Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Glia. Author manuscript; available in PMC 2020 October 01.

Published in final edited form as:

Glia. 2020 October ; 68(10): 2070–2085. doi:10.1002/glia.23827.

Mechanical stretch induces myelin protein loss in oligodendrocytes by activating Erk1/2 in a calcium-dependent manner

Jihyun Kim[1], **Alexandra A. Adams**[1], **Pradeepa Gokina**[1], **Brayan Zambrano**[1], **Jeyanthan Jayakumaran**[1], **Radek Dobrowolski**[1], **Patrice Maurel**[1], **Bryan J. Pfister**[2], **Haesun A. Kim**[1]

[1]Department of Biological Sciences, Rutgers University, Newark, NJ 07102

[2]Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ 07103

Abstract

Myelin loss in brain is a common occurrence in traumatic brain injury (TBI) that results from impact-induced acceleration forces to the head. Fast and abrupt head motions, either resulting from violent blows and/or jolts, cause rapid stretching of the brain tissue, and the long axons within the white matter tracts are especially vulnerable to such mechanical strain. Recent studies have shown that mechanotransduction plays an important role in regulating oligodendrocyte progenitors cell differentiation into oligodendrocytes. However, little is known about the impact of mechanical strain on mature oligodendrocytes and the stability of their associated myelin sheaths. We used an *in vitro* cellular stretch device to address these questions, as well as characterize a mechanotransduction mechanism that mediates oligodendrocytes' responses. Mechanical stretch caused a transient and reversible myelin protein loss in oligodendrocytes. Cell death was not observed. Myelin protein loss was accompanied by an increase in intracellular Ca^{2+} and Erk1/2 activation. Chelating Ca^{2+} or inhibiting Erk1/2 activation was sufficient to block the stretch-induced loss of myelin protein. Further biochemical analyses revealed that the stretch-induced myelin protein loss was mediated by the release of Ca^{2+} from the endoplasmic reticulum (ER) and subsequent Ca^{2+}-dependent activation of Erk1/2. Altogether, our findings characterize an Erk1/2-dependent mechanotransduction mechanism in mature oligodendrocytes that destabilizes the myelination program.

Keywords

myelin; oligodendrocytes; TBI; Erk1/2; calcium; MBP; mechanotransduction

Introduction

Myelin, and its organization into the myelin sheath, allows for the rapid transmission of nerve impulses, as well as for the trophic support of the underlying axons. Therefore, proper

Corresponding Author: Haesun A. Kim, Rutgers University, Department of Biological Sciences, Boyden Hall Rm 206, 195 University Ave, Newark NJ 07102, Telephone: (973) 353-1454, Fax: (973) 353-1007, haekim@newark.rutgers.edu.

Data availability statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

myelination as well as myelin maintenance is important for the normal function of the nervous system. In the central nervous system (CNS), myelin is generated by oligodendrocytes, and the molecular mechanisms that regulate oligodendrocyte differentiation and myelination have been extensively studied. In recent years, mechanotransduction has emerged as an important developmental regulator of the oligodendrocyte lineage (A. Jagielska et al., 2017; Lourenco & Graos, 2016; Makhija et al., 2018; Shimizu et al., 2017).

Mechanotransduction refers to a process by which an extracellular mechanical stimulus is transduced into intracellular biochemical signals that in turn elicit a cellular response. Mechanotransduction plays a significant role in many cell types, by influencing cell behavior during development as well as under pathological conditions. In oligodendrocyte progenitor cells (OPCs), the mechanical tension created by substrate stiffness modulates OPCs differentiation into oligodendrocytes (Anna Jagielska et al., 2012; Kippert, Fitzner, Helenius, & Simons, 2009; Lourenco & Graos, 2016; Urbanski et al., 2016). The spatial constraints and tensile strain applied by mechanical forces induce OPCs differentiation through global changes in gene expression (Hernandez et al., 2016; A. Jagielska et al., 2017; Makhija et al., 2018; Rosenberg, Kelland, Tokar, De La Torre, & Chan, 2008). Neural stem cell differentiation into oligodendrocyte lineage cells is also regulated by ECM-dependent mechanotransduction (Arulmoli et al., 2015). These studies show that stem cells and OPCs are mechano-sensitive, and suggest that local changes in the mechanical environment of cells within the brain, under pathological or trauma-induced conditions, are likely to dysregulate the development and/or differentiation of oligodendrocyte lineage cells. The impact of mechanical strain on mature, differentiated oligodendrocytes has however not been fully elucidated.

Traumatic brain injury (TBI) is brain tissue damage that results from impact-induced violent acceleration forces to the head. White matter tracts are particularly vulnerable to TBI, as fast and abrupt head motions lead to the rapid stretching of the long myelinated axons. Recent advances in neuro-imaging in humans and experimental animals have revealed widespread myelin damage within white matter tracts after TBI. Even mild forms of TBI do result in significant damage to myelin integrity (Jurick et al., 2016; Panenka et al., 2015; Wright et al., 2016). Interestingly, myelin loss in the absence of oligodendrocyte death has also been reported in TBI brains (Mierzwa, Marion, Sullivan, McDaniel, & Armstrong, 2015; Sullivan et al., 2013). Furthermore, myelin detachment and disruption of paranodes organization occurs within few days of injury, an indication of early myelin damage (Marion, Radomski, Cramer, Galdzicki, & Armstrong, 2018). The absence of oligodendrocytes death indicates that an active signaling mechanism may trigger myelin loss in oligodendrocytes. We reasoned that mature oligodendrocytes are responsive to the stretch-induced mechanical strain associated with impact-induced violent head motions, which initiates an intracellular mechanotransduction process that destabilizes myelin.

In this study, we used an *in vitro* cell-stretch device (Magou et al., 2011) to elucidate stretch-induced mechanotransduction in mature oligodendrocytes and determine its impact on myelin protein expression. Our results show that mechanical stretch triggers a transient and reversible myelin protein loss in oligodendrocytes without cell death. The response is

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

initiated by a release of intracellular Ca^{2+} from ER storage, which then activates Erk1/2. Accordingly, blocking the Ca^{2+} release or inhibiting the activation of Erk1/2 attenuates the stretch-induced loss of myelin protein in oligodendrocytes. Furthermore, ectopic increase of Ca^{2+} is sufficient to trigger myelin protein loss in an Erk1/2-dependent manner. Finally, we show that elevating Erk1/2 activity in myelinated oligodendrocytes results in myelin breakdown. Altogether, our study demonstrates a mechanism of mechanotransduction in mature oligodendrocytes that utilizes a Ca^{2+}-dependent activation of Erk1/2 signaling axis to destabilize the myelin.

Materials and Methods

Culture media

The following culture media were used: MEM-C media (10% FBS, 0.6% glucose, 0.1 mg/ml penicillin-streptomycin, 1% glutamate in MEM); N2B2 [DMEM/F-12 (1:1) with 0.6mg/ml BSA, 10ng/ml d-Biotin, 20nM progesterone, 100nM putrescine, 5ng/ml selenium, 50μg/ml apo-transferrin, and 0.1 mg/ml penicillin/streptomycin]; N2S media [66% of N2B2, 33.5% of B104-conditioned media, 5ng/ml FGF-2 and 0.5% FBS]; NB media [Neurobasal medium with 2% B-27 supplement, 0.08% glucose, 1% glutamine, 0.1 mg/ml penicillin/streptomycin, and 50ng/ml nerve growth factor (NGF; Flarlan Laboratories, Inc.); NB-T3 media (NB media with 2% B-27 supplement, 30ng/ml 3,3',5-Triiodo-L-thyronine sodium salt (T3), 1% glutamine, and 0.1 mg/ml penicillin/streptomycin); advanced 293FT media (advanced DMEM with 10% FBS, 1% glutamine, 1% (v/v) chemically defined lipid concentrate and 0.03mM cholesterol]; C10 media (MEM containing 10% FBS, 0.08% glucose, and 50ng/ml NGF).

Antibodies

The following primary antibodies were used for immunofluorescence analyses: mouse anti-pErk1/2 (1:300, Cell Signaling #9106), mouse anti-MBP (1:300, Covance #SMI-94R), rabbit anti-Olig2 (1:10,000, kind gift from Dr. Charles Stiles, Dana-Farber Cancer Institute), and chicken anti-tubulin (1:1000, Aves #TUJ). Secondary antibodies were donkey anti-mouse Alexa 488 (Jackson ImmunoResearch #715-545-151); donkey anti-mouse rhodamine-X (Jackson ImmunoResearch #715-295-151); donkey antichicken Alex 488 (Jackson ImmunoResearch #703-545-155). All secondary antibodies were used at 1:250. The following primary antibodies were used for Western blot analyses: mouse anti-pErk1/2 (1:1000, Cell Signaling #5726), rabbit anti-total Erk1/2 (1:1000, Promega #V114A), rabbit anti-MBP (1:1000, Chemicon #AB980) and mouse anti-ß-actin (1:5000, Sigma #A5441).

Oligodendrocyte progenitor cell isolation and culture

The cerebral cortex from the frontal lobes was dissected out from postnatal day 1-2 rat brains. Meninges were removed from the surface of the isolated brain tissues before dissociation by pipetting in L15 media. Cells were filtered through a 40μm cell strainer. Harvested cells were centrifuged at 200x*g* at room temperature, and resuspended in MEM-C media. Cells were then plated at a density of one brain per T75 flask and cultured (37°C, 5% CO_2) in MEM-C media. The mixed glial cell cultures were replenished with fresh MEM-C media every 5-7 days for about ten to twelve days, at which time oligodendrocyte progenitor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cells (OPCs) were isolated. Briefly, the mixed glial cell cultures were shaken at 200rpm for two hours (37°C) to remove microglial cells, and placed in fresh MEM-C media. Two hours later, the cultures were re-shaken overnight to obtain the OPCs. The following day, the cell suspension containing the OPCs was passed through a 20μm pore-sized nylon mesh. The isolated OPCs that passed through the mesh were centrifuged at 200x*g* for 10 minutes and then plated on a ø100mm tissue culture plate for 10 minutes in N2B2 media to allow contaminating astrocytes to attach while the OPCs remained in suspension. The media was collected from the plate and centrifuged at 200x*g* for 10 minutes. Harvested OPCs were resuspended in N2S media and plated on either PLL-laminin-coated silicone membrane injury wells at a density of $7x10^4$ cells per well, or on PDL-laminin-coated ø10mm glass coverslips (Corning) at a density of $2x10^4$ cells per coverslip. Plating culture media (N2S) was switched to NB-T3 media 24 hrs after plating, and the cultures were kept for 5 days to induce the differentiation of the OPCs into mature oligodendrocytes to be used in later experiments. OPCs differentiation into mature oligodendrocytes was verified by immunostaining for MBP.

Assembly of cellular stretch device and subjecting oligodendrocytes to mechanical stretch

The stretch well in which oligodendrocytes were cultured consisted of two PEEK rings (circular frames), an O-ring and a 3.5x3.5 cm piece of silicone membrane (0.005” gloss/ gloss silicone sheeting, Specialty Manufacturing, Inc.). Assembly and use have been described previously (Magou et al., 2011). Briefly, PEEK rings were cleaned by sonication in Dl water and silicone membranes were rinsed with Dl water. Between the PEEK rings, the inner PEEK ring has a groove for the O-ring insertion. The silicone membrane was placed over the outer PEEK ring and the inner PEEK ring, with O-ring inserted, was placed on top of the silicone membrane and gently pressed into the outer one. Assembled wells were sonicated and autoclaved in Dl water. The wells were air-dried and coated with PLL-laminin before use.

The *in-vitro* stretch device is comprised of a pressure chamber attached to an air pulse generating system. Prior to subjecting the silicone membrane to stretch, a rigid mask with a 2-mm wide by 16-mm long rectangular opening at its center was placed underneath the membrane, thereby restricting the stretch area to the membrane directly overlapping with the rectangular opening. After differentiation of the OPCs into oligodendrocytes, the cultures were stretched by applying a controlled pressure-pulse of air towards the silicone membrane, which resulted in the deformation of the silicon membrane within the rectangular opening. Oligodendrocytes present at the level of the rectangular opening were therefore submitted to stretch, whereas the oligodendrocytes located on the silicon membrane that was supported by the rigid mask did not experienced any stretch (non-stretched). This set-up allowed for experimental oligodendrocytes (stretched) and control oligodendrocytes (non-stretched) to be cultured in one well.

The parameter for the membrane stretch is reported in terms of strain, which is defined as the ratio of membrane length with deformation over original (un-deformed) length. The rate of injury is measured in terms of strain rate, which is defined as the rate at which a particular strain is used to stretch the membrane. In the present study, membrane stretching was

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

performed at the setting of 70% strain at a strain rate of $30s^{-1}$ (equivalent to a stretch injury rise time of 20 ms), similar to our previous studies (Magou, Pfister, & Berlin, 2015).

