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Brain-Derived Neurotrophic Factor Promotes Central Nervous System Myelination via a Direct Effect upon Oligodendrocytes

Junhua Xiao[a, b] Agnes W. Wong[a, b] Melanie M. Willingham[a, b]
Maarten van den Buuse[c] Trevor J. Kilpatrick[a, b] Simon S. Murray[a, b, d]

[a]Centre for Neuroscience, [b]Florey Neuroscience Institutes, [c]Mental Health Research Institute, and [d]Department of Anatomy and Cell Biology, The University of Melbourne, Parkville, Vic., Australia

Key Words
Neurotrophins · Brain-derived neurotrophic factor · TrkB · Myelination · Oligodendrocyte · Central nervous system

Abstract
The extracellular factors that are responsible for inducing myelination in the central nervous system (CNS) remain elusive. We investigated whether brain-derived neurotrophic factor (BDNF) is implicated, by first confirming that BDNF heterozygous mice exhibit delayed CNS myelination during early postnatal development. We next established that the influence of BDNF upon myelination was direct, by acting on oligodendrocytes, using co-cultures of dorsal root ganglia neurons and oligodendrocyte precursor cells. Importantly, we found that BDNF retains its capacity to enhance myelination of neurons or by oligodendrocytes derived from p75NTR knockout mice, indicating the expression of p75NTR is not necessary for BDNF-induced myelination. Conversely, we observed that phosphorylation of TrkB correlated with myelination, and that inhibiting TrkB signalling also inhibited the promyelinating effect of BDNF, suggesting that BDNF enhances CNS myelination via activating oligodendroglial TrkB-FL receptors. Together, our data reveal a previously unknown role for BDNF in potentiating the normal development of CNS myelination, via signalling within oligodendrocytes.
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Introduction

Generation of the insulating myelin sheath is crucial for the development and function of the central nervous system (CNS). Oligodendrocytes, the CNS myelinating cells, develop from oligodendrocyte progenitor cells (OPCs) that arise from stereotyped germinal regions of the CNS. While some OPCs persist in the adult CNS as a population of slowly dividing adult progenitor cells, the majority of progenitors progress through a late progenitor and premyelinating oligodendrocyte stage before maturing into myelinating oligodendrocytes [1–3]. These cells subsequently either upregulate myelin genes and ensheath axons to form myelin membrane, or undergo apoptosis. However, relatively little is known about the nature of the signals that control CNS myelination or how these signals are regulated.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors, which also comprise nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4/5. BDNF signals through two distinct classes of transmembrane receptors: the tropomyosin-related kinase (Trk) receptor B (TrkB) and the structurally unrelated p75 neurotrophin receptor (p75NTR) [4, 5]. BDNF knockout (–/–) mice exhibit cardiorespiratory defects that result in early postnatal death; however, a fraction of these mice are observed to survive for up to

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Dr. Junhua Xiao
Centre for Neuroscience, The University of Melbourne
Parkville, VIC 3010 (Australia)
Tel. +61 3 8344 7572, Fax +61 3 9349 4432
E-Mail xiaoj@unimelb.edu.au

~3 weeks [6, 7]. Interestingly, these mice display marked optic nerve hypomyelination at postnatal day (P) 21, as well as reduced myelin protein mRNA transcripts in hippocampus, cerebellum and cortex [8]. This indicates that BDNF is required for normal CNS myelination; however, the ability to conclusively attribute the hypomyelination to a deficit in BDNF signalling is confounded by their overall failure to thrive, a condition that can also result in the reduction of myelin synthesis [9].

To further investigate the precise role played by BDNF upon CNS myelination, we first analysed myelin development in BDNF heterozygous (+/–) mice, which exhibit a normal lifespan [10–12] and an approximately 40% decrease in BDNF [12–14]. In support of previous findings by Vondran et al. [12], our data demonstrate that BDNF+/– mice display a severe hypomyelinating phenotype in the optic nerve, spinal cord and brain during early postnatal development, suggesting that BDNF plays a key role in potentiating CNS myelination. To verify these in vivo findings and characterize the cellular specificity of the promyelinating effect BDNF was exerting, we utilized an in vitro myelination assay, and found that BDNF enhances myelination via a direct effect upon oligodendrocytes. BDNF retained its promyelinating influence in vitro utilizing either neurons or OPCs derived from p75NTR knockout mice, indicating that p75NTR does not mediate the promyelinating influence of BDNF. Conversely, we observed that phosphorylation of TrkB correlated with myelination, and that blocking TrkB signalling inhibited the promyelinating effect of BDNF, suggesting that BDNF enhances CNS myelination via activating oligodendroglial TrkB-FL receptors. Collectively, these data reveal a previously unknown role for BDNF in potentiating the development of normal CNS myelination via providing signalling within oligodendrocytes.

Materials and Methods

Animals

P2 or P7-8 Sprague-Dawley rats, NGFR(B6) knockout (p75NTR–/–) [15] and wild-type (p75NTR+/+) littermate control mice, BDNF+/– (#002266, B6.129S4-BDNF$^{<tm1Jae>/J}$; Jackson Laboratory) and wild-type (BDNF+/+) littermates were used for histology, primary DRG neuron or OPC culture. All animal procedures were approved by the University of Melbourne Animal Experimentation Ethics Committee.

OPC Culture

Rat OPCs were purified to >99.5% homogeneity from P7-8 rat cortex by immunopanning as previously described [16]. Briefly, cerebral hemispheres were diced and digested with papain (Worthington Biochemical Corp.) at 37°C. Following gentle trituration, cells were resuspended in a panning buffer containing insulin (5 μg/ml; Sigma) and then incubated at room temperature sequentially on three immunopanning dishes: Ran-2, anti-O1 and O4. $O4^+O1^-$ OPCs were released from the final panning dish with trypsin (Sigma). Cultures were maintained in DMEM medium containing SATO base, N-acetylcysteine (60 μg/ml; Sigma), biotin (10 ng/ml; Sigma) and forskolin (5 μM; Sigma) with the presence of NT3 (5 ng/ml; Peprotec) and PDGF (5 ng/ml; Peprotec) [16, 17].

Mouse OPCs were purified to >99% homogeneity from P7-8 mouse (p75NTR+/+ and p75NTR–/–) cortex by immunopanning as previously described [17]. PDGFRα+ OPCs were isolated through sequential panning processes. Cultures were maintained in cultured media described above with the addition of 2% B27 supplement (Gibco) and Trace element B (1:100; Cellgro/Mediatech).

Purified DRG-OPC Co-Cultures

DRG-OPC co-cultures were established based on published techniques [18, 19]. Briefly, rat or mouse NGF-dependent (TrkA+) DRG neurons were purified with antimitotic medium in the presence of NGF (100 ng/ml; Peprotec) for 2–3 weeks. Alternatively, BDNF-dependent (TrkB+) DRG neurons were purified in the presence of BDNF (100 ng/ml; Peprotec). OPCs were seeded onto coverslips containing purified DRGs at a density of 200,000 OPCs per 22-mm coverslip or 100,000 OPCs per well of 4-well chamber slide and incubated overnight to facilitate attachment. DRG-OPC co-cultures were maintained for 14 days in a defined co-culture media containing 50:50 DMEM:neurobasal medium (Gibco) with SATO and 2% B27 supplements (Gibco), N-acetylcysteine (60 μg/ml) and D-biotin (10 μg/ml) in the presence of BDNF (100 ng/ml), the Trk tyrosine kinase inhibitor K252a (100 nM; Biosource), leukaemia inhibitory factor (LIF, 100 ng/ml; Peprotec) or dimethyl sulfoxide as a vehicle, as indicated. For some experiments, rat DRG neurons were co-cultured with mouse OPCs isolated from P7-8 p75NTR–/– or p75NTR+/+ littermates as described [17] in the presence of factors as previously indicated, or rat OPCs were co-cultured with DRG neurons were isolated from P2-3 mouse (p75NTR+/+ and p75NTR–/–) in the presence of indicated factors as previously described [17, 20].

Compartmentalized Co-Cultures

Campenot chambers (CAMP#3; Tyler Research Corp.) were used as previously described [20–23]. DRG neurons were plated into the central compartment, and their axons allowed to extend into the outer (lateral) compartments. The central compartment ('DRG soma + Proximal axon' compartment) contains both DRG soma bodies and their proximal axons, whereas the lateral compartment ('Axon + OPC' compartment) contains only the distal axons [23]. OPCs were then seeded into the lateral compartment and the chambers treated as indicated. The integrity of the seal preventing fluid interchange between compartments was examined quantitatively throughout experiments by monitoring the ability of phenol red in the 'DRG soma + Proximal axon' compartment to diffuse to the phenol red-free 'Axon + OPC' compartment. Media collected every 3 days was measured for its absorbance at 560 nm, with any absorbance from the lateral compartment indicating leakage of phenol red from the central compartment. Only those chambers that displayed both significant axon growth into the lateral compartment and no signs of leakage were further ana-

lysed for the numbers of myelin segments by immunostaining for myelin basic protein (MBP) and βIII-tubulin [20]. Immunocytochemistry and Western blot analysis were used as a direct quantitative readout for myelination [19, 20, 22].

Lentivirus Preparation and Infection in OPCs

To explore the role of oligodendroglial expressed p75NTR in BDNF-induced myelination, a lentiviral shRNA target set against p75NTR (#NM_033217; Open Biosystems, Huntsville, Ala., USA) was utilized to infect OPCs. Lentiviral particles were prepared by co-transfecting viral packaging plasmids and either p75NTR-shRNA or control scrambled shRNA plasmids into HEK293 cells using the Effectene reagent (Qiagen) as described previously [20]. Purified OPCs were infected with concentrated lentiviral particles for 48 h, and then seeded onto NGF-dependent DRG neurons. p75NTR expression and knockdown efficiency were determined in both co-cultures and parallel OPC cultures by Western blot analysis.

