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# The Neurotrophin Receptor p75$^{NTR}$ as a Positive Modulator of Myelination

**José M. Cosgaya,* Jonah R. Chan,* Eric M. Shooter†**


**Schwann cells in developing and regenerating peripheral nerves express elevated levels of the neurotrophin receptor p75$^{NTR}$. Neurotrophins are key mediators of peripheral nervous system myelination. Our results show that myelin formation is inhibited in the absence of functional p75$^{NTR}$ and enhanced by blocking TrkC activity. Moreover, the enhancement of myelin formation by endogenous brain-derived neurotrophic factor is mediated by the p75$^{NTR}$ receptor, whereas TrkC receptors are responsible for neurotrophin-3 inhibition. Thus p75$^{NTR}$ and TrkC receptors have opposite effects on myelination.**


The neurotrophin receptor p75$^{NTR}$ (*1*) is now known to have more diverse functions than that of being a helper for the Trk receptors. We show that the brain-derived neurotrophic factor (BDNF) acts through p75$^{NTR}$ to enhance myelin formation. The neurotrophins, a family of growth factors including nerve growth factor (NGF), BDNF, neurotrophin-3 (NT3), and neurotrophin-4/5 (NT4/5), exert their biological actions mostly in neuronal cells by regulating survival, differentiation, and cell death (*2*). All known neurotrophins bind the receptor p75$^{NTR}$, but others of the Trk family of tyrosine kinase receptors are more selective about which neurotrophin they will bind. NGF binds to TrkA, BDNF and NT4/5 bind to TrkB, and NT3 binds to TrkC. Alternative splicing of the *trkB* and *trkC* genes results in full-length receptor isoforms (TrkB-FL and TrkC-TK$^+$) that contain an intact tyrosine kinase domain and the truncated isoforms (TrkB-T1 and -T2 and TrkC-TK$^-$) that lack the kinase domain (*3*, *4*).

The myelin sheath is a specialized membrane component in the nervous system that maximizes the conduction efficiency and velocity of neuronal action potentials. The myelination program involves a number of signals between the neuronal and myelin-forming cells that include, in the peripheral nervous system (PNS), neuregulins (*5*), adenosine triphosphate (*6*), steroid hormones (*7*), Desert hedgehog (*8*), and the neurotrophins BDNF and NT3 (*9*). Removal of BDNF inhibited myelination, whereas removal of NT3 enhanced myelination in vitro and in vivo (*9*).

To identify the neurotrophin receptors responsible, we determined which receptor mRNAs were present during myelination both in sciatic nerve and in Schwann cell/dorsal root ganglia neuron (SC/DRG) cocultures by nonquantitative reverse transcription–polymerase chain reaction (RT-PCR). The mRNAs for p75$^{NTR}$ and TrkC-TK$^+$ were present in both actively myelinating sciatic nerve and cocultures (Fig. 1A). TrkB-T1 mRNA was also detected in sciatic nerve and in cocultures, whereas only a minute amount of TrkB-FL was observed. Myelination in the sciatic nerve, determined by the expression of the major myelin


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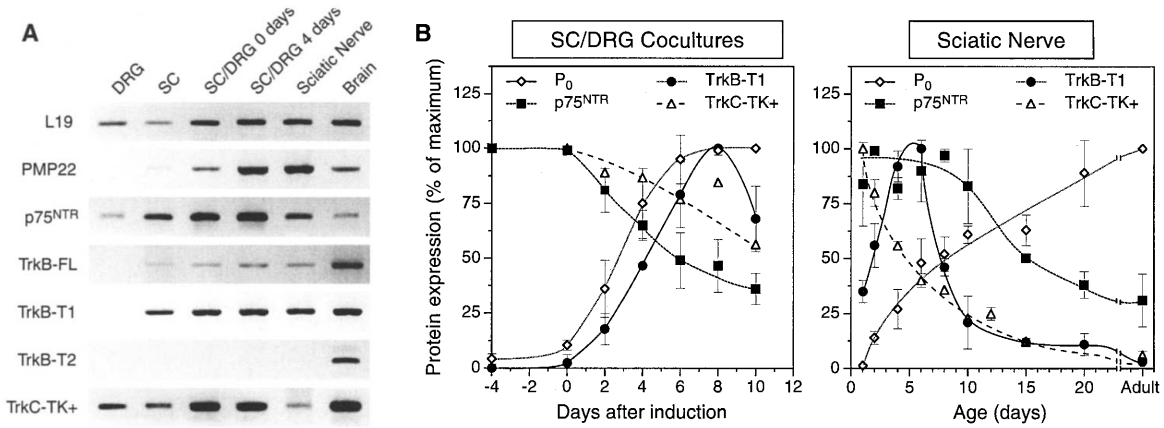


