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Control of Local Protein Synthesis and Initial Events in Myelination by Action Potentials

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Abstract

Formation of myelin, the electrical insulation on axons produced by oligodendrocytes, is controlled by complex cell-cell signaling that regulates oligodendrocyte development and myelin formation on appropriate axons. If electrical activity could stimulate myelin induction, then neurodevelopment and the speed of information transmission through circuits could be modified by neural activity. We find that release of glutamate from synaptic vesicles along axons of mouse dorsal root ganglion neurons in culture promotes myelin induction by stimulating formation of cholesterol-rich signaling domains between oligodendrocytes and axons, and increasing local synthesis of the major protein in the myelin sheath, myelin basic protein, through Fyn kinase-dependent signaling. This axon-oligodendrocyte signaling would promote myelination of electrically active axons to regulate neural development and function according to environmental experience.

Myelin, the multilayered membrane of insulation wrapped around axons by oligodendrocytes, is essential for nervous system function and increases conduction velocity by at least 50 times (1, 2). Unique to vertebrates, formation of the myelin sheath must be highly regulated temporally during development and targeted specifically to appropriate axons. Many axon-derived signals regulate myelination, but there is great interest in the possibility that electrical activity could provide an instructive signal, because activity-dependent regulation of myelinogenesis could control myelination during development according to environmental experience, contribute to learning, and guide regeneration after injury according to functional efficacy (3). Electrical activity has been shown to affect proliferation and differentiation of myelinating glia (4–7), but if electrical activity could regulate subcellular events necessary for myelin induction, then myelin could form preferentially on electrically active axons. Here we test this hypothesis, beginning with the question of how electrical activity in axons might signal to oligodendrocytes to control myelination.

Both neurotransmitters adenosine 5′-triphosphate (ATP) and glutamate (glu) have been implicated in signaling to oligodendrocyte progenitor cells (OPCs). Glutamatergic synapses can form transiently between axons and some OPCs (8, 9). It has been proposed that such synaptic communication between axons and OPCs might stimulate myelin formation on

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individual axons that are electrically active (10). However, glu inhibits OPC proliferation and differentiation in monoculture (11). Electrical activity also causes nonvesicular release of the neurotransmitter ATP from axons through volume-regulated anion channels (12), and ATP released from axons increases myelin formation by regulating OPC differentiation and expression of myelin proteins (5, 7).

Our measurements confirm that electrical activity stimulates both vesicular release of glu and nonvesicular release of ATP from mouse dorsal root ganglion (DRG) neurons in a cell culture preparation equipped with platinum electrodes (13) (fig. S1). Immunocytochemical staining for the postsynaptic protein, PSD-95, failed to provide evidence for postsynaptic specializations on OPCs. However, punctate staining for the synaptic vesicle glycoprotein, synaptophysin, was evident along DRG axons (fig. S2A). Active recycling of vesicles between these sites of vesicle aggregation and the axon membrane was shown by uptake of a lipophilic FM dye (fig. S2, A to C).

To test the hypothesis that release of synaptic vesicles from axons can promote myelination, DRG neurons were treated for 18 hours with botulinum toxin A (BnTX), which cleaves the synaptic vesicle release protein SNAP-25 (25-kD synaptosome–associated protein) (14). SNAP-25 is necessary for synaptic vesicle fusion, and neurotransmitter release is blocked for at least 2 weeks after washing out the toxin from DRG neurons (14). OPCs were added to neuron cultures after washing out the toxin, so that only vesicular release from axons would be impaired, and allowed 5 days to differentiate to a promyelinating oligodendrocyte. DRG axons were then stimulated for 5 hours (9 s at 10 Hz, 5-min intervals) and examined 21 days later (Fig. 1A). Similar experiments used tetanus toxin (TnTX), which blocks vesicular fusion by cleaving a different synaptic vesicle protein, VAMP (vesicle-associated membrane protein or synaptobrevin). Both experiments showed that myelination was suppressed in comparison with cultures where vesicular release from axons was not blocked (Fig. 1, B and C, and fig. S3). No differences in cell differentiation, amount of myelin proteins, or number of oligodendrocytes were evident between cultures stimulated in the presence or absence of BnTX (Fig. 1D and fig. S4), which suggested a requirement for synaptic vesicle release on myelin induction. Although other mechanisms are likely necessary for maintenance of myelin, the increased induction of myelination after only a 5-hour stimulus has consequences that are observed as increased myelination persisting after 16 days.