Immunofluorescence staining

In all cases, cultures were fixed in 4% paraformaldehyde for 20 minutes at room temperature. Double immunostaining for myelin proteins (MBP, PLP, MAG) and Olig2: fixed cultures were washed with PBS, permeabilized with cold-methanol (−20°C, 20 minutes), then washed again with PBS. Cultures were then blocked (10% normal donkey serum, 0.3% Triton X-100, prepared in PBS) for one hour at room temperature and incubated overnight at 4°C with primary antibodies prepared in blocking solution. After washing with PBS, cultures were incubated for one hour at room temperature with secondary antibodies prepared in PBS. Cell nuclei were stained with DAPI for 10 minutes at room temperature. The percentage of MBP (PLP, MAG)-positive oligodendrocytes was calculated as the ratio of MBP and Olig2 co-labeled cells over total Olig2-positive cells. Double immunostaining for phospho-Erk1/2 and Olig2: fixed cultures were washed with TBS, permeabilized in TBST (TBS supplemented with 0.02% Triton X-100) for one hour at room temperature, then washed with TBS. Cultures were blocked (10% normal donkey serum in TBS) for one hour, then incubated for 48 hours at 4°C with primary antibodies prepared in blocking solution. After washing with TBS, the cultures were incubated overnight at 4°C with secondary antibodies prepared in TBS. Cultures were then washed with TBS and cell nuclei stained with DAPI for 10 minutes at room temperature. The percentage of phospho Erk1/2-positive oligodendrocytes was calculated as the ratio of phospho Erk1/2 and Olig2 co-labeled cells over total Olig2-positive cells. Double immunostaining for Tubulin and MBP: fixed cultures were washed with PBS, permeabilized in cold-methanol for 10 minutes at −20°C, and then washed with PBS. Cultures were then blocked (10% normal donkey serum in PBS supplemented with 0.3% Triton X-100) for one hour at room temperature, and then incubated overnight at 4°C with primary antibodies prepared in blocking solution. After washing with PBS, cultures were incubated for one hour at room temperature with secondary antibodies prepared in PBS. Cell nuclei were stained with DAPI for 10 minutes at room temperature. Following washing with PBS, coverslips were mounted on the slides with Fluoromount-G solution (#0100-01, Southern Biotech). Fluorescent images were captured on either a Carl Zeiss MicroImaging LSM 510NLD Meta laser scanning multi-photon confocal microscope (x40/1.3), or a Nikon Eclipse TE2000-U (x20/0.75 and x40/1.30) microscope equipped with a Hamamatsu Photonics camera and the MetaMorph software (Molecular Devices).

TUNEL assay

To detect oligodendrocytes undergoing apoptosis, cultures were first immunostained for Olig2 before detecting apoptotic nuclei by the TUNEL assay. Briefly, cultures were fixed in 4% paraformaldehyde for 20 minutes. After washing with PBS, fixed samples were immunostained for Olig2 as described in the previous section, up to the secondary antibodies incubation step and subsequent washes included. Samples were then post-fixed with 4% paraformaldehyde for 25 minutes at 4°C and washed with PBS. After permeabilization with a 0.2% Triton X-100 solution (prepared in PBS), apoptotic cells were detected using the DeadEnd™ Fluorometric TUNEL system according to the manufacturer's protocol

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(Promega). Briefly, the post-fixed and Triton-permeabilized cultures were washed 2 x 5 minutes with PBS at room temperature. After removal of excess PBS, the cultures were incubated with equilibration buffer for 10 minutes at room temperature, at which time the buffer was replaced by the reaction buffer (5μl of nucleotide mix, 1 μl of the rTdT enzyme in 50μl of equilibration buffer) and the cultures incubated for an additional one hour at 37°C. The reaction was stopped by washing the cultures with 2x SSC for 15 minutes, at room temperature. Cultures were further washed with PBS, stained with DAPI for 10 minutes at room temperature, and mounted on the slides with Fluoromount-G solution.

Ca^{2+} imaging using Fluo-4 AM and analysis

The Ca^{2+} response in oligodendrocytes submitted to stretch was measured using the cell-permeant Ca^{2+} dye Fluo-4 AM (excitation at 488nm, green fluorescence emission). Oligodendrocytes cultured on silicone membrane were washed with PBS and loaded with non-ratiometric Fluo-4 AM (2 mM) mixed with an equal volume of Pluronic-F127 detergent in PBS, for 30 minutes at 37°C. After washing with PBS, the cultures were allowed to sit for 10 minutes prior to imaging in PBS. Images were captured at 2-second intervals for 30 second prior to applying stretch, and image capture (at 2-second intervals) continued for another 2 minutes following the stretch. Image analysis was performed offline using the ImageJ software. In each image (each representing a different time point), every cell was selected as an individual region-of-interest (ROI) using the circle selection tool (all circles were kept constant in size), and the fluorescence integrated intensity (sum of pixel intensities within ROI) measured. In each image, an additional 30 similarly sized circles in areas without cells were measured to obtain an average background fluorescence integrated intensity, which was then subtracted to each cell's fluorescence integrated intensity. Changes in fluorescence intensity of any given cell overtime was defined as a function of $(F_t - F_0) / F_0$ (or $\Delta F/F_0$), where F_t is the fluorescence integrated intensity of that cell at time point "t", and F_0 is the average fluorescence integrated intensity of that cell derived from the 15 frames (30 second time frame) prior to stretch. The average from all cells $\Delta F/F_0$ at each time frame was plotted against time in Figure 4D. A cell was considered responsive to stretch if it displayed, after stretch, a maximum $\Delta F/F_0$ that was strictly 3 standard deviations higher than the mean $\Delta F/F_0$ that was measured from the 15 frames prior to stretch, for that particular cell. To measure the stretch-induced Ca^{2+} response in the absence of extracellular Ca^{2+}, calcium/magnesium-free PBS was used in place of the PBS described above. Images were acquired with a Nikon Eclipse TE-2000S inverted microscope equipped with a CoolSNAP E CCD camera (Teledyne Photometries) and the Nikon imaging software NIS-Elements D. Fluo-4 AM was excited at 488nm via a xenon light source and emitted fluorescence collected at 515nm.

Treatment with pharmacologic agents

For the Ca^{2+} studies, Ca^{2+} ionophore A23187 (Sigma-Aldrich #52665-69-7), Ca^{2+} chelator BAPTA (Sigma-Aldrich #A1076), IP3 receptor inhibitor 2-APB (Sigma-Aldrich #100065) and RyR calcium channel inhibitor ryanodine (Sigma-Aldrich #559276) were used. U0126 (Sigma-Aldrich #19-147) was used to inhibit MEK1/2 and prevent activation of downstream Erk1/2 signaling. To assess the impact of Ca^{2+} on Erk1/2 activation, oligodendrocyte cultures were incubated for 30 minutes either with A23187 (10μM), a combination of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

A23187 and U0126 (10μM), BAPTA (20μM) or a combination of 2-ABP (10μM) and ryanodine (50μM) (all agents prepared in NB-T3 media). A23187-treated cultures were then lysed in lysis buffer (25mM Tris pH 7.4, 1% SDS, 1mM EDTA pH 8.0, 95mM NaCI, 20μM leupeptin, 10μg/ml aprotinin, 1mM PMSF, 1mM sodium orthovanadate, and 10mM sodium fluoride), and analyzed for Erk1/2 activation by Western blotting. BAPTA- and 2-ABP/ryanodine-treated cultures were subjected to stretch and, 30 minutes later, cell lysates were prepared as above to assess Erk1/2 activity. To assess the impact of Ca^{2+} modulation on MBP expression, A23187 (10μM), combination of A23187 and U0126 (10μM each), BAPTA (20μM) or a combination of 2-ABP (10μM) and ryanodine (50μM) was added to oligodendrocytes in the NB-T3 media for 1 hour without (A23187) or after stretch was applied (BAPTA and 2-ABP/ryanodine) and removed. Cultures were then maintained in fresh NB-T3 media for another 24 hours before cells were collected for MBP expression by immunofluorescent staining or Western blot analysis.

Generation of lentivirus and infection of OPCs

Plasmid pBabe-Puro-MEK1-DD, which contains the cDNA encoding for the constitutively active (CA) form of MEK1, was a gift from William Flahn (Addgene plasmid #15268) (Boehm et al., 2007). PCR was used to amplify the MEK1-DD cDNA, and add Xhol / Spel restriction sites at each ends. The PCR product was then inserted into the entry vector pEN_TTmcs (a gift from lain Fraser, Addgene plasmid #25755)(Shin et al., 2006) via the Xhol / Spel restriction sites. LR clonase II (Invitrogen) was then used to mediate recombination of the MEK1-DD-containing entry vector with the doxycycline-inducible pSLIK lentiviral vector (Shin et al., 2006). Lentiviruses were produced as previously described (Yang et al., 2012). Briefly, the pSLIK-MEK1-DD lentiviral vector was transfected into 293FT cells together with packaging plasmids psPAX2 and pMD2.G (gifts from Didier Trono, Addgene plasmids #12260 and #12259, respectively) using the $CaPO_4$ transfection kit from Invitrogen. Five to six hours after transfection, media was removed and changed to fresh advanced 293FT media and cells incubated for 48-60 hours. The virus-containing culture medium was collected and centrifuged at 500xg for 15 minutes at room temperature. The supernatant was collected and centrifuged again at 500xg for 15 minutes. The virus-containing supernatant was used within 24 hours to infect OPCs. Viral media was supplemented with 5ng/ml PDGF and 5ng/ml bFGF-2 and then added onto purified OPCs. Eight to twelve hours later, the media was removed and cells were grown to confluence in N2S media. The induction of MEK-DD expression by doxycycline was verified by Western blotting for activated Erk1/2.

Dorsal root ganglion-OPC myelinating co-cultures

Oligodendrocyte myelinating co-cultures were prepared as previously described (Tyler et al., 2009). Dorsal root ganglion (DRG) neurons were dissected from E15 rat embryos and were dissociated in 0.25 % trypsin for 30 min at 37° C. The dissociated DRGs were plated onto ø10mm glass coverslips coated with growth factor reduced Matrigel (Corning #354230) at the density of one DRG/coverslip, in NB media. 6-8 hours after plating, the cultures were flooded in NB media supplemented with 15μM fluorodeoxyuridine (FUDR; Sigma) to remove non-neuronal proliferating cells. After 3 to 4 days, the cultures were switched to, and maintained in, NB media until the DRG neurites reached the periphery of the coverslips.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Oligodendrocyte progenitor cells were plated onto DRG neurons at a density of 100,000 cells/coverslip in C10 media. The DRG-OPC co-cultures were maintained for 18-21 days, during which time the OPCs differentiated into myelinating oligodendrocytes. During that period the cultures were supplemented with fresh medium every other days. After a sufficient number of myelin segments were formed, cultures were treated with bFGF (3.3 nM) alone or in combination with U0126 and the integrity of the MBP+ myelin segments were assessed 48 hours later.

Statistical Analysis

Data statistical analyses were performed using the GraphPad Prism software (version 8.1.1). The statistical tests used are indicated in the figure legends. Results with p values < 0.05 were considered significant.

Results

Mechanical stretch triggers myelin protein loss in oligodendrocytes

We used an *in vitro* cell-stretch device that has been described previously (Magou et al., 2011). The device uses computer-controlled air pressure to apply mechanical perturbation to cells cultured on a silicone membrane. The silicone membrane is held together by a two-piece circular frame (Figure 1A) that is placed on the stretch device. Prior to the stretch, a flat rigid mask with a 2-mm wide by 16-mm long rectangular opening in the mid-line is placed under the membrane. When air pressure is applied, the mask restricts membrane deformation only within the rectangular opening, causing uniaxial stretch on the cells above. The remaining area covered by the mask is left non-stretched. This feature allows for direct comparison between cell populations that are stretched (central area) and non-stretched (distal area), within the same culture (Magou et al., 2015) (Figure 1B). Immunostaining for myelin basic protein (MBP) and Olig2, a maker of the oligodendrocyte lineage showed that oligodendrocyte progenitor cells were capable of differentiating into mature oligodendrocytes when cultured on Matrigel-coated silicone membrane (Figure 1C). Furthermore, prior to mechanical stretch, 91% ± 2.2 and 92% ± 1.5 of the $Olig2^+$ cells cultured in the central and distal areas, respectively, expressed MBP, indicating that there was no significant difference ($p = 0.8149$, un-paired, two-tailed Student's t-test, $n = 3$) in oligodendrocyte differentiation between the two areas monitored (Figure 1C). These data showed that the experimental setup has in itself no significant effect on oligodendrocytes differentiation and that the cultures are homogeneous across the central and distal areas before applying mechanical stretch-induced injury.

