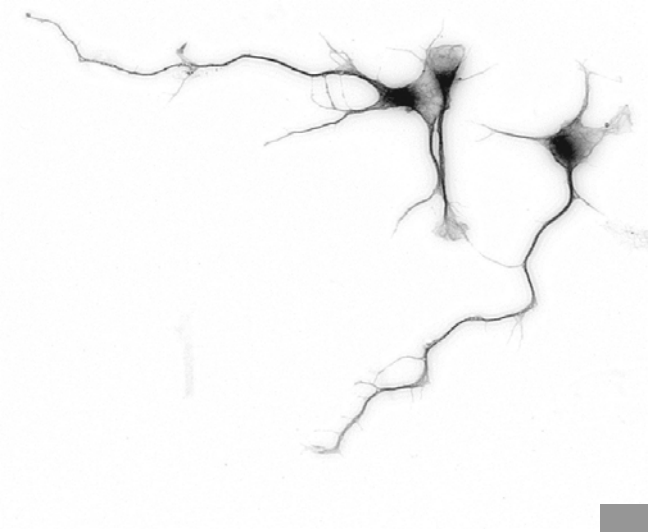


Summary of Results and Discussion

Results and Discussion I

The Role of Myelin-Associated Inhibitors in Axonal Regeneration

Nogo-A and MAG inhibit axonal growth *in vitro* and have been proposed to be one of the main hindrances to axonal regeneration *in vivo* (McKerracher *et al.*, 1994; Mukhopadhyay *et al.*, 1994; Chen *et al.,* 2002; GrandPre *et al.,* 2002; Prinjha *et al.,* 2002; Wong *et al.*, 2003). However, their regulation after axotomy was still unclear when this thesis was started. One of the main contributions of the present work is the characterisation of Nogo-A and MAG regulation after lesion, and the analysis of their contribution to axonal regeneration failure in the same model. This allows us to compare the results obtained to date and provides a more accurate view of what occurs after perforant pathway (PP) axotomy, thus providing more information than isolated reports on different models.

The present thesis demonstrates that both Nogo-A and MAG are key inhibitors of axonal regeneration following axotomy of adult CNS connections, since their regulation after *in vivo* axotomy fits spatially and temporally with this putative role and their blockade strongly promotes regeneration of the perforant pathway after axotomy *in vitro*.

1.1. The developmental expression of MAG and NgR, but not that of Nogo-A, correlates with the loss of axonal regeneration of the PP.

Axonal regeneration in the adult CNS is extremely limited, mainly owing to the presence of growth inhibitory proteins in association with CNS myelin (Schwab, 1998). This seems to be decisive for the PP, whose axons lose their capability to regenerate in a period matching the onset of myelination (Li D. *et al.,* 1995; Savaskan *et al.,* 1999; Prang *et al.,* 2001). We have demonstrated that the developmental loss of regenerative capacity also correlates with the

appearance of MAG expression by oligodendrocytes and the expression of NgR by entorhinal axons, but not with Nogo-A expression (at least not completely, see below).

It has been proposed that the myelination of axons parallels the maturation of their connections. Long projecting cortical neurons are among the first to mature. Thus, the corpus callosum, formed by their axons, is the fist site where MAG expression can be detected, around postnatal day 5 (P5) in mouse (Fig. 1.1). After this stage, transcription of MAG (indicative of myelin synthesis) increases and peaks between P15 and P21, together with synaptogenesis. Thereafter, MAG expression remains constant throughout adulthood. In the grey matter, however, the appearance of MAG expression is delayed (Fig. 1.1). It is first detected in the hippocampus at P10, mainly in areas enriched in oligodendrocytes such as the CA3 stratum radiatum, peaks around the third postnatal week and then decreases and remains steady. Its temporary regulation during development clearly matches myelination onset, indicating that during this period, MAG expression is probably linked to its function in myelin, and is regulated together with other myelin structural proteins, e.g. MBP (Li D. *et al.,* 1995; Savaskan *et al.,* 1999).

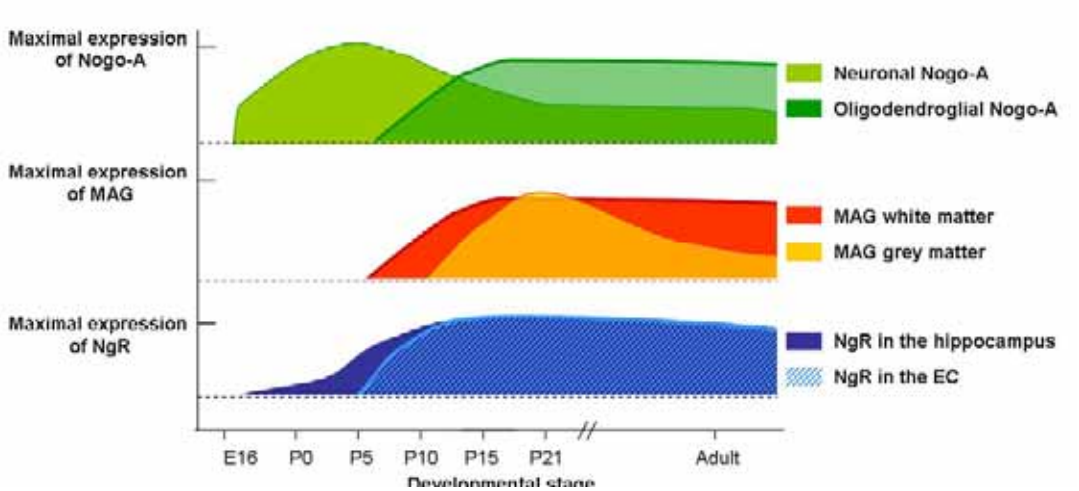


Fig 1.1. Developmental expression of Nogo-A, MAG and NgR. Expression by various cell types/populations has been separated to clarify the analysis of their participation in axonal inhibition. Note that Nogo-A and MAG expression by mature oligodendrocytes, but not that of neuronal Nogo-A, overlaps with NgR expression by entorhinal axons (perforant pathway axons) following the same temporary patterns as the loss of axonal regeneration in this pathway.

In contrast with MAG, which is not expressed when CNS axons can regenerate, the expression of Nogo-A is detected from the first developmental stage analyzed (E12) and is maximal around P0-P5 (figure 1.1 from E16). During these periods, it is restricted to neurons and to a lesser extent to radial glial cells, but is not detected in oligodendrocytes, which are not yet mature. However, as oligodendrocytes become myelinating, they begin to express Nogo-A (presumably exposing it at the myelin surface). This is consistent with data from Hunt *et al.,* (2003), but not from Wang X. *et al.* (2002), who also failed to observe Nogo-A neuronal expression. Oligodendroglial expression of Nogo-A does indeed correlate with MAG expression and with the loss of axonal regeneration capability. Thus, Nogo-A expression by oligodendrocytes may be related to axonal regeneration inhibition, but not Nogo-A expression by neurons or radial glia.

Detection of Nogo-A by immunohistochemistry was highly conditioned by the technique used. In our hands, short fixation times revealed the greatest immunostaining, which was eminently neuronal but permitted the detection of Nogo-A in oligodendrocytes. This variability was not determined by our antibodies but by the protein, as other laboratories have reported Nogo-A expression almost exclusively in oligodendrocytes using long-term fixation (Wang X. *et al.,* 2002) or by selectively modifying citrate buffer pH during antigen retrieval techniques (L. Dupuis, personal communication). In addition, Nogo-A mRNA was typically neuronal in our *in situ* hybridizations, as reported elsewhere. These technical limitations hindered the analysis of Nogo-A expression by oligodendrocytes, and Nogo-A expression regulation after lesion.

The functional role of a protein is determined by its receptor/s and the intracellular signalling it may induce in the target cell. The presence of the putative inhibitors in myelin by itself does not demonstrate these proteins are inhibiting axonal growth. To do this, neurons must express their corresponding receptors and the intracellular machinery necessary to induce growth arrest. Since intracellular molecules involved in transducing myelin-induced inhibition seem to be conserved among different processes (as we shall discuss bellow), the expression of the neuronal receptors to these ligands is the regulatory point that permit neurons become "sensitive" to MAI. Consistent with this, expression of NgR by entorhinal neurons correlates very precisely with the drop of axonal regeneration capability of the perforant pathway and is not detected until P5. Thus, NgR expression could directly determine the regenerative potential of entorhinal neurons once myelination has started. However, as we will discuss bellow, other molecules limit axonal regeneration of the perforant pathway.

1.2. Neuronal Nogo-A and NgR expression is regulated by activity.

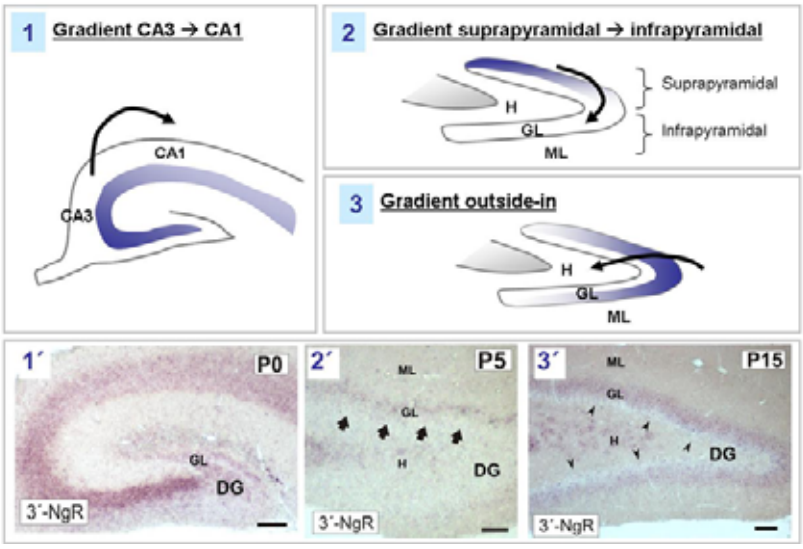


Fig. 1.2. Scheme of the three gradients of neuronal maturation observed in the hippocampus during development. 1-3 represent each one of the gradients explained in the text. Arrows indicate the direction of the maturation gradient. 1´-3´illustrate NgR expression pattern in the same stages represented in 1-3. Arrows in 2´and arrowheads in 3' point to the next granule cells that should express NgR. (Pictures 1´to 3´ have been taken from Mingorance *et al.,* 2004a).

NgR was reported at asymmetric synaptic contacts, with both pre- and postsynaptic localization (Wang X. *et al.,* 2002). When Nogo-A neuronal localization was evident, it was also detected at the postsynaptic density in both symmetric and asymmetric synapses (Liu *et al.,* 2003). These studies suggested that Nogo-A and NgR also regulate structural plasticity at synapses. This prompted us to analyze how Nogo-A and NgR expression is regulated by neuronal activity,

during the development of neuronal connections and in the adult, after kainic acid-induced seizures.

During development, hippocampal neurons maturate in a graded manner, following three defined patters. First, pyramidal neurons maturate. This process starts around perinatal stages in the CA3 pyramidal neurons, which are older than CA1 counterparts, and then progress into the CA1 area (Fig. 1.2.1). Thereafter, as granule cells reach the prospective granule layer in the dentate gyrus, they begin to maturate following a gradient from the suprapyramidal granule layer to the infrapyramidal layer, in an outside-in pattern that coincides with the generation of granule neurons, oldest cells being the first to maturate (Fig 1.2.2 and 3).

Here, we demonstrate that NgR expression in the developing hippocampus closely resembles the gradient of maturation described above, both in pyramidal and granule cells (Fig. 1.2.1´to 3´). One of the mechanisms that may modulate (and induce) NgR expression is synaptic activity. As hippocampal neurons begin to receive afferences and/or connect with postsynaptic targets, they may switch to a state in which expression of NgR allows them to stabilize synapses or to prevent additional sprouting once the right connections have been established through interaction with the MAI (or both). This is consistent with the finding that synaptic stabilization is accompanied during development by the removal (or pruning) of the branches that fail to establish those synapses and the inhibition of additional branching. Certain extracellular molecules, namely CAMs, neurotrophins, integrins and axon guidance molecules, regulate the three processes (Tessier-Lavigne, 1996; Seki and Rutishauser, 1998; Rohrbough *et al.,* 2000; Bagri *et al.,* 2003; Pascual *et al.,* 2004), which indicates that these mechanisms are probably intracellularly related (Rico *et al.,* 2004).

In the mature brain, the control of branch generation (sprouting) to create new synapses and synapse elimination are key mechanisms that ensure fine-tune networks (Rakic *et al.,* 1986). As during development, these mechanisms can be regulated by activity. The hippocampus is one of the brain regions endowed with high plasticity, and hippocampal neurons express high levels of both Nogo-A and NgR. Since both proteins are localized at synapses, they may interact directly, and NgR expression by mature neurons suggests that this interaction leads to synapse stabilization. To test this hypothesis, one or both proteins should be downregulated during processes requiring synaptic plasticity.

In the present work, we confirm that at least some neurons can regulate both Nogo-A and NgR in an activity-dependent manner. The administration of the non-NMDA receptor agonist kainic acid (KA) is used as an animal model for human temporal lobe epilepsy as it induces an increase in neuronal activity (Ben-Ari, 1985). In situ hybridization in rats treated with KA at convulsive doses show that Nogo mRNA is strongly downregulated in the hippocampus, peaking at 24 hours after injection (that was the fist time point analyzed). This downregulation was strikingly manifested in the granule layer, where neurons reduce Nogo-A expression to almost undetectable levels, contrasting with hilar interneurons that maintain high Nogo-A

expression. Similarly, NgR expression is reduced in the granule layer also reaching a minimum 24 hours after lesion, and NgR mRNA levels are partially recovered, but not completely, after 72 hours.

Regulation of Nogo-A, NgR and related proteins in the hippocampus after KA administration

	Normal levels	Regulation	References
Nogo-A (Nogo)	High	No changes	Josephson *et al.,* 2001
		Increase. Peak 5DAL	Meier *et al.,* 2003
		Decrease. Peak 24HAL	Mingorance *et al.,* 2004
NgR	High	Decrease. Peak 4HAL	Josephson *et al.,* 2003
		Decrease. Peak 24HAL	Mingorance *et al.,* 2004
P75	Low	Increase. Peak 3DAL	Roux *et al.,* 1999
Lingo	High	Increase.	Trifunovski *et al.,* 2004
BDNF	Moderated	Increase. Peak 3DAL	Josephson *et al.,* 2003

Table 1.1. Kainic acid-induced regulation of Nogo-A, NgR and related proteins. Our results are compared with reports from other laboratories and complemented with studies about p75, Lingo and BDNF regulation (the later is especially relevant, as it has been proposed to regulate NgR expression).

Interestingly, the time at which Nogo-A and NgR are downregulated coincides with the beginning of kainic acid-induced sprouting and synaptic reorganization of granule cell axons (mossy fibers; Tauck and Nadler, 1985; Cronin and Dudek, 1988). This suggests that Nogo-A and NgR may regulate axonal plasticity, and so their downregulation may allow mossy fiber sprouting. This contrasts with p75 and Lingo regulation, which is overexpressed in the hippocampus following KA treatment (Table 1.1; Roux *et al.,* 1999; Trifunovski *et al.,* 2004), and supports the implication of these receptors in signalling pathways not involving NgR, whose function is negatively regulated by neurons after KA injection. While alternative neuronal functions are well known for p75 (Bandtlow and Dechant, 2004), this is only an assumption for Lingo (supported by its widespread neuronal expression during embryonic development; Carim-Todd *et al.,* 2004), which negatively regulates myelination when expressed by oligodendrocytes (Mi *et al.,* 2005).

Previous studies by Josephson and Meier reported no alterations of Nogo-A expression at 24 hours after KA injection, and no alterations or strong upregulation after 7-5 days (Table 1.1; Josephson *et al.,* 2001; Meier *et al.,* 2003). The doses used in the three studies were similar (10 mg/kg Josephson and Meier, 12-15mg/kg in our work), as well as the animal model (adult rats 200-250g), although our time course was the shortest (12-72 hours). In our study and that of Meier, only animals showing convulsive symptoms were analized, although this was not mentioned in Josephson's manuscript. We have extensively confirmed in the laboratory that animals with the same genetic background and age respond differently to KA injection, depending, among other parameters, on the time the animal is permitted to convulse before sacrifice. Thus, although similar doses were used, Josephson's lack of Nogo-A regulation may correspond to lower seizures in subconvulsive animals. However, mRNA form mice injected with KA at subconvulsive doses (which did not show seizure symptoms) was used for our Northern blot studies, and we observed a similar downregulation of Nogo-A expression (Fig. 1.3B), presuming that Nogo-A expression is regulated similarly in rats and mice. Artefacts due

to differences in animal fixation, which can affect mRNA detection, can be ruled out from our study, since KA injection selectively affected Nogo-A expression in certain layers, like the granule cell layer, while it was not altered in the adjacent hilar interneurons (arrows in Fig. 1.3A).

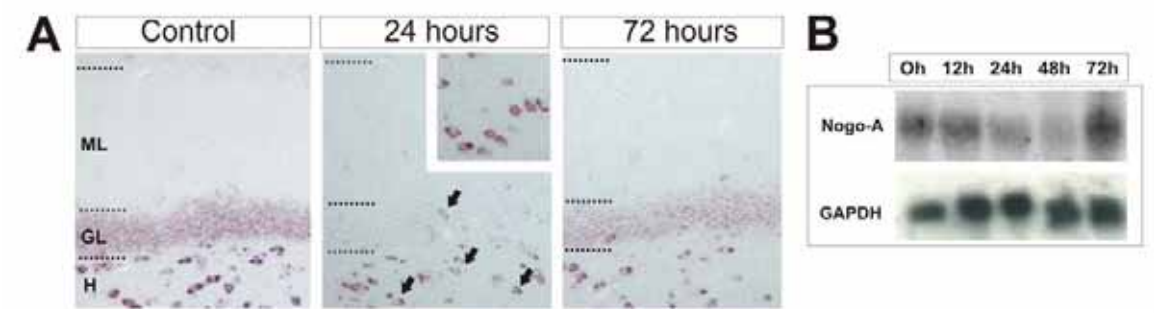


Fig. 1.3. Kainic acid-induced regulation of Nogo-A expression. A) Time course of Nogo-A regulation after KA injection in rats. Arrows point to interneurons that do not downregulate Nogo-A. B) Northern blot of Nogo-A regulation after subconvulsice administration of KA to adult mice. GAPDH is a loading control.

The hippocampus of both sclerotic and non-sclerotic epileptic patients shows elevated levels of neuronal Nogo-A (Bandtlow *et al.,* 2004). In these patients, the hippocampus has undergone extensive remodelling as a result of recurrent seizures and thus these data cannot be directly compared with those obtained after KA injection in experimental animals. However, the upregulation of Nogo-A reported by Meier at 5 days after KA injection is consistent with the hypothesis that Nogo-A has a biphasic regulation after kainic or epileptic seizure (Meier *et al.,* 2003), and after the initial downregulation, Nogo-A levels may remain high for a long time (as observed in patients of chronic epilepsy; Bandtlow *et al.,* 2004). These longer time points were not considered in our analysis.

We would like to highlight that after KA injection, NgR regulation has been shown to follow the same time course as BDNF regulation, but while NgR is strongly downregulated, BDNF is unpregulated (Wetmore *et al.,* 1994; Josephson *et al.,* 2003). In an elegant article, Josephson *et al*. show that this regulation (of both NgR and BDNF) also occurs during learning in rats exposed to running wheels, and proposed that NgR downregulation is essential during learning and memory, as KA injection may, to some extent, simulate neuronal activity during LTP, and NgR downregulation parallels learning and is recovered when the animal has adapted to the running wheel (Josephson *et al.,* 2003).

The function of BDNF in synaptic transmission and plasticity in the hippocampus has been extensively studied. Acute application of exogenous BDNF rapidly enhances neuronal and synaptic activity and transmitter release in primary cultures of embryonic hippocampal neurons (Knipper *et al*. 1994; Levine *et al*. 1995). To assess the possible involvement of BDNF in NgR expression regulation, and confirm the *in vitro* glutamate effect on Nogo-A and NgR levels, we carried out a similar experiment in which long-term hippocampal primary cultures (15 DIV) were treated with glutamate at neurotoxic doses and with BDNF at non-neurotoxic doses (Fig. 1.4). Cultures had been kept in serum-free conditions and consisted primarily of neurons, which after

the first week *in vitro,* had established synaptic contacts and were synaptically active. PD98059, an inhibitor of MEK1 (a downstream activator of Erk1/2), was used to determine whether Erk1/2 blockade regulates Nogo-A and NgR expression, as Erk1/2 is known to mediate the effect of neurotrophins.

