Demyelination in a 3D CNS Environment: Insights from Organotypic Cerebellar Slices

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Introduction

Demyelinating diseases such as multiple sclerosis (MS) involve complex disruptions in myelin integrity and repair. To investigate the cellular mechanisms underlying demyelination and remyelination, we utilized organotypic cerebellar slice (OCS) cultures derived from early postnatal mice. Organotypic slice cultures preserve the native three-dimensional (3D) architecture and cellular diversity of the central nervous system, including neurons, oligodendrocytes, astrocytes, and microglia, in a spatially organized manner marking them uniquely suited for studying complex cell–cell interactions. Additionally, organotypic tissue cultures exhibit responses in a setting that closely mimics *in vivo* conditions, while still allowing for precise experimental manipulation and high-resolution imaging.

Methods

In the current study, OCSs from P6 pups were exposed to cuprizone with or without the additional pro-inflammatory stimulus lipopolysaccharide (LPS) for 72 h to induce demyelination, or alternatively to a more aggressive protocol using lysophosphatidylcholine (LPC) for 18 h. Myelination dynamics were monitored by live-cell imaging with FluoMyelin Red (Invitrogen) using the IncuCyte© system (Sartorius).

At the experimental endpoint on day *in vitro* (DIV) 21, myelin integrity was assessed by immunohistochemistry for myelin basic protein (MBP) on axons visualized with neurofilament-heavy (NF-H) staining, and by automated western blotting for LPC-treated samples. Axonal damage was quantified by measuring neurofilament-light (NF-L) secretion using an immunosorbent assay (Uman Diagnostics).

Finally, microglial and oligodendrocyte populations were analyzed by immunohistochemical staining for IBA1 and Olig2, respectively.

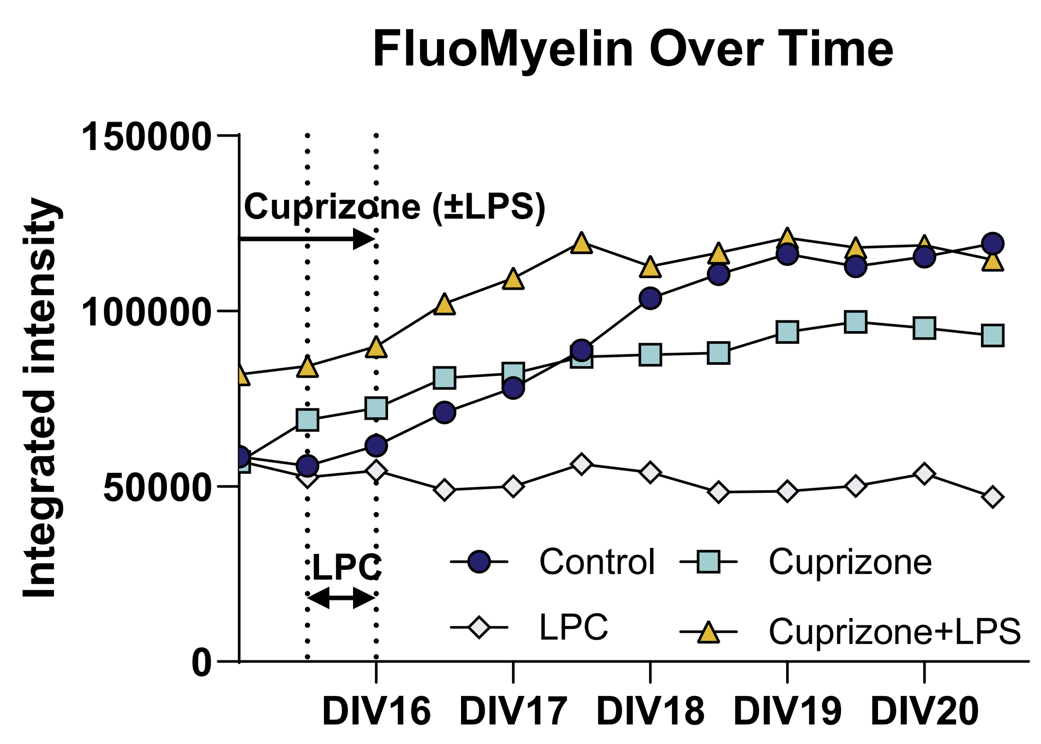
Results and Conclusion

Acute demyelination can be reliably induced by short-term exposure to LPC. LPC treatment not only triggers rapid myelin loss but also results in elevated NF-L levels in the culture supernatant, serving as an indirect biomarker of axonal injury.