The differentiated MBP^+ oligodendrocytes were then subjected to mechanical stretch (70% strain at rate of $30s^{-1}$) and the impact on myelin expression was analyzed 24 hours later. MBP and Olig2 double immunostaining revealed that many oligodendrocytes within the central area lost MBP protein expression (Figure 2A) compared to the oligodendrocytes in the distal area. Quantification showed that mechanical stretch induced a significant 37% decrease in the percentage of MBP^+ cells among the $Olig2^+$ cell population in the central area whereas it had no effect on the $Olig2^+$ cells in the distal area within the same culture (Figure 2B). Indeed the percentage of MBP^+ oligodendrocytes in the distal area after stretch

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(84%; Figure 2B, distal area, + stretch) was not significantly different to that observed before stretch (89%; Figure 2B, distal area, − stretch), nor was it different to that observed before stretch in the central area (87%; Figure 2B, central area, − stretch). We also observed that some of the oligodendrocytes in the central area that retained MBP expression after stretch exhibited an aberrant MBP distribution, such as loss of expression at the cell periphery (Figure 2C) while retaining expression in the perinuclear region. These oligodendrocytes did not show any apparent damage or retraction of the cytoskeletal network (tubulin) following mechanical stretch (Figure 2D). The loss of myelin marker expression by $Olig2^+$ oligodendrocytes is not restricted to MBP. A similarly significant decrease, by 41% and 47% respectively, in the number of MAG^+ (myelin associated glycoprotein) and PLP^+ (proteolipid protein) cells is also observed within the $Olig2^+$ oligodendrocytes population that was submitted to stretch (Figure 2E), central area versus distal area). These data underscore the localized effect of the mechanical stretch to oligodendrocytes in the central area of the culture, and strongly associate mechanical stretch with the loss of myelin protein expression.

The stretch-induced loss of myelin markers is transient and reversible

To determine whether loss of MBP+ oligodendrocytes after stretch was due to cell death, we performed TUNEL analysis. The percentage of TUNEL-positive cells among the $Olig2^+$ cells in the stretched area was extremely low, and no significant difference ($p = 0.1342$, un-paired, two-tailed Student's t-test, $n = 3$) was observed between non-stretched and stretched cultures (0% vs 0.13% ± 0.1, respectively). We also did not observe any aberrant nuclear morphology often associated with cell death in the $Olig2^+$ cells that are losing MBP expression (Figure 3A). These data indicate that the loss of myelin protein expression among the $Olig2^+$ population following mechanical stretch in the central area is not due to oligodendrocytes' death.

The lack of cell death, as well as the apparent lack of physical damage of oligodendrocytes subjected to stretch suggested the possibility that the loss of myelin marker expression might be transient and reversible. Differentiated oligodendrocytes were submitted to mechanical stretch (70% strain at rate of $30s^{-1}$) and the impact on myelin expression was analyzed over time (24 and 48 hours later), by MBP and Olig2 double immunostaining (Figure 3B). Non-stretched sister cultures were also similarly analyzed. As shown above, the number of MBP^+ cells within the $Olig2^+$ population was significantly decreased (by 56%) in the central area 24 hours after stretch, compared to $MBP^+/Olig2^+$ cells within the central area of non-stretched cultures (Figure 3B, t = 24). At 48 hours, the percentage of MBP^+ cells within the stretched area recovered to the levels in non-stretched sister cultures (Figure 3B, t = 48; 72% versus 71%). The number of $MBP^+/Olig2^+$ cells in the central area of non-stretched cultures did not significantly change during the 48 hours experimental period (Figure 3B, compare t = 0 to t = 24 and t = 48, − stretch), indicating that the reappearance of the $MBP^+/Olig2^+$ cells in stretched cultures was not due to an overall increase in oligodendrocyte differentiation over time. Altogether these data, along with the lack of cell death and physical damage, suggest that most of the $Olig2^+$ cells that lost expression of MBP 24 hours after stretch reexpressed the myelin marker within 48 hours post-stretch.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Stretch-induced loss of MBP is mediated by Erk1/2 activation

The myelin protein loss in the absence of cell death prompted us to consider a possibility that the oligodendrocytes response was mediated by an active intracellular event triggered by the mechanical stimulus. In many cell types, Erk1/2 is an important component of mechanotransduction (Martineau & Gardiner, 2001; Ruwhof & van der Laarse, 2000; Ryder et al., 2000). More particularly, Erk1/2 has been shown to disrupt myelin when ectopically activated in white matter oligodendrocytes (Ishii, Furusho, Dupree, & Bansal, 2016). To determine whether Erk1/2 played a role in triggering MBP loss after stretch, we assessed whether mechanical stretch activated Erk1/2 in oligodendrocytes. As shown in Figure 4, panels A and B, basal level of Erk1/2 activation was extremely low (0.3%) in the non-stretched (distal) area. Mechanical stretch initiated a significant Erk1/2 activation in the central, i.e. stretchable area of the culture, as monitored by the immuno-reactvity to phospho-Erk1/2 in $Olig2^+$ oligodendrocytes (38%). Treatment with the MEK1/2 inhibitor U0126 completely abolished stretch-induced Erk1/2 activation (Figure 4B), keeping the number of phospho-Erk1/2 immuno-reactive oligodendrocytes to non-detectable levels. As previously shown, mechanical stretch of the central area induced a substantial and significant (36%) decrease in the number of MBP^+ oligodendrocytes, whereas MBP expression by oligodendrocytes in the distal area was not affected (89% vs 86%, no stretch vs stretch, $p = 0.3633$) (Figure 4C). An intriguing point was the similarity, in the stretched central area, between the number of phospho-Erk1/2 positive oligodendrocytes (38%) and the number of oligodendrocytes that lost MBP expression (36%), which suggested a potential causal relationship. Interestingly, U0126 treatment blocked the stretch-induced loss of MBP expression in oligodendrocytes in the central area, the number of MBP^+ oligodendrocytes (85%) remaining identical to that of non-stretched, untreated cultures (87%; $p = 0.5969$) (Figure 4C). These data indicates that the MBP loss in oligodendrocytes subjected to mechanical stretch was mediated by Erk1/2 activation. The U0126 drug did not affect the oligodendrocytes in the distal area.

Mechanical stretch initiates intracellular Ca^{2+} increase in oligodendrocytes

Mechanical stimuli often trigger an increase in intracellular Ca^{2+} either by influx of extracellular Ca^{2+} through cell surface Ca^{2+} channels, or by release of Ca^{2+} from intracellular stores. Furthermore Ca^{2+}-dependent Erk1/2 phosphorylation has been shown to mediate cellular responses to mechanical stimuli (Gudipaty et al., 2017; Iqbal & Zaidi, 2005; Liu et al., 2008; Momberger, Levick, & Mason, 2006). To determine whether a Ca^{2+} to Erk1/2 signaling axis played a role in mediating oligodendrocytes response to stretch, oligodendrocytes were loaded with the fluorescent Ca^{2+} indicator Fluo-4 AM and then subjected or not to mechanical stretch. Changes in fluorescence integrated intensity in each cell were recorded in 2 seconds increments for 30 seconds before, and 2 minutes after mechanical stretch was applied. Panel A in Figure 5 (+ $[Ca^{2+}]_{extra}$ experiments) shows representative images of Fluo-4 AM fluorescence capture 6 seconds prior and 6 seconds after stretch (− stretch vs + stretch, respectively). While very few cells exhibited changes in fluorescence integrated intensity during the 30 seconds prior to mechanical stretch (2% ± 1.1), stretch induced in a significant increase in the percentage of cells (80%) that exhibited a significant increase in fluorescence integrated intensity above the baseline. Interestingly, with values of 1% ± 0.7 and 76% ± 7.9 (− stretch and + stretch, respectively), the absence of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

calcium in the extracellular media did not affect the number of cells that showed fluorescence integrated intensity above baseline prior mechanical stretch (Figure 5, panels B and C). The response to stretch was rapid, with the maximum change in fluorescence intensity (as an average of all cells at each time point) achieved within 6 seconds after submitting the oligodendrocytes to mechanical stretch (Figure 5D). Fluorescence intensity returned to basal level within 2 minutes post stretch. Presence or absence of calcium in the extracellular media had no effect on fluorescence intensity. All together, these data showed that mechanical stretch induced an immediate increase in intracellular calcium in oligodendrocytes submitted to mechanical stretch, and that intracellular Ca^{2+} storage likely contributed to the Ca^{2+} increase.

Increase in intracellular Ca^{2+} activates Erk1/2 in oligodendrocytes following mechanical stretch

The next step was to determine whether Ca^{2+} increase triggered Erk1/2 activity in oligodendrocytes upon mechanical stretch. To that effect, mechanical stretch was done in the presence of BAPTA, a membrane permeable Ca^{2+} chelator, and Erk1/2 activation was assessed 30 minutes later. Treatment with BAPTA resulted in a 71% reduction in the stretch-induced Erk1/2 activation (Figure 6A). Absence of Ca^{2+} in the extracellular media did not prevent Erk1/2 activation (Figure 6B), suggesting that it was the release of Ca^{2+} from intracellular stores that contributed to the Erk1/2 activation. Supporting this interpretation of the data, stretch-induced activation of Erk1/2 was substantially prevented (down by 70%) when oligodendrocytes were treated with 2-ABP and ryanodine, which respectively blocks the release of stored Ca^{2+} through IP3 and calcium channel receptors on the endoplasmic reticulum (Figure 6C).

Stretch-induced intracellular Ca^{2+} increase triggers myelin protein loss in an Erk1/2-dependent manner

Treating oligodendrocytes with BAPTA or 2-ABP/ryanodine during the first hour following mechanical stretch was sufficient to block the loss of MBP expression observed 24 hours later (Figure 6D). Oligodendrocytes in the distal area (non-stretched) of the same culture were not affected by the drug treatment. These results strongly suggest that the loss of MBP expression in stretched oligodendrocytes results from, in part, the activation of Erk1/2 by Ca^{2+} released from internal stores upon mechanical stretch.

We then determined whether a transient Ca^{2+} increase was sufficient to mimic the stretch response in oligodendrocytes. Treatment of oligodendrocytes with the membrane permeable Ca^{2+} ionophore A23187 was sufficient to activate Erk1/2 within 30 minutes (Figure 6E). Furthermore a transient one-hour treatment with A23187 was sufficient to induce a significant down-regulation (by 31%) of MBP expression 24 hours later (Figure 6, panels F and G), indicating that an early Ca^{2+}-dependent event initiated the MBP loss. Co-treatment with the MEK1/2 inhibitor U0126 completely prevented the A23187-induced MBP loss (Figure 6, panels F and G). Altogether, our data showed that a Ca^{2+}-dependent Erk1/2 activity contributed to myelin protein loss in oligodendrocytes.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ectopic activation of Erk1/2 is sufficient to induce myelin protein loss in oligodendrocytes

Next, we investigated the direct effect of Erk1/2 activation in mature oligodendrocytes. We generated OPCs that expressed a constitutively active form of MEK1 (MEK1-DD) under the control of a doxycycline-inducible promoter. The MEK1-DD-transduced OPCs were differentiated into mature oligodendrocyte, after which the cultures were treated with doxycycline to induce MEK1-DD expression and thus Erk1/2 activation (Figure 7A). Loss of MBP expression was detected in the doxycycline treated cultures (Figure 7A), and immunostaining analysis showed that ectopic activation of Erk1/2 coincided with MBP loss in individual oligodendrocytes (Figure 7B), indicating that ectopic Erk1/2 activity contributed to the myelin protein loss.

Finally, we investigated whether Erk1/2 activity induced by an extracellular stimulus could induce the breakdown of myelinated segments formed by oligodendrocytes. We treated myelinating oligodendrocytes/DRG neurons co-cultures with FGF-2 (3.3 nM), an oligodendrocyte mitogen that is known to activate the Erk1/2 pathway. As shown in Figure 7C, FGF-2 induced a strong and rapid (within 15 minutes) activation of Erk1/2 that lasted at least 6 hours. Within forty-eight hours of FGF-2 treatment, extensive myelin segments breakdown (73%) was observed in the FGF-2 treated cultures (Figure 7, panels E and F). Inhibiting Erk1/2 activation with U0126 (Figure 7D) blocked FGF-2-induced myelin breakdown in a concentration-dependent manner. These results indicate that activation of Erk1/2 signaling pathway by extracellular stimuli could contribute to myelin breakdown in oligodendrocytes. More important, the data suggest that targeted inhibition of Erk1/2 pathway provides protection to the myelin against the extracellular stimuli.