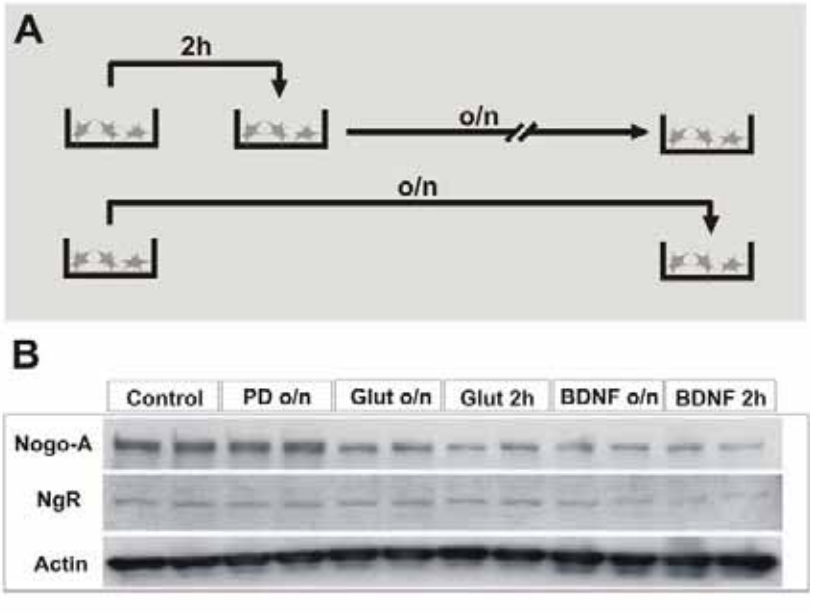


Fig. 1.4. Regulation of Nogo-A and NgR by glutamate and BDNF in hippocampal cultures. A) After 15DIV, Glutamate (100 µM), BDNF (50 ng/ml) or PD98059 (50 µM) were added to the culture medium of hippocampal cultures (consisting primarily in neurons). In a first group of cultures, the drug was left in the culture medium for 15 hours (over night o/n). In a second group, culture medium was changed after two hours and cells were allowed to grow in absence of drug overnight. Both groups of cultures were lisated at the same time and analyzed by western blotting. B) Nogo-A and NgR protein levels after treatment. While Nogo-A expression is affected by both treatments, only BDNF modifies NgR expression. Chronic treatment with PD98059 has no effect on Nogo-A and NgR expression. The experiment was performed in duplicated.

Although Josephson *et al.* did not implicate BDNF in NgR regulation, the same laboratory demonstrated one year later that NgR is downregulated 24 hours after BDNF delivery into rat hippocampus, thus linking both processes (Josephson *et al.,* 2003; Trifunovski *et al.,* 2004). Our *in vitro* assay also shows that NgR is downregulated in cells treated with BDNF at doses known to stimulate synaptic activity but not toxicity. This is particularly relevant, since overnight priming of neurons with neurotrophins has been shown to reduce MAI sensitivity (Cai *et al.,* 1999). Toxic doses of glutamate failed to regulate NgR expression at significant levels (Fig. 1.4B). Nogo-A expression, in turn, was strongly regulated by both toxic glutamate treatment and BDNF (when analyzed 12 hours after treatment initiation), further supporting the results obtained *in vivo* after KA injection.

How can Nogo-A and NgR lead to synapse stabilization? Or more precisely, why would neurons downregulate Nogo-A and NgR when plasticity is required? Granule cells express both Nogo-A and NgR, as do pyramidal cells from CA3. While NgR protein has been found both pre- and postsynaptically, Nogo-A has been detected mainly in the postsynaptic spine of the brain (Fig. 1.5C; Wang X. *et al.,* 2002; Liu *et al.,* 2003), but also in the presynaptic terminal of spinal motorneurons, where neuromuscular synaptogenesis is required (Dodd *et al.,* 2004; Esther Stoeckli, personal communication). Thus, NgR-Nogo-A interaction probably takes place in the mossy fiber-CA3 pyramidal neuron synapse. Based on the hypothesis that NgR and Nogo-A downregulation is advantageous for neurons when plasticity is required, we propose two models (illustrated in figure 1.5.)

Electronic microscopy data (Fig. 1.5C) suggest that NgR localises to the synaptic terminals of mossy fibers and Nogo-A is postsynaptic, in CA3 dendrites (and particularly in dendritic spines; Fig 1.5A-C). Since Nogo-A has at least two distinct subcellular localizations, two models (illustrated in fig. 1.5D1 and D2) are possible. First, Nogo-A and NgR may interact at the synapse. Cell adhesion molecules regulate various aspects of synaptogenesis, from initial contact formation to specific target recognition, and regulation of synaptic size and strength (for review Scheiffele, 2003; Washbourne *et al.,* 2004). Hence, Nogo-A and NgR may function, locally at synapses, as adhesion molecules. Nogo-A is known to interact with Caspr, a protein structurally related with neurexins, at paranodes (Nie *et al.,* 2003). Thus, it would be useful to determine whether Nogo-A, from its postsynaptic localization, also interacts with β-neurexins, a family of presynaptic proteins known to play a central role during synapse assembly (Scheiffele, 2003). As Nogo-A and NgR are negatively regulated during synaptic reorganization, their putative role may be synaptic stabilization. Alternatively, Nogo-A and NgR may be active in synapses even if they don't interact directly (Fig. 1.5D2). While NgR may interact with other postsynaptic proteins, leading to synapse stabilization, Nogo-A may play this role from the endoplasmic reticulum (Fig. 1.5E), perhaps regulating calcium levels, as Nogo-A, -B and –C have been reported to form a cluster with channel-like characteristics and a putative calcium-binding domain was identified in the Nogo-A N-terminus domain (Oertle *et al.,* 2003c; Dodd *et al.,* 2005).

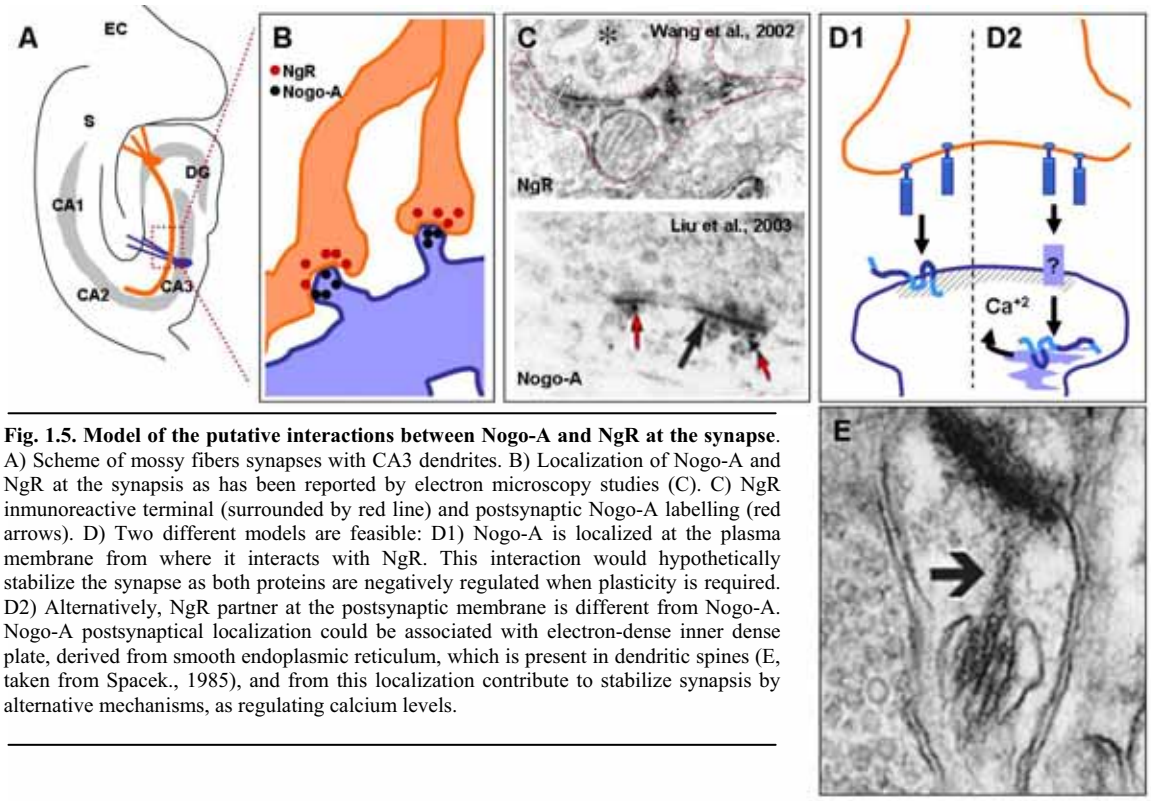


Fig. 1.5. Model of the putative interactions between Nogo-A and NgR at the synapse. A) Scheme of mossy fibers synapses with CA3 dendrites. B) Localization of Nogo-A and NgR at the synapsis as has been reported by electron microscopy studies (C). C) NgR inmunoreactive terminal (surrounded by red line) and postsynaptic Nogo-A labelling (red arrows). D) Two different models are feasible: D1) Nogo-A is localized at the plasma membrane from where it interacts with NgR. This interaction would hypothetically stabilize the synapse as both proteins are negatively regulated when plasticity is required. D2) Alternatively, NgR partner at the postsynaptic membrane is different from Nogo-A. Nogo-A postsynaptical localization could be associated with electron-dense inner dense plate, derived from smooth endoplasmic reticulum, which is present in dendritic spines (E, taken from Spacek., 1985), and from this localization contribute to stabilize synapsis by alternative mechanisms, as regulating calcium levels.

1.3. Nogo-A, MAG and NgR expression is differentially regulated following PP axotomy.

Inhibitors are present in the mature brain in either a constitutive manner or induced following lesion. Class-3 semaphorins are a classical example of the second case (Pasterkamp *et al.,* 1999). MAI, in contrast, are the classic example of constitutive inhibitors. Our studies show that although Nogo-A and MAG are found in association with myelin since myelination begins, both are regulated by axotomy in a spatial and temporary pattern that indicates they can also be regarded as lesion-induced axonal regeneration inhibitors.

1.3.1. MAG OVEREXPRESSION AFTER AXOTOMY: EVIDENCES OF REACTIVE MATURE OLIGODENDROCYTES.

The myelin-associated proteins MAG and MBP have been classically used as mature oligodendrocyte markers. Analysis of MBP expression after electrolytic lesion or axotomy has revealed an increase in the number of mature oligodendrocytes in the hippocampus accompanied by overexpression of MBP (Jensen *et al.,* 2000; Meier *et al.,* 2003). In our study, we demonstrate that in the hippocampus, MAG is also overexpressed and the number of MAG-expressing cells is increased in the denervated SLM around 3 DAL. However, in the entorhinal cortex, the number of mature oligodendrocytes after PP lesion does not increase (or decrease), as assessed by MAG expression. On the contrary, mature oligodendrocytes survive to the axotomy and actively react displaying certain characteristics that lead us to refer to them as “reactive” oligodendrocytes.

Mature oligodendrocytes may have two reactions after lesion: extensive death of mature oligodendrocytes accompanied by downregulation of myelin related genes (Morin-Richaud *et al.,* 1998; Wrathall *et al.,* 1998; Zai and Wrathall, 2005), or, when mature oligodendrocytes survive to axotomy, active reaction and overexpression of these genes (Frei *et al.,* 2000; Jensen *et al.,* 2000; Meier *et al.,* 2003; Li and Blakemore, 2004). The term **reactive oligodendrocytes**, referring to mature oligodendrocytes, was first used in spinal cord injury by Bartholdi and Schwab (see fig. 1.7), who showed transient overexpression of MBP by mature oligodendrocytes around the lesion during the first week following axotomy (Bartholdi and Schwab, 1998). This regulation resembles that observed with MAG hybridization in the entorhinal cortex (Fig. 1.7), indicating that MAG overexpression, as that of MBP, is not an isolated regulation but a sign of glial reactivity. Glia reactivity has been defined as a complex process involving hyperplasia, proliferation, migration and overexpression of certain genes, in response to brain injury (Ridet *et al.,* 1997; Acarin *et al.,* 2001). Only some of these events or all of them occur after lesion, which depends mainly on the type of lesion and the glial cell type. While oligodendrocyte progenitors and microglia proliferate rapidly after transection, proliferation of astrocytes is less frequent and restricted to the proximity of the lesion (Silver and Miller, 2004).

The fourth glial population (separating oligodendrocyte precursor cells from mature oligodendrocytes), formed by mature oligodendrocytes, is probably incapable of reacting after lesion, like the other populations. First, mature oligodendrocytes are postmitotic cells, and in order to proliferate, dedifferentiation would be necessary, which would be manifested as a

decrease in the number of mature oligodendrocytes rather than a proliferative reaction. Second, mature oligodendrocytes are myelinating cells and their processes, enwrapping axons, prevent them from migrating, as the other populations do. Thus, if mature oligodendrocytes undergo a reactive response to axotomy, this would be characterized by hyperplasia and overexpression of certain genes. This is exactly what we found in the entorhinal cortex after PP axotomy. As reactive astrocytes overexpress structural proteins, e.g. GFAP and Vimentin (Yang *et al.,* 1994), mature oligodendrocytes overexpress myelin-related genes, e.g. MAG and MBP, and become hyperplasic during the first week after lesion.

The parallelism between the reactivity of mature oligodendrocytes after axotomy observed in the spinal cord (Fig. 1.7A, C) and in the entorhinal cortex (Fig. 1.7B, D) indicates that the cellular response is the same in both cases. A different process occurs in the hippocampus, where new mature oligodendrocytes are generated after lesion, probably following the differentiation of precursor cells (Fig. 1.7E). It would be useful to establish the factors determining the final outcome of the mature oligodendrocytes population (cell death or survival and reactivity). Although the picture if far from being complete, one of these factors seems to be neuronal survival, as oligodendrocyte death is associated with Wallerian degeneration (Abe *et al.,* 1999). Conversely, as will be discussed below, myelin has a trophic effect on neurons and the survival of mature oligodendrocytes after PP axotomy may contribute to support neuronal survival (Windebank *et al.,* 1985; Sanchez *et al.,* 1996).

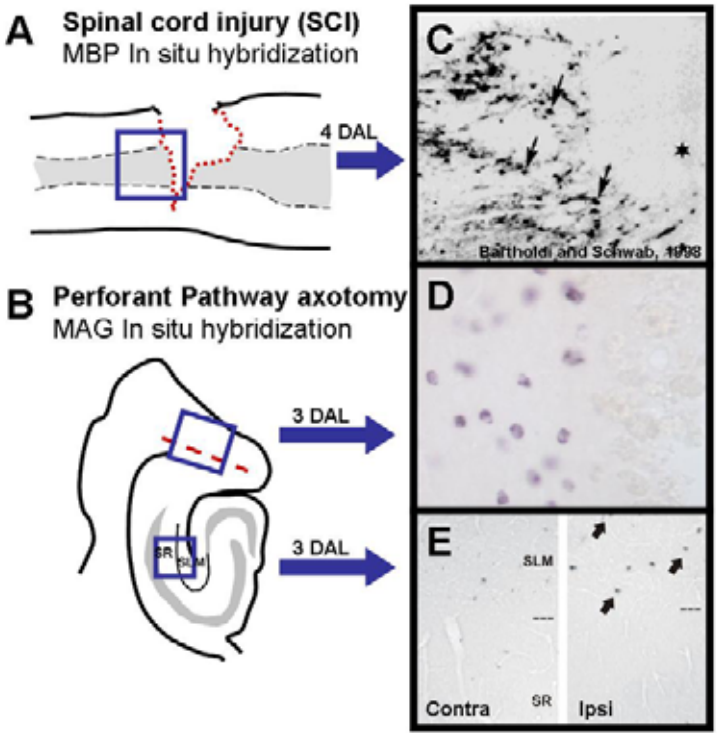


Figure 1.7 Scheme of Spinal Cord Injury (SCI) and Perforant Pathway (PP) axotomy comparing the reactivity of mature oligodendrocytes as assessed by regulation of MBP and MAG expression. A) Scheme of SCI used in Bartholdi and Schwab (1998) indicating the localization of the field shown in C. Lesion corresponds to dorsal laminectomy. B) Scheme of the PP axotomy used in this thesis and also by Dr. B. Finsen's laboratory. Fields D-E are shown as blue squares. C) MBP mRNA increase in oligodendrocytes that delimitate the lesion site (asterisk) 4 days after lesion. D-E) MAG is overexpressed in the entorhinal cortex (D) and the hippocampus (E) after PP axotomy (images show 3 days after lesion).

1.3.2 NOGO-A EXPRESSION AFTER AXOTOMY: RE-EXPRESSION BY ASTROCYTES.

Mature oligodendrocytes overexpress MAG and MBP after lesion, and therefore they could also overexpress Nogo-A. However, as commented in the introduction, the detection of

oligodendrocytic Nogo-A is not easy neither by western blotting nor by *in situ* hybridization (ISH), so descriptions have been made about neuronal Nogo-A regulation after lesion but are lacking about the myelin-associated Nogo-A.

Lesion model	Nogo-A regulation	References
Spinal cord injury (contusion)	Downregulation in the lesion centre	*Josephson et al., 2001*
Spinal cord injury (transection)	Downregulation in the lesion centre Overexpression at the borders	*Wang et al., 2002*
Spinal cord injury (transection)	Downregulation in the lesion centre Slight upregulation in cortical neurons	*Huber et al., 2002*
Spinal cord injury (transection)	Downregulation in the lesion centre Overexpression at the borders (by neurons)	*Hunt et al., 2003*
Optic nerve crush (transection)	Downregulation in the lesion centre Overexpression at the borders	*Hunt et al., 2003*
Electrolytic entorhinal lesion	Overexpression in neuronal cell layers of the hippocampus	*Meier et al., 2003*
Perforant pathway transection	Overexpression at the borders Overexpression by axotomized neurons Overexpression in the dentate gyrus	*This work*
Fimbria-fornix transection	Overexpression at the borders Overexpression by axotomized neurons	*This work*

Table 1.2. Lesion-induced Nogo-A regulation. Nogo-A regulation by injury has been mainly studied by *in situ* hybridization and the data reported normally refer to neuronal Nogo-A. References are given in chronologic order.

During the preparation of this thesis, several articles reported Nogo-A regulation after spinal cord lesions. Most of them described the downregulation of Nogo-A in the lesion centre, probably following the death of Nogo-A expressing cells, but overexpression around the lesion site (table 1.2). Some studies localized the overexpression of Nogo-A in sectioned axons and probably oligodendrocyte processes bordering the lesion (Huber *et al.,* 2002; Hunt *et al.,* 2003). Here, we report Nogo-A overexpression after PP axotomy around the lesion site by ISH. We have also confirmed this overexpression by immunohistochemistry (fig. 1.8). Nogo-A overexpression at the borders of the axotomy is transient, as it was detected by immunohistochemistry from 4 DAL to 7 DAL (fig. 1.8B-C) and by ISH at 3 DAL. The morphology and localization of these Nogo-A-overexpressing cells indicates they are probably glial cells. In contrast, entorhinal projecting neurons also display higher levels of Nogo-A, which accumulates mainly around the cell soma during longer post-axotomy times (fig. 1.8F-H), and is also observed in sectioned axons. This overexpression can be detected, to a lower extent, by ISH.

In the hippocampus, Nogo-A is slightly overexpressed by granule cells around 3 DAL, as also observed by Meier (2003). We also analyzed Nogo-A regulation after fimbria-fornix transection (Fig. 1.9). The fimbria-fornix (FF) constitutes a major afferent and efferent fiber tract connecting the hippocampus with the diencephalon, forebrain, striatum and prefrontal cortex (Cassel *et al*. 1997). Lesioning a fibre tract may facilitate the observation of mature oligodendrocytes, and thus allow us to assess whether they overexpress Nogo-A after lesion. A similar work, performed by David Hunt using optic nerve crush, revealed the upregulation of Nogo-A around

the lesion border but failed to determine the identity of these Nogo-A-overexpressing cells (Hunt *et al.,* 2003; Table 1.2). After FF transection, Nogo-A is also overexpressed around the lesion (Fig. 1.9). Closer examination shows that this overexpression is apparently localized in three cell types, presumably infiltrated fibroblasts (Fig. 1.9C, F), mature oligodendrocytes (Fig. 1.9E) and neurons. Transected axons, with collapsed terminals, were particularly immunoreactive to Nogo-A (Fig. 1.9D, G). Accumulation proximal to lesions is typical of molecules that undergo fast anterograde axonal transport (Tonra *et al.,* 1998) and molecular motors are upregulated after nerve transection (Su *et al.,* 1997). Thus, Nogo-A accumulation in the proximal endbulbs may be linked to an increase in the anterograde transport of vesicles (including Nogo-A associated with ER) to provide the membrane material needed for axonal regeneration (Su *et al.,* 1997).

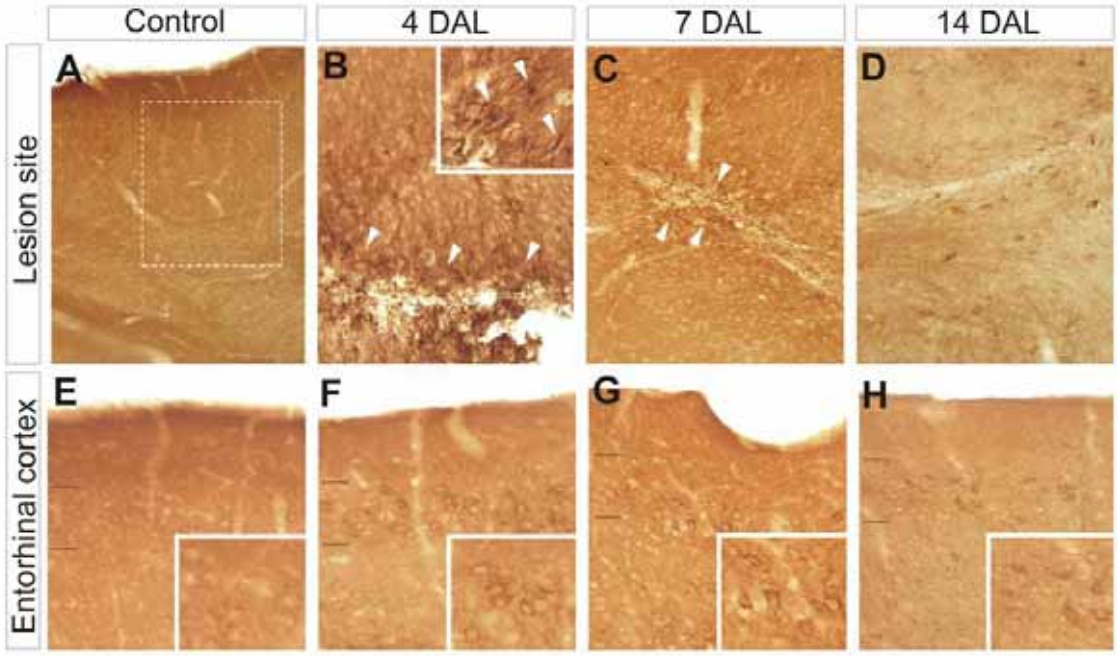


Figure 1.8. Overexpression of Nogo-A in the entorhinal cortex after axotomy of the perforant pathway. After lesion, Nogo-A is overexpressed both around the lesion site (A-D; arrowheads) and by projecting neurons form entorhinal layer II (E-H). Nogo-A increased immunoreactivity in neuronal somas is highlighted in the inserts (E-H).

