Schwab, M. E. (1990). Oligodendrocytes arrest neurite growth by contact inhibition. J Neurosci *10*, 3837-3848.

Bandtlow, C. E., Dlaska, M., Pirker, S., Czech, T., Baumgartner, C., and Sperk, G. (2004). Increased expression of Nogo-A in hippocampal neurons of patients with temporal lobe epilepsy. Eur J Neurosci *20*, 195-206.

Barton, W. A., Liu, B. P., Tzvetkova, D., Jeffrey, P. D., Fournier, A. E., Sah, D., Cate, R., Strittmatter, S. M., and Nikolov, D. B. (2003a). Structure and axon outgrowth inhibitor binding of the Nogo-66 receptor and related proteins. Embo J *22*, 3291-3302.

Bekar, L. K., and Walz, W. (1999). Evidence for chloride ions as intracellular messenger substances in astrocytes. J Neurophysiol *82*, 248-254.

Ben-Ari, Y. (1985). Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience *14*, 375-403.

Benfey, M., and Aguayo, A. J. (1982). Extensive elongation of axons from rat brain into peripheral nerve grafts. Nature *296*, 150-152.

Benson, M. D., Romero, M. I., Lush, M. E., Lu, Q. R., Henkemeyer, M., and Parada, L. F. (2005). Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc Natl Acad Sci U S A *102*, 10694-10699.

Bhat, M. A., Rios, J. C., Lu, Y., Garcia-Fresco, G. P., Ching, W., St Martin, M., Li, J., Einheber, S., Chesler, M., Rosenbluth, J.*, et al.* (2001). Axon-glia interactions and the domain organization of myelinated axons requires neurexin IV/Caspr/Paranodin. Neuron *30*, 369-383.

Bito, H., Furuyashiki, T., Ishihara, H., Shibasaki, Y., Ohashi, K., Mizuno, K., Maekawa, M., Ishizaki, T., and Narumiya, S. (2000). A critical role for a Rho-associated kinase, p160ROCK, in determining axon outgrowth in mammalian CNS neurons. Neuron *26*, 431-441.

Bliss, T. V., and Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31-39.

Bovolenta, P., and Fernaud-Espinosa, I. (2000). Nervous system proteoglycans as modulators of neurite outgrowth. Prog Neurobiol *61*, 113-132.

Brittis, P. A., and Flanagan, J. G. (2001). Nogo domains and a Nogo receptor: implications for axon regeneration. Neuron *30*, 11-14.

Buffo, A., Zagrebelsky, M., Huber, A. B., Skerra, A., Schwab, M. E., Strata, P., and Rossi, F. (2000). Application of neutralizing antibodies against NI-35/250 myelin-associated neurite growth inhibitory proteins to the adult rat cerebellum induces sprouting of uninjured purkinje cell axons. J Neurosci *20*, 2275-2286.

Bunge, R. P. (1968). Glial cells and the central myelin sheath. Physiol Rev *48*, 197-251.

Carbonetto, S., Evans, D., and Cochard, P. (1987). Nerve fiber growth in culture on tissue substrata from central and peripheral nervous systems. J Neurosci *7*, 610-620.

Caroni, P., and Schwab, M. E. (1988). Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. Neuron *1*, 85-96.

Chen, M. S., Huber, A. B., van der Haar, M. E., Frank, M., Schnell, L., Spillmann, A. A., Christ, F., and Schwab, M. E. (2000). Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature *403*, 434-439.

Cheng, H., Cao, Y., and Olson, L. (1996). Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science *273*, 510-513.

Cosgaya, J. M., Chan, J. R., and Shooter, E. M. (2002). The neurotrophin receptor p75NTR as a positive modulator of myelination. Science *298*, 1245-1248.

Cronin, J., and Dudek, F. E. (1988). Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. Brain Res *474*, 181-184.

Crutcher, K. A. (1989). Tissue sections from the mature rat brain and spinal cord as substrates for neurite outgrowth in vitro: extensive growth on gray matter but little growth on white matter. Exp Neurol *104*, 39-54.

Dagerlind, A., Friberg, K., Bean, A. J., and Hokfelt, T. (1992). Sensitive mRNA detection using unfixed tissue: combined radioactive and non-radioactive in situ hybridization histochemistry. Histochemistry *98*, 39-49.