Discussion

In the present study, we demonstrate that physical strain applied by mechanical stretch causes myelin protein loss in differentiated oligodendrocytes. The loss is transient however, and the oligodendrocytes regain the ability to express myelin proteins afterwards. Stretch-induced myelin protein loss is mediated by Ca^{2+}-dependent Erk1/2 activation. Inhibiting intracellular Ca^{2+} increase or blocking Erk1/2 activation both attenuate the oligodendrocyte response. We also show that ectopic Ca^{2+} increase is sufficient to activate Erk1/2 in oligodendrocytes, which in turn leads to myelin protein loss. Erk1/2 activation is sufficient to mimic oligodendrocytes' response to mechanical stimulus, indicating the importance of Erk1/2 signaling in mediating this process. Most importantly, myelin protein loss occurs in the absence of oligodendrocytes' death, indicating an active intracellular mechanism that destabilizes the myelin.

Erk1/2 is a key regulator of myelin homeostasis in oligodendrocytes. During CNS development, Erk1/2 activation in the oligodendrocyte lineage correlates temporally with the period of myelination: the activation level is at the highest during active myelination then declines in the adult CNS (Ishii, Furusho, & Bansal, 2013). *In vivo* studies using oligodendrocyte-specific Erk1/2 knockdown mice showed that while Erk1/2 is dispensable for oligodendrocyte differentiation and the initiation of myelination, it is crucial for the subsequent myelin growth (Ishii, Fyffe-Maricich, Furusho, Miller, & Bansal, 2012). Other studies showed that elevating Erk1/2 activity in oligodendrocytes during active myelination

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

results in thinner myelin formation (Fyffe-Maricich, Schott, Karl, Krasno, & Miller, 2013; Ishii et al., 2013). In adult brain, Erk1/2 activity in oligodendrocyte promotes myelin plasticity and allows new myelin growth (Jeffries et al., 2016), however a prolonged and excessive activation destabilizes the myelin (Ishii et al., 2016). Our findings show that in cultured oligodendrocytes, re-activation of Erk1/2 by an extracellular stimulus, such as mechanical stretch or growth factor stimulation, triggers myelin loss. Traumatic brain injury induces acute Erk1/2 activation in the brain white matter (Raghupathi, Muir, Fulp, Pittman, & McIntosh, 2003). Erk1/2 activation in oligodendrocytes by hypoxia-induced ischemia has also been reported (Wang et al., 2003). It is possible that under a pathologic condition, hyper-elevated Erk1/2 activity in mature oligodendrocytes may be detrimental to the myelin. Furthermore, our data suggest that a targeted inhibition of the Erk1/2 activity may provide myelin protection following such brain insults.

Calcium plays an important role in the translation of mechanical forces into intracellular signals. Our data show that stretch-induced Erk1/2 activation and subsequent myelin protein loss is Ca^{2+}-dependent. Intracellular Ca^{2+} increase occurs immediately after oligodendrocytes are subjected to mechanical stretch. Interestingly, the Ca^{2+} increase was not dependent on the presence of extracellular Ca^{2+}, indicating a contribution from the intracellular stores. In support of this, we show that the inhibition of IP3 receptor and ryanodine-sensitive receptor on the ER blocks stretch-induced Ca^{2+} increase. The mechanism by which mechanical stretch initiates the release of Ca^{2+} from the ER in oligodendrocytes is unknown. Cell surface receptors such as G-protein coupled receptors (GPCRs) and receptor tyrosine kinases activate phospholipase C, which in turn hydrolyze PI(4,5)P2 to produce IP3. IP3 then diffuses into the cytoplasm and binds to IP3 receptors on the ER, promoting the release of stored Ca^{2+}. Mechanosensitive GPCRs that are coupled to PLC activity have been identified in other cell types (Mederos y Schnitzler et al., 2008; Xu et al., 2018; Zou et al., 2004), however the expression of such GPCRs in oligodendrocytes has not been elucidated.

Another possible trigger to release stored Ca^{2+} is stretch-induced integrin signaling. Mechanical tension applied to cells by the extracellular environment is transmitted through integrins (Z. Sun, Guo, & Fassler, 2016), which link components of the ECM to the intracellular cytoskeleton network. Changes in physical properties of the ECM, such as stiffness or deformation, trigger integrin signaling through the activation of associated cytoplasmic adapters and signaling proteins. Calcium-dependent mechanotransduction by integrin has also been reported (Sasamoto et al., 2005). Oligodendrocytes express distinct sets of integrin receptor heterodimers, depending on differentiation state and the composition of the ECM (Colognato & Tzvetanova, 2011). Recent studies have revealed integrin-associated signaling associated with cellular response to mechanical forces applied to oligodendrocyte lineage cells. Stiffness of the ECM substrate regulates OPC survival, migration, proliferation and differentiation (A. Jagielska et al., 2017; Lourenčo et al., 2016; Urbanski et al., 2016). Mechanical stretching affects oligodendrocyte maturation through the formation of focal adhesions (Shimizu et al., 2017), which are platforms for ECM-integrin signaling. ECM-dependent mechanotransduction promotes oligodendroctytes' differentiation and gene expression changes, as well as the differentiation of neural stem cells into the oligodendrocyte lineage (Arulmoli et al., 2015; A. Jagielska et al., 2017). Since

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

we used ECM-coated deformable membrane in the present study, it is possible that an ECM-integrin signaling may be involved in mediating the oligodendrocytes' response to mechanical stretch that results in the myelin protein loss.

The mechanism by which the Ca^{2+}-dependent activation of the Erk1/2 pathway triggers myelin protein loss in oligodendrocyte is unknown. A possible mediator is calpain, a Ca^{2+}-dependent protease which activity has been implicated in myelin loss. Calpain is expressed in oligodendrocyte (Ray et al., 2002) and its activation contributes to myelin retraction and degradation both during development and in mature brain under pathologic conditions (Baraban, Koudelka, & Lyons, 2018; Guyton et al., 2010; Sloane, Hinman, Lubonia, Hollander, & Abraham, 2003). Furthermore, Erk1/2 has been shown to modulate the activity of μ-calpain, the major form of calpain expressed by oligodendrocytes (Glading et al., 2004). A Ca^{2+}-dependent activation of calpain has been shown to trigger paranodal myelin damage following stretch injury of the spinal cord (W. Sun et al., 2012). Therefore, a combined function of Ca^{2+} and Erk1/2 activity may lead to myelin protein loss by promoting calpain activation in oligodendrocytes.

In summary, we demonstrate that a mechanical force applied to oligodendrocytes initiates an intrinsic signaling cascade that triggers myelin protein loss. More importantly, our data indicates that developmentally regulated key signaling pathways in oligodendrocytes, such as Ca^{2+} and Erk1/2, are re-activated in mature oligodendrocytes following mechanical stretch and that the activation destabilizes the myelin. The significance of our findings lies on its implication to the myelin loss associated with traumatic brain injury, in which long myelinated axons are subjected to tissue deformation caused by impact-induced acceleration forces to the brain. While the myelin loss may occur as a direct consequence of oligodendrocytes' death or be secondary to axonal degeneration, myelin loss on intact axons in the absence of oligodendrocyte's death has been reported, especially following mild TBI (Marion et al., 2018; Mierzwa et al., 2015; Sullivan et al., 2013). In the CNS, early myelin loss triggers secondary myelin damage that contributes to long-term demyelination and/or remyelination failure. It is possible that following a mechanical injury to the brain, such as TBI, the Ca^{2+}-dependent activation of Erk1/2 signaling in the pre-existing oligodendrocytes triggers the initial myelin loss. Therefore, inhibiting the Ca^{2+}-Erk1/2 signaling axis may protect the myelin or improve myelin stability following TBI. Another important aspect of our findings is the transient and reversible nature of the myelin loss following mechanical stretch injury. Stretch-induced myelin protein loss occurs independently of oligodendrocyte death and oligodendrocytes eventually regain the ability to express myelin markers. These results provide support to recent studies that suggest that preexisting oligodendrocytes that survive demyelination may participate in myelin repair in adult brain (Duncan et al., 2018; Yeung et al., 2019)(**ref** Duncan 2018, Yeung 2019). In conclusion, early intervention to inhibit myelin loss by inhibiting Ca^{2+}-Erk1/2 signaling may represent a clinically relevant therapeutic strategy in future interventions aimed at improving brain function and/or attenuating the progression of white matter pathology observed in TBI patients. Furthermore, the role of preexisting oligodendrocytes in remyelination should be explored further as an alternative cellular source for myelin repair in adult brain following TBI.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Acknowledgements:

This work was supported by grants from the New Jersey Commission on Brain Injury Research (CBIR11PJT012) and the National Institute of Health (NS109708) to H.A.K. The authors declared no potential conflicts of interest to the research, authorship, and/or publication of this article.

References

Arulmoli Janahan, Pathak Medha M., McDonnell Lisa P., Nourse Jamison L., Tombola Francesco, Earthman James C., & Flanagan Lisa A. (2015). Static stretch affects neural stem cell differentiation in an extracellular matrix-dependent manner. Sci Rep, 5, 8499. doi: 10.1038/srep0849910.1038/srep08499https://www.nature.com/articles/srep08499#supplementary-informationhttps://www.nature.com/articles/srep08499#supplementary-information [PubMed: 25686615]

Baraban M, Koudelka S, & Lyons DA (2018). Ca (2+) activity signatures of myelin sheath formation and growth in vivo. Nat Neurosci., 21(1), 19–23. doi: 10.1038/s41593-41017-40040-x. Epub 42017 Dec 41511. [PubMed: 29230058]

Boehm JS, Zhao JJ, Yao J, Kim SY, Firestein R, Dunn IF, … Hahn WC (2007). Integrative genomic approaches identify IKBKE as a breast cancer oncogene. Cell, 129(6), 1065–1079. doi: 10.1016/j.cell.2007.03.052 [PubMed: 17574021]

Colognato H, & Tzvetanova ID (2011). Glia unglued: how signals from the extracellular matrix regulate the development of myelinating glia. Dev Neurobiol., 71(11), 924–955. doi: 910.1002/dneu.20966. [PubMed: 21834081]

Duncan ID, Radcliff AB, Heidari M, Kidd G, August BK, & Wierenga LA (2018). The adult oligodendrocyte can participate in remyelination. Proc Natl Acad Sci U S A, 115(50), E11807–E11816. doi: 10.1073/pnas.1808064115 [PubMed: 30487224]

Fyffe-Maricich SL, Schott A, Karl M, Krasno J, & Miller RH (2013). Signaling through ERK1/2 controls myelin thickness during myelin repair in the adult central nervous system. J Neurosci, 33(47), 18402–18408. doi: 10.1523/JNEUROSCI.2381-13.2013 [PubMed: 24259565]

Glading A, Bodnar RJ, Reynolds IJ, Shiraha H, Satish L, Potter DA, … Wells A (2004). Epidermal growth factor activates m-calpain (calpain II), at least in part, by extracellular signal-regulated kinase-mediated phosphorylation. Mol Cell Biol., 24(6), 2499–2512. [PubMed: 14993287]

Gudipaty SA, Lindblom J, Loftus PD, Redd MJ, Edes K, Davey CF, … Rosenblatt J (2017). Mechanical stretch triggers rapid epithelial cell division through Piezo1. Nature., 543(7643), 118–121. doi: 110.1038/nature21407. Epub 22017 Feb 21415. [PubMed: 28199303]

Guyton MK, Das A, Samantaray S, Wallace G. C. th, Butler JT, Ray SK, & Banik NL (2010). Calpeptin attenuated inflammation, cell death, and axonal damage in animal model of multiple sclerosis. J Neurosci Res., 55(11), 2398–2408. doi: 2310.1002/jnr.22408.

Hernandez M, Patzig J, Mayoral SR, Costa KD, Chan JR, & Casaccia P (2016). Mechanostimulation Promotes Nuclear and Epigenetic Changes in Oligodendrocytes. J Neurosci, 36(3), 806–813. doi: 10.1523/JNEUROSCI.2873-15.2016 [PubMed: 26791211]

Iqbal J, & Zaidi M (2005). Molecular regulation of mechanotransduction. Biochem Biophys Res Commun, 325(3), 751–755. doi: 710.1016/j.bbrc.2004.1012.1087.