An interesting feature about the regulation of Nogo-A after axotomy is that it is re-expressed by astrocytes. We have shown that during development, Nogo-A is expressed by radial glia cells, which become astrocytes once their developmental role is completed (Culican *et al.,* 1990). After axotomy, reactive astrocytes re-express proteins characteristic of radial glia such as Vimentin and Nestin (Clarke *et al.,* 1994). Similarly, they express Nogo-A transiently after PP axotomy, especially in the deafferented molecular layer of the dentate gyrus. This expression was confirmed by analysis of the C6 cell line derived from a rat astroglioma, whose cells behave as reactive astrocytes (Grobben *et al.,* 2002) and constitutively express Nogo-A. Nogo-A expression by reactive astrocytes is also observed around degenerating neurons in the spinal cord of patients with amyotrophic lateral sclerosis (Luc Dupuis personal communication). Although the expression of Nogo-A in the adult CNS by reactive astrocytes is probably associated with its role in radial glia cells and not *intended* to interact with neuronal NgR and

inhibit axonal regeneration, its presence in the deafferented hippocampal layers may contribute to this effect.

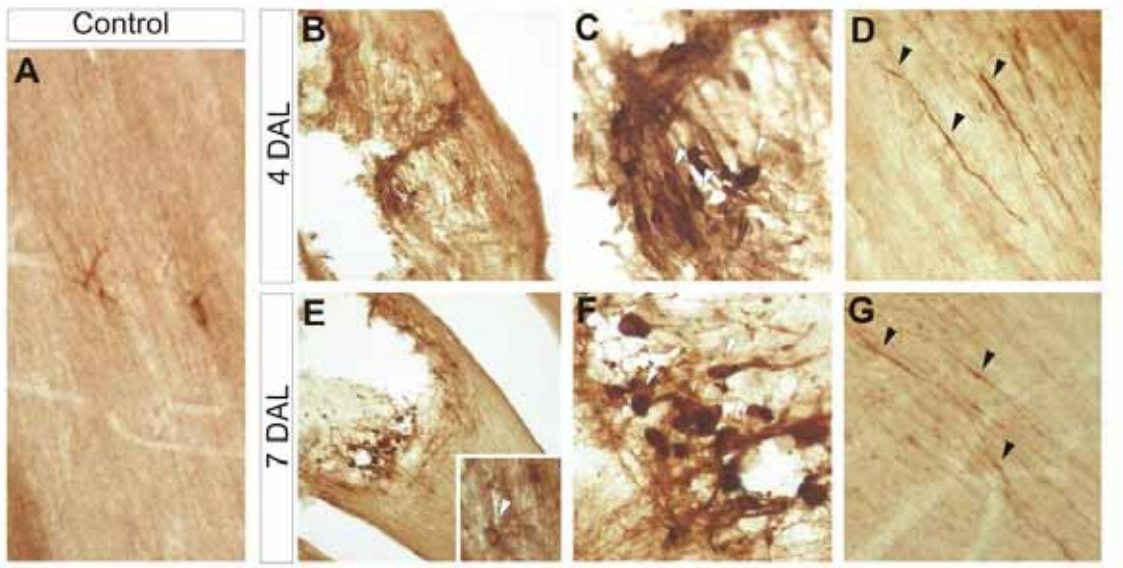


Figure 1.9. Overexpression of Nogo-following fimbria-fornix lesioning. A) In uninjured fimbria, Nogo-A expression follows a fascicular pattern and also labels scattered oligodendrocytes. B-G) 4 and 7 DAL, Nogo-A in expressed at the lesion site by putative infiltrated fibroblasts (C, F), sectioned axons (D, G; arrowheads) and mature oligodendrocytes (insert in E).

1.3.3 NgR BIPHASIC REGULATION AFTER AXOTOMY.

In our study, we show that NgR mRNA is upregulated by granule cell neurons after PP axotomy in the ipsilateral dentate gyrus soon after lesion, and this increase is followed by a decrease in NgR expression below control levels around two weeks after axotomy. This was also observed by Western blotting from total hippocampus. A similar regulation was described by Meier *et al*. (2003), who proposed that long-term NgR downregulation may permit sprouting of granule cell axons, which are known to sprout into the denervated molecular layer in a period matching the downregulation of NgR. This hypothesis is fairly possible, as supported by the downregulation of NgR after KA injection or learning (which also involve sprouting), and the fact that septal neurons, which are the first neuronal population to invade the molecular layer, do not express detectable levels of NgR. In contrast, we failed to detect a clear regulation of NgR mRNA in entorhinal neurons after transection, mainly because NgR expression levels in the adult entorhinal cortex are very low in our ISH. The putative expression of NgR by reactive astrocytes around the lesion site, so far observed by only one group in humans (Satoh et al., 2005), was neither detected in our ISH.

We would like to highlight that although NgR is overexpressed soon after PP axotomy, it has been shown to be subsequently downregulated and in chronic diseases, like amyotrophic lateral sclerosis, neurons even stop expressing NgR (Luc Dupuis personal communication). This may represent a survival response of neurons that try to release themselves from CNS inhibitors in an attempt to overcome their critical situation.

1.3.4 The expression of other inhibitors is also regulated by axotomy.

Although this thesis focuses on MAI, we have also addressed the regulation of other known inhibitory proteins. CSPG are overexpressed by reactive glia after entorhinal cortex lesion, preferentially around the lesion site and along the denervated hippocampal areas. This overexpression is delayed compared with that of MAG and Nogo-A and lasts for months, suggesting that CSPG are the main inhibitors once the overexpression of MAI is over. Another inhibitor whose expression is regulated after entorhinal cortex lesion is Sema 3a (Fig. 1.10). In lesioned organotypic cultures, its protein levels are high soon after lesion and this increase lasts at least 10 days (Fig. 1.10). Although there are no meninges around organotypic cultures, and therefore no infiltration of Sema 3a expressing fibroblasts, Sema 3a is overexpressed by neurons after injury (Pasterkamp and Verhaagen, 2001). In addition, blockade of Sema 3a binding to its receptor, NP-1, promotes axonal regeneration in the same *in vitro* model, pointing to the inhibitory role of Sema 3a after lesion (Montolio *et al*., in preparation). Our results on the blockade of CSPG and Nogo-66/NgR binding are described in the following sections. They are the first direct evidence of the participation of CSPG and Nogo-A in the prevention of PP regeneration.

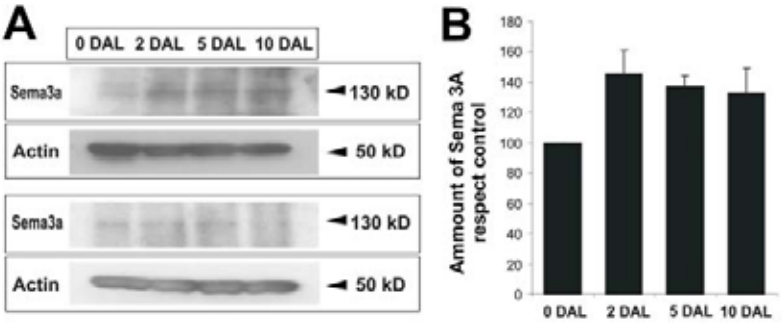


Fig. 1.10. Regulation of Sema 3a protein levels after axotomy *in vitro*. A) Western blotting showing Sema 3a overexpression in lesioned organotypic cultures. Two representative blots from different cultures are shown. B) Quantification of Sema 3a overexpression obtained by measuring band intensity. Axotomy was performed at 15 days *in vitro*. DAL: days after lesion.

1.4 Blockade of Nogo-A and MAG promotes axonal regeneration after PP axotomy in vitro.

The inhibitory role of Nogo-A after SCI has been demonstrated by several *in vivo* studies (see the introduction of this thesis and references within). The most extended Nogo-A blocking reagents have been the antibody IN-1 (which binds to Nogo-A NiG domain) and the NEP1-40 peptide (directed against Nogo-66 domain binding to NgR) (GrandPre *et al.,* 2002; Schwab *et al.,* 2003). In contrast, *in vivo* studies are lacking for MAG, although sialic acid removal by neuraminidase was shown to interfere with MAG binding to its receptors and its capability to inhibit neurite outgrowth *in vitro* (Tang *et al.,* 1997, Vinson *et al.,* 2001; Venkatesh *et al.,* 2005). In this work we have used these tools to assess the contribution of Nogo-A and MAG inhibition to perforant pathway regeneration in organotypic cultures.

Organotypic cultures have been considered as an *ex vivo* technique, as they conserve the cytoarchitecture of the tissue of origin. In addition, we have reported that proliferation, glial activation and regulation of inhibitors finely reproduce what has been described *in vivo*. Thus, the blockade of MAG and Nogo-A in organotypic cultures should closely resemble the *in vivo* blockade, having the advantage of easy access to the lesion site and drug delivery. Table 1.3

summarizes the results obtained from the various blockades assayed in organotypic cultures and complements the blockades listed in table 4.1 of the introduction.

Table 1.3. Regeneration of entorhinal axons following PP axotomy in organotypic cultures with different treatments. Quantification of regenerating axons in given as mean number of axons ± standard error. Buffers in which each drug was dissolved were used as control. [1] Neuraminidase is not specific for MAG and may affect other sialic acid-dependent reactions.

	Target	Control	Acute Treatment	Delayed Treatment
Neuraminidase	MAG[1]	5.7 ± 1.4	23.8 ± 6.7	
IN-1 IgM	NiG (Nogo-A)	4.5 ± 0.9	6.7 ± 2.4	
IN-1 Fab	NiG (Nogo-A)	4.0 ± 0.6	5.7 ± 2.1	
NEP1-40	Loop-66 (Nogo-A)	3.4 ± 1.2	19.7 ± 4.2	17.2 ± 8.2
ChABC	CSPG	3.7 ± 1.3	37.2 ± 8.4	13.8 ± 7.5
NEP1-40 + ChABC	Loop-66 + CSPG	3.8 ± 1.0	43.3 ± 6.9	14.3 ± 5.7

The perforant pathway loses its capability to regenerate in organotypic cultures after 15 days *in vitro* (DIV). **Neuraminidase** treatment of cultures for seven days after axotomy (performed at 15 DAL and starting drug delivery the day after) increases the number of regenerating axons that enter the hippocampus. This increase is at the limit of significance by statistical analysis ($p=0.051$; Student's t-test at 95% confidence level) but clearly important in biological terms, as in control cultures, a mean of 5.7 axons entered the hippocampus (stopping short after) and neuraminidase treatment allowed a mean of 23.8 axons to innervate again the hippocampus (four-fold increase), growing long distances and ending often in the prospective target layers. This result clearly indicates that sialic acid-dependent inhibitors, such as MAG, prevent the axonal regeneration of entorhinal axons *in vitro*. Moreover, neuraminidase did not elicit any major adverse effect on growing axons or interfered with PP termination specificity. A recent article has identified NgR2 as a neuraminic acid-dependent MAG receptor and shown that MAG binding to NgR also depends on neuraminic acids (Venkatesh *et al.,* 2005). This indicates that MAG-induced inhibition totally depends on the presence of neuraminic acids and neuraminidase treatment should fully prevent it. The only published article that had previously reported the effects of neuraminidase in axonal regeneration also used, curiously, hippocampal organotypic cultures (Muller *et al.,* 1994). This article describes the effect of neuraminic acid removal on the regeneration of the CA3-CA1 connection, whose sprouting seems to be dependent on PSA-NCAM. Removal of polysialic acids from PSA-NCAM significantly delayed the sprouting of CA3 axons but did not affect the total degree of regeneration achieved (Muller *et al.,* 1994). At least a subset of entorhinal neurons also express PSA-NCAM, therefore the regeneration accomplished by MAG-specific blockade may be greater than the one observed, as neuraminidase may negatively affect certain growth promoting proteins.

However, **IN-1** delivery failed to clearly increase axonal regeneration, although it did enhance the neurite length of entorhinal axons growing into the hippocampus (Table 1.3). We would like to highlight that these results should not necessarily be understood to reflect the role of the nogo-a specific domain in axonal regeneration, and may respond to differences in tissue penetration, as discussed below.

Targeting of the second inhibitory domain in Nogo, the loop-66, with the antagonistic peptide **NEP1-40,** promotes axonal regeneration of corticospinal axons *in vivo* (Grandpre *et al.,* 2002; Li and Strittmatter, 2003). NEP1-40 seems to be highly specific for Nogo-66-NgR binding, as it does not interfere with MAG or OMgp binding, and has been shown to be extremely innocuous after systemic delivery. Thus, NEP1-40 is so far the most promising therapeutic tool that has been designed to target the MAI (Lee *et al.,* 2003). In our model, acute treatment with NEP1-40 strongly promoted axonal regeneration at a similar rate to neuraminidase, supporting that Nogo-A binding to NgR contributes to prevent axonal regeneration of the perforant pathway (Table 1.3).

1.5 CSPG and Nogo-66 do not have synergistic effects on axonal regeneration.

In addition to their role as myelin-associated inhibitors, CSPG are thought to be the main chemical hindrance to axonal regeneration after lesion. Although HSPG are involved in layer-specific termination of entorhinal axons in the hippocampus (Forster *et al*., 2001), i*n vivo* blockade of CSPG, but not blockade of HSPG, promotes extensive axonal regeneration after various lesion models and enhances functional recovery (Moon *et al.,* 2001; Bradbury *et al.,* 2002; Zuo *et al.,* 2002). Blockade of CSPG is achieved by degradation of chondroitin-sulfate groups with chondroitinase ABC (ChABC), which is extensively used in combinatorial therapies in animal models (e.g. Chau *et al.,* 2004; Fouad *et al.,* 2005).

Acute treatment of axotomized organotypic cultures with **ChABC** promotes a ten-fold increase in the number of regenerating axons and leads to the reinnervation of prospective targets (Table 1.3). In our hands, ChABC induces a degree of regeneration greater than NEP1-40 and more importantly, much more specific. As both drugs have been shown to have very good penetration on tissue (Bradbury *et al.,* 2002; GrandPre *et al.,* 2002) and the concentration used was optimized is preliminary experiments, this difference of efficiency may be due to several reasons. First, NEP1-40 only prevents Nogo-A binding to NgR but does not affect the binding of other MAI, and so its effect in preventing MAI inhibition is partial. Li *et al*. (2004) compared the efficiency of NEP1-40 with that of the NgR ectodomain, which binds to the different NgR ligands and is expected to abolish NgR-mediated inhibition. They reported no increase in functional recovery when using NgR ectodomain compared with NEP1-40 induced recovery, although axonal sprouting was more highly branched with the former (Li *et al.,* 2004). The authors concluded that comparison between the two studies can be used to assess the relative roles of Nogo-66 versus MAG and OMgp, and thus Nogo-A binding to NgR is responsible for the overall myelin inhibition (Li *et al.,* 2004). If this proves true, our NEP1-40/ChABC comparison would show that CSPG play a more determinant role in axonal regeneration failure than myelin. However, we believe that NgR ectodomain treatment underestimates the role of MAG and OMgp, as it is unlikely that a large Fc-NgR ectodomain recombinant protein has the same efficiency as a small 40-aa peptide, which would be more stable and soluble.

Interestingly, if both Nogo-66 and CSPG contribute to preventing axonal regeneration of entorhinal axons and represent the major inhibitors, **combination of NEP1-40 and ChABC** should achieve a much better regenerative degree than any of the blockades alone. Surprisingly, this is not true. As observed in table 1.3, the number of regenerating axons in cultures treated with the best performing concentration of NEP1-40 and ChABC in combination is not significantly greater than that obtained with ChABC alone. We have confirmed that ChABC does not affect MAI regulation and that NEP1-40 does not alter CSGP overexpression in the deafferented hippocampus. We also analyzed the putative effect of both drugs on microglial activation and glial scar development. From these experiments, we ruled out that ChABC or NEP1-40 affects glial reactivity.

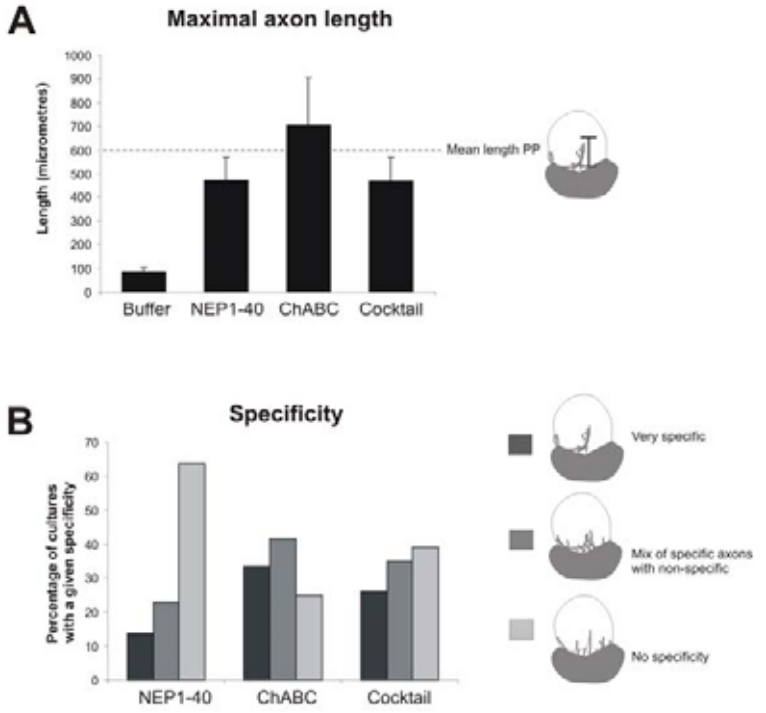


Figure 1.11. Analysis of maximal axon length and regeneration specificity obtained with the different treatments. A) PP axons normally enter up to 600µm into the hippocampus. Although ChABC alone reaches this distance, combination with NEP1-40 has deleterious effects on axonal length in cultures treated with the cocktail. B) Similarly, the strong specificity observed in ChABC treated cultures is diminished when combined with NEP1-40, which promotes an overly unspecific regeneration. Drawings at the right represent the degrees of specificity that were assigned to each culture.

Closer analysis reveals that the delivery of both drugs in combination does not prevent the effect of any of them, as the regeneration pattern achieved by the combined treatment (which will be referred to as “cocktail”) is actually a mix of both drugs when assessed by parameters other than the mean number of axons (Fig. 1.11). Axonal length in ChABC treated cultures reaches the normal length of PP axons, around 600µm (see drawing in fig. 1.11A). However, NEP1-40-treated cultures never displayed axons of such length and prevented them from doing so when added to ChABC in cocktail-treated cultures. Similarly, the high specificity of axonal regeneration observed in ChABC treated cultures was slightly reduced in cocktail-treated ones by the presence of NEP1-40 (Fig. 1.11B). Thus, although the number of regenerating axons was not additive, and resembled that obtained with ChABC alone, this is not due to the inefficiency of NEP1-40 when delivery in combination as the regeneration pattern of ChABC/NEP1-40-treated cultures is intermediate between both treatments.

We would like to underline that IN-1 also induced overall low-specific regeneration. One possible explanation is that MAI prevent axonal regeneration but do not participate in layer specificity. In fact, we observed that the amount of CSPG in the SLM and ML is particularly

lower than that of neighbouring areas, thus creating a permissive corridor for entorhinal axons. After axotomy *in vitro*, this corridor is filled following upregulation of CSPG, and is no longer appreciated. Similarly, regenerating axons (induced by release from MAI) would not grow prefentially along the SLM and ML if these layers are as inhibitory as the rest. On the other hand, ChABC treatment may permit axonal regeneration by removal of inhibitory groups (as NEP1-40 does) and reduce the amount of inhibitors located at the prospective layers, allowing regenerating axons to respond to the putative positive cues located in this layers.

However, why is axonal regeneration not increased, compared with ChABC-induced regeneration, when both Nogo-66/NgR and CSPG are blocked? Two possibilities may account for the lack of synergistic effect. First, inhibitors may only account for one part of axonal regeneration failure and in order to achieve greater regeneration degrees, physical barriers (like glial scar) should be removed or neurons treated to overcome endogenous limitations (e.g. by the activation of growth promoting genes). In this case, the higher effect of ChABC may be sufficient to overcome the "percentage" of axonal regeneration that corresponds to inhibitors, so the blockade of more inhibitors cannot increase axonal regeneration. This has been shown to be at least partially true, as the combination of treatments covering several "obstacles" to regeneration seems to have synergistic effects, e.g. the combination of ChABC with LiCl (a GSK-3β inhibitor; Yick *et al.,* 2004), ChABC and nerve grafts (Krekoski *et al.,* 2001; Fouad *et al.,* 2005), and a dominant negative form of NgR with macrophage-derived factors (Fischer *et al.,* 2004). In contrast, NEP1-40 and ChABC do not have synergistic effects on axonal regeneration because both MAI and CSPG follow the same signalling pathways. In this regard, both CSPG and myelin have been recently shown to activate RhoA via PKC, which is necessary for both their inhibitory effects, indicating that PKC (and downstream cascades) is a common point of signal convergence of the diverse inhibitors (Monnier *et al.,* 2003; Fournier *et al.,* 2003; Schweigreiter *et al.,* 2004; Hasegawa *et al.,* 2004; Sivasankaran *et al.,* 2004). In this case, ChABC reduces PKC activation and reaches an activation threshold, after which the signalling pathway is no longer activated, and ChABC alone suffices to "inhibit" the downstream signalling leading to regeneration inhibition. However, NEP1-40 may not suffice to reach this threshold, as CSPG, MAG and OMgp are still functional. Note that the second hypothesis is not independent of the first, and can be considered a particular case.