Calcium imaging showed that both glu and ATP release can signal electrical activity in axons to OPCs, but the spatiotemporal dynamics of Ca^{2+} signaling differed for the two neurotransmitters (Fig. 2, A and B). Stimulation (10 Hz for 15 s) of DRG neurons induced rapid Ca^{2+} responses in the slender OPC cell processes (Fig. 2, A to E), but the rise time and decay were slower in the OPC soma. This was confirmed by transfecting OPCs with the genetic Ca^{2+} indicator GCaMP2 (Fig. 2, G to I), which allows measurement of Ca^{2+} responses in OPCs independently from responses in axons. Blocking vesicular release with BnTX or blocking glu receptors with a combination of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphonopentanoic acid (AP5), and α-methyl-4-carboxyphenylglycine (MCPG) inhibited Ca^{2+} responses in OPC processes closely associated with axons, but failed to block Ca^{2+} responses in the cell soma (Fig. 2, H and I, and fig. S5). Somatic Ca^{2+} responses were inhibited by the P2 receptor blocker suramin (Fig. 2F and fig. S6). The latency of the Ca^{2+} response to axon stimulation provides evidence for both ectopic release of neurotransmitter and release of neurotransmitter localized to points of axo-glial junctions (fig. S7). We conclude that vesicular release of glu is a major pathway for activity-dependent signaling between axons and OPC processes closely associated with axons and that ATP signaling from axons has a predominant effect on somatic Ca^{2+} responses, presumably because the release mechanism is less restricted to

specialized sites of axo-glial contact (Fig. 2F). Therefore, vesicular release of glu is a likely mechanism to control axo-glial signaling initiating myelination in OPC processes associated with axons.

In human development, oligodendrocytes in cerebral white matter mature 3 months before they begin to form myelin, which suggests that local axon-glial signaling regulates induction of myelin on specific axons (15). Myelinogenesis requires cell recognition, the formation of specialized contacts for intercellular signaling, and the synthesis of large quantities of lipids and specialized proteins necessary for formation of the myelin sheath. Cholesterol-rich microdomains are organizing centers for signaling molecules and cytoskeletal elements controlling trafficking of membrane proteins and receptors to specialized sites of cell-cell contact (16, 17). In oligodendrocytes, specific axon-glial interactions—involving activation of the Src-family kinase Fyn (18), cell adhesion molecule L1, and integrin (16) concentrated in lipid-raft microdomains—regulate subcellular events necessary for induction of myelination on appropriate axons. This includes the local translation of myelin basic protein (MBP) from mRNA in oligodendrocyte processes. It is not known whether electrical activity can control the formation or activity of these signaling complexes.

To test this hypothesis, we monitored trafficking of the transferrin receptor (TfR) into the membrane of OPCs. The TfR is enriched in cholesterol-rich membrane domains, notably at the postsynaptic membrane of neurons (19). OPCs were transfected with TfR-mCherry–superecliptic pHluorin (SEP), which becomes fluorescent only after exocytosis and exposure to neutral pH (20). The results show that stimulation of DRG axons (10 Hz, 9 s, at 5-min intervals for 5 hours) increased TfR surface expression on OPCs (Fig. 3A). This increased trafficking, in turn, required vesicular glu release from axons, as shown by blocking the increased TfR trafficking by stimulation of neurons pretreated with BnTX or in the presence of glu receptor antagonists (Fig. 3, A and B). Activated Fyn kinase (phosphorylated at tyrosine 418) colocalized with the surface expression of TfR receptors (Fig. 3C). Electrical stimulation increased phosphorylation of Fyn kinase (Fig. 3D and fig. S9) and increased the surface expression of cell adhesion molecule L1 (fig. S9A). Both responses required vesicular release, as shown by BnTX treatment before stimulation (Fig. 3D and fig. S9A).

These results indicate that vesicular release of glu from axons stimulates the formation of cholesterol-rich microdomains in oligodendrocytes and activates signaling pathways known to regulate local translation of MBP. This raises the hypothesis that local translation of MBP in oligodendrocytes could be controlled by electrical activity. We focused on MBP because it is the major protein constituent of the myelin sheath and because it is required for formation of myelin. MBP mRNA is transported in RNA granules toward the distal ends of the oligodendrocyte processes where the protein is translated and delivered locally to the plasma membrane for myelin formation (21, 22).