Ishii A, Furusho M, & Bansal R (2013). Sustained activation of ERK1/2 MAPK in oligodendrocytes and schwann cells enhances myelin growth and stimulates oligodendrocyte progenitor expansion. J Neurosci., 33(1), 175–186. . [PubMed: 23283332]

Ishii A, Furusho M, Dupree JL, & Bansal R (2016). Strength of ERK1/2 MAPK Activation Determines Its Effect on Myelin and Axonal Integrity in the Adult CNS. J Neurosci, 36(24), 6471–6487. doi: 10.1523/JNEUROSCI.0299-16.2016 [PubMed: 27307235]

Ishii A, Fyffe-Maricich SL, Furusho M, Miller RH, & Bansal R (2012). ERK1/ERK2 MAPK signaling is required to increase myelin thickness independent of oligodendrocyte differentiation and initiation of myelination. J Neurosci., 32(26), 8855–8864. . [PubMed: 22745486]

Jagielska A, Lowe AL, Makhija E, Wroblewska L, Guck J, Franklin RJM, … Van Vliet KJ (2017). Mechanical Strain Promotes Oligodendrocyte Differentiation by Global Changes of Gene Expression. Front Cell Neurosci, 11, 93. doi: 10.3389/fncel.2017.00093 [PubMed: 28473753]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Jagielska Anna, Norman Adele L., Whyte Graeme, Van Vliet Krystyn J., Guck Jochen, & Franklin Robin J.M. (2012). Mechanical Environment Modulates Biological Properties of Oligodendrocyte Progenitor Cells. Stem Cells and Development, 21(16), 2905–2914. doi: 10.1089/scd.2012.0189 [PubMed: 22646081]

Jeffries MA, Urbanek K, Torres L, Wendell SG, Rubio ME, & Fyffe-Maricich SL (2016). ERK1/2 Activation in Preexisting Oligodendrocytes of Adult Mice Drives New Myelin Synthesis and Enhanced CNS Function. J Neurosci, 36(35), 9186–9200. doi: 10.1523/JNEUROSCI.1444-16.2016 [PubMed: 27581459]

Jurick SM, Bangen KJ, Evangelista ND, Sanderson-Cimino M, Delano-Wood L, & Jak AJ (2016). Advanced neuroimaging to quantify myelin in vivo: Application to mild TBI. Brain Inj, 30(12), 1452–1457. doi: 10.1080/02699052.2016.1219064 [PubMed: 27834545]

Kippert Angelika, Fitzner Dirk, Helenius Jonne, & Simons Mikael. (2009). Actomyosin contractility controls cell surface area of oligodendrocytes. BMC Cell Biology, 10(1), 71. doi: 10.1186/1471-2121-10-71 [PubMed: 19781079]

Liu D, Genetos DC, Shao Y, Geist DJ, Li J, Ke HZ, … Duncan RL (2008). Activation of extracellular-signal regulated kinase (ERK1/2) by fluid shear is Ca(2+)- and ATP-dependent in MC3T3-E1 osteoblasts. Bone., 42(4), 644–652. doi: 610.1016/j.bone.2007.1009.1058. Epub 2007 Oct 1016. [PubMed: 18291742]

Lourenco T, & Graos M (2016). Modulation of Oligodendrocyte Differentiation by Mechanotransduction. Front Cell Neurosci., 10:277.(doi), 10.3389/fncel.2016.00277. eCollection 02016. [PubMed: 27965541]

Lourenčo Tânia, Paes de Faria Joana, Bippes Christian A., Maia João, Lopes-da-Silva José A., Relvas João B., & Grãos Mário. (2016). Modulation of oligodendrocyte differentiation and maturation by combined biochemical and mechanical cues. Sci Rep, 6, 21563. doi: 10.1038/srep2156310.1038/srep21563https://www.nature.com/articles/srep21563#supplementary-informationhttps://www.nature.com/articles/srep21563#supplementary-information [PubMed: 26879561]

Magou GC, Guo Y, Choudhury M, Chen L, Hususan N, Masotti S, & Pfister BJ (2011). Engineering a high throughput axon injury system. J Neurotrauma., 28(11), 2203–2218. doi: 2210.1089/neu.2010.1596. Epub 2011 Oct 2212. [PubMed: 21787172]

Magou GC, Pfister BJ, & Berlin JR (2015). Effect of acute stretch injury on action potential and network activity of rat neocortical neurons in culture. Brain Res. doi: 10.1016/j.brainres.2015.07.056

Makhija E, Jagielska A, Zhu L, Bost AC, Ong W, Chew SY, … Van Vliet KJ (2018). Mechanical Strain Alters Cellular and Nuclear Dynamics at Early Stages of Oligodendrocyte Differentiation. Front Cell Neurosci, 12, 59. doi: 10.3389/fncel.2018.00059 [PubMed: 29559894]

Marion Christina M., Radomski Kryslaine L., Cramer Nathan P., Galdzicki Zygmunt, & Armstrong Regina C. (2018). Experimental Traumatic Brain Injury Identifies Distinct Early and Late Phase Axonal Conduction Deficits of White Matter Pathophysiology, and Reveals Intervening Recovery. The Journal of Neuroscience, 38(41), 8723–8736. doi: 10.1523/jneurosci.0819-18.2018 [PubMed: 30143572]

Martineau LC, & Gardiner PF (2001). Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension. 1985). 91(2), 693–702. doi: 610.1152/jappl.2001.1191.1152.1693.

Mederos y Schnitzler M, Storch U, Meibers S, Nurwakagari P, Breit A, Essin K, … Gudermann T (2008). Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction. EMBO J., 27(23), 3092–3103. doi: 3010.1038/emboj.2008.3233. Epub 2008 Nov 3096. [PubMed: 18987636]

Mierzwa AJ, Marion CM, Sullivan GM, McDaniel DP, & Armstrong RC (2015). Components of myelin damage and repair in the progression of white matter pathology after mild traumatic brain injury. J Neuropathol Exp Neurol, 74(3), 218–232. doi: 10.1097/NEN.0000000000000165 [PubMed: 25668562]

Momberger TS, Levick JR, & Mason RM (2006). Mechanosensitive synoviocytes: a Ca2+ -PKCalpha-MAP kinase pathway contributes to stretch-induced hyaluronan synthesis in vitro. Matrix Biol., 25(5), 306–316. doi: 310.1016/j.matbio.2006.1001.1008. Epub 2006 Mar 1029. [PubMed: 16567084]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Panenka WJ, Lange RT, Bouix S, Shewchuk JR, Heran MK, Brubacher JR, … Iverson GL (2015). Neuropsychological outcome and diffusion tensor imaging in complicated versus uncomplicated mild traumatic brain injury. PLoS One, 10(4), e0122746. doi: 10.1371/journal.pone.0122746 [PubMed: 25915776]

Raghupathi R, Muir JK, Fulp CT, Pittman RN, & McIntosh TK (2003). Acute activation of mitogen-activated protein kinases following traumatic brain injury in the rat: implications for posttraumatic cell death. Exp Neurol, 183(2), 438–448. [PubMed: 14552884]

Ray SK, Neuberger TJ, Deadwyler G, Wilford G, DeVries GH, & Banik NL (2002). Calpain and calpastatin expression in primary oligodendrocyte culture: preferential localization of membrane calpain in cell processes. J Neurosci Res., 70(4), 561–569. doi: 510.1002/jnr.10414. [PubMed: 12404510]

Rosenberg Sheila S., Kelland Eve E., Tokar Eleonora, De La Torre Asia R., & Chan Jonah R. (2008). The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. Proceedings of the National Academy of Sciences, 105(38), 14662–14667. doi: 10.1073/pnas.0805640105

Ruwhof C, & van der Laarse A (2000). Mechanical stress-induced cardiac hypertrophy: mechanisms and signal transduction pathways. Cardiovasc Res., 47(1), 23–37. [PubMed: 10869527]

Ryder JW, Fahlman R, Wallberg-Henriksson H, Alessi DR, Krook A, & Zierath JR (2000). Effect of contraction on mitogen-activated protein kinase signal transduction in skeletal muscle. Involvement Of the mitogen- and stress-activated protein kinase 1. J Biol Chem, 275(2), 1457–1462. [PubMed: 10625698]

Sasamoto A, Nagino M, Kobayashi S, Naruse K, Nimura Y, & Sokabe M (2005). Mechanotransduction by integrin is essential for IL-6 secretion from endothelial cells in response to uniaxial continuous stretch. Am J Physiol Cell Physiol., 288(5), C1012–1022. doi: 1010.1152/ajpcell.00314.02004. Epub 02004 Dec 00321. [PubMed: 15613495]

Shimizu T, Osanai Y, Tanaka KF, Abe M, Natsume R, Sakimura K, & Ikenaka K (2017). YAP functions as a mechanotransducer in oligodendrocyte morphogenesis and maturation. Glia., 65(2), 360–374. doi: 310.1002/glia.23096. Epub 22016 Nov 23093. [PubMed: 27807898]

Shin KJ, Wall EA, Zavzavadjian JR, Santat LA, Liu J, Hwang JI, … Fraser ID (2006). A single lentiviral vector platform for microRNA-based conditional RNA interference and coordinated transgene expression. Proc Natl Acad Sci U S A, 103(31), 13759–13764. doi: 10.1013/pnas.0606119103 [PubMed: 16945906]

Sloane JA, Hinman JD, Lubonia M, Hollander W, & Abraham CR (2003). Age-dependent myelin degeneration and proteolysis of oligodendrocyte proteins is associated with the activation of calpain-1 in the rhesus monkey. J Neurochem., 84(1), 157–168. [PubMed: 12485412]

Sullivan GM, Mierzwa AJ, Kijpaisalratana N, Tang H, Wang Y, Song SK, … Armstrong RC (2013). Oligodendrocyte lineage and subventricular zone response to traumatic axonal injury in the corpus callosum. J Neuropathol Exp Neurol, 72(12), 1106–1125. doi: 10.1097/NEN.0000000000000009 [PubMed: 24226267]

Sun W, Fu Y, Shi Y, Cheng JX, Cao P, & Shi R (2012). Paranodal myelin damage after acute stretch in Guinea pig spinal cord. J Neurotrauma., 29(3), 611–619. doi: 610.1089/neu.2011.2086. Epub 2011 Dec 1085. [PubMed: 21988176]

Sun Z, Guo SS, & Fassler R (2016). Integrin-mediated mechanotransduction. J Cell Biol., 215(4), 445–456. doi: 410.1083/jcb.201609037. Epub 201602016 Nov 201609038. [PubMed: 27872252]

Tyler WA, Gangoli N, Gokina P, Kim HA, Covey M, Levison SW, & Wood TL (2009). Activation of the mammalian target of rapamycin (mTOR) is essential for oligodendrocyte differentiation. J Neurosci., 29(19), 6367–6378. . [PubMed: 19439614]

Urbanski Mateusz M., Kingsbury Lyle, Moussouros Daniel, Kassim Imran, Mehjabeen Saraf, Paknejad Navid, & Melendez-Vasquez Carmen V. (2016). Myelinating glia differentiation is regulated by extracellular matrix elasticity. Sci Rep, 6, 33751. doi: 10.1038/srep3375110.1038/srep33751https://www.nature.com/articles/srep33751#supplementary-informationhttps://www.nature.com/articles/srep33751#supplementary-information [PubMed: 27646171]

Wang X, Zhu C, Qiu L, Hagberg H, Sandberg M, & Blomgren K (2003). Activation of ERK1/2 after neonatal rat cerebral hypoxia-ischaemia. J Neurochem., 86(2), 351–362. [PubMed: 12871576]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wright AD, Jarrett M, Vavasour I, Shahinfard E, Kolind S, van Donkelaar P, … Rauscher A (2016). Myelin Water Fraction Is Transiently Reduced after a Single Mild Traumatic Brain Injury--A Prospective Cohort Study in Collegiate Hockey Players. PLoS One, 11(2), e0150215. doi: 10.1371/journal.pone.0150215 [PubMed: 26913900]

Xu J, Mathur J, Vessieres E, Hammack S, Nonomura K, Favre J, … Patapoutian A (2018). GPR68 Senses Flow and Is Essential for Vascular Physiology. Cell., 173(3), 762–775.e716 doi: 710.1016/j.cell.2018.1003.1076. [PubMed: 29677517]

Yang DP, Kim J, Syed N, Tung YJ, Bhaskaran A, Mindos T, … Kim HA (2012). p38 MAPK activation promotes denervated Schwann cell phenotype and functions as a negative regulator of Schwann cell differentiation and myelination. J Neurosci., 32(21), 7158–7168. doi: 7110.1523/JNEUROSCI.5812-7111.2012. [PubMed: 22623660]

Yeung MSY, Djelloul M, Steiner E, Bernard S, Salehpour M, Possnert G, … Frisen J (2019). Dynamics of oligodendrocyte generation in multiple sclerosis. Nature, 566(7745), 538–542. doi: 10.1038/s41586-018-0842-3 [PubMed: 30675058]

Zou Y, Akazawa H, Qin Y, Sano M, Takano H, Minamino T, … Komuro I (2004). Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. Nat Cell Biol., 6(6), 499–506. doi: 410.1038/ncb1137. Epub 2004 May 1016. [PubMed: 15146194]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Main points

- Mechanical stretch induces myelin protein loss in mature oligodendrocyte by initiating Ca^{2+}-dependent Erk1/2 activation
- Inhibition of Ca^{2+} or Erk1/2 prevents myelin protein loss after stretch injury
- The study has implication on myelin loss associated with traumatic brain injury

