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Characterization of glial trkB receptors: Differential response to injury in the central and peripheral nervous systems

(Schwann cell/astrocyte/oligodendrocyte/spinal cord/regeneration)

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ABSTRACT **_In situ_ hybridization on sections from the adult rat peripheral and central nervous systems demonstrated that trkB mRNA was expressed not only by neurons but also by cells in central nervous system white matter as well as by Schwann cells in the sciatic nerve. _In situ_ hybridization with an oligonucleotide complementary to the trkB tyrosine kinase domain could only demonstrate mRNA in neurons, indicating expression of truncated trkB receptors lacking the tyrosine kinase domain by glial cells. RNA blot analysis was performed on separately cultured central nervous system glial cells to study which cell types express trkB mRNA. Several transcripts encoding truncated trkB receptors were expressed at high levels in O-2A progenitors, astrocytes, and oligodendrocytes, but no trkB mRNA could be detected in microglia. The expression of trkB mRNA by glial cells _in vivo_ was also investigated after injury; strongly elevated levels of mRNA encoding truncated receptors were detected in the glial scar formed after an incision in the spinal cord dorsal funiculus. In contrast, in the cut sciatic nerve, trkB mRNA decreased distal to the transection, and by 3 weeks only very low levels of mRNA could be detected. Immunoelectron microscopy located trkB-like immunoreactivity to axons and Schwann cells in the sciatic nerve. The expression of truncated trkB receptors by astrocytes, oligodendrocytes, and Schwann cells and the altered levels in response to injury indicate that glial trkB receptors may serve an important function in the intact and injured nervous system.**

The first isolated and best characterized neurotrophic factor, nerve growth factor (NGF; ref. 1), is a member of a group of neurotrophic factors called the neurotrophin (NT) family. In addition to NGF this group of related molecules consists of brain-derived neurotrophic factor (BDNF; refs. 2 and 3), NT-3 (4–9), and NT-4 (10–12). The biological effects of the NTs are mediated by binding with high affinity to different, but closely related, cell surface glycoprotein receptors encoded by members of the trk family of protooncogenes. The first identified gene, *trk*, codes for gp140^{trk}, which is a receptor for NGF (13, 14). The *trk*B gene encodes at least two glycoproteins, gp95^{trkB} and gp145^{trkB}, which bind BDNF (15, 16) and NT-4 (11, 12, 17). gp95^{trkB} is a truncated receptor lacking the catalytic tyrosine kinase domain (18, 19). The *trk*C locus seems to encode full-length and kinaseless receptors for NT-3 (20, 21). In addition to these high-affinity receptors, all NTs can also bind with low affinity to a cell surface protein called p75 or the low-affinity NT receptor (LANR) (4, 10, 15, 22, 23). The role of this protein is not fully understood but some data indicate that it is required for signal transduction of NGF (24–26). However, NGF mutants that do not bind to p75 can support neuronal survival and induce neuronal differentiation in neuronal cells that express gp140^{trk} (27). Gene targeting experiments have demonstrated that mice carrying a null mutation in the p75 locus have deficiencies in the sensory nervous system, whereas other neurons seem to be unaffected, indicating an important function for p75 in a subclass of NT-responsive neurons (28).

We have recently reported that astrocytes as well as invading reactive leptomeningeal cells express trkB mRNA and trkB-like immunoreactivity (LI) at high levels after spinal cord injury (29). In this study we have characterized the glial expression of trkB receptors *in vitro* and *in vivo* in the intact animal as well as after injury in the central nervous system (CNS) and in the peripheral nervous system.

MATERIALS AND METHODS

Animals and Surgery. Adult male Wistar rats (n = 20; Alab, Stockholm) were anesthetized with a combination of fluanisone (5 mg/kg), fentanyl (0.1 mg/kg), and midazolam (1.25 mg/kg). In one group of animals the left sciatic nerve was transected and ligated at midthigh level. In another group of animals the midthoracic spinal cord was exposed by a laminectomy, and the dorsal funiculus was cut transversally. To extend the lesion rostrally, a longitudinal 3- to 5-mm-long superficial incision was made in the dorsal funiculus from the transverse transection as described (29).