To visualize local translation of MBP, we developed a genetic construct in which MBP was labeled with a fluorescent protein, kikume green-red (kikGR), which is irreversibly converted from green to red fluorescence by ultraviolet (UV) light (23). After photoconversion, previously synthesized MBP appears red, and newly synthesized MBP will appear as green fluorescent spots. OPCs transfected with a construct containing the MBP coding region showed local MBP translation during a 40-min observation period after stimulation of DRG axons (10 Hz, 10 min), and the newly synthesized protein was inserted into the cell membrane (Fig. 4C and fig. S10). OPCs transfected with a construct containing only the 3′ untranslated region (3′UTR) of MBP also showed local kikGR translation only after stimulation of DRG axons, but the protein was not targeted to the cell membrane (fig. S10). When stimulation was delivered locally by using bipolar electrodes separated by 350 μm, rather than by field stimulation of the entire culture, only oligodendrocytes adjacent to

stimulated axons showed local translation of MBP, and neighboring oligodendrocytes outside the region of stimulation showed no MBP translation (fig. S11). Either pretreating axons with BnTX or stimulation in the presence of glu receptor blockers prevented local synthesis of MBP in response to stimulation. AMPA receptor or P2 receptor blockers were without effect, but AP5 or MCPG significantly inhibited local translation of MBP, which demonstrated that vesicular release of glu from axons acting on *N*-methyl-D-aspartate receptors (NMDARs) and metabotropic glu receptors (mGluRs) stimulates local translation of MBP (Fig. 4, C and D). Suppressing Fyn kinase activity by small interfering RNA (siRNA) transfection into oligodendrocytes completely blocked the electrically induced local translation of MBP (Fig. 4D).

After synthesis, myelin proteins must be restricted to the appropriate membrane regions adjacent to the axon being myelinated. To determine whether vesicular release of neurotransmitter from axons stabilizes mobility of myelin proteins in OPC cell processes, MBP was visualized by using a photoactivated green fluorescent protein (GFP) (24). Illumination with a spot of UV light rendered MBP fluorescent in a discrete 1-μm region of the OPC cell process, so that mobility of the MBP could be monitored by time-lapse confocal microscopy (Fig. 4E and fig. S12). Movement of newly synthesized MBP was highly restricted to discrete regions of OPC cell processes in comparison with OPCs cultured with neurons previously treated with BnTX ($P < 0.005$). Blocking P2 receptor activation with suramin did not prevent the restricted MBP mobility (Fig. 4E and fig. S12). This indicates that vesicular release of glu from axons restricts the mobility of MBP, as would be required to wrap MBP-containing membrane selectively around axons firing action potentials.

Our results suggest that activity-dependent regulation of these subcellular processes in oligodendrocytes initiates myelin formation preferentially on electrically active axons (fig. S13). Although the mechanisms revealed here must be confirmed in vivo, this form of activity-dependent regulation could be important in modifying development of brain circuits according to environmental experience, as myelination of the cerebral cortex continues through at least the first three decades of life (3). Human brain imaging has detected changes in white matter regions after learning (25), although the cellular basis for these changes is unknown. Regulation of myelination by impulse activity in individual axons could regulate neurodevelopment and thus influence information transmission in the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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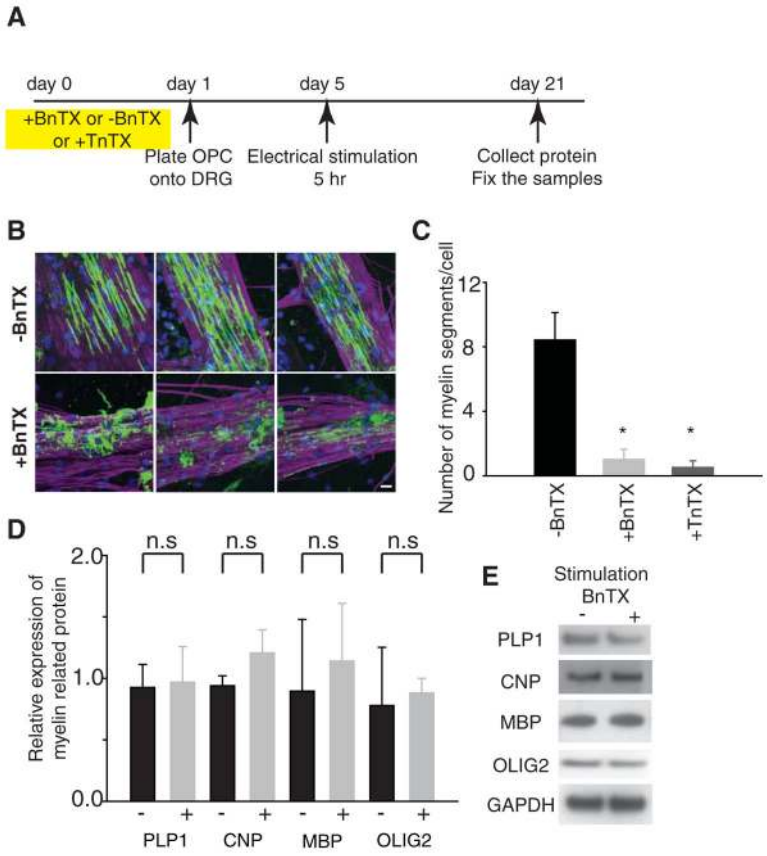