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

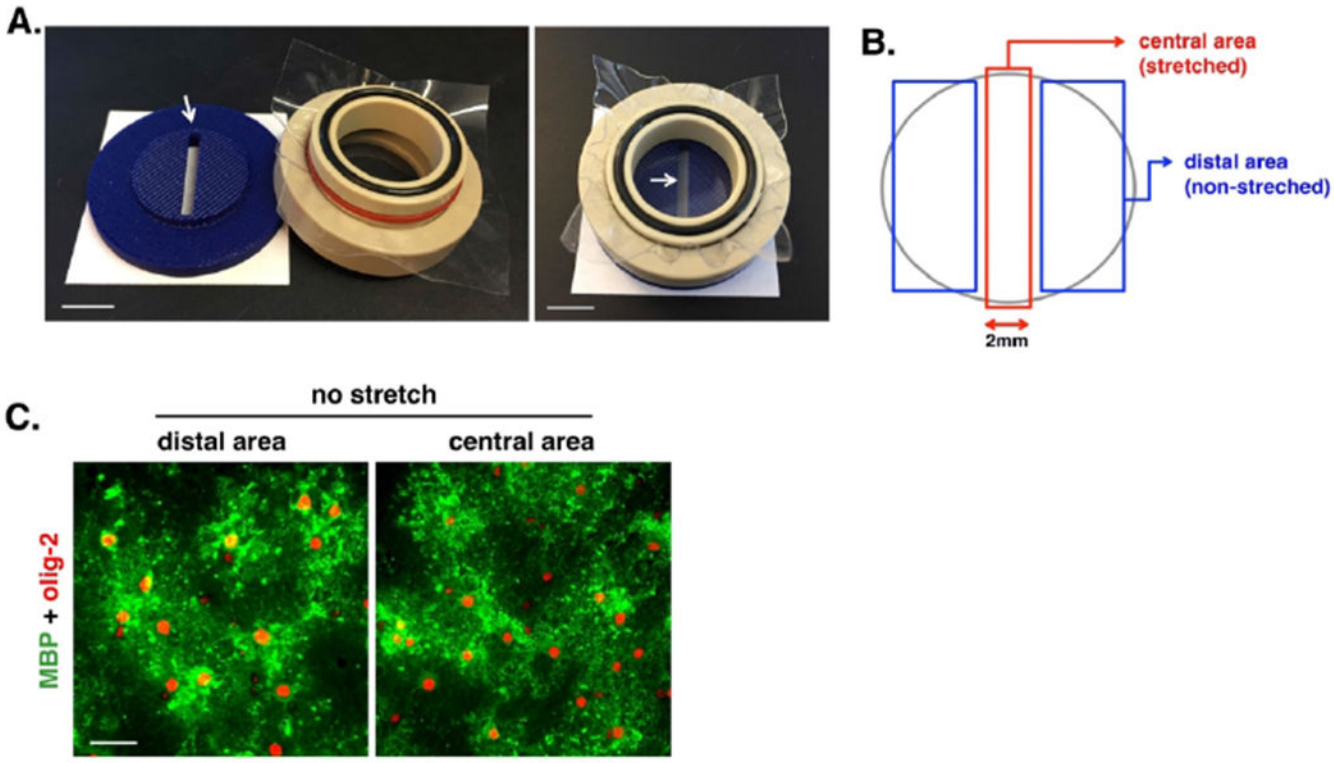


Figure 1. Normal maturation of oligodendrocytes cultured on silicon membrane in the *in vitro* stretch device.

A, Cell culture platform used in the *in vitro* stretch device. The deformable silicone membrane is held together by circular frames. Prior to stretch, a flat circular mask (arrow, left panel) with a 2 mm-wide rectangular gap along the midline is placed under the membrane (arrow, right panel). Scale bar, 1cm. ***B***, As a result, only the cells in the midline area (red) are subjected to stretch; the cells in the distal area (blue) remain non-stretched. ***C***, Double immunostaining for MBP (green) and Olig2 (red) prior to stretch indicates that oligodendrocyte progenitor cells cultured on the Matrigel-coated silicon membrane have differentiated into mature oligodendrocytes in both the central and distal area of the culture.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

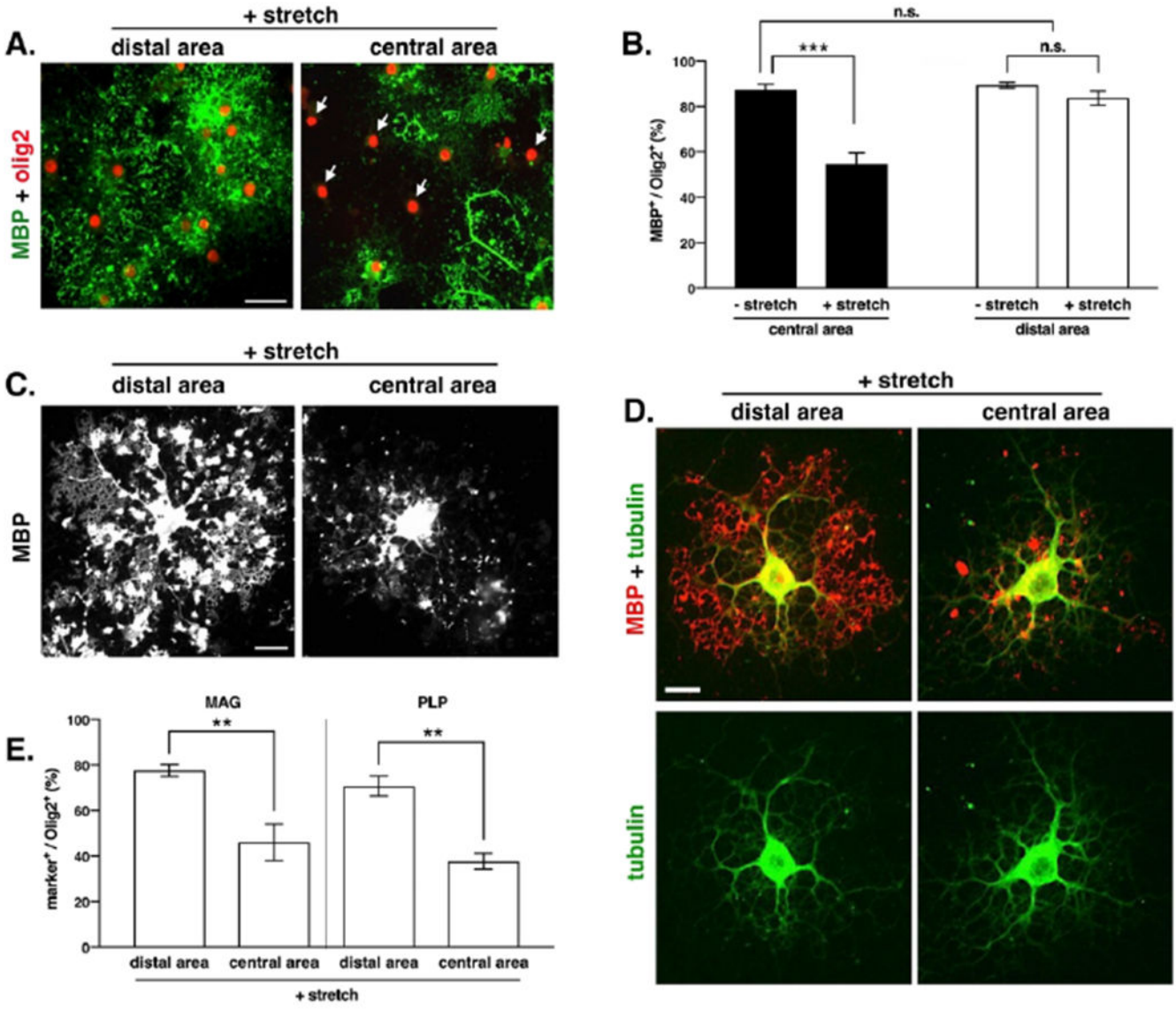


Figure 2. Mechanical stretch triggers myelin protein loss in differentiated oligodendrocytes.

A, Cultures were immunostained for MBP and Olig2, 24 hours after mechanical stretch. Note the decrease in MBP expression by mature oligodendrocytes ($Olig2^+$ cells) subjected to mechanical stretch (arrows, central area), whereas MBP expression by non-stretched mature oligodendrocytes (distal area) is not affected (scale bar, 50μm). ***B***, Quantification of experiments represented in ***A*** (mean ± SEM, n = 3-5). Two-way ANOVA indicates that there is strong interaction between the mechanical stretch and the area of the culture analyzed (p = 0.0051), i.e. that the effect of stretch on oligodendrocytes is different between central and distal areas. Tukey's multiple comparison tests show that mechanical stretch induces a significant decrease (37%) in MBP expression in the central area (***p = 0.0004) where the stretch is applied, but not in the distal area (n.s. = non significant, p = 0.76). The proportion of MBP^+ oligodendrocytes in the distal area, with or without stretch, is comparable to that of the central area of non-stretched cultures (n.s. = not significant, p = 0.9163 and 0.9894,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

respectively). ***C***, Upon mechanical stretch, some oligodendrocytes exhibit a loss of MBP expression at the periphery (scale bar, 20μm). ***D***, The tubulin network (green) within oligodendrocytes undergoing MBP loss appears unaffected following mechanical stretch (scale bar, 20μm). ***E***, Myelinated cultures were double-immunostained for MAG and Olig2 or PLP and Olig2, 24 hours after mechanical stretch, and the number of $Olig2^+$ cells expressing either MAG or PLP quantitatively analyzed (un-paired, two-tailed, Student's t-test). The loss of myelin markers is not limited to MBP expression; MAG and PLP expression are also substantially decreased (**p = 0.0094 and **p = 0.0043, respectively).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

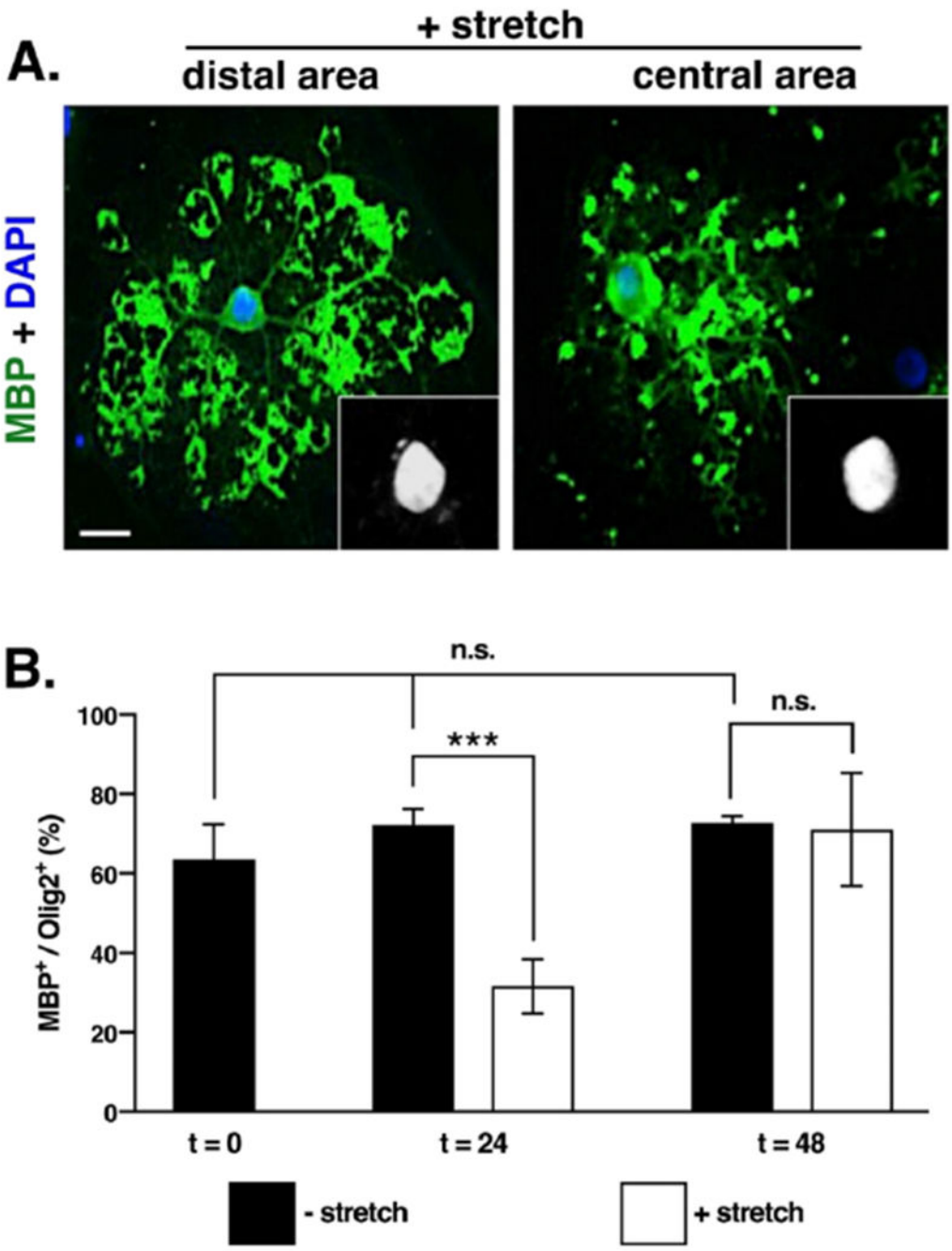


Figure 3. Stretch-induced loss of myelin markers is transient and reversible.