Many treatments that are effective *in vitro* fail to promote axonal regeneration *in vivo* because they need to be started before injury or immediately after, which is normally not feasible in human patients. Therefore, in a last experimental set, we aimed to assess whether entorhinal neurons retain their capability to regenerate for some days, and whether there is a temporary window of effectiveness of ChABC and NEP1-40 beyond the initial hours. To this end, we delayed the beginning of the treatments up to five days after axotomy, and then proceeded as for acute treatments (same doses and every-other-day delivery for a week). The most efficient drug for acute treatments (ChABC) greatly lost its efficiency when treatment was delayed for five days. Although it still promoted slight regeneration when compared to controls, ChABC efficiency fell under the regeneration levels promoted by acute NEP1-40. Moreover, the

efficiency of the combined treatment decreased to similar levels, while NEP1-40 induced the same degree of regeneration in acute and delayed treatments.

The most feasible explanation for the reduced effect of ChABC on axonal regeneration is that the doses used may be insufficient to degrade the growing accumulation of CSPG. When delivered right after lesion, 50 ng/ml of ChABC can degrade the initial amount of CSPG, and this may be enough to permit axonal regeneration during the initial, most critical period for lesioned neurons. After five days, the amount of CSPG accumulated may require higher doses of ChABC to be sufficiently degraded. Unfortunately, we assayed several dilutions of the enzyme (described to be efficient in the literature) and found that doses higher than 100 ng/ml reduced culture viability, as observed by a retraction of the section site that physically prevented axon crossing and culture death with the highest dose (600ng/ml). NEP1-40 showed no apparent toxicity for the cultures, but also lost efficiency at high doses. However, the functional dose used for acute treatment was enough to reduce the binding of Nogo-A to NgR to the same extent when used for delayed treatment. Altogether, we highlight the need to thoroughly test the doses and temporal delivery that optimize the efficiency of each drug and, in addition, the need of selecting treatments that use various mechanisms for combination. To this end,, we should have detailed understanding of how these treatments work, as ChABC and NEP1-40 may appear as complimentary treatments (since they target distinct inhibitors), while the intracellular convergence of this inhibitors makes them ultimately redundant. The present thesis contributes to this critical knowledge, and reports the analysis of two of the most promising drugs available.

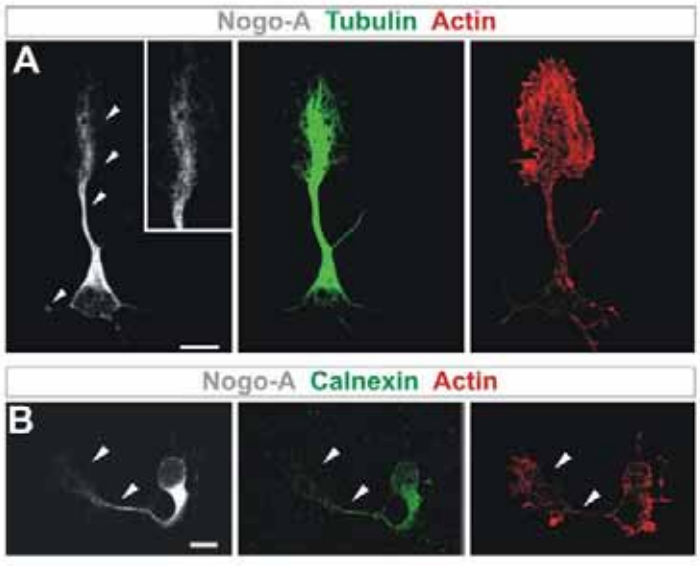
Results and Discussion II

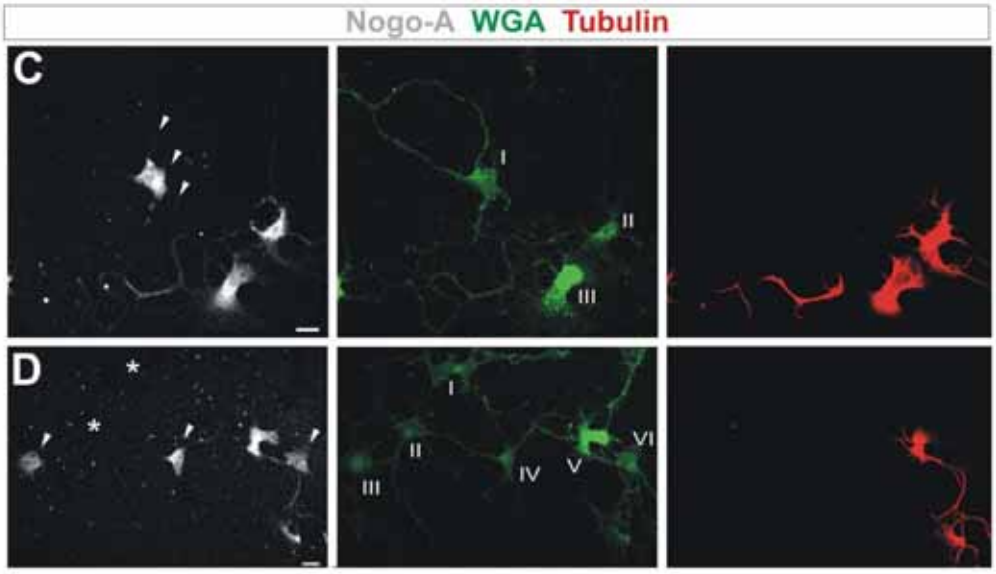
Nogo-A During Neuronal Development

2.1. Nogo-A is expressed by neurons during development

One aspect that remains to be clarified is the neuronal role of Nogo-A. During development, Nogo-A is expressed by most, but not all, of neuronal populations. In fact, it is expressed at higher levels than in postnatal and adult stages. However there are no reports on neuronal

Figure 2.1. Subcellular localization of Nogo-A in developing neurons. Dissociated hippocampal neurons form E13 mice were maintained in serum-containing medium for 15 hours and then fixed to analyze Nogo-A localization. **A) Nogo-A (white) is localized to the neuronal soma, the major neurite (including growth cone) and minor neurites**, but is not detected at the filopodia or lamelipodia, as observed by phalloidin binding to actin (red). **B)** This pattern **coincides with endoplasmic reticulum localization,** as observed by Calnexin immunostaining (green) in permeabilized cultures. **C-D) Nogo-A is also present at the neuronal surface.** WGA, a lectin that binds to glucids at the cell surface and at the Golgi apparatus, was used to assess plasma membrane localization. ICC was performed without permeabilizing agents and membrane integrity was determined by the absence of WGA-Golgi immunostaining and tubulin labelling. Cells II and III in C were permeabilized, but not cell I. All of them express Nogo-A (arrowhead points to non-permeabilized neuron with Nogo-A staining). Not every neuron, however, exposes Nogo-A at the cell surface, like neurons I and II in D (asterisks).





Nogo-A functions. We observed that during developmpent, Nogo-A is expressed by neurons and radial glia cells (Fig. 2.2 A-K). In cultured embryonic neurons, Nogo-A is located at the plasma membrane (Fig. 2.1C-D), but also in association with the endoplasmic reticulum (ER; Fig. 2.1B). Association with the endoplasmic reticulum is evident after Nocodazole treament, which depolimerizes microtubules and causes a retraction of the ER (together with Nogo-A) from neurites to the cell soma (not shown). This association is more manifest in neurites, particularly in the prospective axon and the central domain of the growth cone (Fig. 2.1A), and Nogo-A accumulation in the proximal branch of sectioned axons suggests that this reticular association remains until adulthood, and Nogo-A is transported anterogradely.

2.2. Contact with Nogo-A induces fasciculation in vitro

Nogo-A presence at the plasma membrane suggests it interacts with extracellular receptors. In fact, embryonic hippocampal neurons are sensitive to Nogo-A presence on the substrate, although they fail to express detectable levels of NgR. Hippocampal explants grow radially on ornithine-laminin substrate, and when membrane extract from wild type COS cells is dropped between the two coating agents. However, membrane extract form Nogo-A transfected cells induces a clear fasciculation of embryonic hippocampal explants (Fig. 2.2 L). This may be due to two reasons. First, fasciculation may respond to an *inhibitory* effect of Nogo-A on the substrate, favoring interaxonal adhesion over adhesion to substrate. However, axonal length was not reduced in fasciculated explants (only a small decrease, probably due to increased fasciculation) or in dissociated hippocampal cells, while it was clearly lower in postnatal cerebellar explants and dissociated cerebellar granule cell neurons (CGNs, which express NgR). Second, Nogo-A may directly induce fasciculation. In this regard, Nogo-A is preferentially localized to axon tracts during development (Fig. 2.2 G-K) and when these cells are dissociated, Nogo-A can be detected at the plasma membrane (Fig. 2.1).

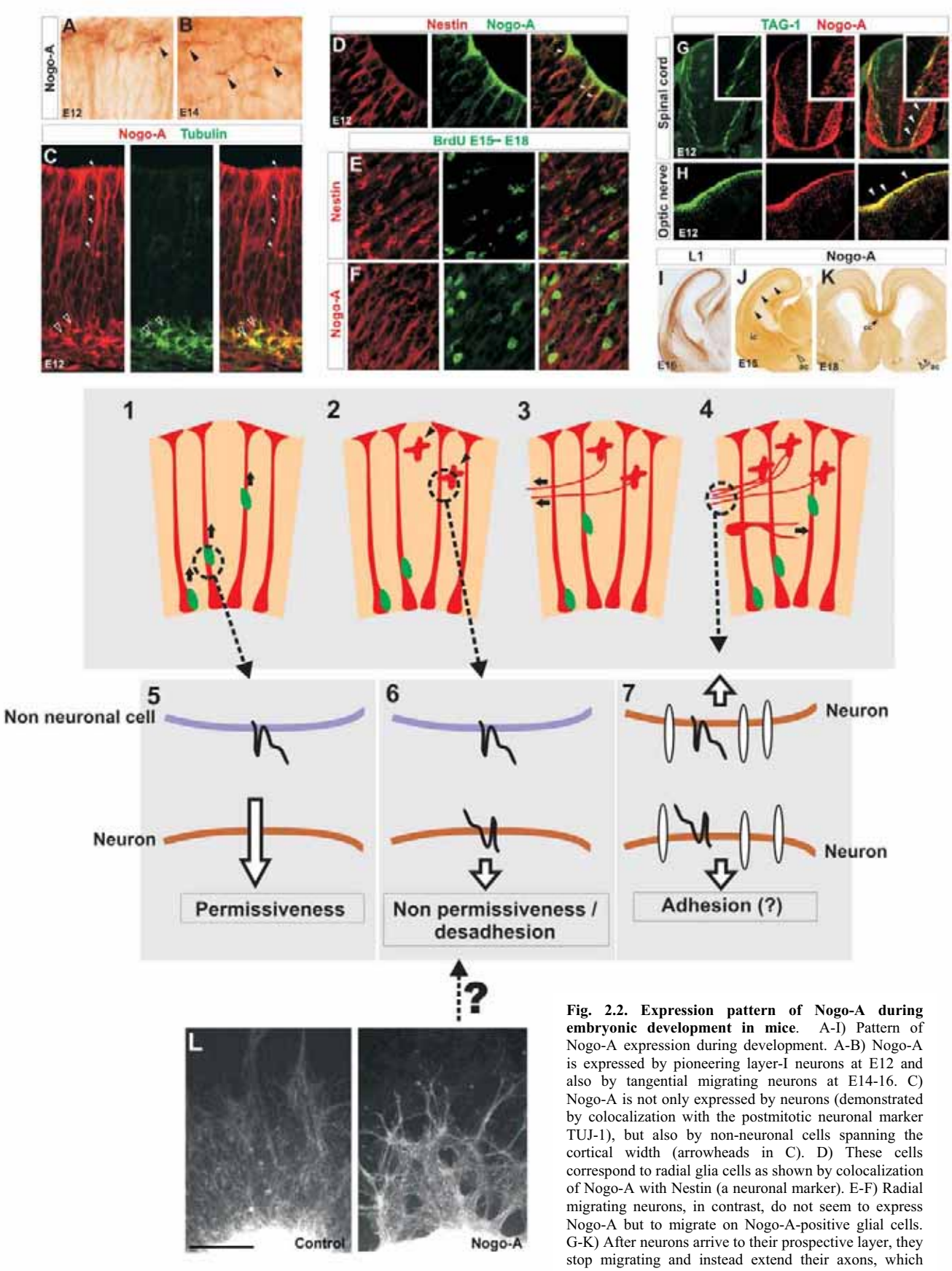


Fig. 2.2. Expression pattern of Nogo-A during embryonic development in mice. A-I) Pattern of Nogo-A expression during development. A-B) Nogo-A is expressed by pioneering layer-I neurons at E12 and also by tangential migrating neurons at E14-16. C) Nogo-A is not only expressed by neurons (demonstrated by colocalization with the postmitotic neuronal marker TUJ-1), but also by non-neuronal cells spanning the cortical width (arrowheads in C). D) These cells correspond to radial glia cells as shown by colocalization of Nogo-A with Nestin (a neuronal marker). E-F) Radial migrating neurons, in contrast, do not seem to express Nogo-A but to migrate on Nogo-A-positive glial cells. G-K) After neurons arrive to their prospective layer, they stop migrating and instead extend their axons, which occasionally form thick axon bundles or tracts. Most of the main axonal tracts are enriched in Nogo-A (G, H, J, K). These tracts are characteristically labelled with antibodies against some cell adhesion molecules such as TAG-1 (G-H), L1 (I) and DCC (not shown). *(Continue)*

(From page 123)
1-4) Schematic representation of cortex development illustrating the expression of Nogo-A (red cells) by certain cell populations, contrasting with cells that do not express Nogo-A (green cells). Initially, radial glia cells span the cortical width and express Nogo-A (1). In contrast, radial migrating neurons, which generate from the subventricular zone (and some of which are generated form radial glia cells; Fishell and Kriegstein, 2003), do not express Nogo-A (see also F). When radial migrating neurons stop migrating and detach from glial cells, they start expressing Nogo-A (2 and A) and some of them extend long projections that are particularly enriched in Nogo-A (G-K, 3). Last, the population of tangential migrating neurons also express high levels of Nogo-A (4). These cells generate in the ganglionic eminences and migrate toward the cortex following paths that at some stages involve migration over Nogo-A/Tag-1-positive tracts.

5-7) Hypothesis of how Nogo-A may participate in cell adhesion. Cells can be separated in non-neuronal (blue) and neuronal cells (brown). When neurons that do not express Nogo-A (i. e. radial migrating neurons) contact with Nogo-A expressing glial cells, the presence of Nogo-A does not seem to prevent cell adhesion and migration. (5). Thus, Nogo-A may be considered permissive when expressed unilaterally. However, no neuron expressing Nogo-A was observed in apposition with radial glia cells. We can propose that this bilateral expression of Nogo-A may result in the deadhesion of the neuron from the Nogo-A source (6). Last, Nogo-A is widely expressed in axonal tracts. As these axons are packed together, the bilateral expression of Nogo-A cannot be repulsive (or induce deadhesion), but instead be permissive and even facilitate cell adhesion (7). The difference between this last situation and the previous one (6) may be the expression, together with Nogo-A, of other cell adhesion molecules by neurons.

L) Nogo-A induces fasciculation *in vitro*. Hippocampal explants express Nogo-A but not NgR. While they display radial growth when cultured on permissive substrates (laminin, collagen, or matrigel), they fasciculate when growing in the presence of Nogo-A. This may correspond to the mechanism explained in (6).

The presence of Nogo-A at the surface of fasciculated axons is inconsistent with an inhibitory or repulsive role *in vivo*. However, this does not explain how the presence of Nogo-A in the substrate induces fasciculation *in vitro*, which fits more with an inhibiting role and sets a dichotomy between the *in vivo* and *in vitro* observations. It could be argued that laminin substrate may switch Nogo-A from adhesive (*in vivo*) to antiadhesive (*in vitro*), as it does with Netrin-1 attraction, probably by changing the intracellular levels of calcium (Song and Poo, 1999; 2001). However, we first observed Nogo-A-induced fasciculation in explants growing on colagen-coated transwells that were droped with mock and Nogo-A membrane extracts, and the experiment was also reproduced in matrigel transwells. Antoher posibility is that the effect of Nogo-A on neurons depends on the context, i.e. on the proteins with which it is co-expressed. This occurs with many receptors, as suggested in figure 2.2 5-7. As for netrin, two receptors are invloved: DCC and Unc-5H (Kennedy, 2000). While binding of Netrin to DCC induces attraction and binding to Unc-5H repulsion, DCC can also transduce repulsion when coexpressed with Unc-5H (Shu *et al*., 2000). Although this example involves receptors, a similar mechanism may occur with Nogo-A as a ligand. Neurons that express high levels of Nogo-A seem to adhere to each other forming fascicles (Fig. 2.2 G-K). However, neurons migrating on radial glia, which express Nogo-A (Fig. 2.2 C-D), do not express it until they stop migrating and detach from the glial cell (Fig. 2.2 F). Neurons may adhere to neuronal Nogo-A, but not to the one expressed by radial glia or COS cells because it needs to be expressed together with other neuronal proteins to induce neurite adhesion. If this proved true, as Nogo-A is expressed by non-neuronal cells in our *in vitro* assay, it would be presented to the neurites *individually* and induce deadhesion from substrate, while it would induce adhesion when expressed by neurons in combination with some/s neuronal protein/s (Fig. 2.2 L). Some candidates for this *partner role* may be TAG-1, L1 and DCC, as they colocalize with Nogo-A in all the tracts where it is expressed, such as the corticothalamic tract (Fig. 2.2 I-J) and the commisural fibres in the embryonic spinal cord (Fig. 2.2 G).

2.3. IN-1 supernatant induces axonal growth regardless of external Nogo-A

Another cue about Nogo-A neuronal role comes from observations about IN-1 effect on neurons. Postnatal CGNs express NgR and are inhibited by the presence of Nogo-A on the substrate, but they also express high levels of Nogo-A. Adding IN-1 supernatant to these substrates enhances axonal length, restoring control length, which may be attributed to a blockade of the inhibitory Nogo-A present in the coating (Fig. 2.3, third column). A similar experiment using DRG neurons was performed by Chen *et al*. in an article describing the cloning of Nogo-A (Fig. 3 in Chen *et al*., 2000). However, axonal length also increases when IN-1 is included in cultures not exposed to Nogo-A (Fig. 2.3 first two columns), indicating that IN-1-induced axonal growth is a direct effect of the antibody on neurons. This control was omited in Chen *et al*. (2000). Although the increase observed in neurons growing on Nogo-A is higher (around 40%), it is significant and very consistant in cells growing on non-inhibitory substrates (around 20%), so that the blockade of Nogo-A in the substrate is combined with the enhancement of neuronal growth through direct binding to neurons.

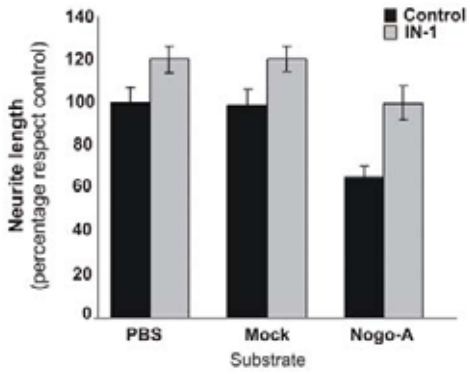


Figure 2.3. Effect of IN-1 on neurite length of P5 CGNs. Dissociated P5 CGNs were planted on coverslips coated with membrane extract form COS cells transfected or not with Nogo-A, and IN-1 antibody or not, and neurite length (a single neurite in CGNs) was measured after 24 hours *in vitro*.

The growth-promoting effect of IN-1 in non-lesioned neurons had been reported elsewhere, but has always been attributed to a release from the constitutive inhibition exherted by (non-neuronal) Nogo-A (Z'Graggen *et al*., 1998; Thallmair *et al*., 1998; Buffo *et al*., 2000). However, our *in vitro* assay allowed us to assess the effect of IN-1 in isolated neurons. Although the specificity of IN-1 for Nogo-A is somehow uncertain, the growth promoting effect seems to be specific, as monoclonal antibodies other than IN-1 raised against Nogo-A induce axonal growth and sprouting in unlesioned Purkinje cells (Buffo *et al*., 2000). Moreover, Purkinje cells express high levels of Nogo-A and al least part of the IN-1 effect may be due to binding to neuronal Nogo-A (as in Fig. 2.3).

How can the binding of IN-1 to neurons promote axon growth? One possibility is that IN-1 binding to neuronal Nogo-A *activates* it, and initiates signaling cascades leading to neurite extension. This effect is known for cell adhesion molecules or receptors that need to dimerize in order to start intracellular signaling (for example L1 and Fas/CD95, Dickson *et al*., 2002; Suda *et al*., 1997). Antibody binding to these proteins induces clustering, as the endogenous ligand would do, and mimics ligand-induced signaling. Likewise, IN-1 binding to Nogo-A at the plasma membrane may induce clustering of Nogo-A. The IN-1 epitope is located in the NiG domain of Nogo-A, which has inhibitory effects on postnatal neurons and fibroblast spreading but whose receptor, like the binding partners for Nogo-A during development, is yet unknown. Although in

the spinal cord, IN-1-induced sprouting of unlesioned corticospinal tract neurons is accompained by the upregulation of growth promoting genes such as GAP-43 (Bareyre *et al*., 2002), GAP-43 was not upregulated in our cultures (not shown).