Cell Cultures. *Glial cultures.* Brains from newborn Wistar rats were enzymatically digested with papain (12 units/ml; Sigma) for 30 min at 37°C. After trituration the cells were collected by centrifugation, resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 100 units of penicillin per ml, and 100 μg of streptomycin per ml, and plated in 75-cm^2 tissue culture flasks (Costar). When the glial cells had reached confluency they were separated in subtypes by their difference in adhesiveness (30). When astrocyte and microglial cultures were confluent the medium was changed to DMEM with 0.5% FCS to minimize the effect of growth factors and cytokines in serum on mRNA expression. Five nanograms of recombinant BDNF per ml (a kind gift from Y.-A. Barde) was added to some astrocyte cultures. Cultures of murine oligodendrocytes and O-2A cells were prepared as described (31, 32).

Hippocampal neuron culture. Hippocampi were outdissected from 17-day-old Wistar rat embryos, and the cells were dissociated as above and plated on poly(DL-ornithine)-coated culture dishes as described (33). All cultures were

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Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; LANR, low-affinity NT receptor; FCS, fetal calf serum; LI, like immunoreactivity; CNS, central nervous system.

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maintained at 37°C in a cell culture incubator with 95% air/5% CO_2.

***In Situ* Hybridization and RNA Blot Hybridization.** Two different synthetic oligonucleotides were used for the trkB *in situ* hybridization, one complementary to nucleotides 1314–1361 of trkB mRNA, detecting truncated and full-length trkB mRNAs. The other probe was complementary to nucleotides 1360–1407 of trkB mRNA and thus only detected mRNA for full-length receptors (34). For detection of LANR mRNA a 50-mer oligonucleotide complementary to the rat LANR was used (35). Cryostat sections were hybridized overnight at 42°C with oligonucleotides labeled at their 3′ end with dATP [α-^{35}S] using terminal deoxynucleotidyltransferase (IBI) to a specific activity of $\approx 1 \times 10^9$ cpm/μg and rinsed; mRNA was detected by radioautography as described (36). For RNA blot hybridization, total RNA was extracted from cell cultures using the method of Chomczynski and Sacchi (37), separated on 1.3% agarose gels, and transferred to nylon filters (Hybond-N, Amersham) by capillary blotting. The filters were hybridized with complementary RNA to rat trkB (38) labeled by *in vitro* transcription in the presence of [α-^{32}P]UTP of an *Eco*RI-linearized plasmid containing the subcloned trkB cDNA. The trkB clone corresponds to the extracellular domain and thus detects all forms of trkB.

Immunoelectron Microscopy. Anesthetized rats were perfused with Tyrode's solution followed by 4% formaldehyde in phosphate buffer. The sciatic nerves were post-fixed for 2 hr and rinsed overnight. ImmunoGold electron microscopy was performed as described (39). Briefly, the nerves were cut on a cryostat and incubated overnight with rabbit antiserum against trkB (Santa Cruz Biotech, Santa Cruz, CA; diluted 1:20), rinsed, incubated with 1-nm gold particle-conjugated goat anti-rabbit antiserum (Auroprobe One, Janssen Biotech; diluted 1:50) for 2 hr at 20°C, and osmicated. Gold labeling was intensified with a silver enhancement reaction (Intense M, Janssen Biotech), and the sections were then dehydrated, embedded, thin-sectioned, contrasted, and examined in a Philips CM12 electron microscope.

RESULTS

CNS Glial Cells and Schwann Cells Express trkB mRNA *in Vivo*. trkB mRNA expression was widespread in the spinal cord, with strongly labeled cells in the grey as well as in the white matter. Cells in all spinal cord laminae expressed trkB mRNA, and glial cells containing high levels of trkB mRNA were evenly distributed throughout the spinal cord white matter (Fig. 1*a*). Hybridization with the oligonucleotide complementary to the trkB tyrosine kinase domain revealed expression of mRNA encoding full-length receptors by cells in grey matter, most likely neurons, but not by glial cells in white matter (Fig. 1 *b–d*). In the sciatic nerve many Schwann cells expressed trkB mRNA, although no mRNA could be detected with the oligonucleotide complementary to the tyrosine kinase domain (Fig. 1*e*). trkB mRNA could not be detected in spinal roots (Fig. 1*a*, ref. 40), indicating regional differences in Schwann cell expression.