**Fig. 1.** p75$^{NTR}$, TrkB, and TrkC are present during development in sciatic nerve and SC/DRG cocultures. (**A**) Expression of neurotrophin receptors, the myelin protein PMP22, and the ribosomal protein L19 was analyzed by RT-PCR from purified rat DRG, SC, premyelinating SC/DRG cocultures before induction of myelination (SC/DRG 0 days), actively myelinating cocultures after 4 days of induction (SC/DRG 4 days), newborn mouse sciatic nerve, and adult mouse brain. (**B**) Protein levels of the myelin protein $P_0$ and the neurotrophin receptors p75$^{NTR}$, TrkC-TK$^+$, and TrkB-T1 were analyzed by Western blot in SC/DRG cocultures and in rat sciatic nerve at the times indicated. The results are presented as the mean ± SD.

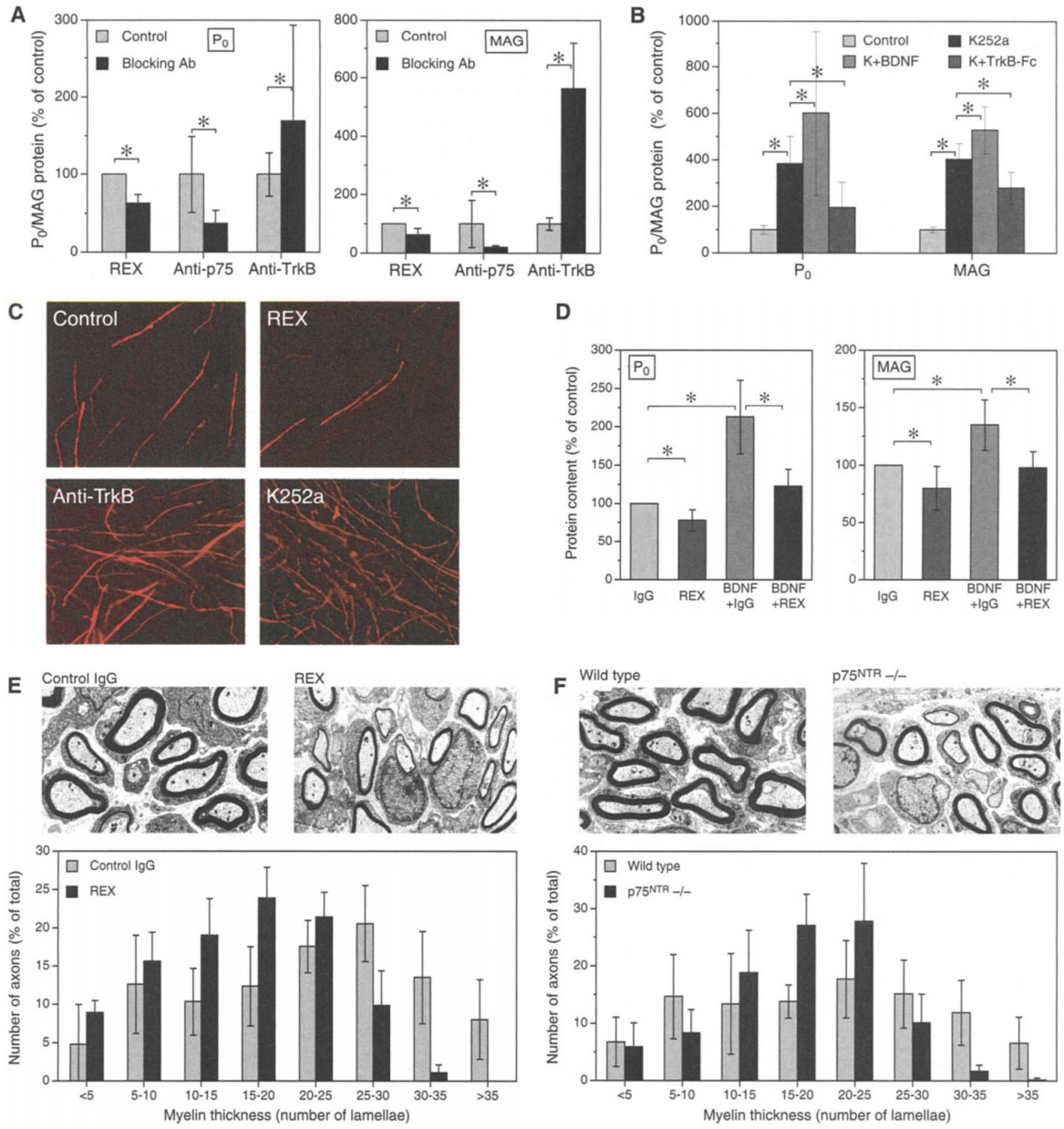


**Fig. 2.** $p75^{NTR}$ and Trk receptors have opposite effects on myelination. Rat SC/DRG cocultures were treated for 6 days with either **(A)** blocking antibodies (Ab) to $p75^{NTR}$ (REX and anti-p75) or to TrkB (anti-TrkB), or **(B)** the Trk tyrosine kinase inhibitor K252a in combination with BDNF or TrkB-Fc at the time of induction. $P_0$ and MAG content was determined by Western blot analysis. Asterisks: (A) $P < 0.01$, (B) $P < 0.05$. **(C)** Mature myelin internodes were visualized by immunocytochemistry with an antibody to $P_0$. Cocultures were maintained in the presence or absence of REX, anti-TrkB, or K252a. **(D)** Blocking antibodies to $p75^{NTR}$ inhibited myelin expression in vivo. Newborn mice were injected with REX ($n = 7$), BDNF and immunoglobulin G (IgG) ($n = 5$), or BDNF and REX ($n = 6$). Four days later the sciatic nerves were isolated and myelin protein expression was analyzed by Western blot. Asterisks: $P < 0.05$. **(E)** Injection of REX decreases the thickness of myelin sheaths from sciatic nerve axons. Newborn mice were injected with REX and the sciatic nerves were extracted and processed for electron microscopy. Representative electron micrographs as well as the myelin thickness distribution from IgG- and REX-treated nerves are shown. The difference in the distribution of the number of lamellae between IgG- and REX-treated samples (21.4 ± 10.0 and 16.4 ± 7.3 lamellae, respectively) is statistically significant ($P < 0.0001$). **(F)** Thick myelin sheaths are absent in sciatic nerve axons from $p75^{NTR-/-}$ mice. Sciatic nerves from 5-day-old wild-type and $p75^{NTR-/-}$ littermate mice were extracted and myelin thickness was analyzed by electron microscopy as in (E). The distributions of the number of lamellae from wild-type and $p75^{NTR-/-}$ mice (20.4 ± 10.4 and 17.9 ± 6.8 lamellae, respectively) are statistically different ($P < 0.0001$).