A, Co-staining of oligodendrocytes with MBP (green) and DAPI (blue) does not reveal the apparition of pycnotic nuclei in oligodendrocytes that are losing MBP expression following stretch (central area), suggesting mechanical stretch at 70% strain and rate of $30s^{-1}$ does not induce cell death (scale bar, 20μm). Lack of oligodendrocyte death was also confirmed by TUNEL assay (see text). ***B***, myelinated cultures were immunostained for MBP and Olig2, 24 and 48 hours (t = 24 and t = 48, respectively) after mechanical stretch (+ stretch). At each time point, sister cultures not subjected to mechanical stretch (− stretch) were also

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

immunostained. Changes in the number of Olig2$^+$ cells expressing MBP in the central area (with and without stretch) were quantitatively analyzed (two-way ANOVA with Tukey's multiple comparison test). As noted before, MBP expression in the central area is significantly reduced 24 hours post-stretch (t = 48, ***p = 0.0001). 48 hours post stretch the number of Olig2$^+$ cells expressing MBP in the central area is not substantially different from that of non-stretched cells (t = 48, n.s., p = 0.9998), nor is it different from non-stretched cells at 24 hours (n.s., p > 0.9999), indicating that the loss of MBP expression at 24 hours is transient and reversible. Of note, the number of non-stretched MBP-expressing Olig2$^+$ cells does not vary significantly over time (compare t = 0 (onset of experiment) with t = 24 and t = 48; n.s., p = 0.7239 and p = 0.6791, respectively).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

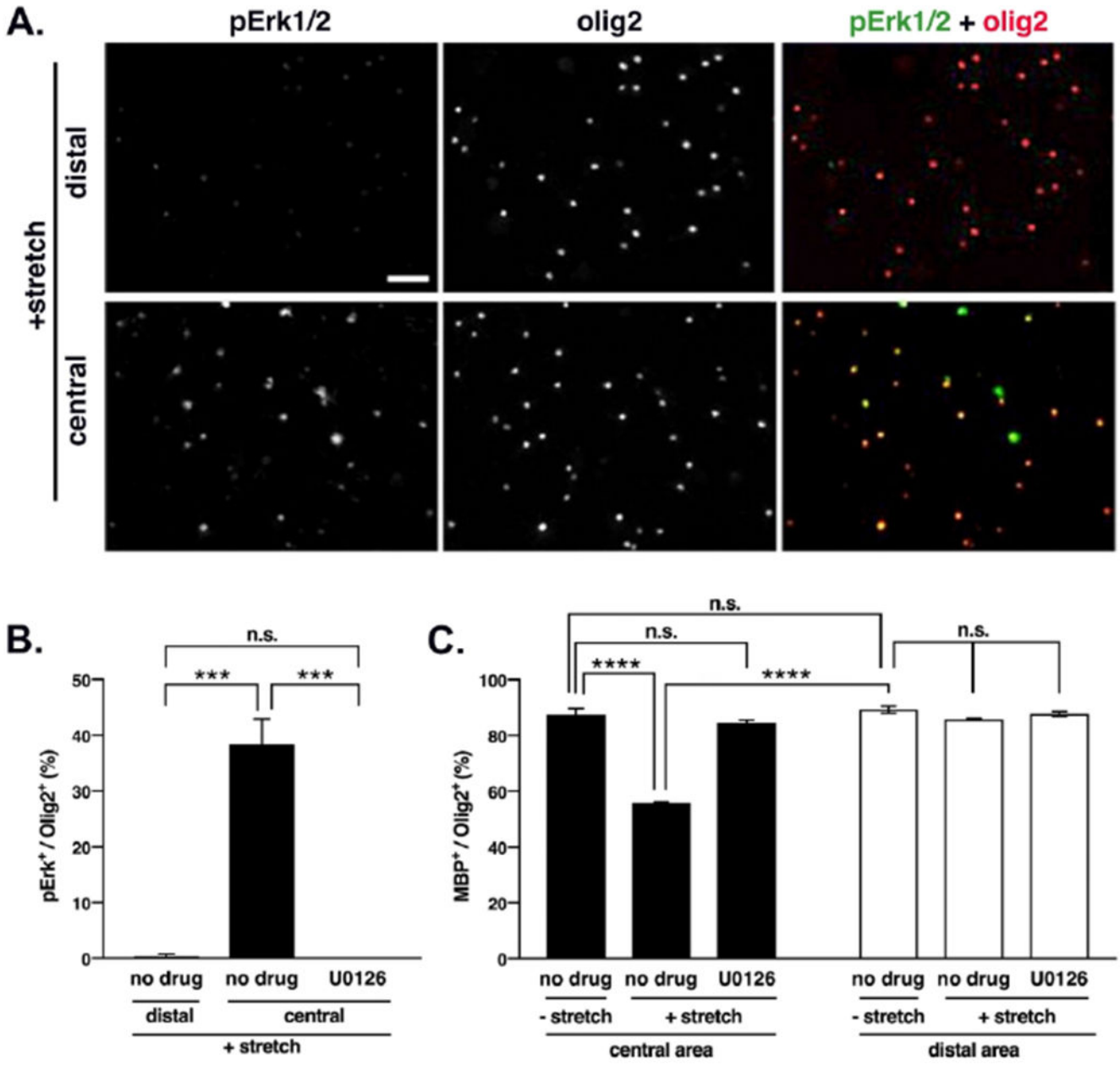


Figure 4. Stretch-induced loss of MBP is mediated by Erk1/2 activation.

A, Oligodendrocytes were immunostained for phospho-Erk1/2 (pErk1/2, green) and Olig2 (red) 30 minutes following mechanical stretch. Erk1/2 activation (p-Erk1/2) is seen in oligodendrocytes that are within the stretched area (central), but not in oligodendrocytes that are within the non-stretched area (distal) (scale bar, 100μm). ***B***, Quantification of the results shown in ***A*** (mean ± SEM, n = 3, one-way ANOVA with Tukey's multiple comparison test). Stretch induces a significant increase in pErk1/2 immunoreactivity in oligodendrocytes (***p = 0.0001) that is as significantly decreased by the MEK1/2 inhibitor U0126 (***p = 0.0001) (n.s. = not significant, p = 0.09952). ***C***, MEK1/2 inhibition blocks the stretch-induced loss of MBP in oligodendrocytes. Cultures were treated with the MEK1/2 inhibitor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

U0126 for 1 hour after the stretch, at which time the inhibitor was removed and the cultures maintained in normal media. 24 hours later, the cultures were quantitatively analyzed for the expression of MBP after co-immunostaining for MBP and Olig2 (mean ± SEM, n = 3). Two-way ANOVA indicates that there is strong interaction between the mechanical stretch and the area of the culture analyzed ($p < 0.0001$), i.e. that the effect of stretch on oligodendrocytes is different between central and distal areas. Tukey's multiple comparison tests show that mechanical stretch induces a significant decrease (36%) in MBP expression in the central area (****$p < 0.0001$) that is abolished by the U0126 drug (****$p < 0.0001$). Oligodendrocytes in the distal area are not affected by either stretch or U0126 drug (n.s., not significant, $p > 0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

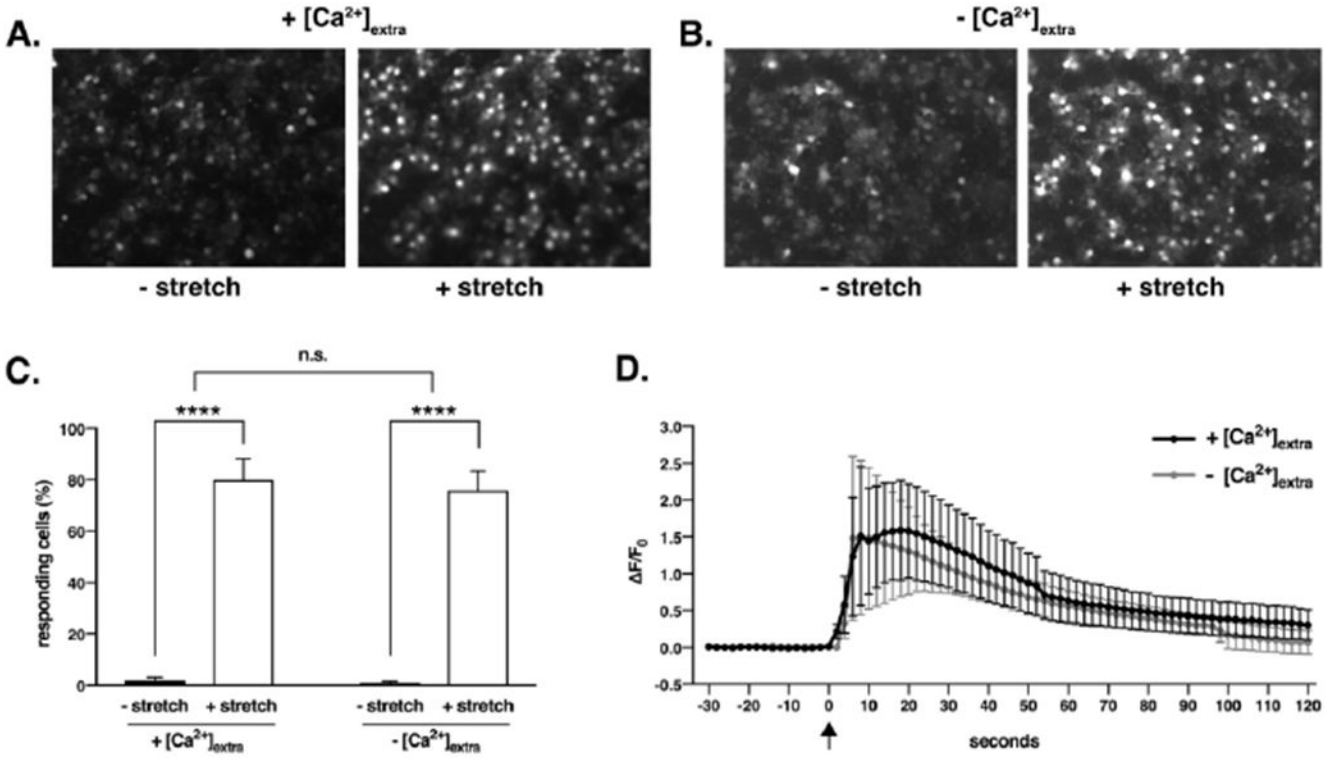


Figure 5. Mechanical stretch induces a rapid increase in intracellular Ca^{2+} in oligodendrocytes.