2.4. Nogo-A does not self-interact, but binds to Nogo-B.

We have suggested that Nogo-A self-interacts at the neuronal surface and IN-1 binding may facilitate this interaction. To test whether this interaction is possible, we cotransfected COS-cells (which do not express Nogo-A) with two Nogo-A constructions carrying different tags: haemaglutinin (HA) and the red fluorescent protein (RFP). Although both constructions were visualized by Western blot as two single bands (RFP molecular weight is about 27 kDa), when immunoprecipitation was performed using anti-HA antibody, the HA tagged Nogo-A was recovered, but not the heavier Nogo-A-RFP (Fig 2.4B). This indicates that Nogo-A does not form homophilic complexes, at least in the ER, as it is hardly detectable at the cell surface in COS cells (GrandPre *et al*., 2000; personal observations) and in the absence of its putative ligand (that is not necessarily expressed in COS cells). However, it does bind to Nogo-B (Fig 2.4C; also reported by Dodd *et al*., 2005). This interaction, which probably takes place in the ER (owing to the patterns of both proteins when transfected in COS cells), prompted us to further study the possible Nogo-A intracellular roles by analysing its intracellular partners. To this end, we contacted John R. Bethea (from the Miami Project to Cure Paralysis), who had recently cloned a Nogo-A interacting protein following a yeast two-hybrid assay (Hu *et al*., 2002). This protein, called NIMP (from Nogo-Interacting Mitochondrial Protein), is a novel mitochondrial protein with unknown function (Hu *et al*., 2002). After performing immunohistochemistry with anti-NIMP antibody in several tissues (covering the complete mouse CNS development, as well as perforant pathway lesions and fimbria-fornix axotomy) and obtaining a complex pattern, which suggested that more than one protein were recognized by the antibody, we tried to reproduce the experiments described in Hu *et al*. to confirm NIMP interaction with Nogo-A and the specificity of the anti-NIMP antibody (Fig. 2.4D-F). Although NIMP antibody recognized a band of the predicted NIMP-RFP molecular weight in lysates from transfected cells, it failed to detect this band following Nogo-A immunoprecipitation (Fig 2.4D). Moreover, the distribution of Nogo-A (by ICC) and NIMP (by RFP fluorescence) in cotransfected COS cells is not overlapped (they are expressed in distinct subcellular compartments), which renders the putative interaction between both proteins, if any, punctual. Unfortunately, NIMP antibody labelling of transfected COS-cells differed greatly from recombinant NIMP localization, as assessed by the fluorescent tag, and not only labelled most of the cell (which may be attributed to the high expression of endogenous NIMP in kidney; Hu *et al*., 2002), but also failed to detect NIMP-RFP enriched mitochondria, thus rendering the immunohistochemical analysis of NIMP pattern in brain tissue arguable. Therefore, although Nogo-A may behave as a cell adhesion molecule by interacting with proteins from this family when exposed at the plasma membrane, it may also associate with proteins in the ER and be involved in ER functioning (e.g. vesicle formation).

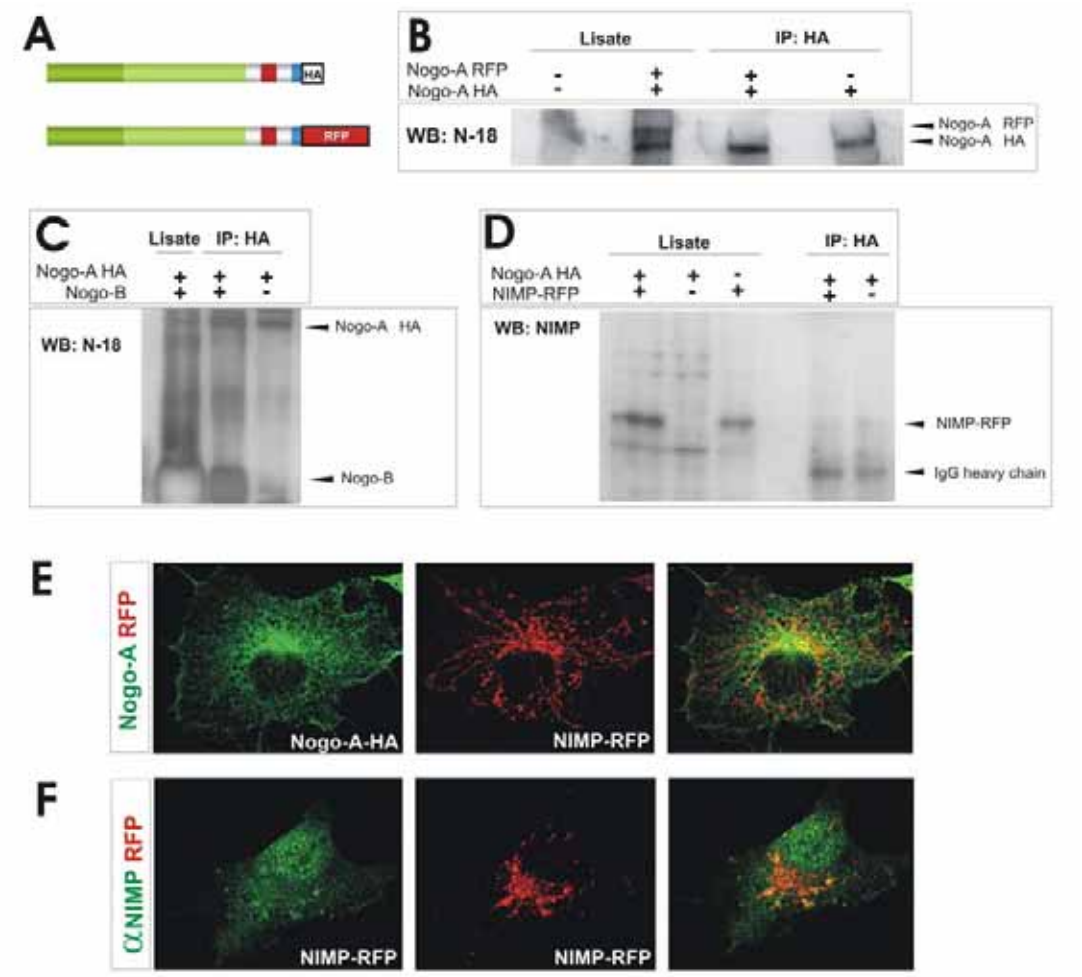


Fig 2.4. Nogo-A intracellular interactions. A-B) Nogo-A does not interact with itself in the endoplasmic reticulum (ER). Two tagged Nogo-A constructions (with HA epitope and RFP) were transfected in COS cells (A). B) Immunoprecipitation against HA epitope pulls down Nogo-A-HA but fails to coprecipitate Nogo-A-RFP. **C) Nogo-A immunoprecipitates with Nogo-B when cotransfected in COS-cells**. Again, HA epitope was used to immunoprecipitate Nogo-A. N-18 antibody recognises both Nogo-A and –B. **D-F) Nogo-A does not interact with NIMP in COS cells.** Antibody against NIMP was tested in Western blotting and efficiently detected a 70kDa band corresponding with transfected NIMP-RFP. However, immunoprecipitation with HA antibody failed to detect NIMP (compared with cells not transfected with NIMP). E) Nogo-A distribution in the ER contrasts with NIMP mitochondrial pattern, as observed by NIMP-fluorescent tag. F) NIMP antibody failed to specifically detect NIMP in transfected cells (as assessed by RFP pattern).

Results and Discussion III

Myelin Contribution to Axonal Regeneration Failure

3.1 Myelin and Evolution.

One interesting analysis that may shed a light on the contribution of MAI to axonal regeneration failure is the extent to which the appearance of MAI during evolution correlates with the loss of axonal regeneration capability. Figure 3.1 illustrates the appearance of the main guidance cue families (blue), and the myelin-associated inhibitors and other myelin proteins (red), during evolution.

Axonal regeneration occurs spontaneously in amphibians and fish but not in mammals. The loss of regenerative capacity during the transition from fish to land vertebrates may be due to the presence of inhibitory molecules in mammalian CNS that are absent in lower vertebrates. However, only Nogo-A specific sequences appear in the evolution approximately when regenerative capacity decreases (Diekmann *et al*., 2005). CSPGs, tenascins and the rest of MAI, in contrast, are already present in fish myelin (Battisti *et al*., 1995; Becker *et al*., 1995; Vourch and Andres, 2004; Diekmann *et al*., 2005). Goldfish oligodendrocytes express rtn4 (including the loop-66) and fish express the corresponding NgR (Klinger *et al*., 2004b), and so both proteins probably interact. As fish neurons can grow over purified fish oligodendrocytes and regenerate after lesion *in vivo* (Stuermer *et al*., 1992), this interaction does not lead to axonal inhibition as it does in mammals (probably because fish neurons follow different signalling pathways). Another possibility is that the regulation of the protein after lesion, but not the presence of the protein itself, determines the final capability to regenerate. Evidence points to the latter hypothesis. Oligodendrocytes in the salamander, for instance, downregulate tenascin-R and MAG following optic nerve crush in the same time window as retinal ganglion regeneration (Becker *et al*., 1999). Moreover, in the peripheral nervous system of lower

vertebrates, inhibitory molecules are rapidly removed after injury. Particularly, myelin debris are rapidly removed from the optic nerve in amphibians (Wilson *et al*., 1992; Sivron and Schwartz, 1995), while in mammals, inhibitors remain for weeks or are even upregulated. Thus, the complex reaction following lesion in mammals, but not the presence of inhibitory molecules, may determine the failure of axonal regeneration.

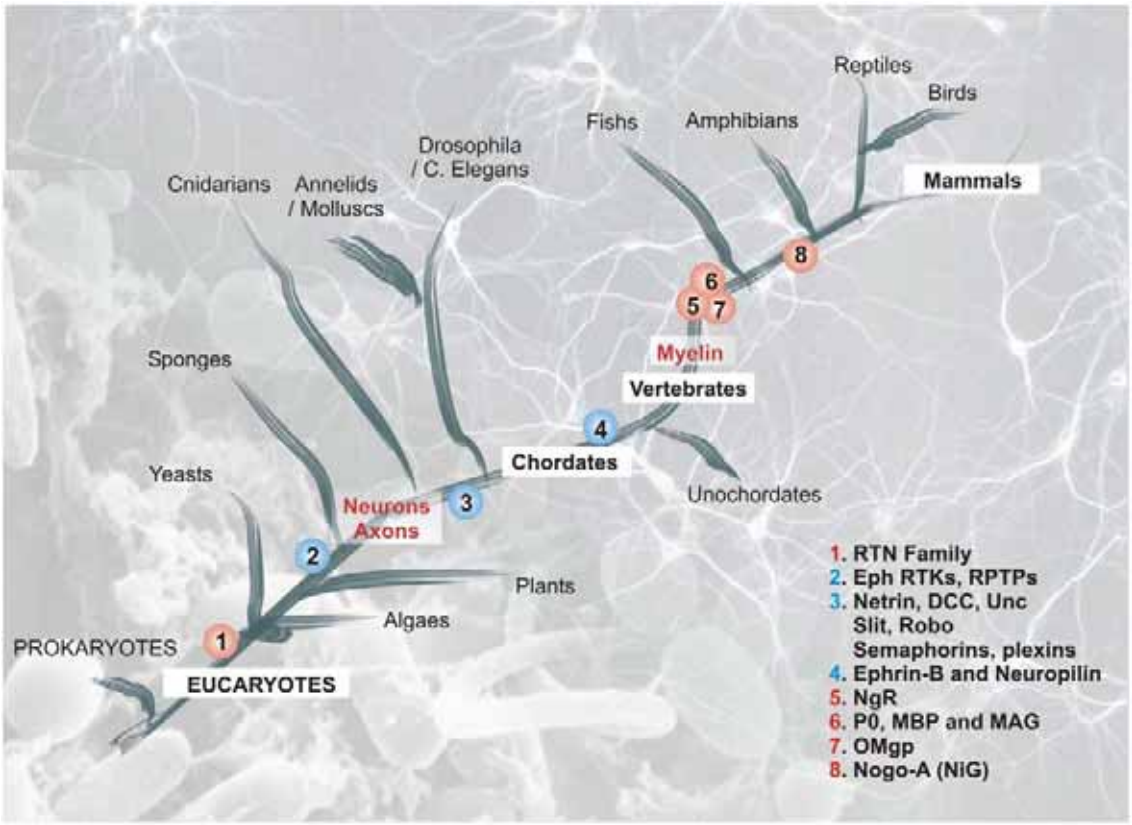


Figure 3.1. Evolutionary tree illustrating the correlation of myelin inhibitors with the loss of axonal regeneration during mammalian evolution. The localization of guidance cues and myelin-related proteins first appearance in the tree are in agreement with previous reports.

3.2 Why inhibition?

One of the first things that one wonders when learning about axonal regeneration is “why”. Why does axonal regeneration not occur spontaneously? And what are the advantages of this? Most probably, spontaneous axonal regeneration was lost during evolution because natural selection favoured it. More complex nervous systems are less capable of regenerating. This occurred during evolution (especially in mammals), but also during normal development and in the same animal when comparing central and PNS. Therefore, CNS complexity may be somehow compromised with regenerative plasticity.

Reactive astrocytes and mature oligodendrocytes probably became the two strongest inhibitors of the CNS by different pathways. We have already mentioned that while the presence of inhibitory molecules in myelin may represent constitutive inhibition of neuronal plasticity, the lesion-induced glial reactivity involving glial scar development and proteoglycan overexpression may be linked to the prevention of secondary cellular degeneration (Faulkner *et*

al., 2004). Therefore, the primary function of MAI would be to stabilize axonal connections and restrict plasticity in normal CNS (McGee et al., 2005), while reactive astrocytes may prevent cavitation after lesion. However, despite these key roles, both oligodendrocytes and astrocytes may restrict axonal regeneration, as a secondary, undesired effect. In fact, this hypothesis may explain why evolution favoured the presence of these restrictive mechanisms in the adult mammal CNS. Mutations associated with the inhibition of neurons by myelin may have led to synaptic disorganization and functional defects that were negatively selected during evolution. Analogously, mutations in astroglial reactivity after lesion may compromise animal survival, as they protect CNS from cavitation, and thus are also negatively selected.

Then, why is MAI expression not downregulated after lesion and restored once axons have regenerated? Releasing axons from constitutive inhibition following lesion may permit axonal regeneration. Actually, this is what we observe when interfering with MAI, their receptors and the intracellular pathways they activate after lesion in diverse models. In this regard, MAG expression is not only maintained after lesion but also strongly increased. This indicates that at least for MAG, there must be a role other than constitutive inhibition that explains this regulation. One possibility is that MAG overexpression, and more generally the reactivity of mature oligodendrocytes after PP axotomy, contributes to maintaining neuronal survival. In this regard, it is known that in many other axotomy models, such as corticospinal tract transection, over 90% of oligodendrocytes die during the first week after lesion (although the population can be recovered following progenitor differentiation in subsequent weeks), which is accompanied by extensive neuronal death (Abe *et al*., 1999). In contrast, the loss of mature oligodendrocytes is not significant in the entorhinal cortex after PP lesion, and we characterized the reactive response of these cells to axotomy. Entorhinal neurons are particularly resistant to axotomy, as only 20% die during the first weeks after lesion (Peterson *et al*., 1996). Oligodendrocyte survival, or the overexpression of certain proteins, may be related to the *increased* survival of entorhinal neurons. In fact, animals deficient in MAG show axonal atrophy in the adulthood, suggesting that MAG has trophic effects on axons/neurons (Windebank *et al*., 1985; Sanchez *et al*., 1996).

Results and Discussion IV

Many inhibitors but one single way to inhibit neurons: intracellular convergence

As the molecular mechanisms that regulate axon growth and axon inhibition began to be revealed, a signalling convergence emerged. The cytoskeleton is the ultimate target of every extracellular molecule regulating axon dynamics. Rho GTPases have a central role in regulating cytoskeletal dynamics, and so their regulation is necessary to modulate it. Thus, the modulation of Rho GTPases activity has been found in the signalling cascades initiated by the majority of guidance cues and inhibitors. This regulation may occur at the beginning of the signalling pathway, as described for Plexins or p75, which bind directly to Rho GTPases, or more downstream, as described for Eph and Robo receptors (Yamashita and Tohyama, 2002; Dickson *et al*., 2002). Key regulatory steps that control Rho GTPases activity are limited (mainly calcium levels, cyclic nucleotide levels and PKC activation), as are the possible ways in which any extracellular molecule modulates axon dynamics. Thus, the finding that inhibitors seem to activate the same pathways is not so surprising; *they are inhibitors because they do so*

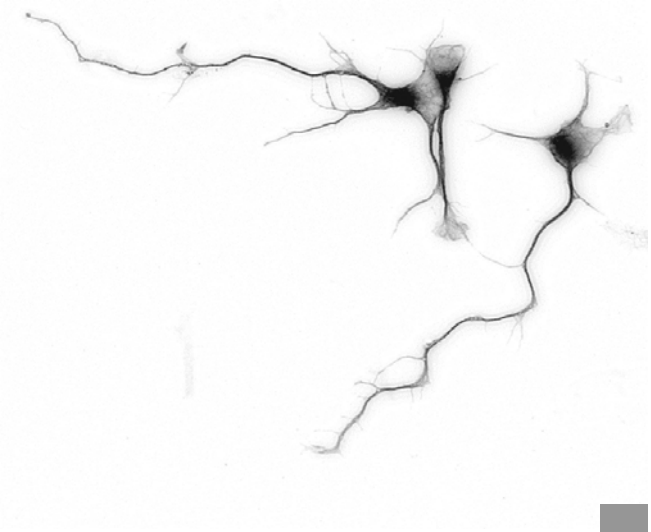
Interestingly, at least two pathways, regulated by distinct key molecules, seem to ultimately converge in the cytoskeleton and regulate growth cone attraction, repulsion and axonal inhibition (Song and Poo, 1999). MAI, as well as BDNF and Netrin, increase calcium intracellular levels and are sensitive to cAMP manipulation (Song *et al*., 1998). On the other hand, Sema 3A and NT3 do not modify calcium levels and are sensitive to cGMP manipulation (Song *et al*., 1998). These manipulations allow us to regulate both growth cone behaviour (e.g. orientation of turning or collapse) and axonal inhibition after lesion in the adult, which indicates not only that the signalling pathways regulating cytoskeleton dynamics converge, but also that these pathways are the same for these developmentally separated processes. The results obtained with ChABC and NEP1-40 combined treatment point to the relevance of this convergence. The targeting of two or more inhibitors is probably be less effective than targeting one inhibitor and provide a permissive substrate (as one of many possible combinations), as many inhibitors may be considered the same thing with a different *face*.

Results and Discussion IV

Development and Regeneration: protein recycling

Although MAI consist of only three proteins, many other inhibitory molecules are present in myelin in a constitutive manner. These include members of the CSPG family, like versican and brevican, and guidance cues like Sema 4D/CD100, Netrin 1and Ephrin B3. Some of them, like Ephrin B3, are exclusively expressed by mature oligodendrocytes, and others, like Netrin 1, are also expressed by neurons. The presence of these guidance cues in the adult is thought to restrict axonal sprouting, leading to inhibition after lesion, probably through the same mechanisms that control axonal chemorepulsion during development. It is quite attractive to propose that the contrary is also true, and molecules restricting plasticity in the mature CNS are probably involved in the control of attraction, repulsion and branching, if expressed during development.

Our results suggest that neuronal Nogo-A is involved in cell adhesion during development. We find an interesting parallelism between Nogo-A and TAG-1, a cell adhesion molecule that is transiently expressed by neurons and regulates fasciculation (and interneuron migration; Denaxa *et al*., 2001; Kyriakopoulou *et al*., 2002; Rougon and Hobert, 2003; McManus *et al*., 2004) during development. As myelination begins, Nogo-A is expressed by myelinating oligodendrocytes and restricted to paranodes, where it regulates neuronal potassium channel sorting (Nie *et al*., 2003). TAG-1, when expressed by myelinating oligodendrocytes, regulates potassium channel sorting in the juxtaparanodes, interacting with a homolog of the binding partner of Nogo-A at the paranodes (Nie *et al*., 2003; Traka *et al*., 2003). Although TAG-1 and Nogo-A have been discovered in different contexts, these proteins seem to share a degree of functional parallelism during development. If this parallelism holds true, and since TAG-1 has been recently found to be expressed in the adult (Wolfer *et al*., 1998; Soares *et al*., 2005), TAG-1 and Nogo-A may also have homologous functions in the mature CNS, either in myelin or when expressed by neurons. Analogously, it is only a matter of time before many proteins that have been discovered in the context of axon guidance or adhesion are found to limit axonal regeneration in the mature CNS.



Conclusions

1. Nogo-A is expressed by hippocampal neurons during development and by myelinating oligodendrocytes, principal neurons and interneurons in the mature CNS. NgR expression is exclusively neuronal and starts during postnatal stages. *In vitro*, Nogo-A induces fasciculation of embryonic explants, but does not inhibit axon outgrowth. This suggests alternative functions for Nogo-A during development.

2. MAG expression by mature oligodendrocytes follows the onset of myelination and parallels the developmental loss of regeneration capability of the perforant pathway.

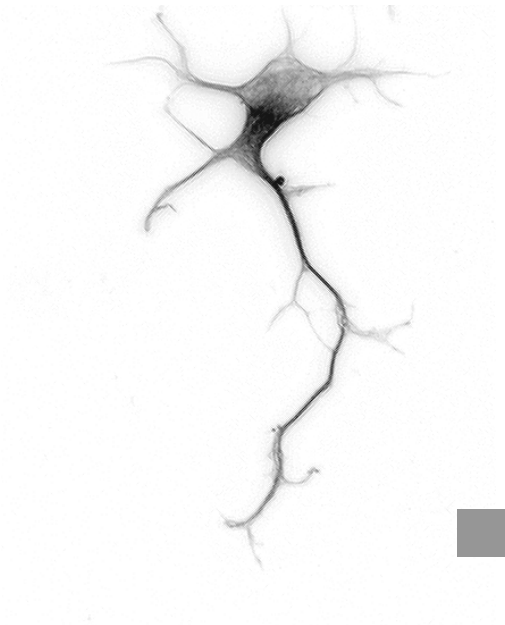
3. Neuronal expression of both Nogo-A and NgR is regulated by neuronal activity. Kainic acid, glutamate (for Nogo-A) and BDNF administration, downregulate Nogo-A and NgR expression. NgR is specifically downregulated by neurons when synaptic remodelling occurs. This indicates that both Nogo-A and NgR may regulate synaptic stabilization and plasticity.