In contrast, cuprizone-induced lesions exhibit a comparatively milder demyelinating phenotype. Interestingly, this effect is reversed upon co-administration of lipopolysaccharide (LPS), which promotes robust microglial activation and oligodendrocyte proliferation, ultimately leading to a significant increase in axonal remyelination.

Taken together, this *ex vivo* model provides a reproducible and accessible platform for investigating the cellular and molecular mechanisms underlying myelin degeneration and repair. Furthermore, it offers a valuable tool for preclinical evaluation of therapeutic strategies aimed at enhancing remyelination and mitigating axonal damage.

Live Monitoring of Myelination



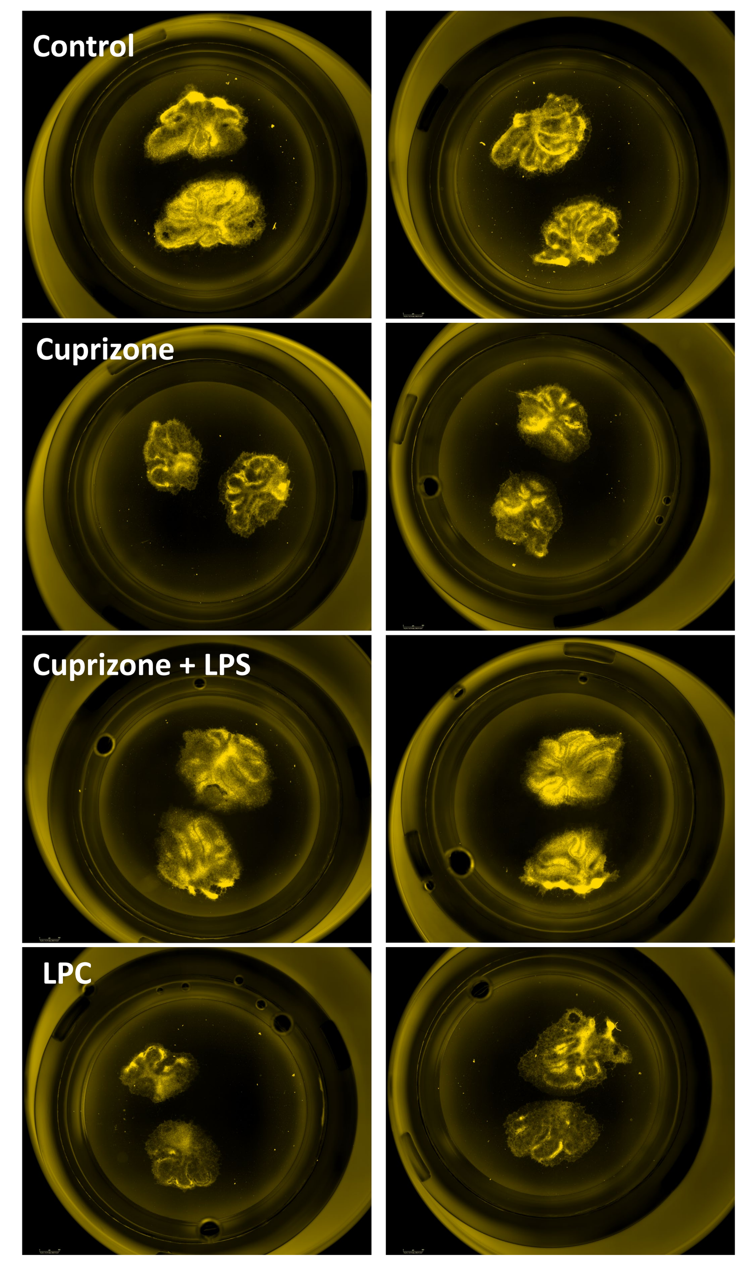


Figure 1: Myelination dynamics of OCS after treatment. OCSs were exposed to cuprizone with or without the additional pro-inflammatory stimulus LPS for 72 or LPC for 18 h. On DIV16 all lesions were removed, and myelination dynamics were monitored by live-cell imaging with FluoMyelin Red (Invitrogen) using the IncuCyte® system (Sartorius). Integrated intensity was assessed on whole well images every 12h until DIV21. n = 5-6 per group. Representative images of all conditions taken on DIV18 are shown on the right. No statistical analysis performed.