Immunocytochemistry

Myelinating co-cultures were fixed with 4% paraformaldehyde (PFA), blocked with 20% fetal calf serum (FCS) in 0.2% Triton X-100 in PBS. Myelinated axonal segments were visualized with an anti-MBP (AB980; Chemicon) antibody, followed by incubation with secondary antibodies (Molecular Probes), and images were captured by Zeiss confocal microscopy (Carl Zeiss, Inc.). Anti-βIII tubulin antibody (G7121; Promega) was used to ensure that myelination occurred under the same approximate axon density. Counts of the number of MBP+ myelin segments in each field, blinded to condition, were performed by counting 4 fields per coverslip or chamber, for at least 3 independent replicates per condition, with 40× magnification per field of vision counted. Groups were statistically analysed using unpaired 2-way t tests and 2-way ANOVA. All data presented as mean ± SEM.

Western Blot Analysis

Lysates generated from CNS tissues, DRG-OPC bulk co-cultures or co-cultures in Campenot chambers were separated by SDS-PAGE and transferred to PVDF membrane. Membranes were probed with specific antibodies against myelin proteins 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) (MAB326; Chemicon), myelin-associated glycoprotein (MAG) (MAB381; Chemicon), MBP (AB980; Chemicon) and myelin oligodendrocyte glycoprotein (MOG) (AF2395; R&D Systems), TrkB (sc8316; Santa Cruz Biotechnology), p75NTR (G323; Promega) or β-actin (A5441; Sigma) then incubated with HRP-conjugated secondary antibodies (Cell Signalling Technology). Each blot is representative of at least 3 independent co-cultures or 3 mice per genotype.

To analyse TrkB activation in BDNF-treated co-cultures, co-cultures were treated with BDNF at increasing concentrations (0–100 ng/ml) over 14 days. Lysates were prepared as above and probed with anti-phospho-TrkB (a gift from Prof. Moses Chao, New York University School of Medicine, USA). All experiments were repeated at least 3 times with similar results.

Quantification of the Western blot bands was achieved by scanning the blots using a Fujifilm LAS-3000 (Fujifilm Corp.). The optical density value for each band was determined using NIH ImageJ, and subsequently corrected to a loading control (actin), then normalized against the control condition. Quantification of phosphorylated TrkB was performed by dividing the intensity of the phosphorylated form by the corresponding intensity of the total TrkB protein. Groups were compared with unpaired 2-way t tests and 2-way ANOVA. All data presented as mean ± SEM.

RT-PCR Analysis

Total RNA from NGF-dependent DRG, BDNF-dependent DRG, primary cultured OPCs and oligodendrocytes was extracted using the RNeasy Mini Kit (Qiagen) and reverse-transcribed using oligo(dT)18 primer and M-MLV reverse transcriptase (Invitrogen). The resultant single strand cDNAs were incubated at 95°C for 1 min, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C, 72°C for 3 min, and a final extension at 72°C for 10 min using TaqMan DNA polymerase (Invitrogen) and primers published previously [20]. GAPDH was used as an internal control.

Proliferation Assay

Proliferation assays on glial cells have been previously described [24]. Briefly, to assay the effect of BDNF on proliferation in the absence of axons, OPCs were plated on poly-D-lysine (PDL) (P-6407, 10 μg/ml; Sigma) coated 8-well chamber slides at a density of 5,000 cells/well. OPCs were maintained in SATO with insulin (10 μg/ml) and forskolin (1 μg/ml) with or without BDNF (100 ng/ml; Peprotec). To study the effect of BDNF on proliferation in the presence of axons, OPCs were seeded on NGF-dependent (TrkA+) DRG neurons in 8-well chamber slides at a density of 5,000 cells/well. Cells were maintained in co-culture media as described above with or without BDNF (100 ng/ml; Peprotec). Cells were labelled with bromodeoxyuridine (BrdU; 15 mM every 24 h) and fixed 24, 48, and 72 h after plating with 4% PFA. For BrdU detection, cells were treated for 30 min at 37°C with 2 N HCl in PBS containing 0.3% Triton X-100 and rinsed twice in 0.1 M sodium tetraborate buffer, pH 8.5, for 10 min at 37°C. After several washes in 0.1 M PBS, cells were double-labelled with rat anti-BrdU-FITC antibody (OBT0030F, 1:100; Immunologicals Direct) and mouse anti-A2B5 IgG hybridoma supernatant as a general marker of OPCs, washed carefully, and incubated for 1 h at 37°C with the appropriate fluorochrome-labelled secondary antibody (Molecular Probes), as well as DAPI to label nuclei. The number of BrdU and A2B5 double-positive cells was counted and their percentage was calculated relative to the total number of DAPI+ cells. Groups were statistically analysed using unpaired 2-way t tests and 2-way ANOVA. All data presented as mean ± SEM.

Live/Dead Assays

To examine the effect of BDNF on cell survival, OPCs were seeded onto NGF-dependent (TrkA+) DRG neurons at a density of 5,000 cells/well in 8-well chamber slides. Cells were maintained in co-culture media for 24, 48 or 72 h. Ethidium homodimer-1 (4 μM; Invitrogen) and calcein AM (4 μM; Invitrogen) were added to each well followed by visualization under a fluorescent microscope (Carl Zeiss). The numbers of live (calcein AM-positive) and dead (ethidium homodimer-1-positive) cells per field ware counted and the proportion of live and dead cells was statistically analysed.

Differentiation Assay

Differentiation assays on glial cells have been previously described [24]. Briefly, to assay the effect of BDNF on differentiation in the absence of axons, OPCs were grown on 8-well chamber slides in the differentiation condition (SATO media without PDGF-AA)

with or without BDNF (100 ng/ml; Peprotec) for 24, 48 and 72 h. Cells were stained for oligodendrocyte lineage markers O1 (1:5; mouse IgG hybridoma supernatant [25]) to label premyelinating oligodendrocytes and for MBP (AB980; Chemicon) to mark mature oligodendrocytes [3, 26], as well as DAPI to label nuclei. The number of O1+ and MBP+ cells was counted and their percentages were calculated relative to the total number of DAPI+ cells.

To study the effect of BDNF on differentiation in the presence of axons, OPCs were seeded on NGF-dependent (TrkA+) DRG neurons in 8-well chamber slides. Cells were maintained in co-culture media as described above with or without BDNF (100 ng/ml; Peprotec), and then stained for MBP (AB980; Chemicon) and GFAP (Z0334; Dako) after 2, 8 and 14 days. The number of total number of MBP+ oligodendrocytes, MBP+ non-myelinating, MBP+ myelinating oligodendrocytes, as well as GFAP+ astrocytes were counted and analysed, as described previously [17]. Groups were statistically analysed using unpaired 2-way t tests and 2-way ANOVA. All data presented as mean ± SEM.

Histology

Mice were anesthetized and perfused transcardially with PBS followed by 4% PFA. The lumbar spinal cord, optic nerve and brain were collected and equilibrated successively in 10 and 30% sucrose in PBS for cryoprotection and then frozen on dry ice. For immunhistochemical analysis, 10-μm cryosections collected on PDL-coated slides were fixed for 30 min in 4% PFA in PBS for 10 min, washed in PBS, and then incubated for 60 min in blocking solution (20% FCS in PBS with 0.2% Triton-X100). Slides were washed in PBS and incubated with anti-MBP (AB980; Chemicon) antibody followed by the appropriate fluorophore-conjugated secondary (Molecular Probes), washed in PBS, and mounted in Dako mounting medium with DAPI. Fluoromyelin (F34652; Invitrogen) staining was performed on 10-μm cryosections according to the manufacturer's instructions.

For electron microscopy (EM), mice were perfused as above, tissues removed, postfixed overnight at 4°C in Karnovsky fixative, and subsequently processed for resin embedding as previously described [27, 28]. Semi-thin sections (0.5 μm) were cut to evaluate quality and orientation. Representative samples were then chosen, and ultrathin sections (90 nm) were cut from the spinal cord ventral column and optic nerve. Images were captured using a Siemens Stereoskop Transmission Electron Microscope (Siemens) at 3,000× magnification.

For cryostat tissues, fluoromyelin- and MBP-stained sections were photographed at 5× magnification and 3 mice per genotype were analysed for each section. For quantification of EM sections, 3 mice were used per genotype and 4 fields were analysed per section. The number of myelinated axons was counted, blinded to conditions, using ImageJ. Groups were compared with unpaired 2-way t tests and 2-way ANOVA. All data presented as mean ± SEM.

Results

BDNF+/– Mice Display CNS Hypomyelination

To more fully investigate the precise role that BDNF plays in regulating CNS myelination, we analysed BDNF+/– mice, which have been widely used [8, 10–12, 14]. Haploinsufficiency in these mice results in a ~40% reduction in the expression of BDNF, and importantly allows these mice to live a normal lifespan [12–14]. Our analyses show that BDNF+/– mice exhibit severe hypomyelination during early postnatal development. Immunohistochemical analysis using MBP and fluoromyelin staining in the spinal cord of BDNF+/– mice at P15 revealed a substantial reduction in myelin protein expression and myelination in the white matter (fig. 1a), although the spinal roots (myelinated by Schwann cells) still stained intensely for MBP as well as fluoromyelin (fig. 1a). A substantial reduction of MBP expression was also observed in the lateral corpus collosum (fig. 1b, arrowheads) of the brain of BDNF+/– mice at P9. We confirmed this hypomyelination qualitatively by electron microscopy (fig. 1c, d). Quantitative analysis revealed a significant decrease in the proportion of myelinated axons in the spinal cord at P9 (fig. 1c, e) and the optic nerve at P15 (fig. 1d, f) of BDNF+/– mice (* $p < 0.05$). Importantly, quantification of mean axonal diameter in both spinal cord and optic nerve revealed no significant differences between genotypes (fig. 1g, h). This phenotype was apparent during the early postnatal period; however, diminished with age, and by P30 myelination of the BDNF+/– mice was histologically indistinguishable from littermate controls (data not shown). In support of the phenotype seen in the early postnatal period, analysis of myelin protein expression by Western blot during this period also showed a significant reduction of myelin protein (MAG, MBP and MOG) expression in both optic nerve and spinal cord of BDNF+/– mice at P9 and P15 (fig. 1g, h, quantitated in online suppl. fig. 1, www.karger.com/doi/10.1159/000323170). However, the reduction in myelin protein expression diminished with age, and by P22 the expression of myelin proteins in BDNF+/– and BDNF+/+ littermates was similar (fig. 1i, j). The expression of endogenous levels of BDNF was also verified by Western blot, with BDNF+/– mice showing a substantial reduction in BDNF expression in comparison to the wild-type littermate mice (fig. 1g, h). Collectively, these data indicate that BDNF haploinsufficiency results in a delay in normal CNS myelination, suggesting that BDNF plays a key role in potentiating normal CNS myelination during early postnatal development.