A and B, Representative images of Fluo-4 AM fluorescence captured 6 seconds prior and 6 seconds after stretch (− stretch vs + stretch, respectively), with calcium present (panel A, + $[Ca^{2+}]_{extra}$) or absent (panel B, − $[Ca^{2+}]_{extra}$) from the extracellular media. ***C***, Quantitative analysis of the data presented in panels A and B (mean ± sem, n = 3). Cells that exhibited an increase in $\Delta F/F_0$, within 2 minutes after mechanical stretch, that was strictly 3 standard deviations higher than the baseline (see Material and Methods for details), were counted and expressed as a % of the total number of cells examined. Two-way ANOVA indicates that there is no interaction between the effect of the mechanical stretch and the presence / absence of calcium in the extracellular media ($p = 0.7939$), i.e. that the effect of stretch on the % of oligodendrocytes that exhibit an increase in Fluo4-AM intensity does not depend on extracellular calcium. Tukey's multiple comparison tests show that in the presence or absence of extracellular calcium, mechanical stretch induces a similar number of oligodendrocytes (80% vs 76%, n.s = not significant, $p = 0.9545$) to exhibit an increase in $\Delta F/F_0$ above baseline (****$p < 0.0001$). ***D***, Change in the average $\Delta F/F_0$ of all cells at a given time point was plotted as a function of time (n = 3, mean ± SEM). The arrow indicates when mechanical stretch was applied. Two-way ANOVA indicates there is no interaction between the effect of the mechanical stretch and the presence / absence of calcium in the extracellular media ($p = 0.9999$). Stretch is the only factor that results in a significant change in $\Delta F/F_0$ across time ($p < 0.0001$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

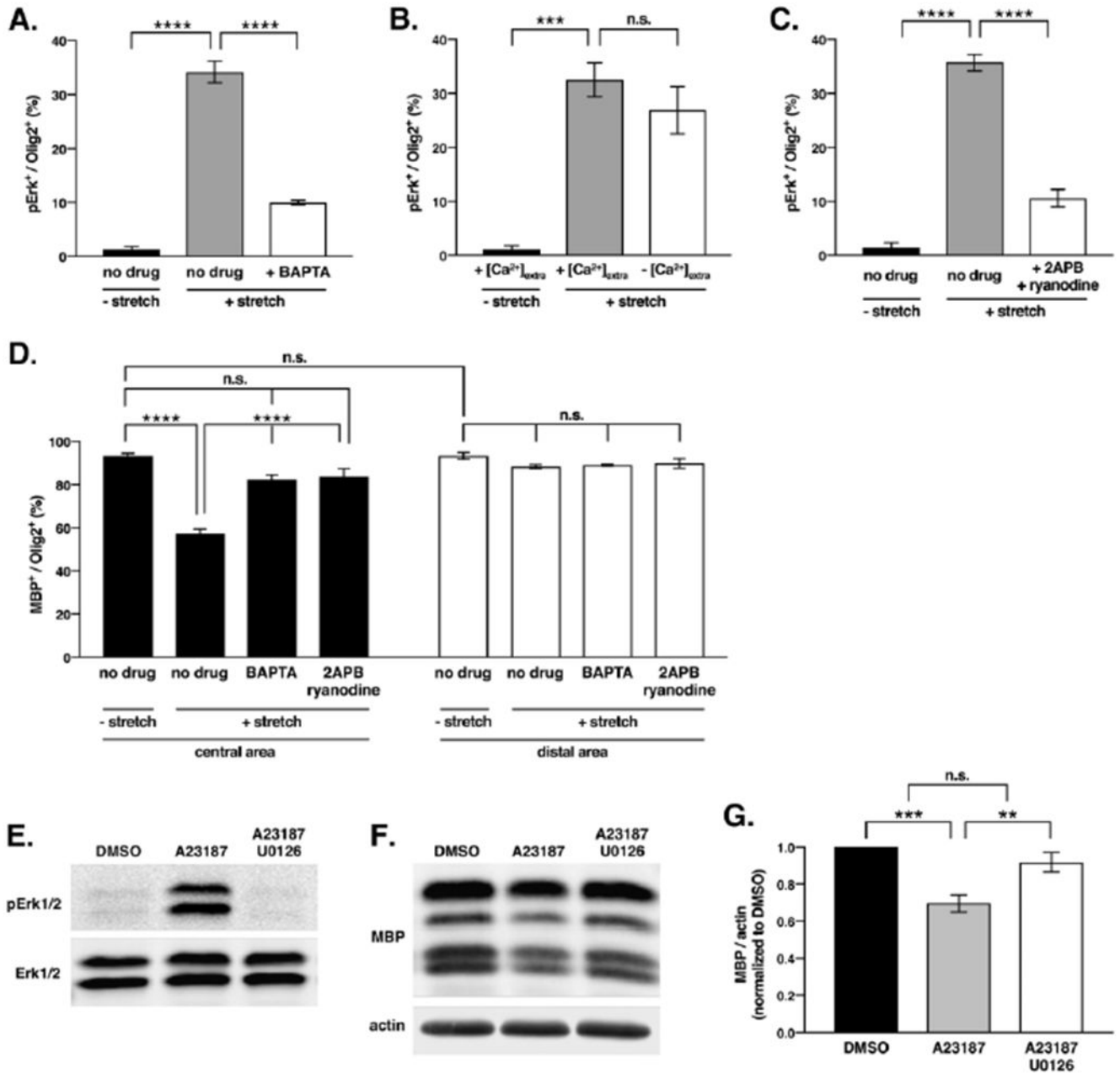


Figure 6. Increase in intracellular Ca^{2+} is sufficient to induce the Erk1/2-dependent loss of MBP in oligodendrocytes.

A, Quantitative analysis of cultures treated or not with BAPTA (20μM) and co-immunostained for pErk1/2 and Olig2 (mean ± SEM, n= 3, one-way ANOVA with Tukey's multiple comparison tests). The chelation of intracellular Ca^{2+} with BAPTA (20μM) substantially abolishes (71% decrease, ****p < 0.0001) the stretch-induced activation of Erk1/2 (****p < 0.0001) in oligodendrocytes. ***B***, Quantitative analysis of cultures stretched in the presence or absence of extracellular calcium and co-immunostained for pErk1/2 and Olig2 (mean ± SEM, n= 3, one-way ANOVA with Tukey's multiple comparison tests). The stretch-induced activation of Erk1/2 (***p = 0.001) in oligodendrocytes is not significantly

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

affected by the presence or absence of extracellular calcium (32% vs 27%, n.s. not significant, p = 0.4574). ***C***, Quantitative analysis of cultures treated, or not, with 2-APB (10μM) and ryanodine (50μM), and co-immunostained for pErk1/2 and Olig2 (mean ± SEM, n= 3, one-way ANOVA with Tukey's multiple comparison tests). The combined treatment of 2-APB and ryanodine significantly blocked (70% decrease, **** $p < 0.0001$) the stretch-induced activation of Erk1/2 (****$p < 0.0001$), which indicates that Ca^{2+} released from ER storage contributes to Erk1/2 activation. ***D***, Quantitative analysis of cultures treated, or not, with either BAPTA (20μM) or a combination of 2-APB (10μM) and ryanodine (50μM), and co-immunostained for MBP and Olig2 (mean ± SEM, n= 3, two-way ANOVA with Tukey's multiple comparison tests). The significant loss of MBP expression in mature oligodendrocytes induced by mechanical stretch (****$p < 0.0001$) is completely abolished by treatment with either BAPTA or 2-ABP/ryanodine (****$p < 0.0001$), treated cultures having a % of MBP+ oligodendrocytes (82% or 84%, respectively) not significantly different form that of untreated and non-stretched cultures (93%, n.s. = not significant, p = 0.0928). ***E***, Western blot analysis of oligodendrocyte cultures treated with Ca^{2+} ionophore A23187 (10μM) for 30 minutes. Increase in intracellular Ca^{2+} is sufficient to activate Erk1/2 in oligodendrocytes. ***F***, Oligodendrocytes were treated with A23187 for 1 hour, after which the ionophore was removed and the cultures maintained in normal media for another 24 hours. MBP expression was then assessed by Western blotting. Transient treatment with A23187 is sufficient to induce the down-regulation of MBP expression in oligodendrocytes 24 hours later. A combined 30 minute-treatment with the MEK1/2 inhibitor U0126 prevents the A23187-induced loss of MBP expression. ***G***, Quantification of the results presented in ***F*** (mean ± SEM, n = 3, one way ANOVA with Tukey's multiple comparison test, ***p = 0.0002, **p = 0.0037, n.s. = not significant p = 0.3436).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

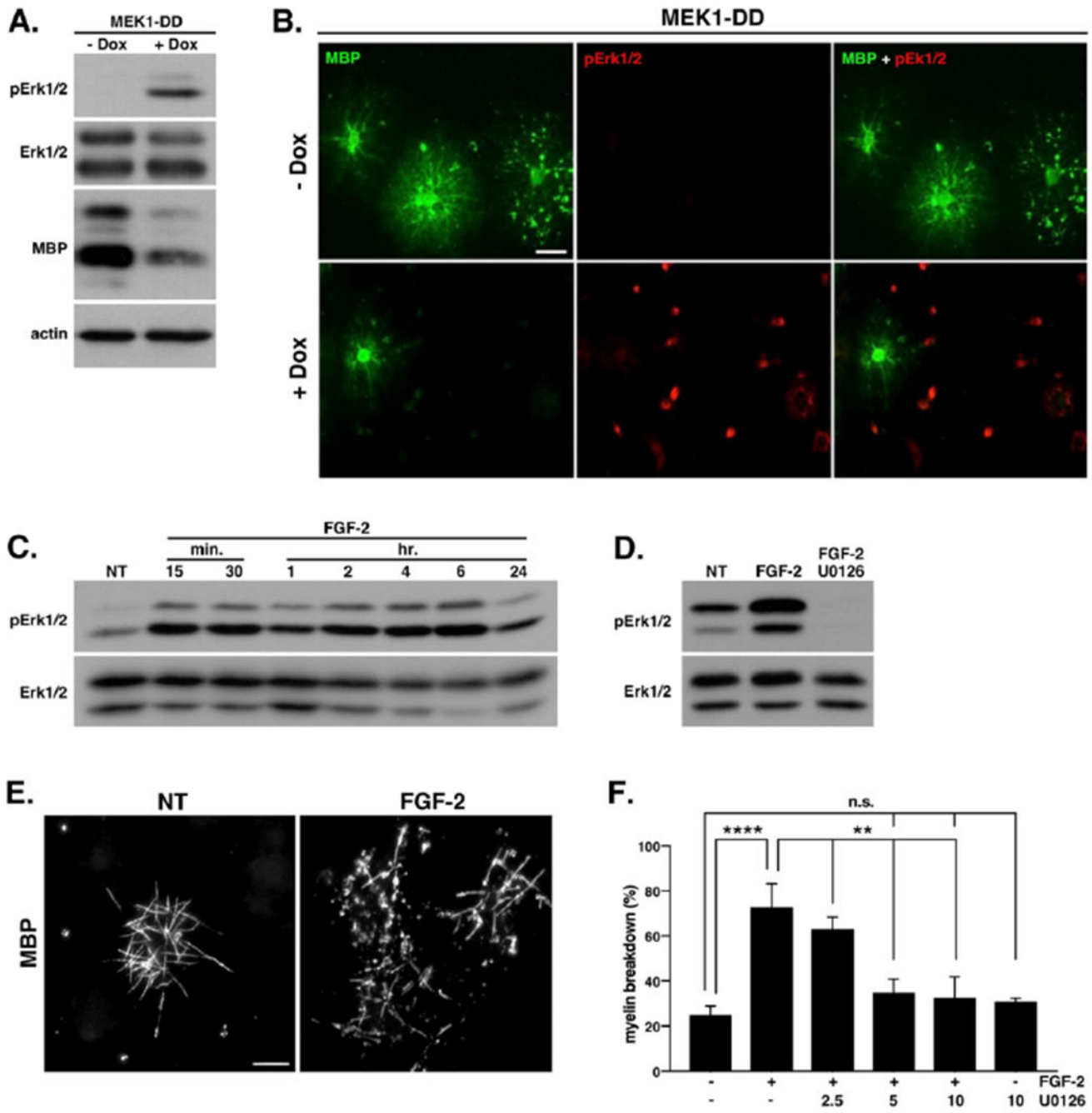


Figure 7. Ectopic activation of Erk1/2 in oligodendrocytes is sufficient to induce myelin protein loss.

A, Doxycycline (Dox) induces activation of Erk1/2 in differentiated oligodendrocytes that are transduced with a constitutively active form of MEK1 (MEK1-DD). ***B***, Differentiated oligodendrocytes transduced with MEK1-DD were treated with doxycycline (Dox) and MBP expression was assessed by immunostaining 48 hours later. Induction of MEK1-DD expression in oligodendrocytes by doxycycline (+ Dox) results in a decrease in the number of MBP^+ cells, concomitant with an increase in cells expressing active Erk1/2 (pErk1/2). ***C***

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and D, Western blot analysis showing the activation of the Erk1/2 pathway (pErk1/2) in oligodendrocytes by exogenous FGF-2 (3.3nM) (panel C) and its inhibition by the MEK1/2 inhibitor U0126 (panel D). ***E***, OPCs were co-cultured with DRG neurons to generate myelin. After myelination (21 days), Erk1/2 activation was induced by FGF-2 treatment. Myelin integrity was assessed by immunostaining for MBP 48 hours later. Clusters of myelin segments are seen in control cultures (NT = not treated), whereas clusters containing fragmented segments are seen in FGF-2-treated cultures, an indication of myelin breakdown (scale bar, 20μm). ***F***, Quantitative analysis of the experiments presented in panel ***E*** (mean ± SEM, n = 3, one way ANOVA with Tukey's multiple comparison test) shows that the significant myelin breakdown (****$p < 0.0001$) is counteracted in a concentration-dependent manner by the U0126 drug (**$p = 0.0037$) until cultures are no different from untreated (no FGF-2, no U0126) cultures (n.s. = not significant, $p > 0.05$).