Astrocytes and Oligodendrocytes, but Not Microglia, Express trkB mRNA *in Vitro*. The high expression of trkB receptors by CNS glial cells demonstrated with *in situ* hybridization prompted us to characterize which glial cell types express trkB mRNA and to investigate which trkB transcripts they express. We therefore made RNA blots from separately cultured O-2A progenitors, astrocytes, microglia, and oligodendrocytes. Several trkB transcripts expressed at high levels could be detected in cultured astrocytes. However, transcripts encoding full-length tyrosine-kinase receptors (4.8 and 9.0 kb; ref. 19) were only detected in neuronal cultures and not in astrocyte cultures (Fig. 2*a*). O-2A cells, the progenitors for oligodendrocytes and type 2 astrocytes, as well as mature oligodendrocytes also expressed trkB mRNA encoding truncated receptors lacking the catalytic domain at high levels (Fig. 2*b*). trkB mRNA could not be detected in microglial cultures (Fig. 2*c*). The presence or absence of serum in the astrocyte medium did not alter the trkB mRNA levels, and no change in mRNA levels could be seen after adding BDNF (5 ng/ml) to the astrocyte cultures (Fig. 2*c*).

trkB mRNA Expression by CNS Glial Cells and Schwann Cells Is Altered by Injury. Strongly increased levels of trkB mRNA could be detected in the injured spinal cord dorsal funiculus white matter after hybridization with the oligonucleotide complementary to the trkB extracellular domain (Fig. 3*a*; 3 weeks postinjury). No trkB mRNA could be detected in the glial scar in adjacent sections hybridized with the oligonucleotide complementary to the tyrosine kinase domain (Fig. 3*b*), indicating that the increase in trkB protein after this type of injury is restricted to truncated receptors (29). No alteration in trkB hybridization was seen in spinal cord segments above or below the lesion. In the cut and ligated sciatic nerve no substantial alteration in the level of trkB hybridization could be detected proximal to the ligature. In contrast, the level of trkB mRNA distal to the ligation was slightly decreased 1 week after the lesion, and 3 weeks after the injury only very low levels of trkB mRNA could be detected (Fig. 4 *a* and *c*). Six months postinjury the trkB mRNA levels were similar to those in the intact sciatic nerve proximal and distal to the ligation. The expression of trkB

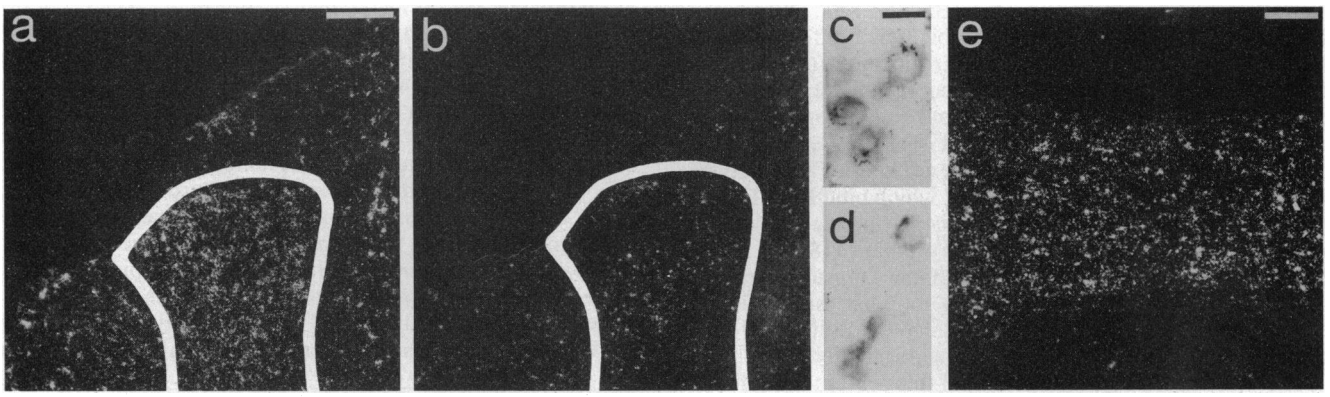

FIG. 1. Dark-field (*a*, *b*, and *e*) and bright-field (*c* and *d*) photomicrographs showing detection of truncated and full-length (*a* and *e*) or only full-length (*b–d*) trkB mRNAs. (*a*) In the adult rat spinal cord, trkB mRNA is expressed in grey matter (dorsal horn, outlined with white line) as well as in white matter. No trkB mRNA can be seen in dorsal roots. The glial trkB mRNA in the white matter encodes truncated receptors, since mRNA encoding full-length receptors only is seen in the grey matter (*b*, adjacent section to *a*). Silver grains were seen over cells in the grey matter (*c*, superficial dorsal horn) but not over cells in the white matter (*d*, dorsal funiculus). (*e*) Strong expression of trkB mRNA by Schwann cells in the sciatic nerve is seen. (Bars = 200 μm in *a*, *b*, and *e* and 10 μm in *c* and *d*.)