BDNF Enhances Oligodendrocyte Myelination in vitro

To verify the above in vivo findings and determine whether the promyelinating effect exerted by BDNF was a direct effect upon myelination, we performed the in vitro myelination assay, a well-established technique which

replicates many of the fundamental processes that occur during myelination in vivo, involving the co-culture of purified populations of DRG neurons with OPCs [18, 19, 22]. DRG neurons are heterogeneous with respect to their neurotrophin dependence and responsiveness. NGF-dependent DRG neurons express TrkA but not full-length TrkB receptors (TrkB-FL) [19, 20, 29], whereas BDNF-dependent DRG neurons express TrkB-FL but not TrkA receptors [20]. To determine the effect that BDNF exerted upon the myelination of these distinct subsets of DRG neurons, we established parallel co-cultures, purifying independent populations of DRG neurons dependent either on NGF or BDNF. The absence of TrkB-FL receptors from NGF-dependent (TrkA+) neurons, and the absence of TrkA receptors from BDNF-dependent (TrkB+) neurons was verified by RT-PCR analysis (fig. 2a). We first investigated whether BDNF could regulate oligodendrocyte myelination of these distinct neuronal populations in vitro. Interestingly, we found that exogenous BDNF produced a similar promyelinating effect upon the myelination of both NGF-dependent (TrkA+) and BDNF-dependent (TrkB+) DRG neurons (fig. 2b–e). BDNF substantially enhanced the expression of the myelin proteins CNPase, MAG and MBP by Western blot analysis of co-culture lysates (fig. 2b, c, * $p < 0.01$) and the formation of MBP+ myelinated axonal segments as analysed by immunocytochemistry to a similar extent (fig. 2d, e, * $p < 0.001$). An approximately 2-fold increase in myelin protein (MBP) expression (fig. 2c), as well as a 2.4-fold increase in the number of MBP+ myelinated axonal segments (fig. 2e) was detected in both co-culture systems following treatment with exogenous BDNF (100 ng/nl). As NGF-dependent (TrkA+) DRG neurons do not express TrkB-FL (fig. 2a) [19, 20, 29], our data indicated that BDNF was able to enhance myelination in the absence of neuronal TrkB-FL. However, as both NGF- and BDNF-dependent DRG neurons express both p75NTR and truncated TrkB-1 (TrkB-T1) receptors (fig. 2a) [20], this indicated that BDNF could enhance myelination via acting either on the DRG neurons, or the oligodendroglial cells which express all BDNF receptors (fig. 2a) [30, 31].

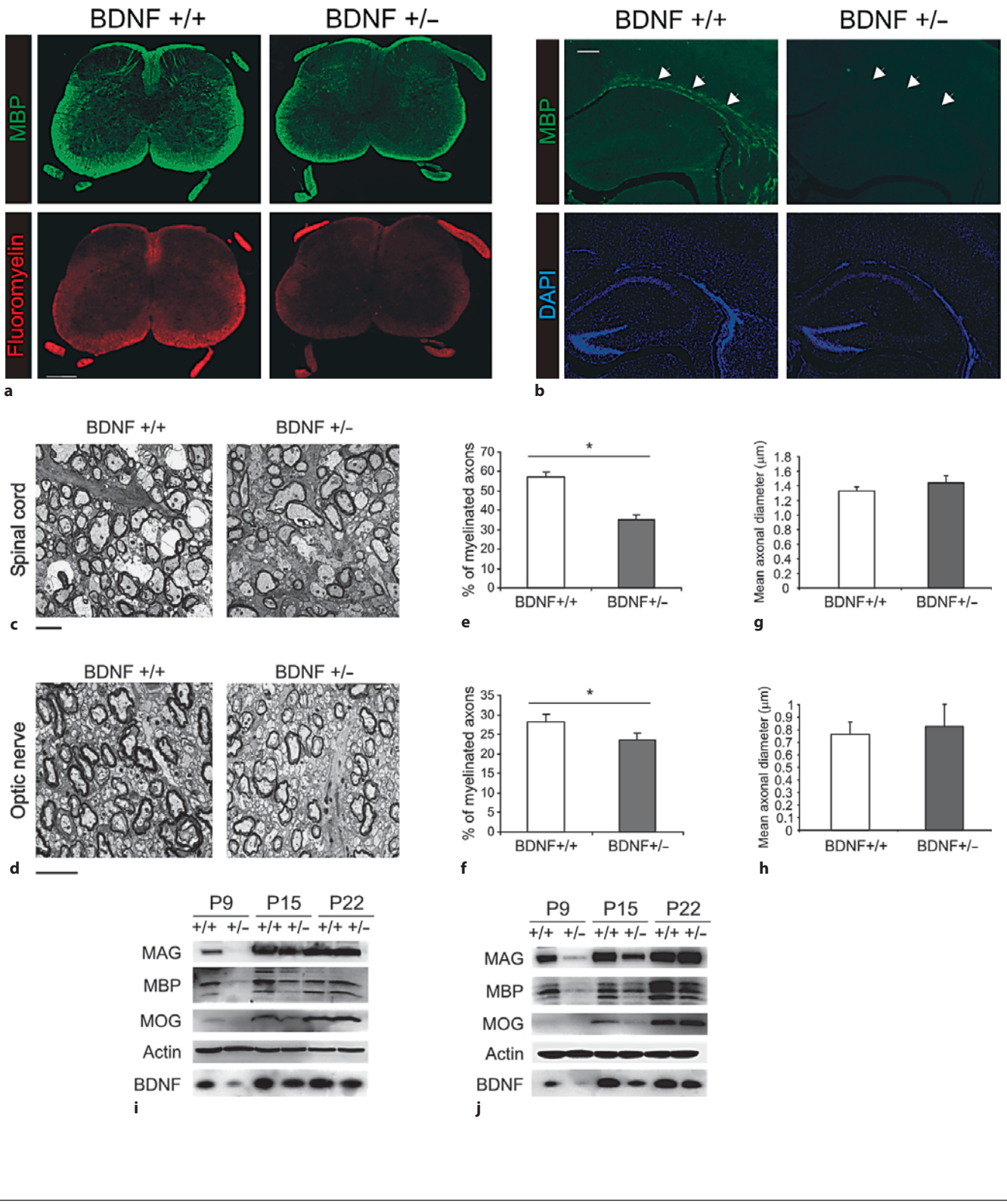
BDNF Exerts a Specific Effect upon Oligodendrocyte Myelination in vitro

Studies from Barres' laboratory [32] have demonstrated that BDNF does not support the survival, proliferation and differentiation of OPCs derived from optic nerve in vitro. We further investigated this issue in the context of OPCs derived from the cortex, by performing BrdU incorporation assays of primary cortical OPCs in either the absence or presence of axons (fig. 3). We found that BDNF did not promote the proliferation of isolated cortical OPCs in vitro in either the absence (fig. 3a) or presence of axons (fig. 3b) at 24, 48 and 72 h time points. To rule out the possibility of BDNF exerting an influence upon cell death or survival in the co-cultures, quantitative LIVE/DEAD assays were carried out, and revealed no significant difference between control and BDNF-treated myelinating co-cultures at 24, 48 and 72 h time point (fig. 3c). It is noticed that the basal level of OPC proliferation is higher in the presence of axons than in the absence of axons (fig. 3), suggesting that the presence of axons has an a posteriori effect upon OPC proliferation independent of the provision of BDNF, and that the development of oligodendrocytes is highly contextual [1]. Together, our data consistently indicate that promyelinating effect of BDNF was not due to an accelerated proliferation of OPCs.