Fig. 1.

Release of synaptic vesicles from axons promotes myelination. (**A**) Synaptic vesicle release from DRG neurons was blocked by adding BnTX or TnTX to neuron cultures, and OPCs were added after washing out the toxin. Five days later, axons were stimulated for 5 hours (10 Hz, 9 s at 5-min intervals), and they were examined 21 days later. (**B** and **C**) Myelin formation was greatly reduced in cultures in which vesicular release was blocked (MBP, green; neurofilament, purple). Scale bar, 10 μm ($P < 0.005$, $n = 7$). (**D** and **E**) OPCs had differentiated into oligodendrocytes regardless of whether vesicular release was blocked during electrical stimulation (black bar, −BnTX; gray bar, +BnTX), as indicated by protein expression (D and E) for myelin proteins [proteolipid protein 1 (PLP1), MBP, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP)] and the transcription factor *Olig2*.

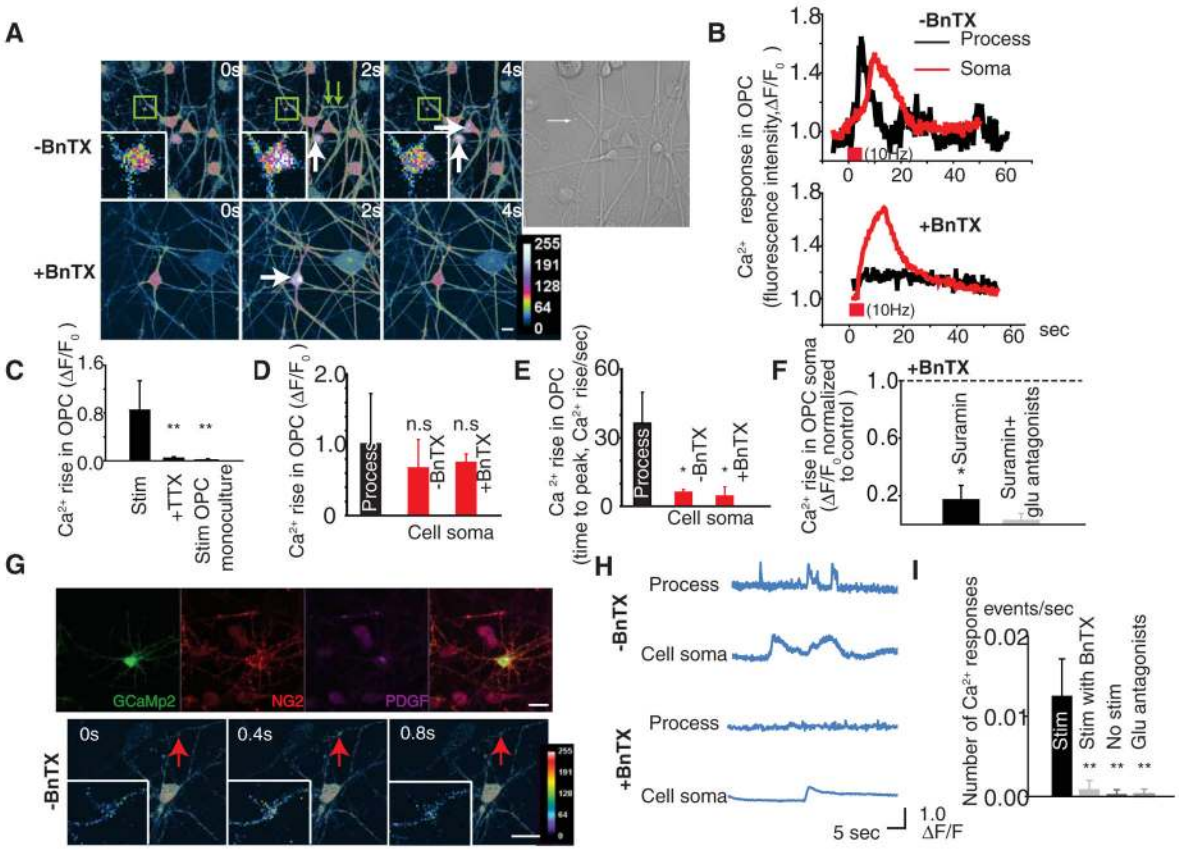

Fig. 2.

Electrical activity in axons is signaled to OPCs by the neurotransmitters glu and ATP. The two neurotransmitters are released through different mechanisms and produce different spatiotemporal Ca^{2+} responses in OPCs. (**A**) Ca^{2+} responses were seen in the cell body of OPCs (white arrows), using the Ca^{2+} indicator Oregon Green 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid 1, AM ester form (BAPTA-1 AM), in response to electrical stimulation of cocultures when synaptic vesicle release was blocked with BnTX, but responses in OPC cell processes (green box and arrows) were only seen when vesicular release was not blocked. Scale bar, 10 μm. (**B**) Plot