David, S., and Aguayo, A. J. (1981). Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. Science *214*, 931-933.

DeBellard, M. E., Tang, S., Mukhopadhyay, G., Shen, Y. J., and Filbin, M. T. (1996). Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein. Mol Cell Neurosci *7*, 89-101.

Dergham, P., Ellezam, B., Essagian, C., Avedissian, H., Lubell, W. D., and McKerracher, L. (2002). Rho signaling pathway targeted to promote spinal cord repair. J Neurosci *22*, 6570-6577.

Diez, M., Schweinhardt, P., Petersson, S., Wang, F. H., Lavebratt, C., Schalling, M., Hokfelt, T., and Spenger, C. (2003). MRI and in situ hybridization reveal early disturbances in brain size and gene expression in the megencephalic (mceph/mceph) mouse. Eur J Neurosci *18*, 3218-3230.

Domeniconi, M., Cao, Z., Spencer, T., Sivasankaran, R., Wang, K., Nikulina, E., Kimura, N., Cai, H., Deng, K., Gao, Y.*, et al.* (2002). Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. Neuron *35*, 283-290.

Dupuis, L., Gonzalez de Aguilar, J. L., di Scala, F., Rene, F., de Tapia, M., Pradat, P. F., Lacomblez, L., Seihlan, D., Prinjha, R., Walsh, F. S.*, et al.* (2002). Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis. Neurobiol Dis *10*, 358-365.

Engert, F., and Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature *399*, 66-70.

Erschbamer, M. K., Hofstetter, C. P., and Olson, L. (2005). RhoA, RhoB, RhoC, Rac1, Cdc42, and Tc10 mRNA levels in spinal cord, sensory ganglia, and corticospinal tract neurons and long-lasting specific changes following spinal cord injury. J Comp Neurol *484*, 224-233.

Faulkner, J. R., Herrmann, J. E., Woo, M. J., Tansey, K. E., Doan, N. B., and Sofroniew, M. V. (2004). Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci *24*, 2143-2155.

Filbin, M. T. (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat Rev Neurosci *4*, 703-713.

Fournier, A. E., GrandPre, T., and Strittmatter, S. M. (2001). Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature *409*, 341-346.

Fournier, A. E., Takizawa, B. T., and Strittmatter, S. M. (2003). Rho kinase inhibition enhances axonal regeneration in the injured CNS. J Neurosci *23*, 1416-1423.

GrandPre, T., Li, S., and Strittmatter, S. M. (2002). Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature *417*, 547-551.

GrandPre, T., Nakamura, F., Vartanian, T., and Strittmatter, S. M. (2000). Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature *403*, 439-444.

Gruner, J. A. (1992). A monitored contusion model of spinal cord injury in the rat. J Neurotrauma *9*, 123-126; discussion 126-128.

Habib, A. A., Marton, L. S., Allwardt, B., Gulcher, J. R., Mikol, D. D., Hognason, T., Chattopadhyay, N., and Stefansson, K. (1998). Expression of the oligodendrocyte-myelin glycoprotein by neurons in the mouse central nervous system. J Neurochem *70*, 1704-1711.

He, Z., and Koprivica, V. (2004). The Nogo signaling pathway for regeneration block. Annu Rev Neurosci *27*, 341-368.

Hennig, J., Nauerth, A., and Friedburg, H. (1986). RARE imaging: a fast imaging method for clinical MR. Magn Reson Med *3*, 823-833.

Hirata, K., and Kawabuchi, M. (2002). Myelin phagocytosis by macrophages and nonmacrophages during Wallerian degeneration. Microsc Res Tech *57*, 541-547.

Houle, J. D., Tom, V. J., Mayes, D., Wagoner, G., Phillips, N., and Silver, J. (2006). Combining an autologous peripheral nervous system "bridge" and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J Neurosci *26*, 7405-7415.

Huang, E. J., and Reichardt, L. F. (2003). Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem *72*, 609-642.

Huang, J. K., Phillips, G. R., Roth, A. D., Pedraza, L., Shan, W., Belkaid, W., Mi, S., Fex-Svenningsen, A., Florens, L., Yates, J. R., 3rd, and Colman, D. R. (2005). Glial membranes at the node of Ranvier prevent neurite outgrowth. Science *310*, 1813-1817.