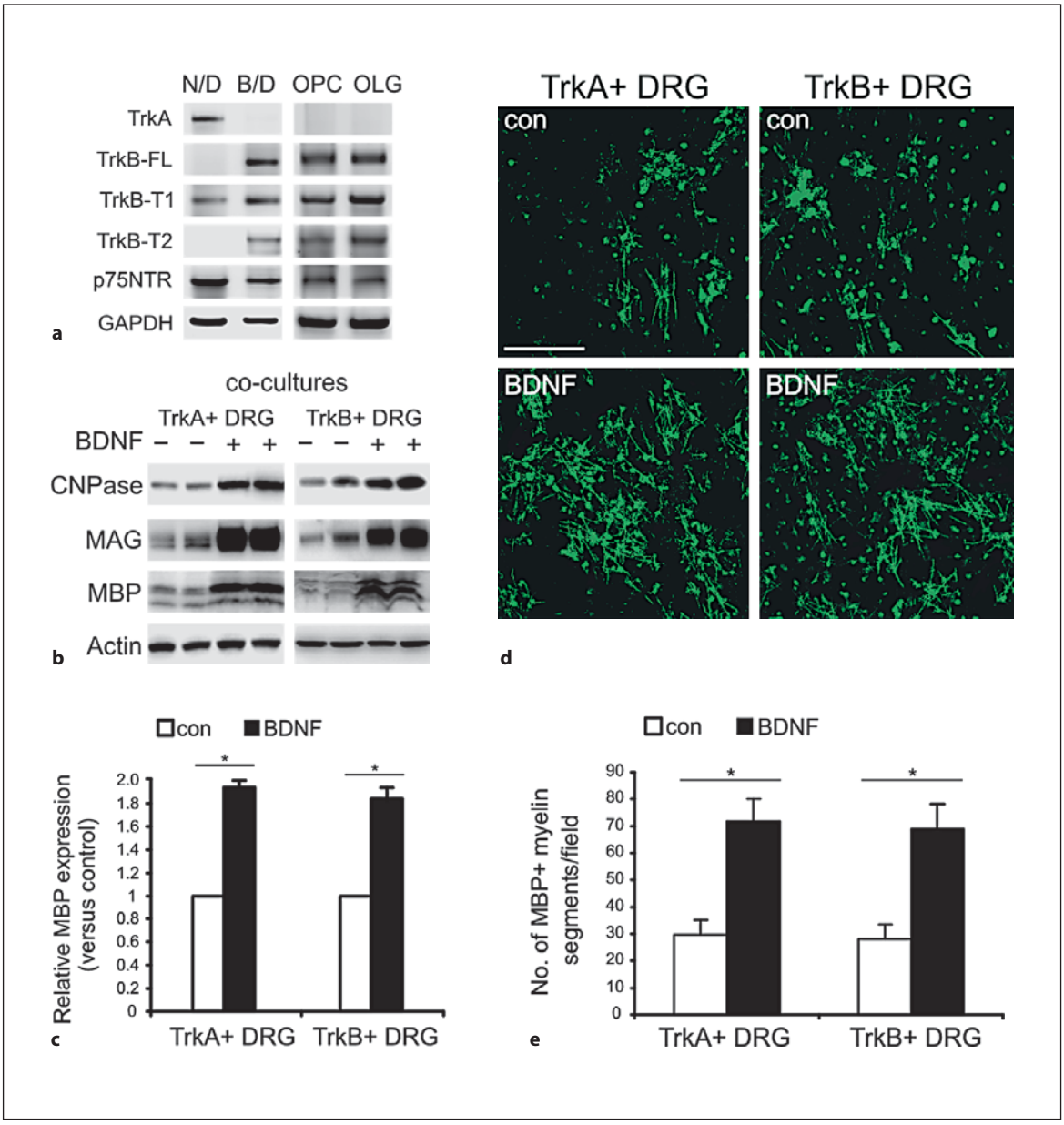
We next investigated whether BDNF was influencing the differentiation of OPCs into oligodendrocytes. This was initially assessed in the absence of axons (fig. 4a, b), revealing that BDNF did not enhance the differentiation

Fig. 1. BDNF+/– mice display CNS hypomyelination. **a**, **b** Representative images of P15 spinal cord (transverse section, **a**) and P9 brain (coronal section, **b**) of BDNF+/– and wild-type (+/+) littermate control mice stained with MBP, fluoromyelin and DAPI. There is a clear reduction in the intensity of MBP and fluoromyelin staining in the white matter of spinal cord and brain (arrowheads indicate lateral corpus collosum) in BDNF+/– mice. Scale bar: 25 μm. **c**, **d** Representative EM images of P9 spinal cord ventral column (**c**) and P15 optic nerve (**d**) in BDNF+/+ and BDNF+/– mice. Wild-type (+/+) tissues show robust myelination, while substantially fewer myelinated axons are observed in BDNF+/– mice in comparison to wild-type littermate controls. Scale bar: 2 μm. **e**, **f** Quantification of the proportion of myelinated axons of P9 spinal cord ventral column (**e**) and P15 optic nerve (**f**) in BDNF+/+ and BDNF+/– mice. There is a significant reduction in the proportion of myelinated axons observed in BDNF+/– mice in comparison to wild-type littermate controls (* $p < 0.05$, data = mean ± SEM, n = 3 mice per genotype). **g**, **h** Quantification of mean axonal diameter in P9 spinal cord ventral column (**g**) and P15 optic nerve (**h**) of BDNF+/+ and BDNF+/– mice. There are no significant differences in the axonal diameter between two genotypes (data = mean ± SEM, n = 3 mice per genotype). **i**, **j** Western blot analysis of myelin protein expression during development from lysates generated from spinal cord (**g**) and optic nerve (**h**) of BDNF+/+ and BDNF+/– mice. During early development (P9 and P15), BDNF+/– mice exhibit clear reductions in myelin protein (MAG, MBP and MOG) expression compared to wild-type littermate controls.

Color version available online



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Fig. 2. BDNF enhances oligodendrocyte myelination of NGF- and BDNF-dependent DRG neurons in vitro. **a** RT-PCR analysis of TrkB and p75NTR gene transcript expression in primary cultures of NGF-dependent DRG neurons (N/D), BDNF-dependent DRG neurons (B/D), OPCs and oligodendrocytes (OLG). The gene transcript of full-length (FL) TrkB is only present in BDNF-dependent DRG neurons as well as OPCs and oligodendrocytes, but absent from NGF-dependent DRG neurons. The gene transcripts of both truncated (T1 and T2) TrkB and p75NTR are present in both subtype of DRG neurons, as well as OPCs and oligodendrocytes. The gene transcript of TrkA is only present in NGF-dependent DRG neurons. **b**, **c** Western blot (**b**) and densitometric (**c**) analysis of co-cultures containing either NGF-dependent (TrkA+) or BDNF-dependent (TrkB+) DRG neurons. BDNF exerts a similar positive influence upon oligodendrocyte myelination of both neuronal subtypes. A significant increase in myelin protein (MBP) expression was detected in both co-culture systems with exogenous BDNF (100 ng/nl) (* $p = 0.0015$ for TrkA+ neurons, * $p = 0.0032$ for TrkB+ neurons, data = mean ± SEM, n = 3 independent experiments). **d**, **e** Qualitative (**d**) and quantitative analysis (**e**) of the number of MBP+ myelinated axonal segments in co-cultures containing either NGF-dependent (TrkA+) or BDNF-dependent (TrkB+) DRG neurons. A significant increase in the number of MBP+ myelin segments was detected in both co-culture systems with exogenous BDNF (100 ng/nl) (* $p = 1.4 \times 10^{-6}$ for TrkA+ neurons, * $p = 5.7 \times 10^{-6}$ for TrkB+ neurons, data = mean ± SEM, n = 3 independent experiments). Scale bar: 100 μm.

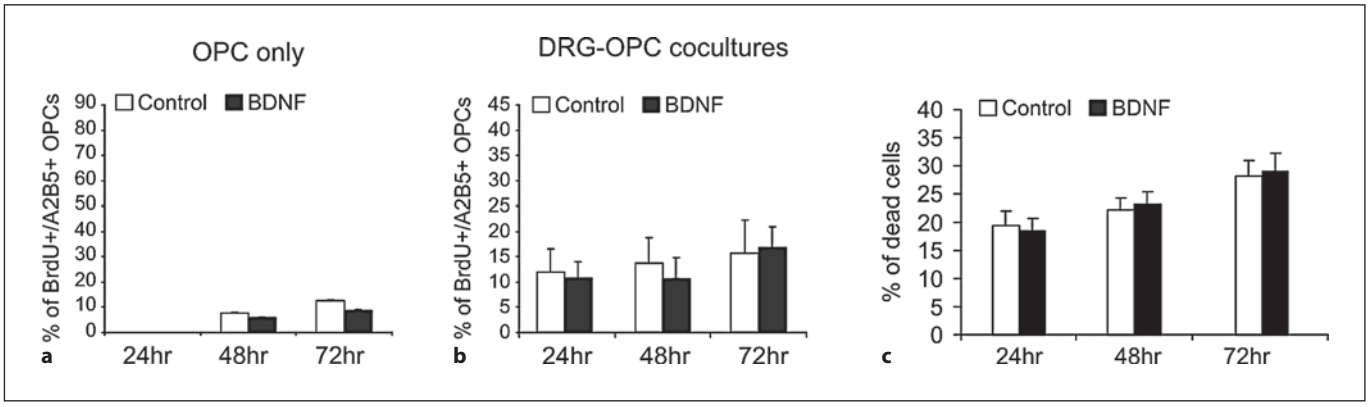


Fig. 3. BDNF has no effect on the proliferation and survival of OPCs. **a** BDNF (100 ng/ml) did not increase OPC proliferation in the absence of axons. Quantification of the percentage of BrdU+/A2B5+ OPCs indicated that there was no significant difference in the proportion of labelled cells between control and BDNF-treated primary OPC cultures at 24, 48 and 72 h time point (data = mean ± SEM, n = 3, $p > 0.05$). **b** BDNF did not increase the OPC proliferation in the presence of axons. Quantification of the percentage of BrdU+/A2B5+ OPCs indicated that there was no significant difference in the proportion of labelled cells between control and BDNF-treated myelinating co-cultures at 24, 48 and 72 h time point (data = mean ± SEM, n = 3, $p > 0.05$). **c** Live and dead assay of OPCs at indicated time points. In vitro quantification of the percentage of ethidium homodimer-1-positive dead cells revealed that that there was no significant difference in the proportion of labelled cells between control and BDNF-treated myelinating co-cultures at 24, 48 and 72 h time point (data = mean ± SEM, n = 3).