protein $P_0$, begins immediately after birth and continues over ~20 days. Myelination in cocultures occurs over 6 to 8 days after induction (Fig. 1B). The expression profile of the neurotrophin receptors was similar during myelination in the sciatic nerve and in coculture (Fig. 1). On the protein level, $p75^{NTR}$ and TrkC-$TK^+$ were present at high levels during myelination in both systems, decreasing only at later time points (Fig. 1B). TrkB-T1 protein levels correlated with active myelination, being induced at the initiation of myelination both in vitro and in vivo, reaching a peak at the time of maximum myelin accumulation and diminishing afterwards. TrkB-T1 expression may be an indicator of PNS myelination, while TrkB-FL protein levels in sciatic nerve during myelination were at least 100 times lower than that of TrkB-T1 (*10*, *11*). Therefore, $p75^{NTR}$, TrkB-T1, and full-length TrkC receptors are likely to be the major mediators of neurotrophin actions during PNS myelination.

We analyzed functions of $p75^{NTR}$ and TrkB-T1 by adding specific blocking antibodies to SC/DRG cocultures (*12*). Two different $p75^{NTR}$ blocking antibodies [REX (*13*) and anti-p75] inhibited the accumulation of two major myelin proteins, myelin-associated glycoprotein (MAG) and $P_0$ (Fig. 2A), and also inhibited the formation of mature myelin internodes as shown by $P_0$ immunocytochemistry (Fig. 2C). In contrast, an antibody that blocks BDNF binding to TrkB (*14*) had the opposite effect, increasing myelin protein accumulation (Fig. 2A) and the number of mature myelin internodes (Fig. 2C). The blockade of all Trk-mediated tyrosine kinase activity by addition of the inhibitor K252a also produced an increase in myelin protein accumulation (Fig. 2B) and mature myelin internode formation (Fig. 2C). This result is reminiscent of the effect obtained with the NT3 scavenger TrkC-Fc (*9*) and, most likely, could be attributed to the inhibition of TrkC activity. In the presence of Schwann cells,