of Ca^{2+} responses in the soma (red) and cell processes (black) of OPCs shown in (A). Note the absence of responses in OPC cell process on neurons when vesicular release was blocked with BnTx. Red bar, 10 Hz field stimulation. (**C**) No Ca^{2+} response was produced when action potentials were blocked with tetrodotoxin (TTX), or when stimulation was delivered to OPCs in monoculture (*$P < 0.005$, n = five dishes in each category). (**D**) The peak Ca^{2+} concentration and (**E**) rate of Ca^{2+} rise after stimulation were not statistically different in the cell body of OPCs (red) on neurons treated with BnTX. No Ca^{2+} responses were evident in the cell processes (black) after stimulating neurons treated with BnTX (n = 13 dishes for each condition). (**F**) Somatic Ca^{2+} responses were inhibited by the P2 receptor blocker suramin (50 μM) and were completely blocked by a combination of suramin and glu receptor antagonists AP5 (50 μM) and CNQX (20 μM). (**G**) The genetic Ca^{2+} reporter GCaMP2 transfected into OPCs (green) enabled Ca^{2+} responses to be measured specifically in OPCs. Immunological staining of chondroitin sulfate proteoglycan 4 (NG2, red) and platelet-derived growth factor receptor (PDGFR) (purple). (**H**) Ca^{2+} responses were not seen when GCaMP2 was used in OPC cell processes and vesicular release was blocked by BnTX. (**I**) Summary data showing Ca^{2+} responses measured by GCaMP2. Glu antagonists were 20

μM CNQX + 50 μM AP5 + 500 μM MCPG [**$P < 0.001$ (black bar); $n = 45$ cells.] Ca^{2+} responses after 5 hours of stimulation were similar to acute responses (fig. S8).