Huber, A. B., Weinmann, O., Brosamle, C., Oertle, T., and Schwab, M. E. (2002). Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. J Neurosci *22*, 3553-3567.

Hunt, D., Coffin, R. S., Prinjha, R. K., Campbell, G., and Anderson, P. N. (2003). Nogo-A expression in the intact and injured nervous system. Mol Cell Neurosci *24*, 1083-1102.

Hunt, D., Mason, M., Campbell, G., Coffin, R., and Anderson, P. (2002). Nogo receptor mRNA expression in intact and regenerating CNS neurons. Mol Cell Neurosci *20*, 537.

Josephson, A., Trifunovski, A., Scheele, C., Widenfalk, J., Wahlestedt, C., Brene, S., Olson, L., and Spenger, C. (2003). Activity-induced and developmental downregulation of the Nogo receptor. Cell Tissue Res *311*, 333-342.

Josephson, A., Trifunovski, A., Widmer, H. R., Widenfalk, J., Olson, L., and Spenger, C. (2002). Nogo-receptor gene activity: Cellular localization and developmental regulation of mRNA in mice and humans. J Comp Neurol *453*, 292-304.

Josephson, A., Widenfalk, J., Widmer, H. W., Olson, L., and Spenger, C. (2001). NOGO mRNA expression in adult and fetal human and rat nervous tissue and in weight drop injury. Exp Neurol *169*, 319-328.

Karnezis, T., Mandemakers, W., McQualter, J. L., Zheng, B., Ho, P. P., Jordan, K. A., Murray, B. M., Barres, B., Tessier-Lavigne, M., and Bernard, C. C. (2004). The neurite outgrowth inhibitor Nogo A is involved in autoimmune-mediated demyelination. Nat Neurosci *7*, 736-744.

Khan, U., Starega, U., and Seeley, P. J. (1990). Selective growth of hippocampal neurites on cryostat sections of rat brain. Brain Res Dev Brain Res *54*, 87-92.

Kim, J. E., Bonilla, I. E., Qiu, D., and Strittmatter, S. M. (2003a). Nogo-C is sufficient to delay nerve regeneration. Mol Cell Neurosci *23*, 451-459.

Kim, J. E., Li, S., GrandPre, T., Qiu, D., and Strittmatter, S. M. (2003b). Axon regeneration in young adult mice lacking Nogo-A/B. Neuron *38*, 187-199.

Kim, J. E., Liu, B. P., Park, J. H., and Strittmatter, S. M. (2004). Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. Neuron *44*, 439-451.

Kullander, K., Croll, S. D., Zimmer, M., Pan, L., McClain, J., Hughes, V., Zabski, S., DeChiara, T. M., Klein, R., Yancopoulos, G. D., and Gale, N. W. (2001). Ephrin-B3 is the midline barrier that prevents corticospinal tract axons from recrossing, allowing for unilateral motor control. Genes Dev *15*, 877-888.

Lai, C., Watson, J. B., Bloom, F. E., Sutcliffe, J. G., and Milner, R. J. (1987). Neural protein 1B236/myelin-associated glycoprotein (MAG) defines a subgroup of the immunoglobulin superfamily. Immunol Rev *100*, 129-151.

LeDoux, J. E. (1993). Emotional memory systems in the brain. Behav Brain Res *58*, 69-79.

Lee, J. K., Kim, J. E., Sivula, M., and Strittmatter, S. M. (2004). Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. J Neurosci *24*, 6209-6217.

Li, C., Tropak, M. B., Gerlai, R., Clapoff, S., Abramow-Newerly, W., Trapp, B., Peterson, A., and Roder, J. (1994). Myelination in the absence of myelin-associated glycoprotein. Nature *369*, 747-750.

Li, S., and Strittmatter, S. M. (2003). Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord injury. J Neurosci *23*, 4219-4227.

Liesi, P., and Silver, J. (1988). Is astrocyte laminin involved in axon guidance in the mammalian CNS? Dev Biol *130*, 774-785.

Liu, B. P., Fournier, A., GrandPre, T., and Strittmatter, S. M. (2002). Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. Science *297*, 1190-1193.

Lu, B., and Figurov, A. (1997). Role of neurotrophins in synapse development and plasticity. Rev Neurosci *8*, 1-12.

Luo, L. (2000). Rho GTPases in neuronal morphogenesis. Nat Rev Neurosci *1*, 173-180.