of OPCs, as the proportion of both O1+ premyelinating oligodendrocytes (fig. 4a) and MBP+ mature oligodendrocytes (fig. 4b) remained unaffected by BDNF. We then assessed whether BDNF was influencing the differentiation of OPCs into oligodendrocytes in the presence of axons via analysis of OPC differentiation in co-cultures treated with BDNF over 2, 8 or 14 days (fig. 4c–f) through determination of the number of MBP+ oligodendrocytes. We found that BDNF had no effect upon the total number of MBP+ oligodendrocytes compared to the control cultures (fig. 4c), suggesting that BDNF has no effect upon the differentiation of OPCs into oligodendrocytes. Nevertheless, it remained possible that BDNF could exert a specific influence upon the conversion of MBP+ premyelinating oligodendrocytes into mature myelinating oligodendrocytes. To investigate this, MBP+ oligodendrocytes were subcharacterized morphologically, in co-cultures treated with BDNF over 2, 8 or 14 days (fig. 4d, e), as previously described [17]. Myelinating oligodendrocytes were identified as MBP+ cells with multiple smooth MBP+ segments ensheathing axons, as described previously [17]. Non-myelinating oligodendroytes were identified as irregular, stellate-shaped MBP+ cells, with no evidence of axonal ensheathment, as described previously [17]. Interestingly, while the number of differentiated MBP+ non-myelinating oligodendrocytes was unaffected by BDNF after 2 or 8 days, a significant reduction in the number of these cells was observed after 14 days in comparison to control cultures (fig. 4d). Importantly, after 8 and 14 days, BDNF significantly increased the number of MBP+ myelinating oligodendrocytes compared to control cultures (fig. 4e). As the total number of MBP+ oligodendrocytes remains unaffected by BDNF (fig. 4c), our data suggest that BDNF was exerting its influence directly upon myelination via enhancing the maturation of premyelinating oligodendrocytes into myelinating cells. Thus our data strongly indicate that the effect of BDNF upon cortical oligodendrocytes is highly contextual, requiring concurrent neuronal interaction and are selective to axonal ensheathment and myelination, with no influence upon OPC proliferation being delineated. In addition, BDNF had no effect upon the number of GFAP+ astrocytes in the co-cultures (fig. 4f). Collectively, these data strongly suggest that BDNF exerts a specific influence upon myelination, promoting the maturation of premyelinating oligodendrocytes into myelinating cells.

BDNF Enhances CNS Myelination via a Direct Effect upon Oligodendrocytes

To further investigate the cell type upon which BDNF was acting in order to promote myelination, in vitro myelination assays were performed in compartmentalized Campenot chambers containing NGF-dependent DRG neurons and OPCs. In this system, myelination was as-

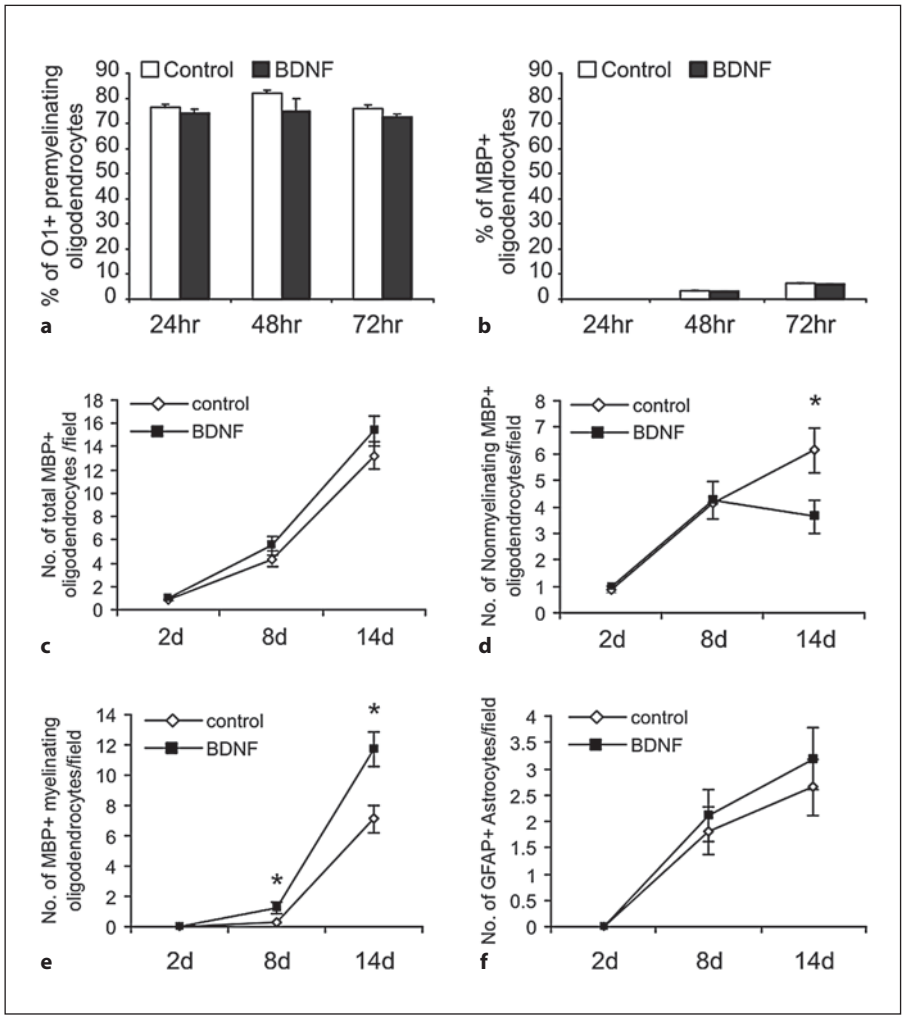
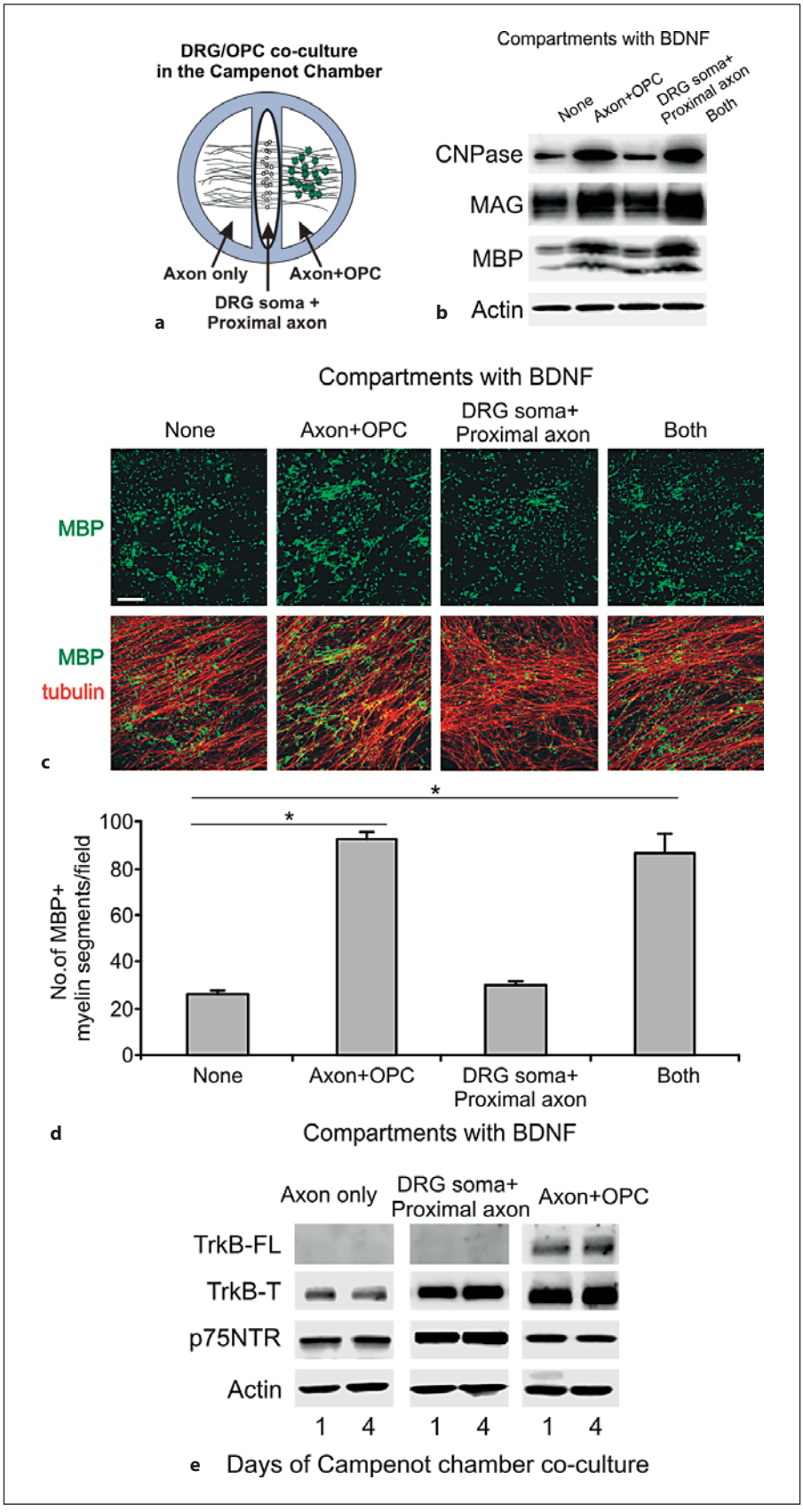


Fig. 4. BDNF enhances oligodendrocyte maturation in myelinating co-cultures. **a**, **b** Analysis of OPC differentiation in the absence of axons, as assessed by immunocytochemistry in either the absence or presence of BDNF (100 ng/ml) in primary OPC cultures under differentiative conditions. Quantification of the percentage of O1+ premyelinating oligodendrocytes (**a**) or MBP+ mature oligodendrocytes (**b**) indicated that there was no significant difference in the proportion of labelled cells between control and BDNF-treated OPC primary cultures at 24, 48 and 72 h time point (data = mean ± SEM, n = 3, $p > 0.05$). **c–f** Time-course analysis of OPC differentiation in the presence of axons, as assessed by immunocytochemistry in either the absence or presence of BDNF in myelinating co-cultures. Quantification of the total number of MBP+ oligodendrocytes (**c**), MBP+ non-myelinating (**d**), MBP+ myelinating oligodendrocytes (**e**), as well as GFAP+ astrocytes (**f**) per field after 2, 8 and 14 days (**d**) of co-culture in the absence or presence of BDNF (100 ng/ml). BDNF significantly increased the number of MBP+ myelinating oligodendrocytes at day 8 and day 14 without affecting the total number of differentiated MBP+ oligodendrocytes (* $p < 0.05$, data = mean ± SEM, n = 3 independent experiments).

