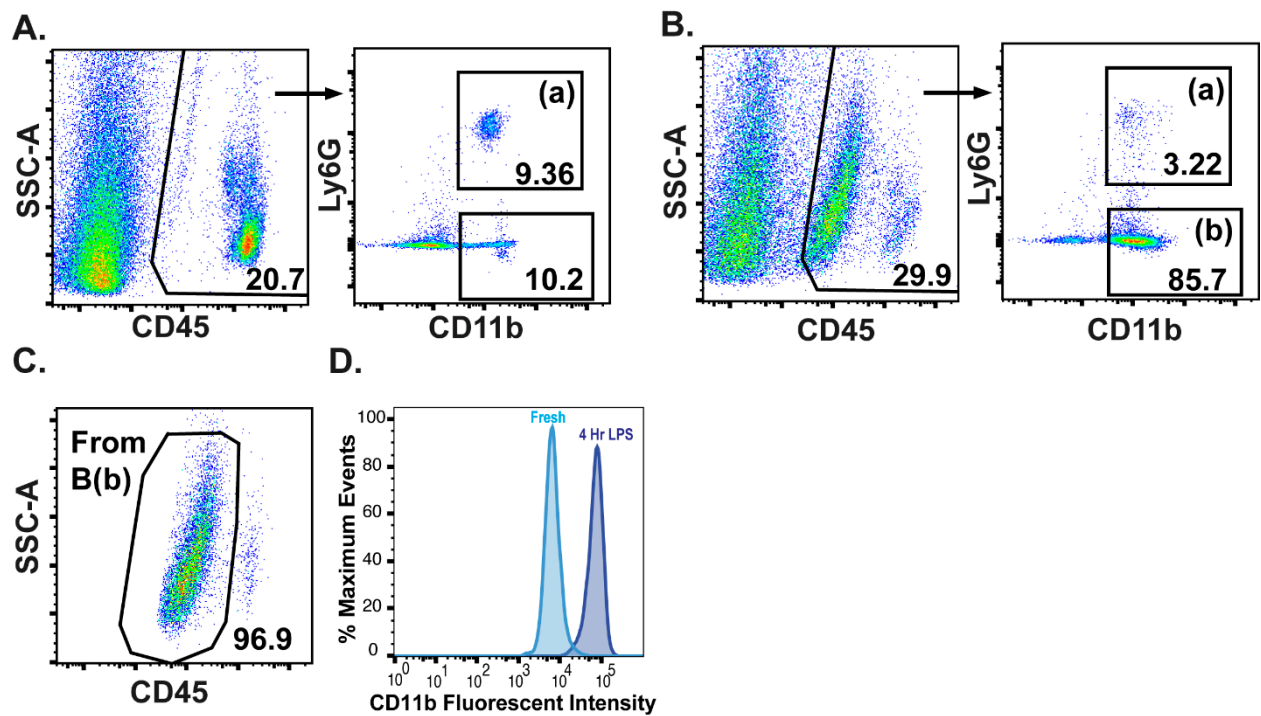
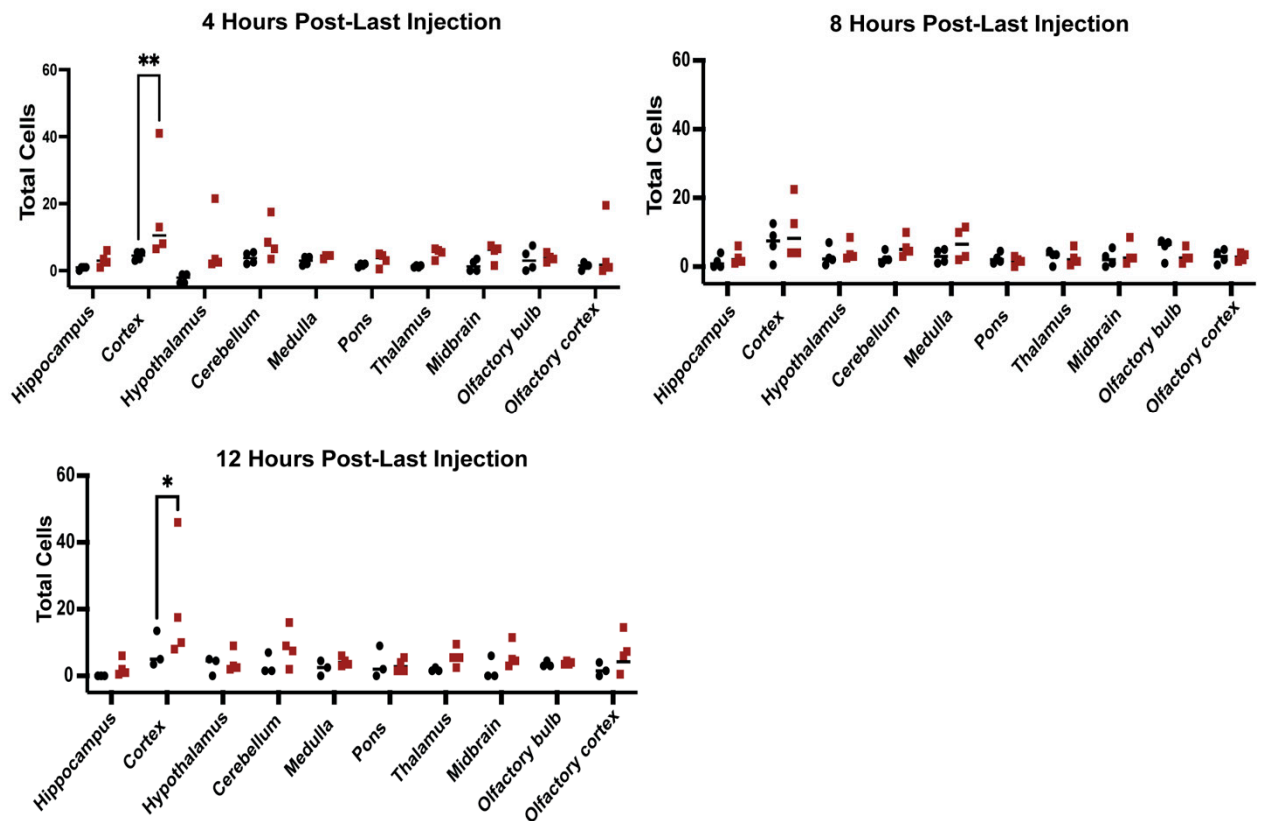