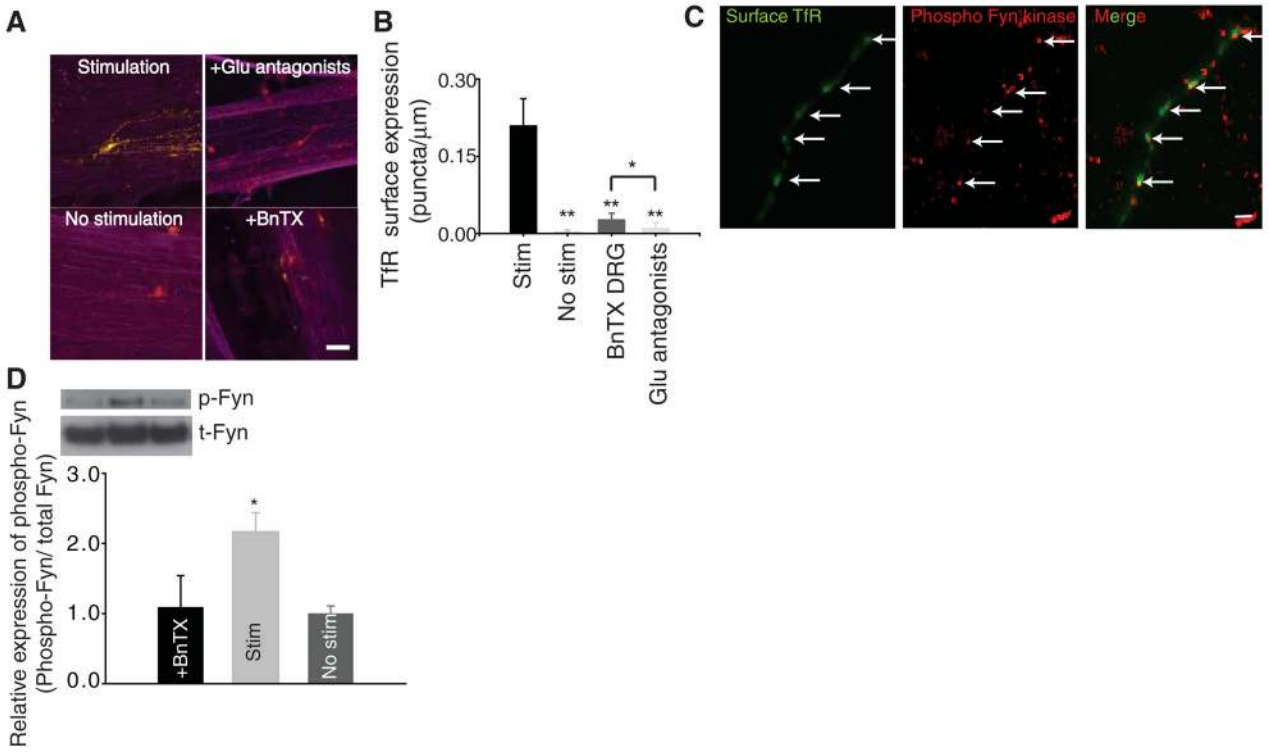

Fig. 3.

Formation of axon-oligodendrocyte signaling domains by electrical stimulation. Trafficking of TfR into cholesterol-rich membrane domains of OPCs was increased by the vesicular release of glu from neurons induced by electrical stimulation. (**A**) Punctate expression of TfR on the membrane (yellow) was greatly increased by 5 hours of electrical stimulation (10 Hz, 9 s, at 5-min intervals) of neurons (purple, neurofilament), but BnTX treatment or glu receptor antagonists (20 μM CNQX + 50 μM AP5), strongly inhibited trafficking of TfR into the membrane induced by electrical stimulation. Scale bar, 10 μm. (**B**) Stimulation of neurons treated with BnTX or in the presence of glu receptor antagonists inhibited trafficking of TfR receptor into cholesterol-rich membrane domains after stimulation (*$P <$ 0.005; **$P <$ 0.001; $n = 21$ cells from 21 dishes in each condition). (**C**) Phosphorylated Fyn kinase (red) colocalized with TfR receptor (green), consistent with axo-glial signaling localized at cholesterol-rich microdomains. (**D**) Electrical stimulation increased phosphorylation of Fyn kinase (p-Fyn), and this was blocked by BnTX treatment.