NF-L Secretion and Confocal Imaging

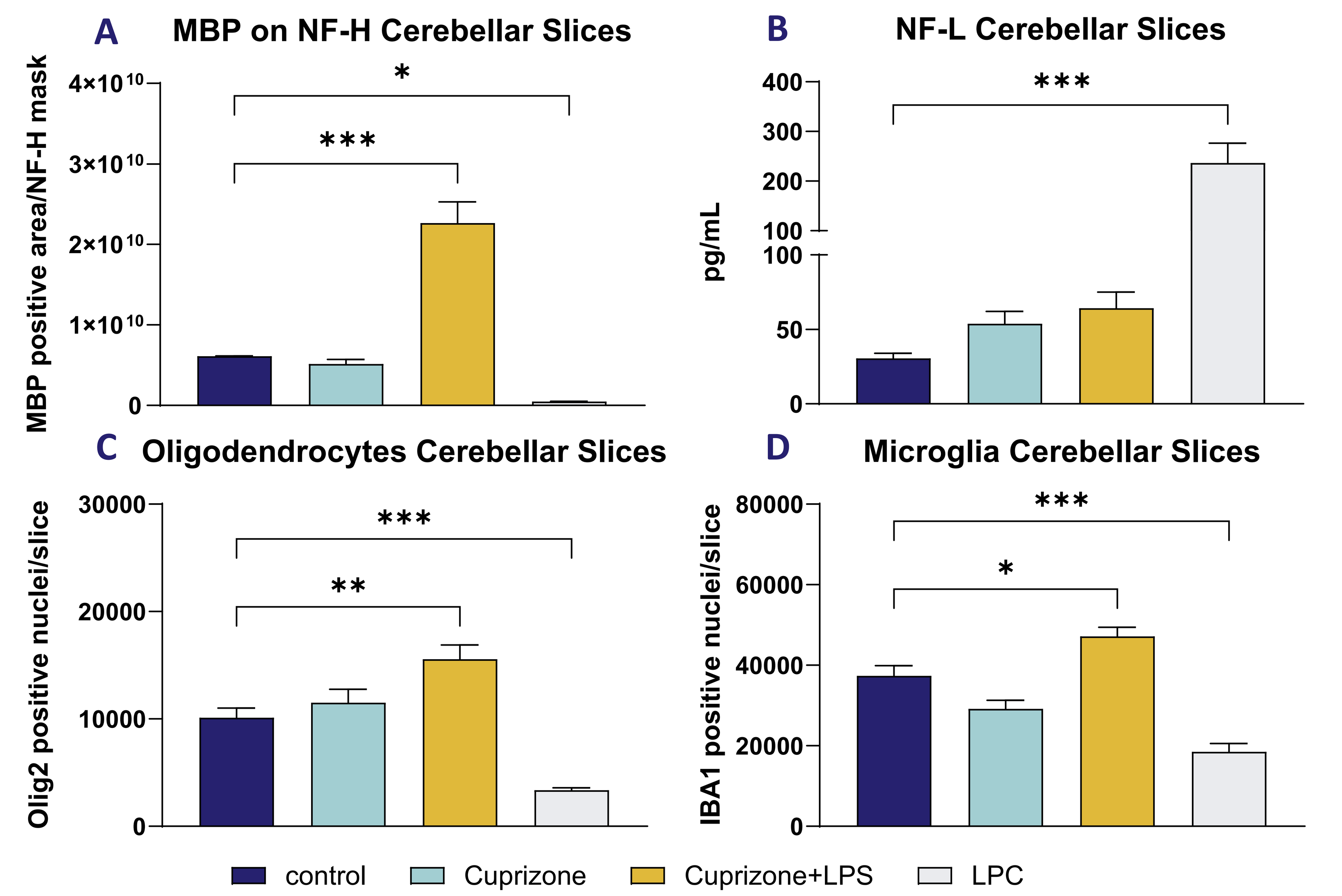


Figure 2: Analysis of myelination, NF-L secretion and glial cell counts. On DIV21 OCS were subject to immunohistological analysis and supernatant was examined for NF-L secretion. The area of MBP and NF-H colocalization **(A)** was analyzed as marker for myelination. NF-L secretion **(B)** as marker for axonal