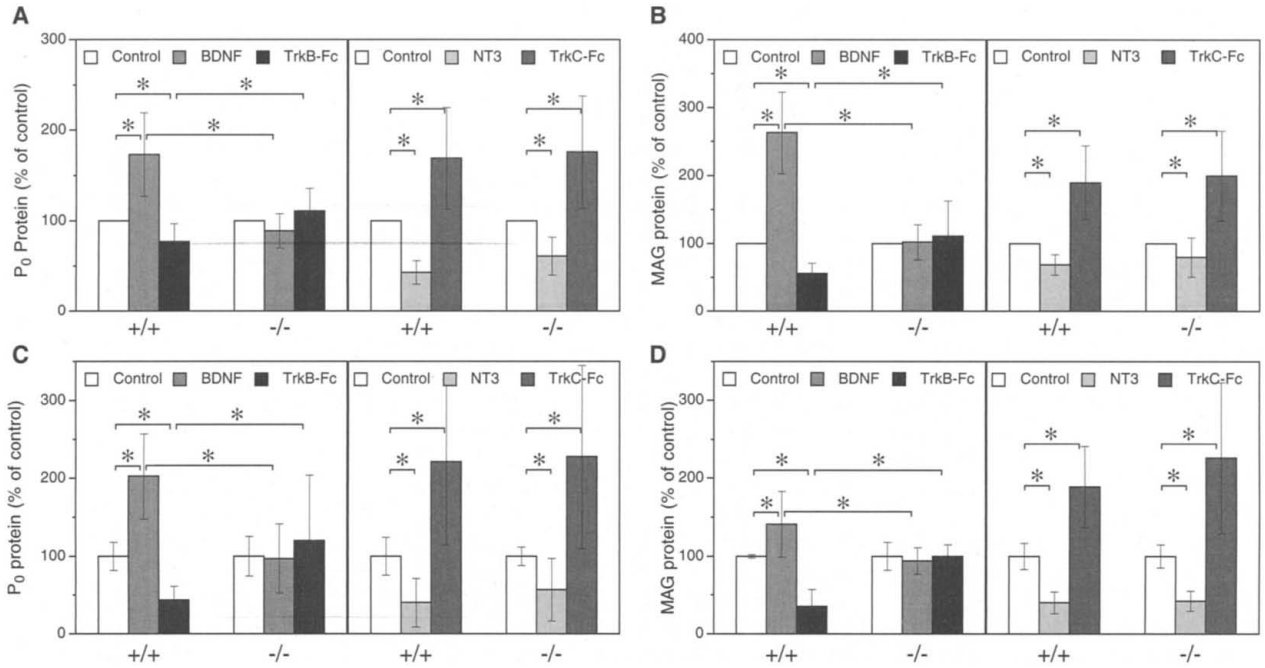


**Fig. 3.** The modulation of endogenous BDNF levels does not affect the myelination process in the $p75^{NTR-/-}$ mice. (**A** and **B**) Newborn wild-type (+/+) and $p75^{NTR-/-}$ mice were injected with BDNF (wild-type, $n = 10$; $p75^{NTR-/-}$, $n = 25$) and NT3 (wild-type, $n = 13$; $p75^{NTR-/-}$, $n = 14$) or the scavengers TrkB-Fc (wild-type, $n = 13$; $p75^{NTR-/-}$, $n = 20$) and TrkC-Fc (wild-type, $n = 14$; $p75^{NTR-/-}$, $n = 20$). The levels of (A) $P_0$ and (B) MAG were analyzed by Western blot 4 days later. Asterisks: $P < 0.01$. (**C** and **D**) Wild-type and $p75^{NTR-/-}$ SC/DRG cocultures were established from embryonic day 13 mouse embryos obtained through crossing heterozygous ($p75^{NTR+/-}$) mice. Levels of (C) $P_0$ and (D) MAG were determined by Western blot 6 days after initiation of myelination in the presence of the different factors. Asterisks: $P < 0.01$.