4. Nogo-A and MAG are transiently overexpressed by reactive astrocytes and mature oligodendrocytes respectively in response to perforant pathway axotomy. NgR expression undergoes biphasic regulation after lesion in the deafferented granule neurons, and its subsequent downregulation coincides with the reactive sprouting of granule neurons. This agrees with a possible role of MAG, Nogo-A and NgR in preventing axonal regeneration.

5. The blockade of MAG and both Nogo-A inhibitory domains successfully promotes axonal regeneration of the perforant pathway *in vitro*.

6. Chondrotin sulphate proteoglycans are overexpressed after axotomy of the perforant pathway and their blockade permits axonal regeneration of the transected connection.

7. The combined blockade of Nogo-A binding to NgR and chondrotin sulphate proteoglycans activity does not have synergistic effects on perforant pathway regeneration



References

1. 1 (2005) Hospital Nacional de Paralíticos de Toledo. España.

2. Abe, Y., Yamamoto, T., Sugiyama, Y., Watanabe, T., Saito, N., Kayama, H., and Kumagai, T. (1999) Apoptotic cells associated with Wallerian degeneration after experimental spinal cord injury: a possible mechanism of oligodendroglial death. J Neurotrauma 16, 945-952

3. Acarin, L., Gonzalez, B., and Castellano, B. (2001) Glial activation in the immature rat brain: implication of inflammatory transcription factors and cytokine expression. Prog Brain Res 132, 375-389

4. Amaral DG, W. M. (1995) The hippocampal formation, Paxinos G (Ed), Academic Press

5. Asher, R. A., Morgenstern, D. A., Moon, L. D., and Fawcett, J. W. (2001) Chondroitin sulphate proteoglycans: inhibitory components of the glial scar. Prog Brain Res 132, 611-619

6. Bagri, A., Cheng, H. J., Yaron, A., Pleasure, S. J., and Tessier-Lavigne, M. (2003) Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 113, 285-299

7. Bahr, M., Vanselow, J., and Thanos, S. (1988) In vitro regeneration of adult rat ganglion cell axons from retinal explants. Exp Brain Res 73, 393-401

8. Bandtlow, C., and Dechant, G. (2004) From cell death to neuronal regeneration, effects of the p75 neurotrophin receptor depend on interactions with partner subunits. Sci STKE 2004, pe24

9. Bandtlow, C., Schiweck, W., Tai, H. H., Schwab, M. E., and Skerra, A. (1996) The Escherichia coli-derived Fab fragment of the IgM/kappa antibody IN-1 recognizes and neutralizes myelin-associated inhibitors of neurite growth. Eur J Biochem 241, 468-475

10. 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

11. Bandtlow, C. E., Schmidt, M. F., Hassinger, T. D., Schwab, M. E., and Kater, S. B. (1993) Role of intracellular calcium in NI-35-evoked collapse of neuronal growth cones. Science 259, 80-83

12. Bandtlow, C. E., and Schwab, M. E. (2000) NI-35/250/nogo-a: a neurite growth inhibitor restricting structural plasticity and regeneration of nerve fibers in the adult vertebrate CNS. Glia 29, 175-181

13. Bandtlow, C. E., and Zimmermann, D. R. (2000) Proteoglycans in the developing brain: new conceptual insights for old proteins. Physiol Rev 80, 1267-1290

14. Bareyre, F. M., Haudenschild, B., and Schwab, M. E. (2002) Long-lasting sprouting and gene expression changes induced by the monoclonal antibody IN-1 in the adult spinal cord. J Neurosci 22, 7097-7110

15. Bareyre, F. M., Kerschensteiner, M., Raineteau, O., Mettenleiter, T. C., Weinmann, O., and Schwab, M. E. (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. Nat Neurosci 7, 269-277

16. Barker, P. A. (2004) p75NTR is positively promiscuous: novel partners and new insights. Neuron 42, 529-533

17. 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. (2003) Structure and axon outgrowth inhibitor binding of the Nogo-66 receptor and related proteins. Embo J 22, 3291-3302

18. Bartsch, U. (1996) Myelination and axonal regeneration in the central nervous system of mice deficient in the myelin-associated glycoprotein. J Neurocytol 25, 303-313

19. Bartsch, U., Kirchhoff, F., and Schachner, M. (1989) Immunohistological localization of the

adhesion molecules L1, N-CAM, and MAG in the developing and adult optic nerve of mice. J Comp Neurol 284, 451-462

20. Battisti, W. P., Wang, J., Bozek, K., and Murray, M. (1995) Macrophages, microglia, and astrocytes are rapidly activated after crush injury of the goldfish optic nerve: a light and electron microscopic analysis. J Comp Neurol 354, 306-320

21. Bechmann, I., and Nitsch, R. (1997) Astrocytes and microglial cells incorporate degenerating fibers following entorhinal lesion: a light, confocal, and electron microscopical study using a phagocytosis-dependent labeling technique. Glia 20, 145-154

22. Bechmann, I., and Nitsch, R. (2000) Involvement of non-neuronal cells in entorhinal-hippocampal reorganization following lesions. Ann N Y Acad Sci 911, 192-206

23. Becker, C. G., Becker, T., Meyer, R. L., and Schachner, M. (1999) Tenascin-R inhibits the growth of optic fibers in vitro but is rapidly eliminated during nerve regeneration in the salamander Pleurodeles waltl. J Neurosci 19, 813-827

24. Becker, T., Becker, C. G., Niemann, U., Naujoks-Manteuffel, C., Bartsch, U., Schachner, M., and Roth, G. (1995) Immunohistological localization of tenascin-C in the developing and regenerating retinotectal system of two amphibian species. J Comp Neurol 360, 643-657

25. Bellen, H. J., Lu, Y., Beckstead, R., and Bhat, M. A. (1998) Neurexin IV, caspr and paranodin--novel members of the neurexin family: encounters of axons and glia. Trends Neurosci 21, 444-449

26. 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

27. Berger, T., and Frotscher, M. (1994) Distribution and morphological characteristics of oligodendrocytes in the rat hippocampus in situ and in vitro: an immunocytochemical study with the monoclonal Rip antibody. J Neurocytol 23, 61-74

28. Bertrand, J., Winton, M. J., Rodriguez-Hernandez, N., Campenot, R. B., and McKerracher, L. (2005) Application of rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and regeneration of retinal ganglion cell axons in the optic nerve of adult rats. J Neurosci 25, 1113-1121

29. Bonfanti, L., Strettoi, E., Chierzi, S., Cenni, M. C., Liu, X. H., Martinou, J. C., Maffei, L., and Rabacchi, S. A. (1996) Protection of retinal ganglion cells from natural and axotomy-induced cell death in neonatal transgenic mice overexpressing bcl-2. J Neurosci 16, 4186-4194

30. Borisoff, J. F., Chan, C. C., Hiebert, G. W., Oschipok, L., Robertson, G. S., Zamboni, R., Steeves, J. D., and Tetzlaff, W. (2003) Suppression of Rho-kinase activity promotes axonal growth on inhibitory CNS substrates. Mol Cell Neurosci 22, 405-416

31. Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., Fawcett, J. W., and McMahon, S. B. (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416, 636-640

32. Bregman, B. S., Kunkel-Bagden, E., Schnell, L., Dai, H. N., Gao, D., and Schwab, M. E. (1995) Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. Nature 378, 498-501

33. Bregman, B. S., McAtee, M., Dai, H. N., and Kuhn, P. L. (1997) Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. Exp Neurol 148, 475-494

34. Brosamle, C., Huber, A. B., Fiedler, M., Skerra, A., and Schwab, M. E. (2000) Regeneration of lesioned corticospinal tract fibers in the adult rat induced by a recombinant, humanized IN-1 antibody fragment. J Neurosci 20, 8061-8068

35. Brose, K., Bland, K. S., Wang, K. H., Arnott, D., Henzel, W., Goodman, C. S., Tessier-

Lavigne, M., and Kidd, T. (1999) Slit proteins bind Robo receptors and have an evolutionarily conserved role in repulsive axon guidance. Cell 96, 795-806

36. 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

37. Cai, D., Deng, K., Mellado, W., Lee, J., Ratan, R. R., and Filbin, M. T. (2002) Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro. Neuron 35, 711-719

38. Cai, D., Qiu, J., Cao, Z., McAtee, M., Bregman, B. S., and Filbin, M. T. (2001) Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. J Neurosci 21, 4731-4739

39. Cai, D., Shen, Y., De Bellard, M., Tang, S., and Filbin, M. T. (1999) Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron 22, 89-101

40. Carim-Todd, L., Escarceller, M., Estivill, X., and Sumoy, L. (2003) LRRN6A/LERN1 (leucine-rich repeat neuronal protein 1), a novel gene with enriched expression in limbic system and neocortex. Eur J Neurosci 18, 3167-3182

41. Caroni, P., Savio, T., and Schwab, M. E. (1988) Central nervous system regeneration: oligodendrocytes and myelin as non-permissive substrates for neurite growth. Prog Brain Res 78, 363-370

42. Caroni, P., and Schwab, M. E. (1988) Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. J Cell Biol 106, 1281-1288

43. 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

44. Carulli, D., Laabs, T., Geller, H. M., and Fawcett, J. W. (2005) Chondroitin sulfate proteoglycans in neural development and regeneration. Curr Opin Neurobiol 15, 116-120

45. Cassel, J. C., Duconseille, E., Jeltsch, H., and Will, B. (1997) The fimbria-fornix/cingular bundle pathways: a review of neurochemical and behavioural approaches using lesions and transplantation techniques. Prog Neurobiol 51, 663-716

46. Chao, M. V. (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4, 299-309

47. 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

48. 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

49. Condic, M. L. (2002) Neural development: axon regeneration derailed by dendrites. Curr Biol 12, R455-457

50. 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

51. 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

52. David, S., and Lacroix, S. (2003) Molecular approaches to spinal cord repair. Annu Rev Neurosci 26, 411-440

53. Davies, S. J., and Silver, J. (1998) Adult axon regeneration in adult CNS white matter. Trends Neurosci 21, 515

54. Dawson, M. R., Levine, J. M., and Reynolds, R. (2000) NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res 61, 471-479

55. de Wit, J., and Verhaagen, J. (2003) Role of semaphorins in the adult nervous system. Prog Neurobiol 71, 249-267

56. 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

57. Del Rio, J. A., Heimrich, B., Borrell, V., Forster, E., Drakew, A., Alcantara, S., Nakajima, K., Miyata, T., Ogawa, M., Mikoshiba, K., Derer, P., Frotscher, M., and Soriano, E. (1997) A role for Cajal-Retzius cells and reelin in the development of hippocampal connections. Nature 385, 70-74

58. Del Rio, J. A., Martinez, A., Auladell, C., and Soriano, E. (2000) Developmental history of the subplate and developing white matter in the murine neocortex. Neuronal organization and relationship with the main afferent systems at embryonic and perinatal stages. Cereb Cortex 10, 784-801

59. del Rio, J. A., Martinez, A., Fonseca, M., Auladell, C., and Soriano, E. (1995) Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. Cereb Cortex 5, 13-21

60. del Rio, J. A., Sole, M., Borrell, V., Martinez, A., and Soriano, E. (2002) Involvement of Cajal-Retzius cells in robust and layer-specific regeneration of the entorhino-hippocampal pathways. Eur J Neurosci 15, 1881-1890

61. del Rio, J. A., and Soriano, E. (1989) Immunocytochemical detection of 5'-bromodeoxyuridine incorporation in the central nervous system of the mouse. Brain Res Dev Brain Res 49, 311-317

62. Del Turco, D., Woods, A. G., Gebhardt, C., Phinney, A. L., Jucker, M., Frotscher, M., and Deller, T. (2003) Comparison of commissural sprouting in the mouse and rat fascia dentata after entorhinal cortex lesion. Hippocampus 13, 685-699

63. Deller, T., Frotscher, M., and Nitsch, R. (1995) Morphological evidence for the sprouting of inhibitory commissural fibers in response to the lesion of the excitatory entorhinal input to the rat dentate gyrus. J Neurosci 15, 6868-6878

64. Deller, T., Frotscher, M., and Nitsch, R. (1996) Sprouting of crossed entorhinodentate fibers after a unilateral entorhinal lesion: anterograde tracing of fiber reorganization with Phaseolus vulgaris-leucoagglutinin (PHAL). J Comp Neurol 365, 42-55

65. Deller, T., Haas, C. A., Naumann, T., Joester, A., Faissner, A., and Frotscher, M. (1997) Up-regulation of astrocyte-derived tenascin-C correlates with neurite outgrowth in the rat dentate gyrus after unilateral entorhinal cortex lesion. Neuroscience 81, 829-846

66. Deller, T., Nitsch, R., and Frotscher, M. (1995) Phaseolus vulgaris-leucoagglutinin tracing of commissural fibers to the rat dentate gyrus: evidence for a previously unknown commissural projection to the outer molecular layer. J Comp Neurol 352, 55-68

67. Deller, T., Nitsch, R., and Frotscher, M. (1996) Layer-specific sprouting of commissural fibres to the rat fascia dentata after unilateral entorhinal cortex lesion: a Phaseolus vulgaris leucoagglutinin tracing study. Neuroscience 71, 651-660

68. Dickson, B. J. (2002) Molecular mechanisms of axon guidance. Science 298, 1959-1964

69. Dickson, T. C., Mintz, C. D., Benson, D. L., and Salton, S. R. (2002) Functional binding

interaction identified between the axonal CAM L1 and members of the ERM family. J Cell Biol 157, 1105-1112

70. Diekmann, H., Klinger, M., Oertle, T., Heinz, D., Pogoda, H. M., Schwab, M. E., and Stuermer, C. A. (2005) Analysis of the reticulon gene family demonstrates the absence of the neurite growth inhibitor Nogo-A in fish. Mol Biol Evol 22, 1635-1648

71. Dodd, D. A., Niederoest, B., Bloechlinger, S., Dupuis, L., Loeffler, J. P., and Schwab, M. E. (2005) Nogo-A, -B, and -C are found on the cell surface and interact together in many different cell types. J Biol Chem 280, 12494-12502

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

73. Dou, C. L., and Levine, J. M. (1994) Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. J Neurosci 14, 7616-7628

74. Drojdahl, N., Fenger, C., Nielsen, H. H., Owens, T., and Finsen, B. (2004) Dynamics of oligodendrocyte responses to anterograde axonal (Wallerian) and terminal degeneration in normal and TNF-transgenic mice. J Neurosci Res 75, 203-217

75. Drojdahl, N., Hegelund, I. V., Poulsen, F. R., Wree, A., and Finsen, B. (2002) Perforant path lesioning induces sprouting of CA3-associated fibre systems in mouse hippocampal formation. Exp Brain Res 144, 79-87

76. Eby, M. T., Jasmin, A., Kumar, A., Sharma, K., and Chaudhary, P. M. (2000) TAJ, a novel member of the tumor necrosis factor receptor family, activates the c-Jun N-terminal kinase pathway and mediates caspase-independent cell death. J Biol Chem 275, 15336-15342

77. Einheber, S., Zanazzi, G., Ching, W., Scherer, S., Milner, T. A., Peles, E., and Salzer, J. L. (1997) The axonal membrane protein Caspr, a homologue of neurexin IV, is a component of the septate-like paranodal junctions that assemble during myelination. J Cell Biol 139, 1495-1506

78. Ellezam, B., Dubreuil, C., Winton, M., Loy, L., Dergham, P., Selles-Navarro, I., and McKerracher, L. (2002) Inactivation of intracellular Rho to stimulate axon growth and regeneration. Prog Brain Res 137, 371-380

79. Emerick, A. J., Neafsey, E. J., Schwab, M. E., and Kartje, G. L. (2003) Functional reorganization of the motor cortex in adult rats after cortical lesion and treatment with monoclonal antibody IN-1. J Neurosci 23, 4826-4830

80. Eyupoglu, I. Y., Bechmann, I., and Nitsch, R. (2003) Modification of microglia function protects from lesion-induced neuronal alterations and promotes sprouting in the hippocampus. Faseb J 17, 1110-1111

81. Fagan, A. M., and Gage, F. H. (1994) Mechanisms of sprouting in the adult central nervous system: cellular responses in areas of terminal degeneration and reinnervation in the rat hippocampus. Neuroscience 58, 705-725

82. 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

83. Fiedler, M., Horn, C., Bandtlow, C., Schwab, M. E., and Skerra, A. (2002) An engineered IN-1 F(ab) fragment with improved affinity for the Nogo-A axonal growth inhibitor permits immunochemical detection and shows enhanced neutralizing activity. Protein Eng 15, 931-941

84. Filbin, M. T. (1996) The Muddle with MAG. Mol Cell Neurosci 8, 84-92

85. Fischer, D., He, Z., and Benowitz, L. I. (2004) Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J Neurosci 24, 1646-1651

86. Fontana, X., Nacher, J., Soriano, E., and Del Rio, J. A. (2005) Cell Proliferation in the Adult

Hippocampal Formation of Rodents and its Modulation by Entorhinal and Fimbria-Fornix Afferents. Cereb Cortex

87. Fouad, K., Dietz, V., and Schwab, M. E. (2001) Improving axonal growth and functional recovery after experimental spinal cord injury by neutralizing myelin associated inhibitors. Brain Res Brain Res Rev 36, 204-212

88. Fouad, K., Klusman, I., and Schwab, M. E. (2004) Regenerating corticospinal fibers in the Marmoset (Callitrix jacchus) after spinal cord lesion and treatment with the anti-Nogo-A antibody IN-1. Eur J Neurosci 20, 2479-2482

89. Fouad, K., Schnell, L., Bunge, M. B., Schwab, M. E., Liebscher, T., and Pearse, D. D. (2005) Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J Neurosci 25, 1169-1178

90. Fournier, A. E., Gould, G. C., Liu, B. P., and Strittmatter, S. M. (2002) Truncated soluble Nogo receptor binds Nogo-66 and blocks inhibition of axon growth by myelin. J Neurosci 22, 8876-8883

91. 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

92. 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

93. Frail, D. E., and Braun, P. E. (1984) Two developmentally regulated messenger RNAs differing in their coding region may exist for the myelin-associated glycoprotein. J Biol Chem 259, 14857-14862

94. Freyaldenhoven, T. E., Ali, S. F., and Schmued, L. C. (1997) Systemic administration of MPTP induces thalamic neuronal degeneration in mice. Brain Res 759, 9-17

95. Friedman, H. V., Bresler, T., Garner, C. C., and Ziv, N. E. (2000) Assembly of new individual excitatory synapses: time course and temporal order of synaptic molecule recruitment. Neuron 27, 57-69

96. Gahwiler, B. H. (1984) Slice cultures of cerebellar, hippocampal and hypothalamic tissue. Experientia 40, 235-243
97. Gahwiler, B. H. (1987) Organotypic slice cultures: a model for interdisciplinary studies. Prog Clin Biol Res 253, 13-18

98. Gahwiler, B. H., Capogna, M., Debanne, D., McKinney, R. A., and Thompson, S. M. (1997) Organotypic slice cultures: a technique has come of age. Trends Neurosci 20, 471-477

99. Gall, C., Rose, G., and Lynch, G. (1979) Proliferative and migratory activity of glial cells in the partially deafferented hippocampus. J Comp Neurol 183, 539-549

100. Gallo, G., and Letourneau, P. C. (2004) Regulation of growth cone actin filaments by guidance cues. J Neurobiol 58, 92-102

101. Gao, Y., Nikulina, E., Mellado, W., and Filbin, M. T. (2003) Neurotrophins elevate cAMP to reach a threshold required to overcome inhibition by MAG through extracellular signal-regulated kinase-dependent inhibition of phosphodiesterase. J Neurosci 23, 11770-11777

102. Giniger, E. (2002) How do Rho family GTPases direct axon growth and guidance? A proposal relating signaling pathways to growth cone mechanics. Differentiation 70, 385-396

103. Goldberg, J. L., Espinosa, J. S., Xu, Y., Davidson, N., Kovacs, G. T., and Barres, B. A. (2002) Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. Neuron 33, 689-702

104. Goldberg, J. L., Klassen, M. P., Hua, Y., and Barres, B. A. (2002) Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. Science 296, 1860-1864

105. Goldberg, J. L., Vargas, M. E., Wang, J. T., Mandemakers, W., Oster, S. F., Sretavan, D. W., and Barres, B. A. (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci 24, 4989-4999

106. Grados-Munro, E. M., and Fournier, A. E. (2003) Myelin-associated inhibitors of axon regeneration. J Neurosci Res 74, 479-485

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

108. 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

109. Haas, C. A., Rauch, U., Thon, N., Merten, T., and Deller, T. (1999) Entorhinal cortex lesion in adult rats induces the expression of the neuronal chondroitin sulfate proteoglycan neurocan in reactive astrocytes. J Neurosci 19, 9953-9963

110. 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

111. Hagino, S., Iseki, K., Mori, T., Zhang, Y., Hikake, T., Yokoya, S., Takeuchi, M., Hasimoto, H., Kikuchi, S., and Wanaka, A. (2003) Slit and glypican-1 mRNAs are coexpressed in the reactive astrocytes of the injured adult brain. Glia 42, 130-138

112. Hall, E. D., and Springer, J. E. (2004) Neuroprotection and Acute Spinal Cord Injury: A Reappraisal. Neurorx 1, 80-100