sessed by Western blot analysis of co-culture lysates and immunocytochemical analysis of myelinated axonal segments. In these cultures that contain a physical barrier to diffusion, BDNF can be applied specifically into the central compartment ('DRG soma + Proximal axon') that contain the DRG soma and proximal axons, or the lateral compartment that contain axons and OPCs ('Axon + OPC') (fig. 5a) [19, 20, 22, 23]. We found that the addition of BDNF to the 'Axon + OPC' compartment substantially enhanced myelin protein (CNPase, MAG and MBP) expression (fig. 5b) and also significantly increased the number of MBP+ myelinated axonal segments (qualitatively represented in fig. 5c, quantitated in fig. 5d). This indicated that BDNF could be acting directly upon either the axons or OPCs to promote myelination. In contrast, when BDNF was added to the 'DRG soma + Proximal axon' compartment, no significant change in myelin protein expression or the number of myelinated axonal segments was observed (fig. 5b–d). This indicated that BDNF acting upon either the DRG soma or the proximal axons alone is insufficient to promote myelination. The addition of BDNF to both compartments produced no additive effect in comparison with addition to the 'Axon + OPC' compartment, alone (fig. 5b–d). Collectively, these data indicate that BDNF is unable to promote myelination when acting upon the axons alone, but that it acts directly upon the oligodendrocytes to promote myelination.

We complemented these observations by assessing for the expression of BDNF receptors in individual compartments of the Campenot chamber co-cultures (fig. 5e). Western blot analysis was performed on lysates generated from 'Axon only', 'DRG soma + Proximal axon' and 'Axon + OPC' compartments at 1 and 4 days after seeding with



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Fig. 5. BDNF promotes CNS myelination via a direct mechanism. **a** Diagram of a Campenot chamber co-culture. NGF-dependent (TrkA+) DRG neurons are plated into the central compartment ('DRG soma + Proximal axon'), and axons extend to the outer (lateral) compartments. OPCs are seeded into the lateral 'Axon + OPC' compartment. **b** Western blot analysis of co-culture lysates collected from the 'Axon + OPC' compartment of Campenot chamber. Compared to the control condition (None), BDNF applied to the 'Axon + OPC' compartment promoted myelin protein (CNPase, MAG and MBP) expression, whereas when applied to the 'DRG soma + Proximal axon' compartment had no effect. **c**, **d** Qualitative (**c**) and quantitative (**d**) analysis of the number of MBP+ myelinated axonal segments in the 'Axon + OPC' compartment of Campenot chamber co-cultures. Compared to the control condition (None), BDNF significantly increased the number of myelinated axonal segments when applied to the 'Axon + OPC' compartment (* $p = 0.003$ vs. 'None'). The addition of BDNF to the 'DRG soma + Proximal axon' compartment had no effect upon myelin formation. Concurrent addition of BDNF to both 'DRG soma + Proximal axon' and 'Axon + OPC' compartments ('Both') had no additive effect upon the number of myelinated axonal segments above that observed when BDNF was applied to the 'Axon + OPC' compartment alone (* $p = 0.02$ vs. 'None', $p = 0.79$ vs. 'Axon + OPC'). Scale bar: 100 μm (data = mean ± SEM, n = 3 independent experiments). **e** Western blot analysis of full-length (FL) TrkB, truncated (T) TrkB and p75NTR expression in protein lysates generated from the 'Axon only', 'DRG soma + Proximal axon' and 'Axon + OPC' compartment of Campenot chamber co-cultures at indicated days. The expression of TrkB-FL is only detected in lysates generated from 'Axon + OPC' compartment, but absent from either 'Axon only' or 'DRG soma + Proximal axon' compartment, whereas the expression of TrkB-T and p75NTR is present in lysates generated from all three compartments.

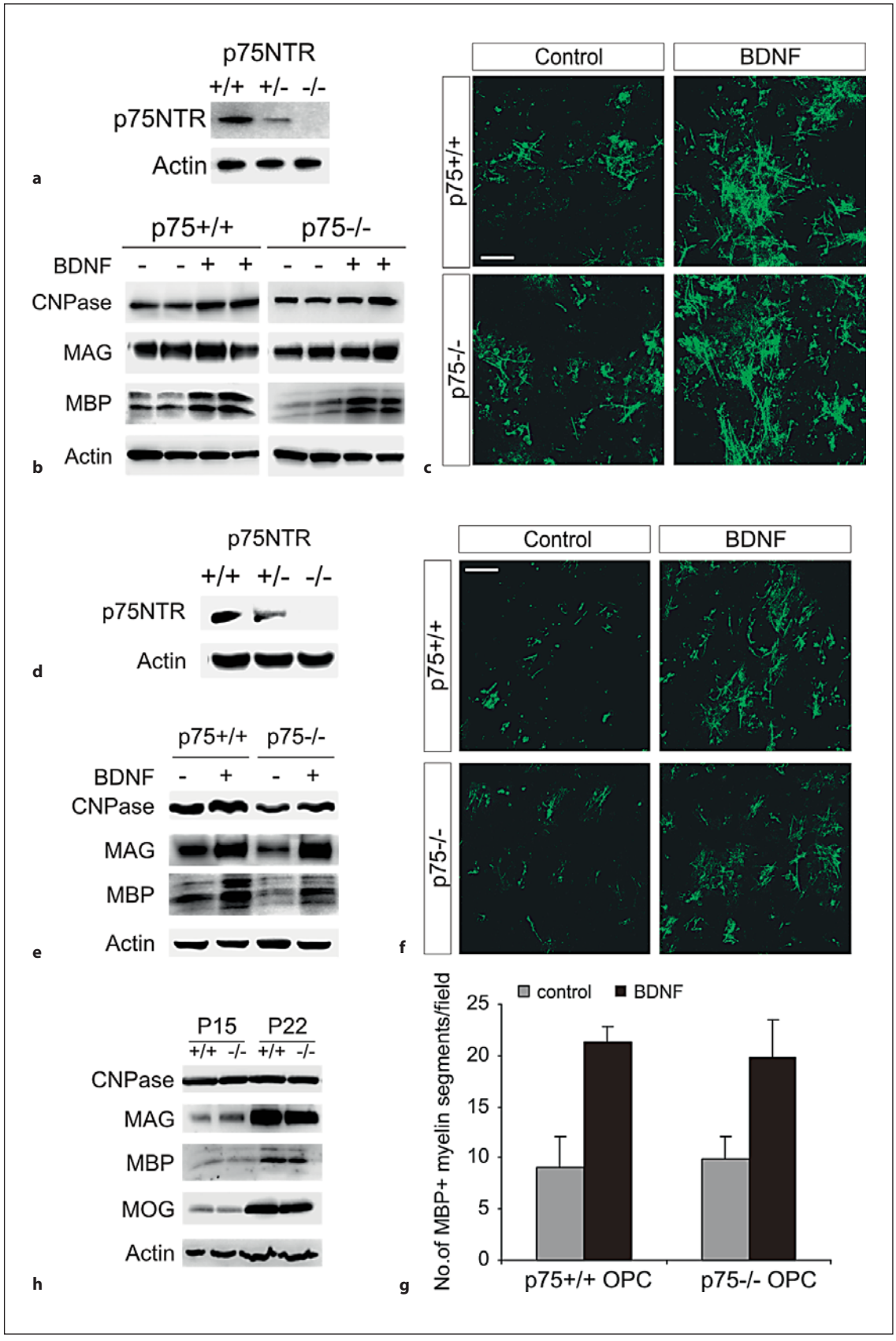
OPCs (fig. 5e). Our data show that TrkB-FL expression is only present in the 'Axon + OPC' compartment, but is absent from the 'Axon only' and 'DRG soma + Proximal axon' compartments. On the other hand, TrkB-T and p75NTR were present in all three compartments, with TrkB-T showing qualitatively lower expression in the 'Axon only' compartment than other two compartments (fig. 5e). Thus, the topography of BDNF's promyelinating influence within the Campenot chambers correlates with the expression profile of TrkB-FL, with the perspective that the influence is mediated via oligodendrocytes rather than via neurons.

p75NTR Expression Is Not Required for BDNF-Induced Myelination

While p75NTR plays a key role in mediating BDNF-induced Schwann cell myelination [20, 33], it remains unknown whether the expression of p75NTR is also required for BDNF-induced CNS myelination. To definitively confirm that neuronal expressed p75NTR was playing no role in BDNF induced myelination, we isolated NGF-dependent DRG neurons from p75NTR–/– mice and wild-type (p75NTR+/+) littermates for in vitro myelination assays (fig. 6a–c). Expression of p75NTR in DRG cultures was verified by Western blot analysis (fig. 6a). We found that BDNF retained its capacity to enhance myelin protein expression (fig. 6b, quantitated in online suppl. fig. 2a), as well as to promote the formation of myelinated axonal segments (fig. 6c) utilizing DRG neurons derived from p75NTR–/– mice. Thus, our data indicate neuronal expression of p75NTR does not mediate the promyelinating influence of BDNF.