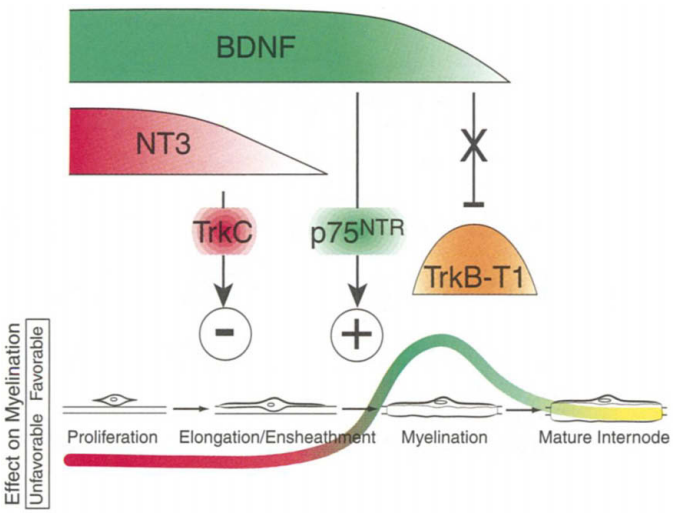


**Fig. 4.** Actions of endogenous neurotrophins and their receptors throughout myelination. During glial proliferation, elongation, and ensheathment, NT3 levels decrease whereas TrkC and $p75^{NTR}$ remain constant. The activation of TrkC by NT3 during these phases prevents the myelination program from proceeding. When myelination is initiated, NT3 protein levels have already become undetectable, thereby removing its inhibitory action. At the same time, BDNF acts as a positive modulator of myelination through the activation of $p75^{NTR}$. Once active myelination is under way, extracellular BDNF is removed through its binding to the increased levels of TrkB-T1. After myelination is complete, all the neurotrophins and their receptors are downregulated.

DRG neurons in culture become NGF independent, and the addition of K252a at the start of myelination does not affect neuronal or glial survival as determined by terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) analysis. In the presence of K252a, BDNF and TrkB-Fc are still able to modulate myelination (Fig. 2B), indicating that BDNF effects are not mediated by the tyrosine kinase activity of the TrkB-FL receptor. These results suggest that $p75^{NTR}$ is the functional receptor that mediates the enhancement of myelination by endogenous BDNF, whereas TrkB-T1 acts in an inhibitory fashion, most likely by decreasing the availability of endogenous BDNF by competing with $p75^{NTR}$ for its binding.

The function of $p75^{NTR}$ was also analyzed during the development of the sciatic nerve in vivo. Newborn mice were injected subcutaneously with REX and/or BDNF along the sciatic nerve (*12*). BDNF enhanced $P_0$ and MAG expression, whereas REX had an inhibitory effect (Fig. 2D). REX also blocked the enhancement achieved with BDNF. Electron microscopy analysis showed a decrease in myelin thickness in the sciatic nerves treated with REX when compared with the contralateral control nerves (Fig. 2E). Sciatic nerves from $p75^{NTR-/-}$ mice (*15*) also displayed less-than-normal myelin thickness (Fig. 2F). Although more than 20% of the axons in control nerves had myelin sheaths with more than 30 wraps of myelin, very few axons presented such a degree of myelination if $p75^{NTR}$ function was blocked either by REX treatment or by genetic deletion ($p75^{NTR-/-}$ mice). Thus functional $p75^{NTR}$ is necessary for proper myelination of the sciatic nerve during development. Sciatic nerves from adult $p75^{NTR-/-}$ mice showed a large reduction in the number of myelinated axons (more than 50%), suggesting that the developmental decrease in myelination persists into adulthood. However, the selective decrease in specific neuronal populations in $p75^{NTR-/-}$ mice complicates this analysis, and a more thorough examination is still required.

The involvement of $p75^{NTR}$ in the control of myelin formation by BDNF was further demonstrated in studies with $p75^{NTR-/-}$ mutants, both in vivo and in vitro. Injection of BDNF along the sciatic nerves of wild-type mice enhanced $P_0$ and MAG protein expression (Fig. 3, A and B). Likewise, removal of endogenous BDNF by injecting the neurotrophin scavenger TrkB-Fc resulted in the reduction of myelin

protein expression. In contrast, neither BDNF nor TrkB-Fc was able to modulate myelin protein expression when injected into the $p75^{NTR-/-}$ mice, in agreement with the premise that $p75^{NTR}$ is the functional receptor for BDNF. The lack of BDNF activity was in sharp contrast with that of NT3. In both wild-type and $p75^{NTR-/-}$ mice, injection with NT3 inhibited myelination and injection with TrkC-Fc enhanced myelination to the same degree. Similar conclusions were obtained with mouse SC/DRG cocultures (*12*). Myelin protein expression was enhanced by BDNF and decreased by TrkB-Fc in myelinating cocultures from wild-type embryos (Fig. 3, C and D), whereas neither BDNF nor TrkB-Fc had any effect in cocultures from $p75^{NTR-/-}$ embryos. Furthermore, NT3 inhibited and TrkC-Fc enhanced myelination in both wild-type and $p75^{NTR-/-}$ cocultures with the same efficiency, again indicating that $p75^{NTR}$ is the functional receptor for BDNF but not for NT3.