113. Harper, J. M., Krishnan, C., Darman, J. S., Deshpande, D. M., Peck, S., Shats, I., Backovic, S., Rothstein, J. D., and Kerr, D. A. (2004) Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. Proc Natl Acad Sci U S A 101, 7123-7128

114. Hasan, S. J., Keirstead, H. S., Muir, G. D., and Steeves, J. D. (1993) Axonal regeneration contributes to repair of injured brainstem-spinal neurons in embryonic chick. J Neurosci 13, 492-507

115. Hasegawa, Y., Fujitani, M., Hata, K., Tohyama, M., Yamagishi, S., and Yamashita, T. (2004) Promotion of axon regeneration by myelin-associated glycoprotein and Nogo through divergent signals downstream of Gi/G. J Neurosci 24, 6826-6832

116. Hauben, E., Ibarra, A., Mizrahi, T., Barouch, R., Agranov, E., and Schwartz, M. (2001) Vaccination with a Nogo-A-derived peptide after incomplete spinal-cord injury promotes recovery via a T-cell-mediated neuroprotective response: comparison with other myelin antigens. Proc Natl Acad Sci U S A 98, 15173-15178

117. He, X. L., Bazan, J. F., McDermott, G., Park, J. B., Wang, K., Tessier-Lavigne, M., He, Z., and Garcia, K. C. (2003) Structure of the Nogo receptor ectodomain: a recognition module implicated in myelin inhibition. Neuron 38, 177-185

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

119. Henley, J., and Poo, M. M. (2004) Guiding neuronal growth cones using Ca2+ signals. Trends Cell Biol 14, 320-330

120. Hisaoka, T., Morikawa, Y., Kitamura, T., and Senba, E. (2003) Expression of a member of tumor necrosis factor receptor superfamily, TROY, in the developing mouse brain. Brain Res Dev Brain Res 143, 105-109

121. Horner, P. J., and Gage, F. H. (2000) Regenerating the damaged central nervous system. Nature 407, 963-970

122. Hu, W. H., Hausmann, O. N., Yan, M. S., Walters, W. M., Wong, P. K., and Bethea, J. R.

(2002) Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). J Neurochem 81, 36-45

123. Huang, D. W., McKerracher, L., Braun, P. E., and David, S. (1999) A therapeutic vaccine approach to stimulate axon regeneration in the adult mammalian spinal cord. Neuron 24, 639-647

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

125. Huber, A. B., and Schwab, M. E. (2000) Nogo-A, a potent inhibitor of neurite outgrowth and regeneration. Biol Chem 381, 407-419

126. 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

127. 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

128. Hunt, D., Mason, M. R., Campbell, G., Coffin, R., and Anderson, P. N. (2002) Nogo receptor mRNA expression in intact and regenerating CNS neurons. Mol Cell Neurosci 20, 537-552

129. Jalink, K., van Corven, E. J., Hengeveld, T., Morii, N., Narumiya, S., and Moolenaar, W. H. (1994) Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. J Cell Biol 126, 801-810

130. Jay, D. G., and Keshishian, H. (1990) Laser inactivation of fasciclin I disrupts axon adhesion of grasshopper pioneer neurons. Nature 348, 548-550

131. Jensen, M. B., Gonzalez, B., Castellano, B., and Zimmer, J. (1994) Microglial and astroglial reactions to anterograde axonal degeneration: a histochemical and immunocytochemical study of the adult rat fascia dentata after entorhinal perforant path lesions. Exp Brain Res 98, 245-260

132. Jensen, M. B., Hegelund, I. V., Lomholt, N. D., Finsen, B., and Owens, T. (2000) IFNgamma enhances microglial reactions to hippocampal axonal degeneration. J Neurosci 20, 3612-3621

133. Jensen, M. B., Hegelund, I. V., Poulsen, F. R., Owens, T., Zimmer, J., and Finsen, B. (1999) Microglial reactivity correlates to the density and the myelination of the anterogradely degenerating axons and terminals following perforant path denervation of the mouse fascia dentata. Neuroscience 93, 507-518

134. Jin, Z., and Strittmatter, S. M. (1997) Rac1 mediates collapsin-1-induced growth cone collapse. J Neurosci 17, 6256-6263

135. Johnson, P. W., Abramow-Newerly, W., Seilheimer, B., Sadoul, R., Tropak, M. B., Arquint, M., Dunn, R. J., Schachner, M., and Roder, J. C. (1989) Recombinant myelin-associated glycoprotein confers neural adhesion and neurite outgrowth function. Neuron 3, 377-385

136. 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

137. 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

138. 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

139. Kantor, D. B., Chivatakarn, O., Peer, K. L., Oster, S. F., Inatani, M., Hansen, M. J., Flanagan, J. G., Yamaguchi, Y., Sretavan, D. W., Giger, R. J., and Kolodkin, A. L. (2004) Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. Neuron 44, 961-975

140. Kartje, G. L., Schulz, M. K., Lopez-Yunez, A., Schnell, L., and Schwab, M. E. (1999) Corticostriatal plasticity is restricted by myelin-associated neurite growth inhibitors in the adult rat. Ann Neurol 45, 778-786

141. Kelm, S., Schauer, R., and Crocker, P. R. (1996) The Sialoadhesins--a family of sialic acid-dependent cellular recognition molecules within the immunoglobulin superfamily. Glycoconj J 13, 913-926

142. Kennedy, T. E. (2000) Cellular mechanisms of netrin function: long-range and short-range actions. Biochem Cell Biol 78, 569-575

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

144. Klinger, M., Diekmann, H., Heinz, D., Hirsch, C., Hannbeck von Hanwehr, S., Petrausch, B., Oertle, T., Schwab, M. E., and Stuermer, C. A. (2004) Identification of two NOGO/RTN4 genes and analysis of Nogo-A expression in Xenopus laevis. Mol Cell Neurosci 25, 205-216

145. Klinger, M., Taylor, J. S., Oertle, T., Schwab, M. E., Stuermer, C. A., and Diekmann, H. (2004) Identification of Nogo-66 receptor (NgR) and homologous genes in fish. Mol Biol Evol 21, 76-85

146. Knipper, M., da Penha Berzaghi, M., Blochl, A., Breer, H., Thoenen, H., and Lindholm, D. (1994) Positive feedback between acetylcholine and the neurotrophins nerve growth factor and brain-derived neurotrophic factor in the rat hippocampus. Eur J Neurosci 6, 668-671

147. Kobe, B., and Kajava, A. V. (2001) The leucine-rich repeat as a protein recognition motif. Curr Opin Struct Biol 11, 725-732

148. Kovac, A. D., Kwidzinski, E., Heimrich, B., Bittigau, P., Deller, T., Nitsch, R., and Bechmann, I. (2004) Entorhinal cortex lesion in the mouse induces transsynaptic death of perforant path target neurons. Brain Pathol 14, 249-257

149. Kuja-Panula, J., Kiiltomaki, M., Yamashiro, T., Rouhiainen, A., and Rauvala, H. (2003) AMIGO, a transmembrane protein implicated in axon tract development, defines a novel protein family with leucine-rich repeats. J Cell Biol 160, 963-973

150. Kursula, P., Lehto, V. P., and Heape, A. M. (2000) S100beta inhibits the phosphorylation of the L-MAG cytoplasmic domain by PKA. Brain Res Mol Brain Res 76, 407-410

151. Kursula, P., Lehto, V. P., and Heape, A. M. (2001) The small myelin-associated glycoprotein binds to tubulin and microtubules. Brain Res Mol Brain Res 87, 22-30

152. Lassmann, H., Bartsch, U., Montag, D., and Schachner, M. (1997) Dying-back oligodendrogliopathy: a late sequel of myelin-associated glycoprotein deficiency. Glia 19, 104-110

153. Levine, E. S., Dreyfus, C. F., Black, I. B., and Plummer, M. R. (1995) Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc Natl Acad Sci U S A 92, 8074-8077

154. Levine, J. M., Reynolds, R., and Fawcett, J. W. (2001) The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24, 39-47

155. Li, D., Field, P. M., and Raisman, G. (1995) Failure of axon regeneration in postnatal rat entorhinohippocampal slice coculture is due to maturation of the axon, not that of the pathway or target. Eur J Neurosci 7, 1164-1171

156. Li, G., and Blakemore, W. F. (2004) The number of cells expressing the myelin-supporting oligodendrocyte marker PLP-exon 3b remains unchanged in Wallerian degeneration. J Neurotrauma 21, 1044-1049

157. Li, S., Kim, J. E., Budel, S., Hampton, T. G., and Strittmatter, S. M. (2005) Transgenic inhibition of Nogo-66 receptor function allows axonal sprouting and improved locomotion after spinal injury. Mol Cell Neurosci 29, 26-39

158. Li, S., Liu, B. P., Budel, S., Li, M., Ji, B., Walus, L., Li, W., Jirik, A., Rabacchi, S., Choi, E., Worley, D., Sah, D. W., Pepinsky, B., Lee, D., Relton, J., and Strittmatter, S. M. (2004) Blockade of Nogo-66, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein by soluble Nogo-66 receptor promotes axonal sprouting and recovery after spinal injury. J Neurosci 24, 10511-10520

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

160. Li, W., Walus, L., Rabacchi, S. A., Jirik, A., Chang, E., Schauer, J., Zheng, B. H., Benedetti, N. J., Liu, B. P., Choi, E., Worley, D., Silvian, L., Mo, W., Mullen, C., Yang, W., Strittmatter, S. M., Sah, D. W., Pepinsky, B., and Lee, D. H. (2004) A neutralizing anti-Nogo66 receptor monoclonal antibody reverses inhibition of neurite outgrowth by central nervous system myelin. J Biol Chem 279, 43780-43788

161. Lighthall, J. W. (1988) Controlled cortical impact: a new experimental brain injury model. J Neurotrauma 5, 1-15

162. Lighthall, J. W., Dixon, C. E., and Anderson, T. E. (1989) Experimental models of brain injury. J Neurotrauma 6, 83-97

163. Lin, H., Wada, K., Yonezawa, M., Shinoki, K., Akamatsu, T., Tsukui, T., and Sakamoto, C. (2003) Tomoregulin ectodomain shedding by proinflammatory cytokines. Life Sci 73, 1617-1627

164. Lin, J. C., Ho, W. H., Gurney, A., and Rosenthal, A. (2003) The netrin-G1 ligand NGL-1 promotes the outgrowth of thalamocortical axons. Nat Neurosci 6, 1270-1276

165. 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

166. Luo, Z., Diaz, B., Marshall, M. S., and Avruch, J. (1997) An intact Raf zinc finger is required for optimal binding to processed Ras and for ras-dependent Raf activation in situ. Mol Cell Biol 17, 46-53

167. Lynch, G., Rose, G., and Gall, C. (1977) Anatomical and functional aspects of the septo-hippocampal projections. Ciba Found Symp, 5-24

168. Manitt, C., Thompson, K. M., and Kennedy, T. E. (2004) Developmental shift in expression of netrin receptors in the rat spinal cord: predominance of UNC-5 homologues in adulthood. J Neurosci Res 77, 690-700

169. Marillat, V., Cases, O., Nguyen-Ba-Charvet, K. T., Tessier-Lavigne, M., Sotelo, C., and Chedotal, A. (2002) Spatiotemporal expression patterns of slit and robo genes in the rat brain. J Comp Neurol 442, 130-155

170. Marin, O., Plump, A. S., Flames, N., Sanchez-Camacho, C., Tessier-Lavigne, M., and Rubenstein, J. L. (2003) Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. Development 130, 1889-1901

171. Martini, R., and Schachner, M. (1986) Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. J Cell Biol 103, 2439-2448

172. Matthews, D. A., Cotman, C., and Lynch, G. (1976) An electron microscopic study of

lesion-induced synaptogenesis in the dentate gyrus of the adult rat. II. Reappearance of morphologically normal synaptic contacts. Brain Res 115, 23-41

173. Matthews, D. A., Cotman, C., and Lynch, G. (1976) An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. I. Magnitude and time course of degeneration. Brain Res 115, 1-21

173. McGee AW, Yang Y, Fischer QS, Daw NW, Strittmatter SM. (2005) Experience-driven plasticity of visual cortex limited by myelin and Nogo receptor. Science 309:2222-6.

174. McKerracher, L., David, S., Jackson, D. L., Kottis, V., Dunn, R. J., and Braun, P. E. (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron 13, 805-811

175. McMahon, S. B., Armanini, M. P., Ling, L. H., and Phillips, H. S. (1994) Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. Neuron 12, 1161-1171

176. Meier, S., Brauer, A. U., Heimrich, B., Schwab, M. E., Nitsch, R., and Savaskan, N. E. (2003) Molecular analysis of Nogo expression in the hippocampus during development and following lesion and seizure. Faseb J 17, 1153-1155

177. Merkler, D., Metz, G. A., Raineteau, O., Dietz, V., Schwab, M. E., and Fouad, K. (2001) Locomotor recovery in spinal cord-injured rats treated with an antibody neutralizing the myelin-associated neurite growth inhibitor Nogo-A. J Neurosci 21, 3665-3673

178. Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R. L., McCoy, J. M., and Pepinsky, R. B. (2004) LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 7, 221-228

179. Mikol, D. D., Alexakos, M. J., Bayley, C. A., Lemons, R. S., Le Beau, M. M., and Stefansson, K. (1990) Structure and chromosomal localization of the gene for the oligodendrocyte-myelin glycoprotein. J Cell Biol 111, 2673-2679

180. Mikol, D. D., and Stefansson, K. (1988) A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. J Cell Biol 106, 1273-1279

181. Mingorance, A., Fontana, X., Sole, M., Burgaya, F., Urena, J. M., Teng, F. Y., Tang, B. L., Hunt, D., Anderson, P. N., Bethea, J. R., Schwab, M. E., Soriano, E., and del Rio, J. A. (2004) Regulation of Nogo and Nogo receptor during the development of the entorhino-hippocampal pathway and after adult hippocampal lesions. Mol Cell Neurosci 26, 34-49

182. Mingorance, A., Fontana, X., Soriano, E., and Del Rio, J. A. (2005) Overexpression of myelin-associated glycoprotein after axotomy of the perforant pathway. Mol Cell Neurosci 29, 471-483

183. Mingorance A, S. E., del Río JA (2004) Nogo, myelin and axonal regeneration. Contributions to Science 2, 499-512

184. Monnier, P. P., Sierra, A., Schwab, J. M., Henke-Fahle, S., and Mueller, B. K. (2003) The Rho/ROCK pathway mediates neurite growth-inhibitory activity associated with the chondroitin sulfate proteoglycans of the CNS glial scar. Mol Cell Neurosci 22, 319-330

185. 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

186. Moon, L. D., Asher, R. A., Rhodes, K. E., and Fawcett, J. W. (2001) Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci 4, 465-466

187. Moreau-Fauvarque, C., Kumanogoh, A., Camand, E., Jaillard, C., Barbin, G., Boquet, I., Love, C., Jones, E. Y., Kikutani, H., Lubetzki, C., Dusart, I., and Chedotal, A. (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

188. Morgenstern, D. A., Asher, R. A., and Fawcett, J. W. (2002) Chondroitin sulphate proteoglycans in the CNS injury response. Prog Brain Res 137, 313-332

189. Morin-Richaud, C., Feldblum, S., and Privat, A. (1998) Astrocytes and oligodendrocytes reactions after a total section of the rat spinal cord. Brain Res 783, 85-101

190. 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

191. Nadler, J. V., Cotman, C. W., and Lynch, G. S. (1977) Histochemical evidence of altered development of cholinergic fibers in the rat dentate gyrus following lesions. I. Time course after complete unilateral entorhinal lesion at various ages. J Comp Neurol 171, 561-587

192. Nadler, J. V., Cotman, C. W., Paoletti, C., and Lynch, G. S. (1977) Histochemical evidence of altered development of cholinergic fibers in the rat dentate gyrus following lesions. II. Effects of partial entorhinal and simultaneous multiple lesions. J Comp Neurol 171, 589-604

193. Neumann, S., Bradke, F., Tessier-Lavigne, M., and Basbaum, A. I. (2002) Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. Neuron 34, 885-893

194. Nguyen Ba-Charvet, K. T., Brose, K., Marillat, V., Kidd, T., Goodman, C. S., Tessier-Lavigne, M., Sotelo, C., and Chedotal, A. (1999) Slit2-Mediated chemorepulsion and collapse of developing forebrain axons. Neuron 22, 463-473

195. Nguyen-Ba-Charvet, K. T., and Chedotal, A. (2002) Role of Slit proteins in the vertebrate brain. J Physiol Paris 96, 91-98

196. Nguyen-Ba-Charvet, K. T., Picard-Riera, N., Tessier-Lavigne, M., Baron-Van Evercooren, A., Sotelo, C., and Chedotal, A. (2004) Multiple roles for slits in the control of cell migration in the rostral migratory stream. J Neurosci 24, 1497-1506

197. 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., Ng, Y. K., Faivre-Sarrailh, C., Popko, B., Ling, E. A., Schachner, M., Watanabe, K., Pallen, C. J., Tang, B. L., and Xiao, Z. C. (2003) Nogo-A at CNS paranodes is a ligand of Caspr: possible regulation of K(+) channel localization. Embo J 22, 5666-5678

198. 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

199. Nishiyama, M., Hoshino, A., Tsai, L., Henley, J. R., Goshima, Y., Tessier-Lavigne, M., Poo, M. M., and Hong, K. (2003) Cyclic AMP/GMP-dependent modulation of Ca2+ channels sets the polarity of nerve growth-cone turning. Nature 423, 990-995

200. O'Connor, T. P., and Bentley, D. (1993) Accumulation of actin in subsets of pioneer growth cone filopodia in response to neural and epithelial guidance cues in situ. J Cell Biol 123, 935-948

201. Oertle, T., Klinger, M., Stuermer, C. A., and Schwab, M. E. (2003) A reticular rhapsody: phylogenic evolution and nomenclature of the RTN/Nogo gene family. Faseb J 17, 1238-1247

202. Oertle, T., Merkler, D., and Schwab, M. E. (2003) Do cancer cells die because of Nogo-B? Oncogene 22, 1390-1399

203. Oertle, T., and Schwab, M. E. (2003) Nogo and its paRTNers. Trends Cell Biol 13, 187-194

204. 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., Kaupmann, K., Vallon, R., and Schwab, M. E.

(2003) Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. J Neurosci 23, 5393-5406

205. Ono, T., Sekino-Suzuki, N., Kikkawa, Y., Yonekawa, H., and Kawashima, S. (2003) Alivin 1, a novel neuronal activity-dependent gene, inhibits apoptosis and promotes survival of cerebellar granule neurons. J Neurosci 23, 5887-5896

206. Oudega, M., Rosano, C., Sadi, D., Wood, P. M., Schwab, M. E., and Hagg, T. (2000) Neutralizing antibodies against neurite growth inhibitor NI-35/250 do not promote regeneration of sensory axons in the adult rat spinal cord. Neuroscience 100, 873-883

207. Papadopoulos, C. M., Tsai, S. Y., Alsbiei, T., O'Brien, T. E., Schwab, M. E., and Kartje, G. L. (2002) Functional recovery and neuroanatomical plasticity following middle cerebral artery occlusion and IN-1 antibody treatment in the adult rat. Ann Neurol 51, 433-441

208. 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

209. Pascual, M., Pozas, E., Barallobre, M. J., Tessier-Lavigne, M., and Soriano, E. (2004) Coordinated functions of Netrin-1 and Class 3 secreted Semaphorins in the guidance of reciprocal septohippocampal connections. Mol Cell Neurosci 26, 24-33

210. Pasterkamp, R. J., Giger, R. J., Ruitenberg, M. J., Holtmaat, A. J., De Wit, J., De Winter, F., and Verhaagen, J. (1999) Expression of the gene encoding the chemorepellent semaphorin III is induced in the fibroblast component of neural scar tissue formed following injuries of adult but not neonatal CNS. Mol Cell Neurosci 13, 143-166

211. Pasterkamp, R. J., and Verhaagen, J. (2001) Emerging roles for semaphorins in neural regeneration. Brain Res Brain Res Rev 35, 36-54

212. Pearse, D. D., Pereira, F. C., Marcillo, A. E., Bates, M. L., Berrocal, Y. A., Filbin, M. T., and Bunge, M. B. (2004) cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. Nat Med 10, 610-616

213. Peterson, D. A., Lucidi-Phillipi, C. A., Eagle, K. L., and Gage, F. H. (1994) Perforant path damage results in progressive neuronal death and somal atrophy in layer II of entorhinal cortex and functional impairment with increasing postdamage age. J Neurosci 14, 6872-6885

214. Pignot, V., Hein, A. E., Barske, C., Wiessner, C., Walmsley, A. R., Kaupmann, K., Mayeur, H., Sommer, B., Mir, A. K., and Frentzel, S. (2003) Characterization of two novel proteins, NgRH1 and NgRH2, structurally and biochemically homologous to the Nogo-66 receptor. J Neurochem 85, 717-728

215. 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

216. Plump, A. S., Erskine, L., Sabatier, C., Brose, K., Epstein, C. J., Goodman, C. S., Mason, C. A., and Tessier-Lavigne, M. (2002) Slit1 and Slit2 cooperate to prevent premature midline crossing of retinal axons in the mouse visual system. Neuron 33, 219-232

217. 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

218. Prang, P., Del Turco, D., and Kapfhammer, J. P. (2001) Regeneration of entorhinal fibers in mouse slice cultures is age dependent and can be stimulated by NT-4, GDNF, and modulators of G-proteins and protein kinase C. Exp Neurol 169, 135-147

219. Prins, M. L., Lee, S. M., Cheng, C. L., Becker, D. P., and Hovda, D. A. (1996) Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure. Brain Res Dev Brain Res 95, 272-282

220. Properzi, F., Asher, R. A., and Fawcett, J. W. (2003) Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury. Biochem Soc Trans 31, 335-336