Maekawa, M., Ishizaki, T., Boku, S., Watanabe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohashi, K., Mizuno, K., and Narumiya, S. (1999). Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science *285*, 895-898.

Martin, K. C., and Kandel, E. R. (1996). Cell adhesion molecules, CREB, and the formation of new synaptic connections. Neuron *17*, 567-570.

Massey, J. M., Hubscher, C. H., Wagoner, M. R., Decker, J. A., Amps, J., Silver, J., and Onifer, S. M. (2006). Chondroitinase ABC digestion of the perineuronal net promotes functional collateral sprouting in the cuneate nucleus after cervical spinal cord injury. J Neurosci *26*, 4406-4414.

Matus, A., Brinkhaus, H., and Wagner, U. (2000). Actin dynamics in dendritic spines: a form of regulated plasticity at excitatory synapses. Hippocampus *10*, 555-560.

McGee, A. W., Yang, Y., Fischer, Q. S., Daw, N. W., and Strittmatter, S. M. (2005). Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science *309*, 2222-2226.

McKeon, R. J., Schreiber, R. C., Rudge, J. S., and Silver, J. (1991). Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci *11*, 3398-3411.

Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B.*, et al.* (2004). LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci *7*, 221-228.

Mikol, D. D., Gulcher, J. R., and Stefansson, K. (1990). The oligodendrocyte-myelin glycoprotein belongs to a distinct family of proteins and contains the HNK-1 carbohydrate. J Cell Biol *110*, 471-479.

Montag, D., Giese, K. P., Bartsch, U., Martini, R., Lang, Y., Bluthmann, H., Karthigasan, J., Kirschner, D. A., Wintergerst, E. S., Nave, K. A., and et al. (1994). Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. Neuron *13*, 229-246.

Moorman, S. J. (1996). The inhibition of motility that results from contact between two oligodendrocytes in vitro can be blocked by pertussis toxin. Glia *16*, 257-265.

Moreau-Fauvarque, C., Kumanogoh, A., Camand, E., Jaillard, C., Barbin, G., Boquet, I., Love, C., Jones, E. Y., Kikutani, H., Lubetzki, C.*, et al.* (2003). The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J Neurosci *23*, 9229-9239.

Mukhopadhyay, G., Doherty, P., Walsh, F. S., Crocker, P. R., and Filbin, M. T. (1994). A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron *13*, 757-767.

Nie, D. Y., Zhou, Z. H., Ang, B. T., Teng, F. Y., Xu, G., Xiang, T., Wang, C. Y., Zeng, L., Takeda, Y., Xu, T. L.*, et al.* (2003). Nogo-A at CNS paranodes is a ligand of Caspr: possible regulation of K(+) channel localization. Embo J *22*, 5666-5678.

Niederost, B., Oertle, T., Fritsche, J., McKinney, R. A., and Bandtlow, C. E. (2002). Nogo-A and Myelin-Associated Glycoprotein Mediate Neurite Growth Inhibition by Antagonistic Regulation of RhoA and Rac1. J Neurosci *22*, 10368-10376.

Oertle, T., van der Haar, M. E., Bandtlow, C. E., Robeva, A., Burfeind, P., Buss, A., Huber, A. B., Simonen, M., Schnell, L., Brosamle, C.*, et al.* (2003). Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. J Neurosci *23*, 5393-5406.

Park, J. B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, X. L., Garcia, K. C., and He, Z. (2005). A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron *45*, 345-351.

Park, J. H., Gimbel, D. A., GrandPre, T., Lee, J. K., Kim, J. E., Li, W., Lee, D. H., and Strittmatter, S. M. (2006). Alzheimer precursor protein interaction with the Nogo-66 receptor reduces amyloid-beta plaque deposition. J Neurosci *26*, 1386-1395.

Peles, E., Nativ, M., Lustig, M., Grumet, M., Schilling, J., Martinez, R., Plowman, G. D., and Schlessinger, J. (1997). Identification of a novel contactin-associated transmembrane receptor with multiple domains implicated in protein-protein interactions. Embo J *16*, 978-988.

Petersson, S., Persson, A. S., Johansen, J. E., Ingvar, M., Nilsson, J., Klement, G., Arhem, P., Schalling, M., and Lavebratt, C. (2003). Truncation of the Shaker-like voltage-gated potassium channel, Kv1.1, causes megencephaly. Eur J Neurosci *18*, 3231-3240.