As p75NTR is also present on oligodendroglial cells (fig. 2a), it remains unknown whether the expression of p75NTR by oligodendrocytes is also required for BDNF-induced CNS myelination. To investigate this, we first used a lentiviral-based shRNA approach for the specific knockdown of p75NTR in OPCs, in order to compare the degree of myelination in co-cultures containing these cells with co-cultures that contain oligodendrocytes that have either not been infected (control), or alternatively infected with scrambled shRNA. We found that BDNF maintains its capacity to enhance myelination by the oligodendrocytes expressing reduced levels of p75NTR (data not shown). To definitively confirm that oligodendrocyte expressed p75NTR was playing no role in BDNF induced myelination, we next isolated OPCs from p75NTR–/– mice and wild-type (p75NTR+/+) littermates for in vitro myelination assays. Expression of p75NTR in OPC cultures was verified by Western blot analysis (fig. 6d). Importantly, BDNF retained its capacity to enhance myelin protein expression (fig. 6e, quantitated in online suppl. fig. 2b, c), as well as promote the formation of myelinated axonal segments (fig. 6f, g) utilizing OPCs derived from p75NTR–/– mice, indicating oligodendroglial expression of p75NTR does not mediate the promyelinating influence of BDNF. In support of this, Western blot analysis of myelin protein expression in optic nerve lysates from p75NTR–/– mice indicate no significant difference in the level of myelin protein (CNPase, MAG, MBP and MOG) expression at P15 and P22 in comparison to littermate control mice (fig. 6h, quantitated in online suppl. fig. 2d–f), suggesting CNS myelin development is normal in these mice. Thus, our data demonstrate that neither neuronal nor oligodendroglial expression of p75NTR is essential for the promyelinating influence of BDNF.

Fig. 6. p75NTR is not necessary for BDNF-induced myelination. **a–c** BDNF enhances myelination of p75NTR–/– DRG neurons. Western blot analysis of NGF-dependent DRG neuron culture lysates derived wild-type (p75NTR+/+), p75NTR+/– and p75NTR–/– littermate mice. p75NTR expression was absent in DRG neurons derived from p75NTR–/– mice. (**b**, **c**) Western blot analysis (**b**) and MBP+ immunostaining (**c**) of co-cultures containing DRG neurons derived from wild-type (p75NTR+/+) and p75NTR–/– mice co-cultured with rat OPCs. No qualitative difference in myelin protein expression (**b**) or MBP+ myelin formation (**c**) occurred between co-cultures of wild-type (p75NTR+/+) or p75NTR–/– DRG neurons following BDNF treatment. Scale bar: 100 μm (n = 3 independent experiments). **d–g** BDNF enhances myelination by p75NTR–/– oligodendrocytes. (**d**) Western blot analysis of wild-type (p75NTR+/+), p75NTR+/– and p75NTR–/– OPC cultures lysates derived from littermate mice. p75NTR expression was absent in OPC cultures derived from p75NTR–/– mice. (**e–g**) Western blot (**e**), MBP+ immunocytochemical (**f**) and quantitative analysis of the number of MBP+ myelin segments (**g**) of co-cultures containing OPCs derived from wild-type (p75NTR+/+) and p75NTR–/– mice co-cultured with rat DRG neurons. No qualitative difference upon myelin protein expression or quantitative change in the number of MBP+ myelin segments ($p = 0.42$) occurred following BDNF treatment of either co-culture. BDNF retained the capacity to enhance myelin formation to a similar degree in co-cultures using either wild-type (p75NTR+/+) or p75NTR–/– OPCs. Scale bar: 100 μm (data = mean ± SEM, n = 3 independent experiments). **h** Western blot analysis of myelin proteins (CNPase, MAG, MBP and MOG) from optic nerve lysates of p75NTR+/+ and p75NTR–/– mice. No substantial differences were observed in the expression of myelin proteins between wild-type (p75NTR+/+) and p75NTR–/– mice (n = 3 mice per genotype).



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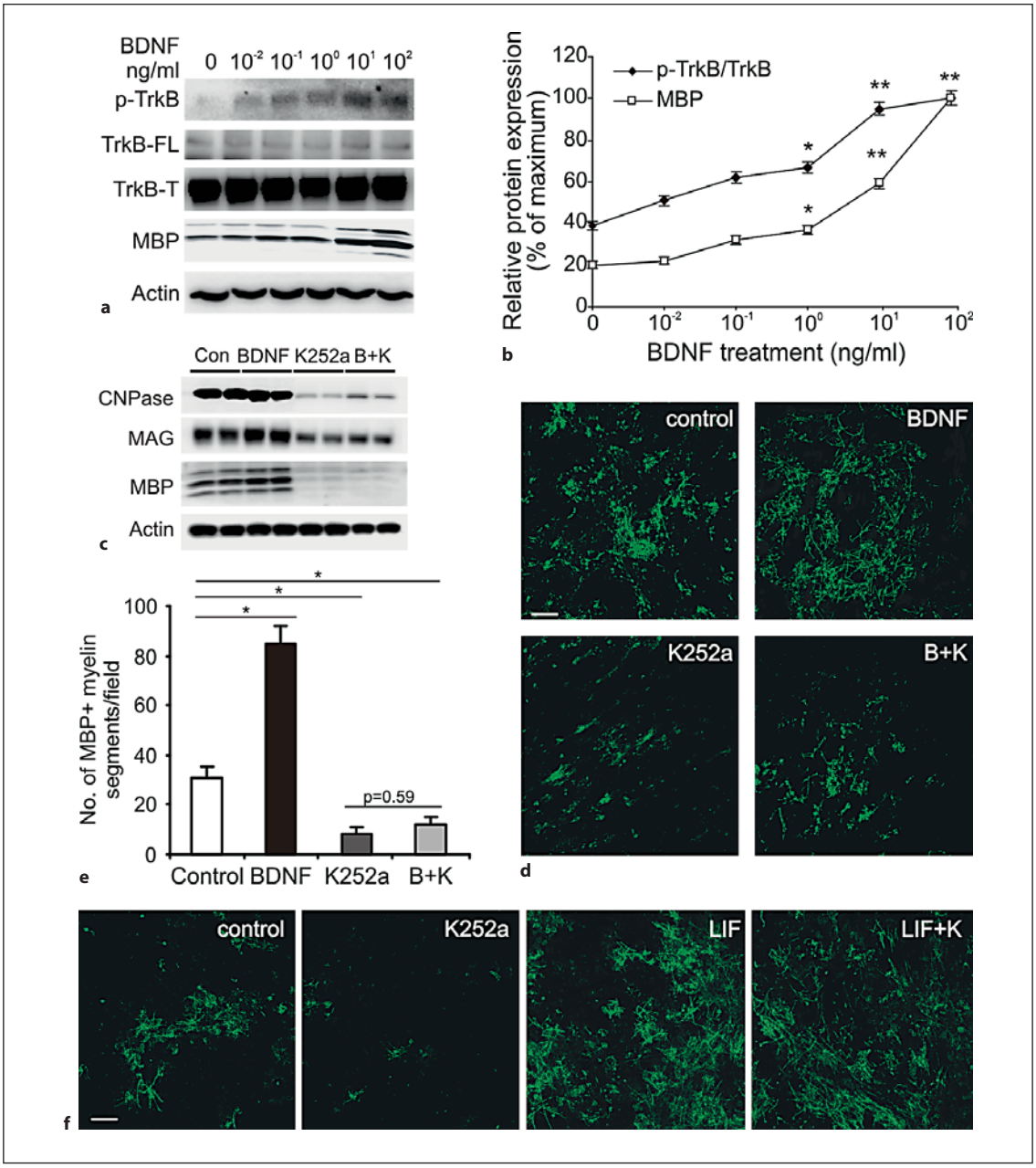


Fig. 7. Oligodendroglial TrkB-FL signalling mediates BDNF-induced myelination. **a**, **b** TrkB phosphorylation correlates with myelination. Western blot analysis (**a**) of in vitro myelinating co-cultures treated with BDNF for 14 days, at the indicated concentrations (0–100 ng/ml). Phosphorylation of TrkB in the co-cultures was potentiated with increasing concentrations of BDNF. Densitometric analysis of Western blot bands (**b**) indicate that the TrkB phosphorylation (relative to total TrkB) correlated with an increase in MBP expression (* $p < 0.05$, ** $p < 0.01$ vs. untreated control condition '0'). **c–e** Inhibition of TrkB signalling blocks the promyelinating effect of BDNF. Western blot analysis (**c**), MBP+ immunostaining (**d**) and quantitative analysis of the number of MBP+ myelin segments (**e**) of co-cultures treated with BDNF (100 ng/ml), K252a (100 nM) or BDNF and K252a concurrently (B + K). BDNF enhanced myelin protein expression and significantly increased the number of MBP+ myelinated axonal segments (* $p = 0.0011$) compared to the control untreated condition. K252a alone inhibited the basal level of myelin protein expression and the number of myelinated segments (* $p = 0.0004$ vs. control). BDNF was unable to promote myelination in the presence of K252a (B + K) (* $p = 0.002$ vs. control, $p = 0.59$ vs. K252a alone). Scale bar: 100 μm (data = mean ± SEM, n = 3 independent experiments). **f** Representative images of MBP+ immunostaining of co-cultures treated with LIF (10 ng/ml), K252a (100 nM) or LIF and K252a concurrently (LIF+K). LIF substantially increases the number of myelinated axonal segments in either the absence or presence of K252a, indicating that promyelinating effect of LIF was preserved in the presence of K252a.