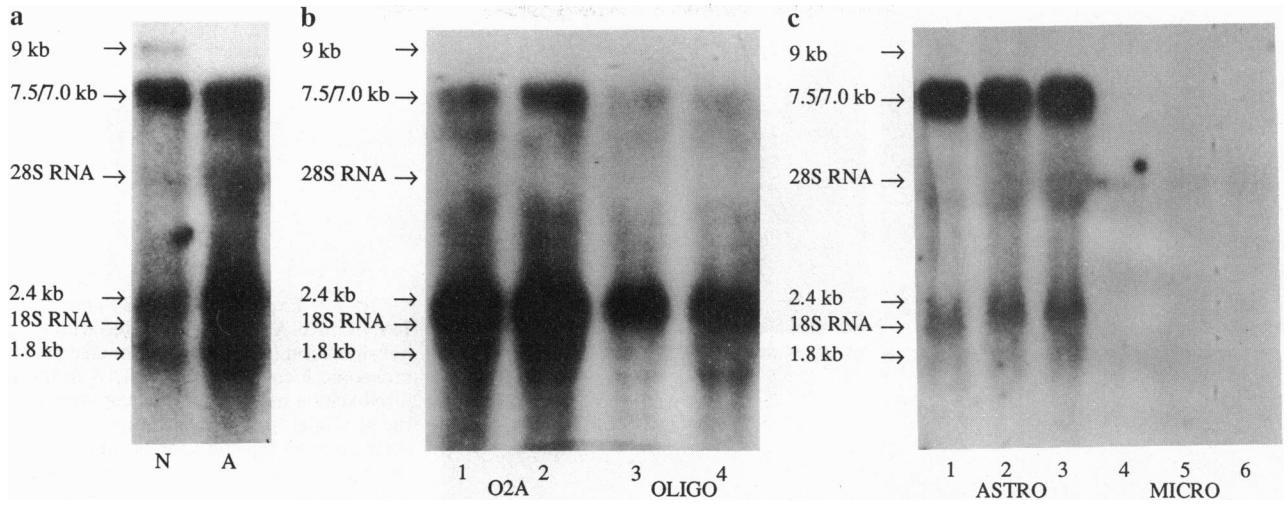

FIG. 2. RNA blot analysis of cell cultures. (*a*) Several trkB transcripts were detected in hippocampal neurons (N) and in hippocampal astrocytes (A). Note that the 9-kb transcript, encoding a full-length tyrosine kinase receptor, is expressed only by neurons and not by astrocytes. (*b*) O-2A progenitors (O2A, lanes 1 and 2) as well as mature oligodendrocytes (OLIGO, lanes 3 and 4) also contained several trkB transcripts encoding truncated receptors. (*c*) In contrast, no trkB mRNA could be detected in microglia (MICRO, lanes 4–6). The level of trkB mRNA in astrocytes (ASTRO, lanes 1–3) was not dependent on the concentration of serum in the medium (lane 1, 0.5% FCS; lane 2, 10% FCS) and was not affected by the presence of 5 ng of BDNF per ml (lane 3).

mRNA in the injured sciatic nerve was different from the expression of mRNA for the low-affinity NT receptor—i.e., LANR mRNA could not be detected or only detected at low levels in the intact sciatic nerve or proximal to the transection in the cut sciatic nerve but was abundantly expressed distal to the transection in the cut sciatic nerve 1 and 3 weeks postinjury (Fig. 4 *b* and *d*).

All specific hybridization was abolished in sections in which an excess of unlabeled probe (400 times) was added. No change in hybridization pattern was seen when an unrelated probe was used for competition (growth hormone-releasing factor).

trkB-LI in the Sciatic Nerve. Widespread trkB-LI was seen in the sciatic nerve with labeling of Schwann cells and axons. Schwann cells associated with myelinated and unmyelinated axons were labeled. However, some Schwann cells lacked immunoreactivity, indicating that a subpopulation of Schwann cells expresses trkB receptors. At the ultrastructural level the immunoreactivity was localized to Schwann cell cytoplasm and surfaces and to axonal membranes (Fig. 4*e*).