injury. Olig2 positive cells per slice area as marker for oligodendrocytes **(C)** and IBA1 positive cells as marker for microglial cells **(D).** Mean + SEM. One-way ANOVA followed by Bonferroni's *post hoc* test. n = 5-6 per group. *p <0.05, **p <0.01, ***p <0.001.

Confocal Imaging and WES Analysis

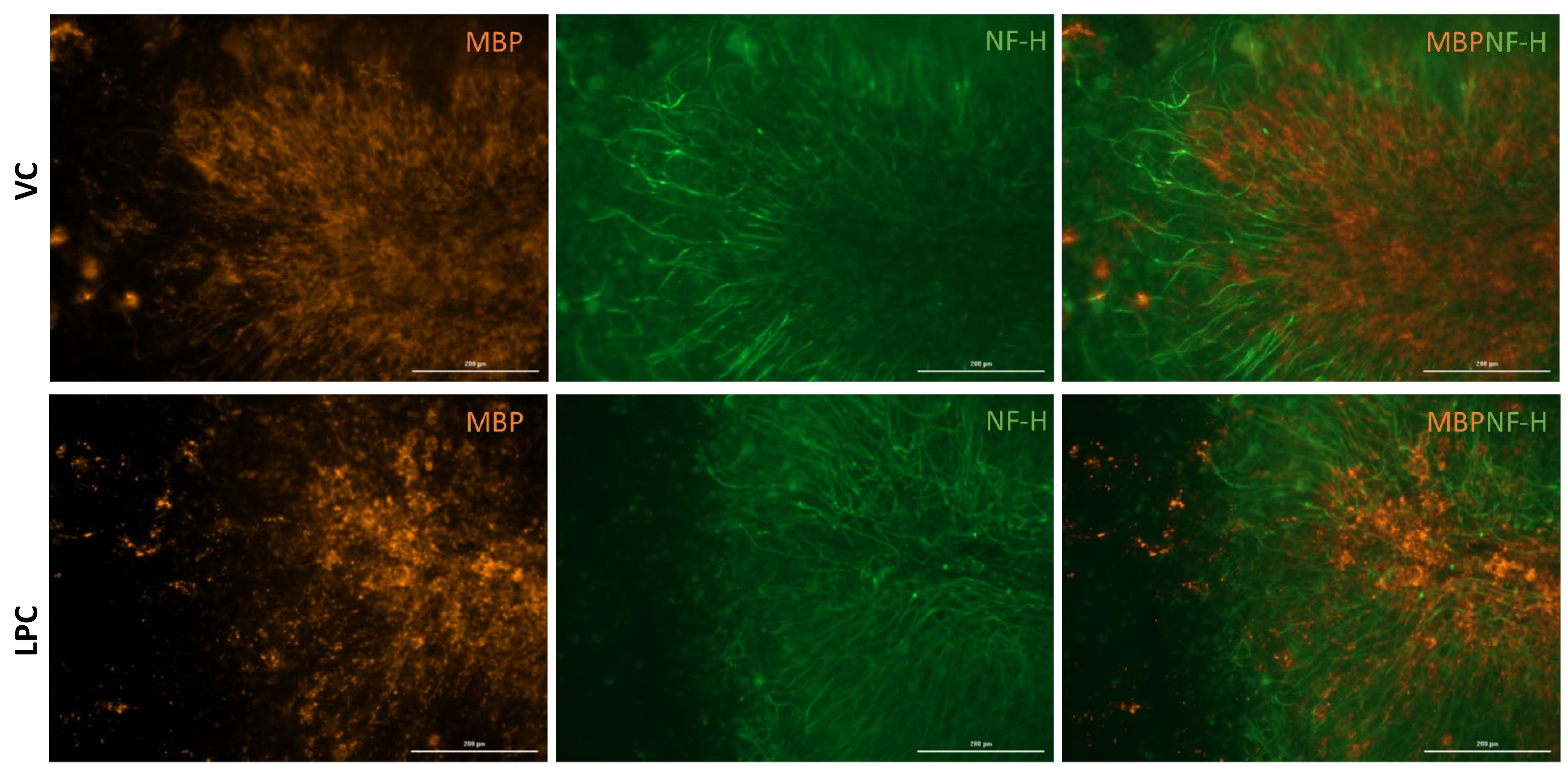
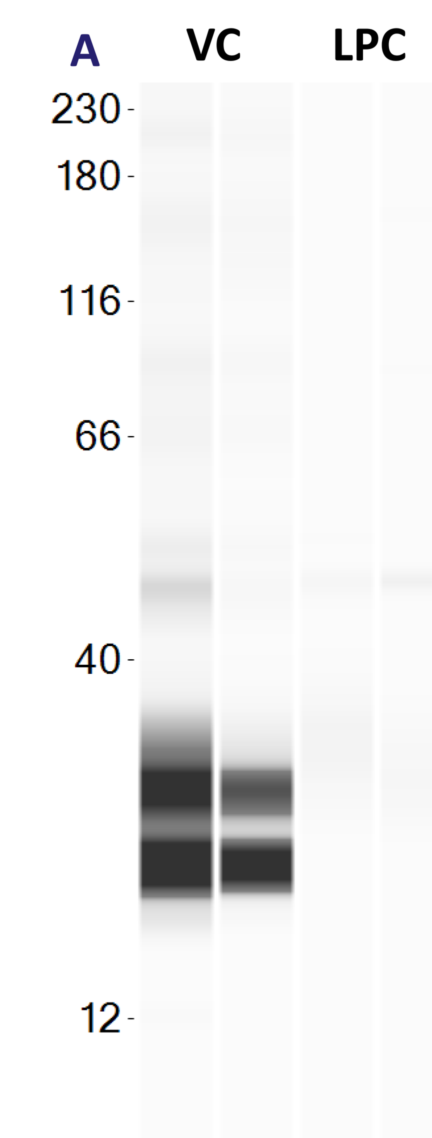