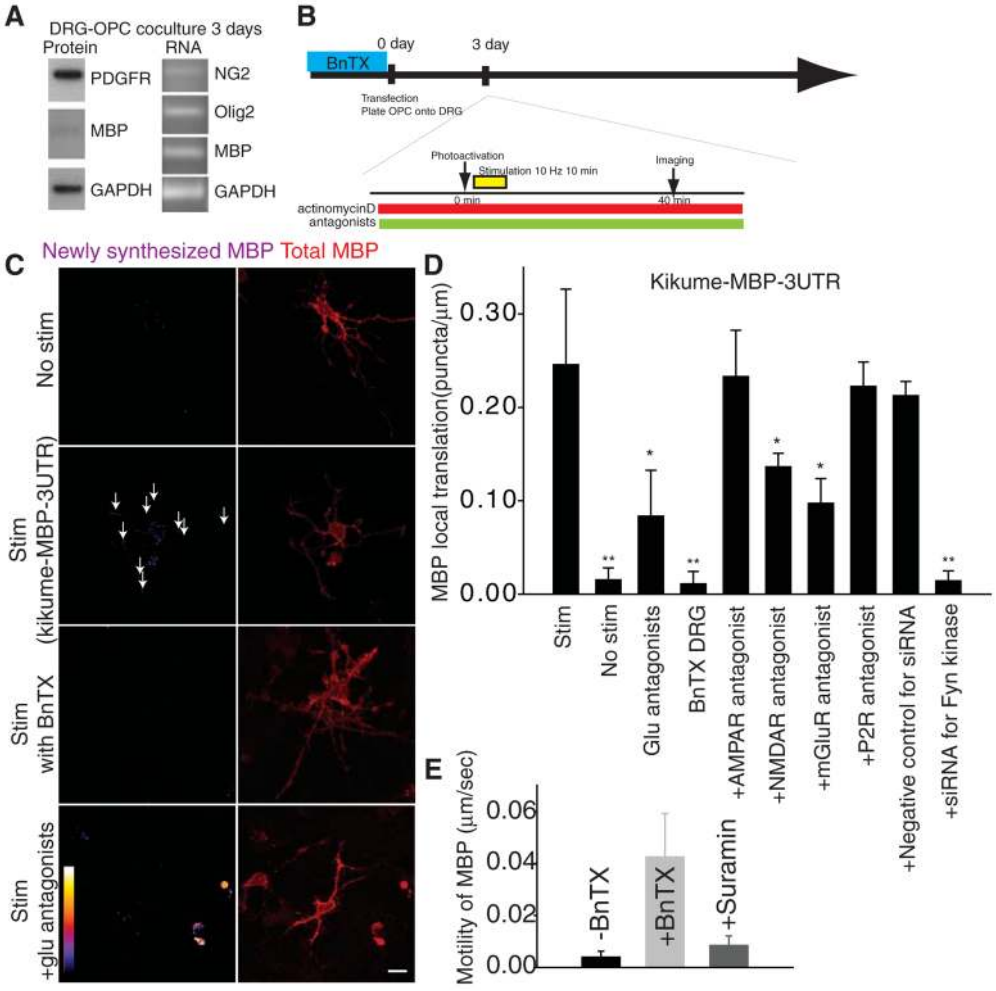

Fig. 4.

Action potentials induce local translation of MBP by vesicular release of glu. (**A**) Experiments were carried out on rat OPCs before expressing MBP protein, although MBP mRNA was present. (**B**) Local translation of MBP was studied by transfecting OPCs with kikume protein. Actinomycin D was used to block transcription and the appearance of newly synthesized green-fluorescent MBP was monitored. (**C**) Electrical stimulation induced local translation in OPCs transfected with kikume-MBP-3′UTR (white arrows), which was inhibited by pre-treating axons with BnTX or stimulation in the presence of glu receptor antagonists (20 μM CNQX + 50 μM AP5 + 500 μM MCPG) (see also fig. S10). Scale bar, 10 μm. Pixel intensity is shown on an 8-bit pseudocolor scale. (**D**) Statistical analysis shows that the local translation was strongly increased by electrical stimulation and that blocking vesicular release with BnTX significantly decreased stimulus-induced local MBP translation, as did stimulation in the presence of NMDA or mGluR receptor antagonists or suppressing Fyn kinase with siRNA. AMPA receptor and P2 receptor antagonists were without significant effect (*$P < 0.005$; **$P < 0.001$; $n = 32$ cells for each condition, one cell sampled per dish). (**E**) MBP mobility was monitored using a photoactivatible GFP–MBP (fig. S12). The mobility of newly synthesized MBP was increased by blocking vesicular release from axons, but not by blocking P2 receptors with suramin ($P < 0.001$; $n = 17$ cells from 17 dishes for each condition).