DISCUSSION

In this study we report that adult rat CNS glial cells and Schwann cells express mRNA encoding truncated trkB receptors *in vivo*. RNA blots of cultured CNS glial cells demonstrated that astrocytes and oligodendrocytes, but not microglia, expressed several trkB transcripts. However, transcripts encoding full-length tyrosine kinase receptors (4.8 and 9.0 kb; ref. 19) were only detected in neurons and not in astrocytes or oligodendrocytes, although it cannot be excluded that glial cells express levels of these transcripts below the detection limit. After transection of the sciatic nerve, trkB mRNA was strongly decreased distal to the lesion, in contrast to after spinal cord injury, when the expression of trkB mRNA for truncated receptors increased markedly at the injury site.

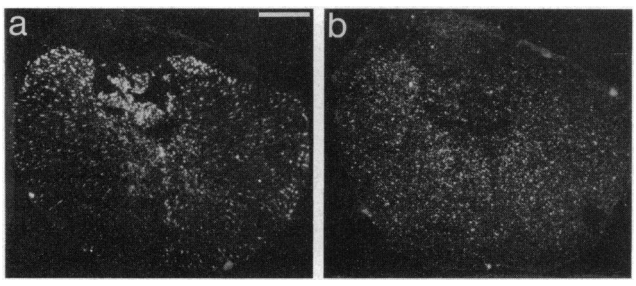

FIG. 3. Detection of trkB mRNA in the rat spinal cord with *in situ* hybridization 3 weeks after a dorsal funiculus transection. Strongly elevated mRNA levels are detected in the glial scar after hybridization with an oligonucleotide detecting all splice forms of trkB (*a*), but no expression of mRNA encoding full-length receptors can be detected at the lesion in an adjacent section hybridized with an oligonucleotide complementary to the tyrosine kinase domain (*b*). (Bar = 500 μm.)

It is unlikely that the truncated trkB receptors expressed by astrocytes, oligodendrocytes, and Schwann cells are involved in signal transduction by the ligands BDNF and NT-4, since the receptors lack the catalytic tyrosine kinase domain. However, the high expression in the normal nervous system and the altered expression after injury suggest that this cell surface protein may serve an important function. Truncated trkB receptors have been implicated in cell adhesion (19). This is supported by the presence of immunoglobulin-like domains and leucine-rich motifs in the extracellular part (41), as well as by the fact that D*trk*, a *Drosophila* gene closely related to the mammalian *trk* family, encodes a cell adhesion molecule expressed in the nervous system (42).

After transection of a peripheral nerve, the synthesis of NGF, BDNF, and LANR increases distal to the injury (43–45). NGF and LANR synthesis is rapidly up-regulated (46), whereas BDNF levels increase after an initial delay, reaching a peak 3–4 weeks after the injury (44). The decrease in trkB mRNA in the denervated distal nerve stump seems to follow a time course similar to that of the increase of BDNF mRNA, whereas the increase in LANR mRNA is more rapid. Thus, the denervated Schwann cells decrease the expression of trkB mRNA in parallel to the increased synthesis of the ligand for the receptor. A possible rationale for this could be to avoid high-affinity binding of BDNF to Schwann cell trkB receptors. This could enable BDNF to bind to the LANR on the Schwann cell surface, as suggested for NGF (47). Axonal growth cones bearing full-length trkB receptors could then bind BDNF linked to Schwann cell LANR and internalize BDNF because of the higher affinity of the axonal receptor.

The strong expression of trkB in the injured CNS, in contrast to the decrease in the injured peripheral nervous