Figure 3: Representative images of vehicle-treated (VC) or LPC-treated OCSs 24h post-lesion. Images show representative labeling of myelin basic protein (MBP, red) and axonal marker NF-H (green).



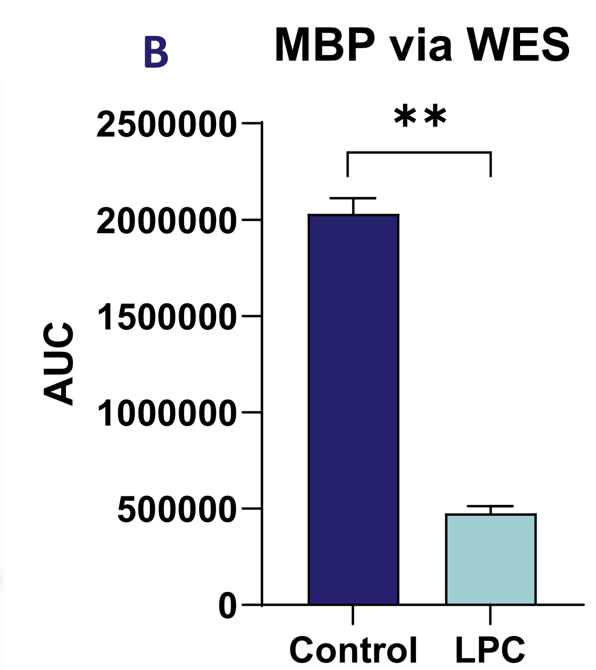


Figure 4: Quantification of myelin basic protein via WES. Vehicle-treated (VC) or LPC-treated OCSs were harvested in RIPA buffer 24h post-lesion on DIV17 and investigated via automated western blotting WES for MBP level. Representative lane-view **(A)** of 2 lanes per condition as well as quantification of the area under the curve (AUC) of the bands **(B).** Mean + SEM. Unpaired two-tailed T-Test. n = 4 per group. **p <0.01.

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