Our results demonstrate that neurotrophins are key mediators of PNS myelination and that different receptors are implicated in the positive and negative modulation by BDNF and NT3, respectively. A model illustrating their roles during myelination is depicted in Fig. 4. The binding of neurotrophins to $p75^{NTR}$ and Trk receptors activates divergent intracellular pathways, with Trk receptors preferentially activating prosurvival and mitogenic pathways (*2*). NT3 has been described as a prosurvival factor for SCs (*16*) and could, therefore, be acting like other ligands of tyrosine kinase receptors, such as neuregulins or fibroblast growth factor 2, by keeping the SCs in a proliferative, premyelinogenic state (*5*). On the other hand, less is known about the roles of $p75^{NTR}$. Most of the studies have focused on its pro- and anti-apoptotic functions in neurons and the intracellular signaling pathways that are activated after NGF binding (*2*, *17*). Although our results show that mature forms of neurotrophins modulate myelination, it may be possible that secreted proneurotrophins, which act as $p75^{NTR}$-specific ligands (*18*), could also regulate myelination through $p75^{NTR}$. The complete ablation of all $p75^{NTR}$ isoforms (*19*), including a splice variant that is unable to bind neurotrophins, produces a larger decrease in the number of neurons and SCs present in the sciatic nerve compared with the traditional $p75^{NTR-/-}$ mice. This suggests an additional neurotrophin-independent role for this receptor. It remains unknown whether this potential role is accompanied by a greater decrease in myelin. The DRG neurons used in this study were maintained in NGF and the sensory fibers that grew and survived were NGF dependent, which can constitute yet another layer of complexity in the interplay of neurotrophins and their receptors. Whether NGF and TrkA signaling contributes to myelination remains to be determined.

Our results offer an example of how neurotrophins promote different effects according to whether $p75^{NTR}$ or Trk is activated. Other instances in which such behavior has been documented include cell death or survival decisions in different neuronal types (*2*) and the differential regulation of neurotransmitter release by sympathetic neurons that produces a switch between excitatory and inhibitory neurotransmission (*20*).

An interesting characteristic of $p75^{NTR}$ is its high level of expression in SCs during development and in demyelination and remyelination paradigms (*21*). After nerve injury, the increase in $p75^{NTR}$ expression is accompanied by an upregulation of BDNF (*22*) and a decrease in NT3 expression (*10*). Aside from any effects on neuronal survival and axonal regrowth, these responses might also indicate a function in the myelination program. Our results indicating that $p75^{NTR}$ regulates the myelination process in the PNS allow for the possibility of using specific $p75^{NTR}$ agonists as therapeutic agents in instances in which increased myelination is required, such as peripheral neuropathies or nerve injury. Such compounds could mimic the promyelinating effects of BDNF without adverse collateral consequences in its neuronal counterparts.

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**Supporting Online Material**

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Materials and Methods

References and Notes


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# Reactivation of Ocular Dominance Plasticity in the Adult Visual Cortex


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**In young animals, monocular deprivation leads to an ocular dominance shift, whereas in adults after the critical period there is no such shift. Chondroitin sulphate proteoglycans (CSPGs) are components of the extracellular matrix (ECM) inhibitory for axonal sprouting. We tested whether the developmental maturation of the ECM is inhibitory for experience-dependent plasticity in the visual cortex. The organization of CSPGs into perineuronal nets coincided with the end of the critical period and was delayed by dark rearing. After CSPG degradation with chondroitinase-ABC in adult rats, monocular deprivation caused an ocular dominance shift toward the nondeprived eye. The mature ECM is thus inhibitory for experience-dependent plasticity, and degradation of CSPGs reactivates cortical plasticity.**


Cortical circuits are sensitive to experience during well-defined intervals of early postnatal development called critical periods (*1*). After the critical period, plasticity is reduced or absent. Monocular deprivation (MD) is a classic model of experience-dependent plasticity. MD during the critical period results in a shift of ocular dominance (OD) of cortical neurons in favor of the nondeprived eye (*2*, *3*). No OD shift is seen after MD in adult