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J. W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. Science *298*, 1248-1251.

Pot, C., Simonen, M., Weinmann, O., Schnell, L., Christ, F., Stoeckle, S., Berger, P., Rulicke, T., Suter, U., and Schwab, M. E. (2002). Nogo-A expressed in Schwann cells impairs axonal regeneration after peripheral nerve injury. J Cell Biol *159*, 29-35.

Prinjha, R., Moore, S. E., Vinson, M., Blake, S., Morrow, R., Christie, G., Michalovich, D., Simmons, D. L., and Walsh, F. S. (2000). Inhibitor of neurite outgrowth in humans. Nature *403*, 383-384.

Ramon y Cajal, S. (1991) Degeneration and Regeneration of the Nervous Ssytem. Translation of the 1928 Spanish edition by R.M. May. New York: Oxford Univ. Press. 976 pp.

Reindl, M., Khantane, S., Ehling, R., Schanda, K., Lutterotti, A., Brinkhoff, C., Oertle, T., Schwab, M. E., Deisenhammer, F., Berger, T., and Bandtlow, C. E. (2003). Serum and cerebrospinal fluid antibodies to Nogo-A in patients with multiple sclerosis and acute neurological disorders. J Neuroimmunol *145*, 139-147.

Richardson, P. M., McGuinness, U. M., and Aguayo, A. J. (1980). Axons from CNS neurons regenerate into PNS grafts. Nature *284*, 264-265.

Risau, W., and Wolburg, H. (1990). Development of the blood-brain barrier. Trends Neurosci *13*, 174-178.

Rosenzweig, E. S., and Barnes, C. A. (2003). Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog Neurobiol *69*, 143-179.

Sagot, Y., Swerts, J. P., and Cochard, P. (1991). Changes in permissivity for neuronal attachment and neurite outgrowth of spinal cord grey and white matters during development: a study with the 'cryoculture' bioassay. Brain Res *543*, 25-35.

Salzer, J. L., Holmes, W. P., and Colman, D. R. (1987). The amino acid sequences of the myelin-associated glycoproteins: homology to the immunoglobulin gene superfamily. J Cell Biol *104*, 957-965.

Savio, T., and Schwab, M. E. (1989). Rat CNS white matter, but not gray matter, is nonpermissive for neuronal cell adhesion and fiber outgrowth. J Neurosci *9*, 1126-1133.

Schafer, M., Fruttiger, M., Montag, D., Schachner, M., and Martini, R. (1996). Disruption of the gene for the myelin-associated glycoprotein improves axonal regrowth along myelin in C57BL/Wlds mice. Neuron *16*, 1107-1113.

Schmidt-Kastner, R., Humpel, C., Wetmore, C., and Olson, L. (1996). Cellular hybridization for BDNF, trkB, and NGF mRNAs and BDNF-immunoreactivity in rat forebrain after pilocarpine-induced status epilepticus. Exp Brain Res *107*, 331-347.

Schnell, L., and Schwab, M. E. (1990). Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. Nature *343*, 269-272.

Schwab, M. E., Kapfhammer, J. P., and Bandtlow, C. E. (1993). Inhibitors of neurite growth. Annu Rev Neurosci *16*, 565-595.

Schwab, M. E., and Thoenen, H. (1985). Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. J Neurosci *5*, 2415-2423.

Shao, Z., Browning, J. L., Lee, X., Scott, M. L., Shulga-Morskaya, S., Allaire, N., Thill, G., Levesque, M., Sah, D., McCoy, J. M., *et al.* (2005). TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. Neuron *45*, 353-359.

Shyu, B. C., Andersson, S. A., and Thoren, P. (1984). Spontaneous running in wheels. A microprocessor assisted method for measuring physiological parameters during exercise in rodents. Acta Physiol Scand *121*, 103-109.

Silver, J., and Miller, J. H. (2004). Regeneration beyond the glial scar. Nat Rev Neurosci *5*, 146-156.

Simonen, M., Pedersen, V., Weinmann, O., Schnell, L., Buss, A., Ledermann, B., Christ, F., Sansig, G., van der Putten, H., and Schwab, M. E. (2003). Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. Neuron *38*, 201-211.