TrkB-FL Signalling Is Required for BDNF to Promote Myelination in vitro

We next investigated the role of oligodendroglial TrkB in myelination in vitro. Firstly, to determine whether the activation of TrkB was regulated by BDNF during myelination in vitro, NGF-dependent DRG-OPC co-cultures were either untreated, or treated with BDNF over a concentration range from 10 pg/ml to 100 ng/ml for 14 days, and the lysates probed for phospho-TrkB (p-TrkB) (fig. 7a, b). Phosphorylation of TrkB receptors was apparent in BDNF-treated co-cultures, with a clear-cut BDNF concentration-dependent increase in TrkB phosphorylation being observed (fig. 7a, b). Importantly, expression of the myelin protein MBP was positively correlated with the concentration of BDNF (fig. 7a, b). As NGF-dependent DRG neurons do not express TrkB-FL (fig. 2a, 5e) [20] and BDNF is not able to enhance myelination via TrkB-T receptors that are also present on the NGF-dependent DRG neurons (fig. 2a, 5), our data strongly suggest that BDNF directly activates oligodendroglial expressed TrkB-FL receptors to positively regulate myelination.

We next investigated whether TrkB-FL signalling is required for the promyelinating effect of BDNF by using the Trk tyrosine kinase inhibitor K252a (100 nM) in the myelinating co-cultures, with and without exogenous BDNF (100 ng/ml). Importantly, K252a blocked the promyelinating influence of BDNF, as assessed by expression of the myelin proteins CNPase, MAG and MBP (fig. 7c), as well as the number of myelinated axonal segments as shown by MBP immunostaining (fig. 7d, quantitated in fig. 7e). This strongly suggests that the promyelinating effect of BDNF is mediated by activation of oligodendroglial TrkB-FL receptors. Interestingly, K252a also markedly reduced the extent to which the control co-cultures expressed myelin proteins (CNPase, MAG and MBP) and formed myelinated axonal segments (fig. 7c–e). To rule out the possibility that the observed reduction in myelination was due to K252a inducing oligodendroglial cell death, we performed live/dead assays with and without K252a (100 nM) in co-cultures for 24 and 48 h. We found that the total number of living cells was not affected by the addition of K252a (data not shown), indicating that K252a produces no significant toxic affect upon co-cultures containing OPCs. To rule out the possibility of K252a exerting a non-specific inhibitory effect upon myelination, we treated co-cultures with LIF (10 ng/ml), a commonly used factor that promotes both oligodendrocyte survival [34, 35] and myelination [22]. We found that the promyelinating effect of LIF was preserved in the presence of K252a (fig. 7f). Together, these data strongly suggest that the promyelinating effect of BDNF is mediated via a direct effect upon oligodendroglial expressed TrkB-FL receptors, and that BDNF not only has pharmacological effects but that it also has endogenous promyelinating activity within the co-cultures.

Discussion

In this study, we confirmed that BDNF is required to potentiate CNS myelination during early postnatal development. Utilizing in vitro myelinating and compartmentalized co-cultures, our data indicate that BDNF enhances myelination via a direct action upon oligodendrocytes. Using a series of knockdown, knockout and pharmacological strategies, we also identified that BDNF signals its promyelinating effect within oligodendrocytes via TrkB-FL, but not p75NTR. Our findings therefore identify a novel role for BDNF in CNS myelination mediated via direct activation of TrkB-FL receptors expressed by oligodendrocytes.

BDNF Regulates Oligodendroglial Maturation and Myelination during Development

Analysis of BDNF–/– mice has suggested that BDNF might be required for normal CNS myelination [8, 36]. However, these mice also display reduced postnatal growth, poor health and, in most cases, early postnatal lethality [8, 12, 13], such that it is difficult to be sure as to whether the effect upon myelination is either direct or indirect, secondary to a failure to thrive. Our analysis of postnatal myelin development in BDNF+/– mice, which survive a normal lifespan, has helped to clarify this issue and indicates that BDNF haploinsufficiency results in a delay in normal myelination within the spinal cord, optic nerve and corpus collosum. This is concordant with recently published findings from the Dreyfus laboratory [12], which identified that expression of myelin proteins MAG, MBP and PLP is reduced in the basal forebrain of BDNF+/– mice [12]. Interestingly, the Dreyfus laboratory identified that this reduction was permanent, evident not only at P14 but also in the adult, whereas our data indicate that myelination is delayed, but that by ~P22 expression of myelin proteins in the BDNF+/– mice is indistinguishable to that of controls within the spinal cord and optic nerve. This is, at first pass, difficult to reconcile, but could be possibly accounted for by regional differences in the influence that BDNF was exerting upon oligodendrocytes [31], and consistent with recent reports that there are regional differences in the developmental origins of

oligodendrocytes [2, 37]. Despite these differences in the age of animal analysed, the conclusions drawn from these observations both argue for a significant role for BDNF in the potentiation of normal CNS myelination.

Further insight into the influence that BDNF exerts upon CNS myelination has been recently afforded through generation of mice in which BDNF has been conditionally deleted from postmitotic neurons [38]. In these mice at 2 months of age, neither the number nor density of Olig2+ oligodendrocytes in the striatum, or myelination of optic nerve axons was different to controls, suggesting that CNS myelination in the adult is not dependent upon neuronal BDNF. However, neurons are not the only source of BDNF in the CNS, and this may be of particular relevance in the developmental context. In particular, while analysis of mice with neuronal-specific deletion of BDNF reveals essentially no BDNF expression in 2-month-old mice [38], it remains unclear whether glial derived BDNF could be expressed and influence CNS myelination in the early postnatal period when myelination is most active. The strategy of conditionally deleting BDNF from postmitotic neurons therefore does not account for the influence that glial cell expression of neurotrophins could exert. It has been long known that astrocytes and microglia secrete neurotrophins, including BDNF, in vitro [39–43] and during normal development in vivo [44, 45], as well as following CNS injury or disease [46–49]. Such expression may be highly developmentally regulated and reflect changing trophic interactions between different cell types within the CNS during postnatal development. Generation of mutant mice with conditional deletion of BDNF in other CNS cell types, such as astrocytes, could help clarify this issue.

Glial Signals Are Sufficient to Mediate the Promyelinating Effect of BDNF

The influence that BDNF exerts upon PNS myelination has been extensively investigated [19, 20, 23, 33, 50]; however, its precise role in regulating CNS myelination remains relatively obscure [8]. Insights from the analysis of peripheral myelination have highlighted the importance that neuronally expressed receptors can play in regulating myelination. Both NGF and BDNF have been found to regulate myelination by Schwann cells, although not by acting directly on these cells, but rather by activating neuronal receptors to influence the capacity of axons to regulate the myelination process [19, 20]. The influence that BDNF exerts upon PNS myelination is even more intriguing, as it is able to either promote or inhibit Schwann cell myelination, depending on the subset of DRG neurons being myelinated [20]. In contrast to these effects in the peripheral nervous system, we now report that BDNF exerts a consistent, promyelinating effect upon oligodendrocyte myelination of distinct subsets of DRG neurons independent of whether these neurons are TrkA or TrkB responsive. This is attributable to the fact that in contrast to the effects that BDNF exerts upon neurons to regulate Schwann cell myelination, it enhances oligodendrocyte myelination via direct influence upon oligodendrocytes. The fact that BDNF utilizes distinct mechanisms to influence myelination by Schwann cells and oligodendrocytes supports the view that central and peripheral myelination are regulated via unique and mutually exclusive mechanisms.

Cells in the oligodendroglial lineage express the BDNF receptors TrkB and p75NTR [30, 31]. Our data indicate that p75NTR plays no role in mediating the promyelinating effect of BDNF. This is supported by genetically, as there are no reports of a CNS myelin deficit in p75NTR knockout mice. On the other hand, the promyelinating effect of BDNF was lost in the presence of the Trk tyrosine kinase inhibitor K252a, indicating that TrkB is most likely mediating the promyelinating effect. The TrkB gene locus gives rise to three distinct gene products [51], all of which are expressed in oligodendroglial cells. The full-length isoform (TrkB-FL) is a bona fide receptor tyrosine kinase, whereas the two additional isoforms (T1 and T2) contain a truncated kinase-deficient intracellular domain. As K252a inhibits tyrosine phosphorylation of Trk receptors, the data suggest that TrkB-FL mediates the promyelinating effect of BDNF.

Interestingly, we found that BDNF exerted no effect upon the survival, proliferation or differentiation of cortical OPCs when cultured alone in vitro. These results concur with those of Barres et al. [32] who also found that BDNF does not support the survival, proliferation or differentiation of OPCs derived from optic nerve in vitro. Thus our data strongly indicate that the effect of BDNF upon cortical oligodendrocytes is highly contextual, requiring concurrent neuronal interaction and are selective to axonal ensheathment and myelination, with no influence upon oligodendrocyte survival or OPC proliferation being delineated.

In contrast to this, in vitro analysis of OPCs derived from the basal forebrain has shown that BDNF increases their proliferation [52] as well as potentiates their differentiation to MBP, PLP and MAG-expressing oligodendrocytes [53, 54]. The reasons for these disparities are uncertain, and may reflect regional specificity in the responsiveness of OPCs, given that we and Barres studied

OPC isolated from the optic nerve and cortex, whereas Van't Veer and colleagues [52–54] studied OPCs derived from the basal forebrain, further supporting the view that there are regionally specific effects of BDNF on oligodendrocytes [31]. Interestingly, these authors have shown that BDNF-dependent proliferation and differentiation of basal forebrain OPCs in vitro requires TrkB-FL and activation of the MAPK signalling pathway. Thus, it would be interesting to identify the effect of BDNF upon these cells in the context of myelination in vitro, and the role that MAPK signalling plays in oligodendrocyte proliferation, differentiation and myelination. Clarification of these issues warrants further investigation.

Myelination is a complex biological process, and the full complement of factors that influence this process has yet to be elucidated. Our data have identified that BDNF plays an important role in promoting CNS myelination via activating TrkB-FL receptors expressed by oligodendrocytes. As such, these observations have provided new insight into the cellular and molecular mechanisms that control CNS myelination. Ultimately, future studies aimed at identifying key signalling targets of BDNF within oligodendroglial cells will allow specific insight into the molecular events that expedite myelination, and whether these mechanisms are relevant not only to development but also in the context of human demyelinating diseases.

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