221. Prydz, K., and Dalen, K. T. (2000) Synthesis and sorting of proteoglycans. J Cell Sci 113 Pt 2, 193-205

222. Qiu, J., Cai, D., Dai, H., McAtee, M., Hoffman, P. N., Bregman, B. S., and Filbin, M. T. (2002) Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 34, 895-903

223. Qiu, J., Cai, D., and Filbin, M. T. (2000) Glial inhibition of nerve regeneration in the mature mammalian CNS. Glia 29, 166-174

224. Raineteau, O., Fouad, K., Bareyre, F. M., and Schwab, M. E. (2002) Reorganization of descending motor tracts in the rat spinal cord. Eur J Neurosci 16, 1761-1771

225. Raineteau, O., Fouad, K., Noth, P., Thallmair, M., and Schwab, M. E. (2001) Functional switch between motor tracts in the presence of the mAb IN-1 in the adult rat. Proc Natl Acad Sci U S A 98, 6929-6934

226. Raineteau, O., Z'Graggen, W. J., Thallmair, M., and Schwab, M. E. (1999) Sprouting and regeneration after pyramidotomy and blockade of the myelin-associated neurite growth inhibitors NI 35/250 in adult rats. Eur J Neurosci 11, 1486-1490

227. Rakic, P., Bourgeois, J. P., Eckenhoff, M. F., Zecevic, N., and Goldman-Rakic, P. S. (1986) Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science 232, 232-235

228. Ramón y Cajal, S. (1928) Degeneration and Regeneration of the Nervous System.

229. Ramon-Cueto, A., Plant, G. W., Avila, J., and Bunge, M. B. (1998) Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. J Neurosci 18, 3803-3815

230. Rhodes, K. E., and Fawcett, J. W. (2004) Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? J Anat 204, 33-48

231. Rhodes, K. E., Moon, L. D., and Fawcett, J. W. (2003) Inhibiting cell proliferation during formation of the glial scar: effects on axon regeneration in the CNS. Neuroscience 120, 41-56

232. Rico, B., Beggs, H. E., Schahin-Reed, D., Kimes, N., Schmidt, A., and Reichardt, L. F. (2004) Control of axonal branching and synapse formation by focal adhesion kinase. Nat Neurosci 7, 1059-1069

233. Ridet, J. L., Malhotra, S. K., Privat, A., and Gage, F. H. (1997) Reactive astrocytes: cellular and molecular cues to biological function. Trends Neurosci 20, 570-577

234. Rohrbough, J., Grotewiel, M. S., Davis, R. L., and Broadie, K. (2000) Integrin-mediated regulation of synaptic morphology, transmission, and plasticity. J Neurosci 20, 6868-6878

235. Roonprapunt, C., Huang, W., Grill, R., Friedlander, D., Grumet, M., Chen, S., Schachner, M., and Young, W. (2003) Soluble cell adhesion molecule L1-Fc promotes locomotor recovery in rats after spinal cord injury. J Neurotrauma 20, 871-882

236. Roux, P. P., Colicos, M. A., Barker, P. A., and Kennedy, T. E. (1999) p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. J Neurosci 19, 6887-6896

237. Rush, R. A. (1984) Immunohistochemical localization of endogenous nerve growth factor. Nature 312, 364-367

238. Sanchez, I., Hassinger, L., Paskevich, P. A., Shine, H. D., and Nixon, R. A. (1996) Oligodendroglia regulate the regional expansion of axon caliber and local accumulation of neurofilaments during development independently of myelin formation. J Neurosci 16, 5095-5105

239. Sandvig, A., Berry, M., Barrett, L. B., Butt, A., and Logan, A. (2004) Myelin-, reactive glia-, and scar-derived CNS axon growth inhibitors: expression, receptor signaling, and correlation with axon regeneration. Glia 46, 225-251

240. Savaskan, N. E., Plaschke, M., Ninnemann, O., Spillmann, A. A., Schwab, M. E., Nitsch, R., and Skutella, T. (1999) Myelin does not influence the choice behaviour of entorhinal axons but strongly inhibits their outgrowth length in vitro. Eur J Neurosci 11, 316-326

241. Schachner, M., and Bartsch, U. (2000) Multiple functions of the myelin-associated glycoprotein MAG (siglec-4a) in formation and maintenance of myelin. Glia 29, 154-165

242. Scheiffele, P. (2003) Cell-cell signaling during synapse formation in the CNS. Annu Rev Neurosci 26, 485-508

243. Schnell, L., Schneider, R., Kolbeck, R., Barde, Y. A., and Schwab, M. E. (1994) Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion. Nature 367, 170-173

244. 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

245. Schnell, L., and Schwab, M. E. (1993) Sprouting and regeneration of lesioned corticospinal tract fibres in the adult rat spinal cord. Eur J Neurosci 5, 1156-1171

246. Schwab, M. E. (1991) Regeneration of lesioned CNS axons by neutralisation of neurite growth inhibitors: a short review. Paraplegia 29, 294-298

247. Schwab, M. E. (1998) Regenerative Nerve Fiber Growth in the Adult Central Nervous System. News Physiol Sci 13, 294-298

248. Schwab, M. E. (2002) Repairing the injured spinal cord. Science 295, 1029-1031

249. Schwab, M. E., and Caroni, P. (1988) Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth and fibroblast spreading in vitro. J Neurosci 8, 2381-2393

250. Schweigreiter, R., Walmsley, A. R., Niederost, B., Zimmermann, D. R., Oertle, T., Casademunt, E., Frentzel, S., Dechant, G., Mir, A., and Bandtlow, C. E. (2004) Versican V2 and the central inhibitory domain of Nogo-A inhibit neurite growth via p75NTR/NgR-independent pathways that converge at RhoA. Mol Cell Neurosci 27, 163-174

251. Seki, T., and Rutishauser, U. (1998) Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocampus. J Neurosci 18, 3757-3766

252. Selzer, M. E. (2003) Promotion of axonal regeneration in the injured CNS. Lancet Neurol 2, 157-166

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

254. Shearer, M. C., Niclou, S. P., Brown, D., Asher, R. A., Holtmaat, A. J., Levine, J. M., Verhaagen, J., and Fawcett, J. W. (2003) The astrocyte/meningeal cell interface is a barrier to neurite outgrowth which can be overcome by manipulation of inhibitory molecules or axonal signalling pathways. Mol Cell Neurosci 24, 913-925

255. Shen, Y. J., DeBellard, M. E., Salzer, J. L., Roder, J., and Filbin, M. T. (1998) Myelin-associated glycoprotein in myelin and expressed by Schwann cells inhibits axonal regeneration and branching. Mol Cell Neurosci 12, 79-91

256. Shimizu, I., Oppenheim, R. W., O'Brien, M., and Shneiderman, A. (1990) Anatomical and

functional recovery following spinal cord transection in the chick embryo. J Neurobiol 21, 918-937

257. Sicotte, M., Tsatas, O., Jeong, S. Y., Cai, C. Q., He, Z., and David, S. (2003) Immunization with myelin or recombinant Nogo-66/MAG in alum promotes axon regeneration and sprouting after corticospinal tract lesions in the spinal cord. Mol Cell Neurosci 23, 251-263

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

259. 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

260. Sivasankaran, R., Pei, J., Wang, K. C., Zhang, Y. P., Shields, C. B., Xu, X. M., and He, Z. (2004) PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. Nat Neurosci 7, 261-268

261. Sivron, T., and Schwartz, M. (1994) Nonpermissive nature of fish optic nerves to axonal growth is due to presence of myelin-associated growth inhibitors. Exp Neurol 130, 411-413

262. Snyder, E. Y., Park, K. I., Flax, J. D., Liu, S., Rosario, C. M., Yandava, B. D., and Aurora, S. (1997) Potential of neural "stem-like" cells for gene therapy and repair of the degenerating central nervous system. Adv Neurol 72, 121-132

263. Sole, M., Fontana, X., Gavin, R., Soriano, E., and del Rio, J. A. (2004) Bcl-2 overexpression does not promote axonal regeneration of the entorhino-hippocampal connections in vitro after axotomy. Brain Res 1020, 204-209

264. Song, H., Ming, G., He, Z., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., and Poo, M. (1998) Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. Science 281, 1515-1518

265. Song, H., and Poo, M. (2001) The cell biology of neuronal navigation. Nat Cell Biol 3, E81-88

266. Song, H. J., Ming, G. L., and Poo, M. M. (1997) cAMP-induced switching in turning direction of nerve growth cones. Nature 388, 275-279

267. Song, H. J., and Poo, M. M. (1999) Signal transduction underlying growth cone guidance by diffusible factors. Curr Opin Neurobiol 9, 355-363

268. 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

269. Soriano, E., Del Rio, J. A., Martinez, A., and Super, H. (1994) Organization of the embryonic and early postnatal murine hippocampus. I. Immunocytochemical characterization of neuronal populations in the subplate and marginal zone. J Comp Neurol 342, 571-595

270. Spencer, T., and Filbin, M. T. (2004) A role for cAMP in regeneration of the adult mammalian CNS. J Anat 204, 49-55

271. Spillmann, A. A., Bandtlow, C. E., Lottspeich, F., Keller, F., and Schwab, M. E. (1998) Identification and characterization of a bovine neurite growth inhibitor (bNI-220). J Biol Chem 273, 19283-19293

272. Steiner, P., Kulangara, K., Sarria, J. C., Glauser, L., Regazzi, R., and Hirling, H. (2004) Reticulon 1-C/neuroendocrine-specific protein-C interacts with SNARE proteins. J Neurochem 89, 569-580

273. Steward, O. (1976) Reinnervation of dentate gyrus by homologous afferents following entorhinal cortical lesions in adult rats. Science 194, 426-428

274. Steward, O., Cotman, C., and Lynch, G. (1976) A quantitative autoradiographic and

electrophysiological study of the reinnervation of the dentate gyrus by the contralateral entorhinal cortex following ipsilateral entorhinal lesions. Brain Res 114, 181-200

275. Steward, O., Cotman, C. W., and Lynch, G. S. (1974) Growth of a new fiber projection in the brain of adult rats: Re-innervation of the dentate gyrus by the contralateral entorhinal cortex following ipsilateral entorhinal lesions. Exp Brain Res 20, 45-66

276. Steward, O., Torre, E. R., Phillips, L. L., and Trimmer, P. A. (1990) The process of reinnervation in the dentate gyrus of adult rats: time course of increases in mRNA for glial fibrillary acidic protein. J Neurosci 10, 2373-2384

277. Steward, O., and Vinsant, S. L. (1978) Collateral projections of cells in the surviving entorhinal area which reinnervate the dentate gyrus of the rat following unilateral entorhinal lesions. Brain Res 149, 216-222

278. Stoppini, L., Buchs, P. A., and Muller, D. (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Methods 37, 173-182

279. Stuermer, C. A., Bastmeyer, M., Bahr, M., Strobel, G., and Paschke, K. (1992) Trying to understand axonal regeneration in the CNS of fish. J Neurobiol 23, 537-550

280. Suda, T., and Nagata, S. (1997) Why do defects in the Fas-Fas ligand system cause autoimmunity? J Allergy Clin Immunol 100, S97-101

281. Super, H., Martinez, A., Del Rio, J. A., and Soriano, E. (1998) Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. J Neurosci 18, 4616-4626

282. Tacke, R., and Martini, R. (1990) Changes in expression of mRNA specific for cell adhesion molecules (L1 and NCAM) in the transected peripheral nerve of the adult rat. Neurosci Lett 120, 227-230

283. Tamura, A., Asano, T., and Sano, K. (1980) Correlation between rCBF and histological changes following temporary middle cerebral artery occlusion. Stroke 11, 487-493

284. Tanaka, M., Sato, S., and Miyatake, T. (1984) Identification of myelin-associated glycoprotein (MAG) on the cell surface of mononuclear cells in human peripheral blood by a heterologous antiserum. Jpn J Exp Med 54, 9-15

285. Tang, S., Qiu, J., Nikulina, E., and Filbin, M. T. (2001) Soluble myelin-associated glycoprotein released from damaged white matter inhibits axonal regeneration. Mol Cell Neurosci 18, 259-269

286. Tang, S., Shen, Y. J., DeBellard, M. E., Mukhopadhyay, G., Salzer, J. L., Crocker, P. R., and Filbin, M. T. (1997) Myelin-associated glycoprotein interacts with neurons via a sialic acid binding site at ARG118 and a distinct neurite inhibition site. J Cell Biol 138, 1355-1366

287. Tang, S., Woodhall, R. W., Shen, Y. J., deBellard, M. E., Saffell, J. L., Doherty, P., Walsh, F. S., and Filbin, M. T. (1997) Soluble myelin-associated glycoprotein (MAG) found in vivo inhibits axonal regeneration. Mol Cell Neurosci 9, 333-346

288. Tatagiba, M., Brosamle, C., and Schwab, M. E. (1997) Regeneration of injured axons in the adult mammalian central nervous system. Neurosurgery 40, 541-546; discussion 546-547

289. 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

290. Tessier-Lavigne, M., and Goodman, C. S. (1996) The molecular biology of axon guidance. Science 274, 1123-1133

291. Thon, N., Haas, C. A., Rauch, U., Merten, T., Fassler, R., Frotscher, M., and Deller, T. (2000) The chondroitin sulphate proteoglycan brevican is upregulated by astrocytes after entorhinal cortex lesions in adult rats. Eur J Neurosci 12, 2547-2558

292. Tozaki, H., Kawasaki, T., Takagi, Y., and Hirata, T. (2002) Expression of Nogo protein by growing axons in the developing nervous system. Brain Res Mol Brain Res 104, 111-119
293. Trifunovski, A., Josephson, A., Ringman, A., Brene, S., Spenger, C., and Olson, L. (2004) Neuronal activity-induced regulation of Lingo-1. Neuroreport 15, 2397-2400

294. Tropak, M. B., Johnson, P. W., Dunn, R. J., and Roder, J. C. (1988) Differential splicing of MAG transcripts during CNS and PNS development. Brain Res 464, 143-155

295. Tropak, M. B., and Roder, J. C. (1997) Regulation of myelin-associated glycoprotein binding by sialylated cis-ligands. J Neurochem 68, 1753-1763

296. Venkatesh, K., Chivatakarn, O., Lee, H., Joshi, P. S., Kantor, D. B., Newman, B. A., Mage, R., Rader, C., and Giger, R. J. (2005) The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. J Neurosci 25, 808-822

297. Vinson, M., Strijbos, P. J., Rowles, A., Facci, L., Moore, S. E., Simmons, D. L., and Walsh, F. S. (2001) Myelin-associated glycoprotein interacts with ganglioside GT1b. A mechanism for neurite outgrowth inhibition. J Biol Chem 276, 20280-20285

298. von Meyenburg, J., Brosamle, C., Metz, G. A., and Schwab, M. E. (1998) Regeneration and sprouting of chronically injured corticospinal tract fibers in adult rats promoted by NT-3 and the mAb IN-1, which neutralizes myelin-associated neurite growth inhibitors. Exp Neurol 154, 583-594

299. Vourc'h, P., and Andres, C. (2004) Oligodendrocyte myelin glycoprotein (OMgp): evolution, structure and function. Brain Res Brain Res Rev 45, 115-124

300. Vourc'h, P., Dessay, S., Mbarek, O., Marouillat Vedrine, S., Muh, J. P., and Andres, C. (2003) The oligodendrocyte-myelin glycoprotein gene is highly expressed during the late stages of myelination in the rat central nervous system. Brain Res Dev Brain Res 144, 159-168

301. Vyas, A. A., Patel, H. V., Fromholt, S. E., Heffer-Lauc, M., Vyas, K. A., Dang, J., Schachner, M., and Schnaar, R. L. (2002) Gangliosides are functional nerve cell ligands for myelin-associated glycoprotein (MAG), an inhibitor of nerve regeneration. Proc Natl Acad Sci U S A 99, 8412-8417

302. Vyas, A. A., and Schnaar, R. L. (2001) Brain gangliosides: functional ligands for myelin stability and the control of nerve regeneration. Biochimie 83, 677-682

303. Walsh, F. S., and Doherty, P. (1997) Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. Annu Rev Cell Dev Biol 13, 425-456

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

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

306. Washbourne, P., Dityatev, A., Scheiffele, P., Biederer, T., Weiner, J. A., Christopherson, K. S., and El-Husseini, A. (2004) Cell adhesion molecules in synapse formation. J Neurosci 24, 9244-9249

307. Watari, A., and Yutsudo, M. (2003) Multi-functional gene ASY/Nogo/RTN-X/RTN4: apoptosis, tumor suppression, and inhibition of neuronal regeneration. Apoptosis 8, 5-9

308. Weibel, D., Cadelli, D., and Schwab, M. E. (1994) Regeneration of lesioned rat optic nerve fibers is improved after neutralization of myelin-associated neurite growth inhibitors. Brain Res 642, 259-266

309. Wen, Z., Guirland, C., Ming, G. L., and Zheng, J. Q. (2004) A CaMKII/calcineurin switch controls the direction of Ca(2+)-dependent growth cone guidance. Neuron 43, 835-846
310. Wetmore, C., Olson, L., and Bean, A. J. (1994) Regulation of brain-derived neurotrophic factor (BDNF) expression and release from hippocampal neurons is mediated by non-NMDA type glutamate receptors. J Neurosci 14, 1688-1700

311. Wiessner, C., Bareyre, F. M., Allegrini, P. R., Mir, A. K., Frentzel, S., Zurini, M., Schnell, L., Oertle, T., and Schwab, M. E. (2003) Anti-Nogo-A antibody infusion 24 hours after experimental stroke improved behavioral outcome and corticospinal plasticity in normotensive and spontaneously hypertensive rats. J Cereb Blood Flow Metab 23, 154-165

312. Wilson, M. A., Gaze, R. M., Goodbrand, I. A., and Taylor, J. S. (1992) Regeneration in the Xenopus tadpole optic nerve is preceded by a massive macrophage/microglial response. Anat Embryol (Berl) 186, 75-89

313. Windebank, A. J., Wood, P., Bunge, R. P., and Dyck, P. J. (1985) Myelination determines the caliber of dorsal root ganglion neurons in culture. J Neurosci 5, 1563-1569

314. Wong, E. V., David, S., Jacob, M. H., and Jay, D. G. (2003) Inactivation of myelin-associated glycoprotein enhances optic nerve regeneration. J Neurosci 23, 3112-3117

315. Woodhams, P. L., and Atkinson, D. J. (1996) Regeneration of entorhino-dentate projections in organotypic slice cultures: mode of axonal regrowth and effects of growth factors. Exp Neurol 140, 68-78

316. Woodhams, P. L., Atkinson, D. J., and Raisman, G. (1993) Rapid decline in the ability of entorhinal axons to innervate the dentate gyrus with increasing time in organotypic co-culture. Eur J Neurosci 5, 1596-1609

317. Wrathall, J. R., Li, W., and Hudson, L. D. (1998) Myelin gene expression after experimental contusive spinal cord injury. J Neurosci 18, 8780-8793

318. Xu, X. M., Guenard, V., Kleitman, N., Aebischer, P., and Bunge, M. B. (1995) A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp Neurol 134, 261-272

319. Xu, X. M., Zhang, S. X., Li, H., Aebischer, P., and Bunge, M. B. (1999) Regrowth of axons into the distal spinal cord through a Schwann-cell-seeded mini-channel implanted into hemisected adult rat spinal cord. Eur J Neurosci 11, 1723-1740

320. Yamaguchi, Y., and Pasquale, E. B. (2004) Eph receptors in the adult brain. Curr Opin Neurobiol 14, 288-296

321. 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

322. 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

323. Yang, H. Y., Lieska, N., Shao, D., Kriho, V., and Pappas, G. D. (1994) Proteins of the intermediate filament cytoskeleton as markers for astrocytes and human astrocytomas. Mol Chem Neuropathol 21, 155-176

324. Yick, L. W., So, K. F., Cheung, P. T., and Wu, W. T. (2004) Lithium chloride reinforces the regeneration-promoting effect of chondroitinase ABC on rubrospinal neurons after spinal cord injury. J Neurotrauma 21, 932-943

325. Yim, S. H., and Quarles, R. H. (1992) Biosynthesis and expression of the myelin-associated glycoprotein in cultured oligodendrocytes from adult bovine brain. J Neurosci Res 33, 370-378

326. Yin, X., Crawford, T. O., Griffin, J. W., Tu, P., Lee, V. M., Li, C., Roder, J., and Trapp, B.

D. (1998) Myelin-associated glycoprotein is a myelin signal that modulates the caliber of myelinated axons. J Neurosci 18, 1953-1962

327. Zaczek, R., Nelson, M., and Coyle, J. T. (1981) Kainic acid neurotoxicity and seizures. Neuropharmacology 20, 183-189

328. Zagrebelsky, M., Buffo, A., Skerra, A., Schwab, M. E., Strata, P., and Rossi, F. (1998) Retrograde regulation of growth-associated gene expression in adult rat Purkinje cells by myelin-associated neurite growth inhibitory proteins. J Neurosci 18, 7912-7929

329. Zai, L. J., and Wrathall, J. R. (2005) Cell proliferation and replacement following contusive spinal cord injury. Glia 50, 247-257

330. 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

331. 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

332. Zheng, J. Q. (2000) Turning of nerve growth cones induced by localized increases in intracellular calcium ions. Nature 403, 89-93

333. Zhu, Y., Li, H., Zhou, L., Wu, J. Y., and Rao, Y. (1999) Cellular and molecular guidance of GABAergic neuronal migration from an extracortical origin to the neocortex. Neuron 23, 473-485

334. Zimmer, J., Laurberg, S., and Sunde, N. (1986) Non-cholinergic afferents determine the distribution of the cholinergic septohippocampal projection: a study of the AChE staining pattern in the rat fascia dentata and hippocampus after lesions, X-irradiation, and intracerebral grafting. Exp Brain Res 64, 158-168