Song, X. Y., Zhong, J. H., Wang, X., and Zhou, X. F. (2004). Suppression of p75NTR does not promote regeneration of injured spinal cord in mice. J Neurosci *24*, 542-546.

Sperk, G., Lassmann, H., Baran, H., Seitelberger, F., and Hornykiewicz, O. (1985). Kainic acid-induced seizures: dose-relationship of behavioural, neurochemical and histopathological changes. Brain Res *338*, 289-295.

Stiedl, O., Misane, I., Spiess, J., and Ogren, S. O. (2000). Involvement of the 5-HT1A receptors in classical fear conditioning in C57BL/6J mice. J Neurosci *20*, 8515-8527.

Strittmatter, S. M. (2002). Modulation of axonal regeneration in neurodegenerative disease: focus on Nogo. J Mol Neurosci *19*, 117-121.

Tang, B. L. (2003). Inhibitors of neuronal regeneration: mediators and signaling mechanisms. Neurochem Int *42*, 189-203.

Tauck, D. L., and Nadler, J. V. (1985). Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. J Neurosci *5*, 1016-1022.

Thallmair, M., Metz, G. A., Z'Graggen, W. J., Raineteau, O., Kartje, G. L., and Schwab, M. E. (1998). Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions. Nat Neurosci *1*, 124-131.

Tropea, D., Caleo, M., and Maffei, L. (2003). Synergistic effects of brain-derived neurotrophic factor and chondroitinase ABC on retinal fiber sprouting after denervation of the superior colliculus in adult rats. J Neurosci *23*, 7034-7044.

Wang, K. C., Kim, J. A., Sivasankaran, R., Segal, R., and He, Z. (2002a). p75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature *420*, 74-78.

Wang, K. C., Koprivica, V., Kim, J. A., Sivasankaran, R., Guo, Y., Neve, R. L., and He, Z. (2002b). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature *417*, 941-944.

Wang, X., Chun, S. J., Treloar, H., Vartanian, T., Greer, C. A., and Strittmatter, S. M. (2002c). Localization of Nogo-A and Nogo-66 receptor proteins at sites of axon-myelin and synaptic contact. J Neurosci *22*, 5505-5515.

West, M. J., and Gundersen, H. J. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. J Comp Neurol *296*, 1-22.

Westergaard, N., Sonnewald, U., and Schousboe, A. (1995). Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. Dev Neurosci *17*, 203-211.

Widenfalk, J., Olson, L., and Thoren, P. (1999). Deprived of habitual running, rats downregulate BDNF and TrkB messages in the brain. Neurosci Res *34*, 125-132.

Wiesel, T. N., and Hubel, D. H. (1963a). Effects Of Visual Deprivation On Morphology And Physiology Of Cells In The Cats Lateral Geniculate Body. J Neurophysiol *26*, 978-993.

Wiesel, T. N., and Hubel, D. H. (1963b). Single-Cell Responses In Striate Cortex Of Kittens Deprived Of Vision In One Eye. J Neurophysiol *26*, 1003-1017.

Wong, S. T., Henley, J. R., Kanning, K. C., Huang, K. H., Bothwell, M., and Poo, M. M. (2002). A p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. Nat Neurosci. *5,* 1302-1308

Yamashita, T., Higuchi, H., and Tohyama, M. (2002). The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. J Cell Biol *157*, 565-570.

Yamashita, T., and Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. Nat Neurosci *6*, 461-467.

Yiu, G., and He, Z. (2003). Signaling mechanisms of the myelin inhibitors of axon regeneration. Curr Opin Neurobiol *13*, 545-551.

Yiu, G., and He, Z. (2006). Glial inhibition of CNS axon regeneration. Nat Rev Neurosci *7*, 617-627.

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., and Lindholm, D. (1990). Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. Embo J *9*, 3545-3550.

Zheng, B., Atwal, J., Ho, C., Case, L., He, X. L., Garcia, K. C., Steward, O., and Tessier-Lavigne, M. (2005). Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. Proc Natl Acad Sci U S A *102*, 1205-1210.

Zheng, B., Ho, C., Li, S., Keirstead, H., Steward, O., and Tessier-Lavigne, M. (2003). Lack of enhanced spinal regeneration in Nogo-deficient mice. Neuron *38*, 213-224.