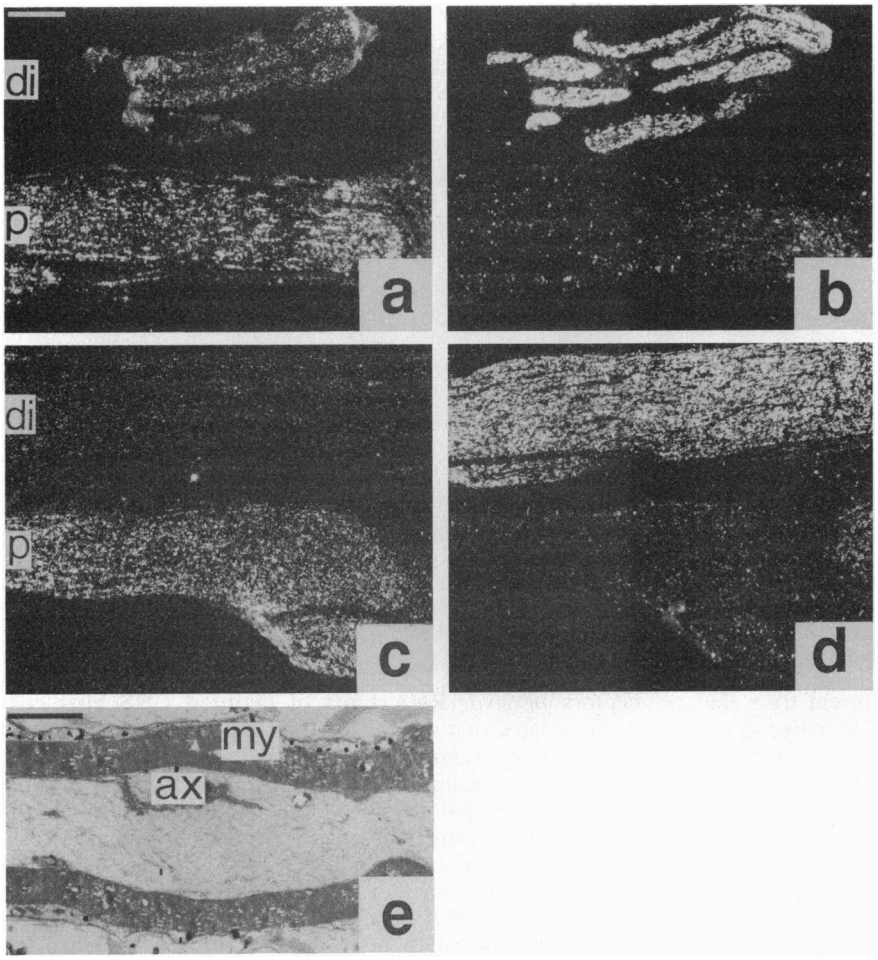

FIG. 4. Expression of trkB (*a* and *c*) and LANR mRNA (*b* and *d*) detected with *in situ* hybridization (*a–d*) and immunoelectron microscopic localization of trkB-LI in the injured sciatic nerve (*e*). Adjacent sections of the proximal (p) and distal (di) stump of cut sciatic nerves 1 (*a* and *b*) or 3 (*c* and *d*) weeks postinjury are shown. The level of trkB mRNA in the sciatic nerve is not substantially altered proximal to the transection 1 or 3 weeks after the transection (*a* and *c*). In contrast, a slight decrease in trkB mRNA is seen in the denervated nerve distal to the injury after 1 week (*a*), and after 3 weeks the mRNA level has decreased to barely detectable levels (*c*). The high expression of trkB mRNA in the intact sciatic nerve and proximal to a transection is opposite to the expression of LANR mRNA, which is very low in the intact nerve and proximal to a lesion but strongly increased in denervated Schwann cells distal to a transection (*b* and *d*). (*e*) A Schwann cell ensheaths an axon (ax) with myelin (my) in the proximal stump 12 days after the nerve was cut. Goldsilver particles indicating trkB-LI are localized to the Schwann cell cytoplasm and cell membrane. (Bars = 500 μm in *a–d* and 2 μm in *e*.)

system, could suggest that truncated trkB receptors are involved in inhibition of axon regrowth in the CNS. However, the increased levels of trkB are confined to the lesion area where substantial axonal sprouting is seen after spinal cord trauma (48–50). The close relation between axonal sprouts in the injured spinal cord and trkB-expressing astrocytes suggests that truncated trkB receptors may guide axonal sprouts by binding NTs to their surface, thereby producing a NT-laden substratum on which to grow (29).

The addition of BDNF to the astrocyte cultures did not influence the expression of trkB mRNA, and addition of serum to the medium did not alter the level of trkB mRNA in astrocytes, possibly indicating that this gene is not regulated by growth factors or cytokines present in serum. In a previous study we demonstrated that preferentially cells in direct contact with axonal sprouts showed trkB-LI (29). This could indicate that trkB expression in astrocytes and Schwann cells is induced by axonal contact, as opposed to the LANR in Schwann cells, which is down-regulated by axonal contact (43).

In conclusion, this study demonstrates that astrocytes, oligodendrocytes, and Schwann cells express high levels of truncated trkB receptors, but trkB mRNA could not be detected in cultured microglia. Strongly increased levels of trkB transcripts encoding truncated receptors were seen at the lesion after spinal cord injury, whereas Schwann cell expression was strongly decreased distal to a sciatic nerve transection. The high expression of truncated trkB receptors by glial cells in the intact nervous system and the altered expression after injury indicate important functions for this protein, which may be elucidated in the future by, for example, gene targeting experiments.

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