# Peripheral Low Level Chronic LPS Injection as a Model of Neutrophil Activation in the Periphery and Brain in Mice

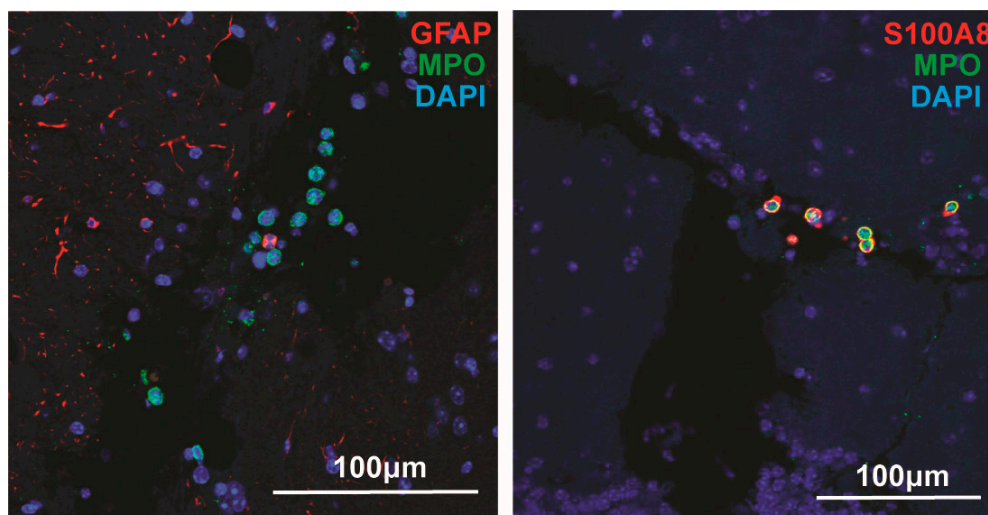
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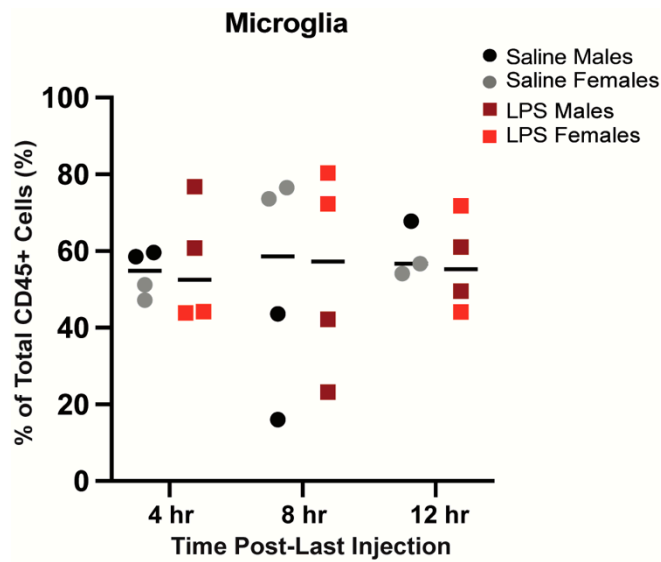
**Figure S1. Identification of neutrophils in blood and brain tissue.** Example gating strategy in C57BL/6J mouse blood (A) and brain tissue following saline injection (B). (A,B) Samples are first gated on total cells, singlets (FSC-A vs. FSC-H), and live cells. Neutrophils are identified as CD45+CD11b+Ly6G+ (a). (C) Microglia are identified as CD11b+Ly6G- and CD45-intermediate. (D) Shift in CD11b median fluorescent intensity on blood neutrophils following LPS exposure.



**Figure S2. Neutrophils in mouse brain regions following LPS and saline injections.** Neutrophils counted via anti-MPO staining and given as a total within the brain region. Each datapoint represents an average count from 2 sagittal sections. Statistical significance was assessed between the LPS and saline groups within the same hour post-last injection by 2way ANOVA followed by Tukey's multiple comparisons test with multiplicity adjusted P values represented as \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure S3.** MPO staining colocalizes with S100A8, a protein highly expressed in neutrophils, and stains for cells with multilobed nuclei. **(Left)** Mouse brain stained with anti-MPO (green), DAPI (blue), and GFAP (red) demonstrating cells with distinct multilobed nuclei near areas of inflammation in mouse brain tissue (cerebellum 40X). **(Right)** Image of mouse cerebellum (40X) stained with anti-MPO (green), DAPI (blue), and S100A8 (red).



**Figure S4. Assessment of microglia frequencies in the brain following chronic low-level LPS injections.** Microglia were assessed via flow cytometry and gated as described in Supplementary Fig 1. Statistical significance was assessed between the LPS and saline groups across timepoints by 2-way ANOVA followed by post-hoc assessments within each timepoint using Tukey's multiple comparisons test.