NF-kB and microglia activation in the PMC following LPS treatment

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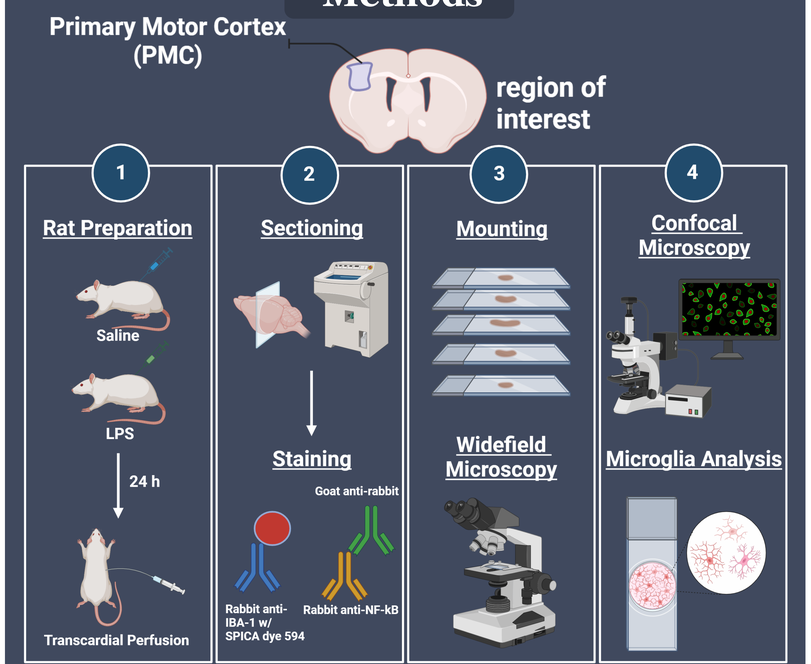
Introduction

- Goal: investigate inflammatory pathways typically seen in the onset of ALS
- ALS impacts motor function (Brotman et al., 2023)
- Canonical inflammatory NF-kB pathway in microglia is upregulated in patients w/ ALS (Frakes et al., 2014)
- Constitutive NF-kB activity in wildtype microglia has previously been shown to lead to upper motor neuron death
- Upper motor neurons are housed in the primary motor cortex (PMC) beside microglia, where microglia can potentially activate into M1 inflammatory morphology (Purves et. al., 2001; Migliarini et. al., 2021)
- Overview: LPS (mimics stress)-> activation of NF-kB downstream of TLRs -> activation of microglia -> upper motor neuron death via activated microglia (Brotman et al., 2023; Lopez et al., 2023; Frakes et al., 2014; Jung et al., 2022; Okun et al., 2009)

Hypothesis

Acute stress will activate NF-kB in microglia within the PMC in LPS-treated rats.

Methods



The experimental process involved the cryosectioning of 16 adult female rat brain tissues for immunohistochemistry, encompassing mounting, antibody staining, widefield microscopy, confocal microscopy, and microglia analysis.

Figures

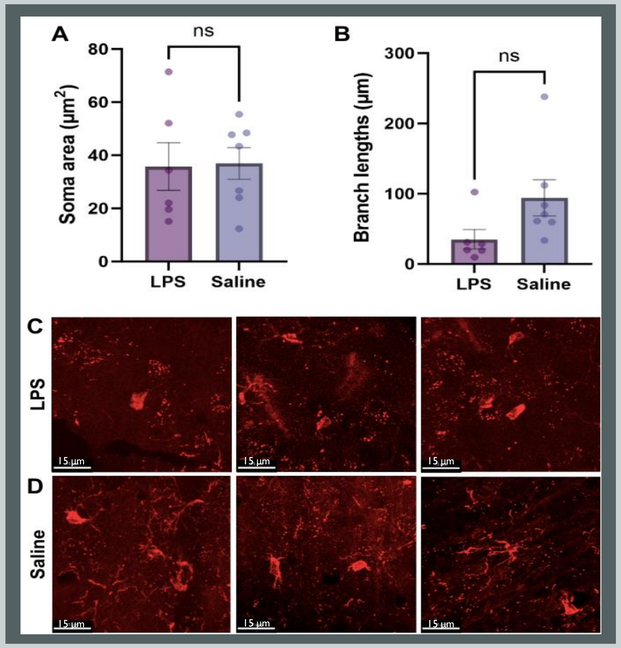


Figure 1. Confocal microscopy of PMC microglia in the LPS treated and saline treated groups. Quantification of mean soma areas (A) and branch lengths (B) for both groups, with error bars representing standard error of the mean (SEM) and a sample size of n = 6-8 for each group. The conducted statistical test was an unpaired t-test at a significance level of 0.05. Representative images (63X) of IBA-1 in the PMC of LPS group (C) and the saline group (D). * $p < 0.05$.

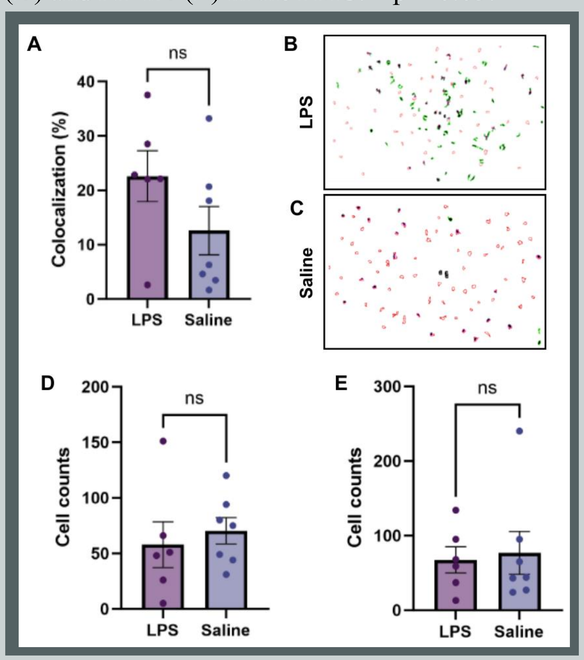


Figure 2. Colocalization analysis of NF-kB with IBA-1 in the PMC (A) with representative images taken from ImageJ (B & C). Cell counts of IBA-1 (D) and NF-kB (E) in the PMC. * $p < 0.05$.

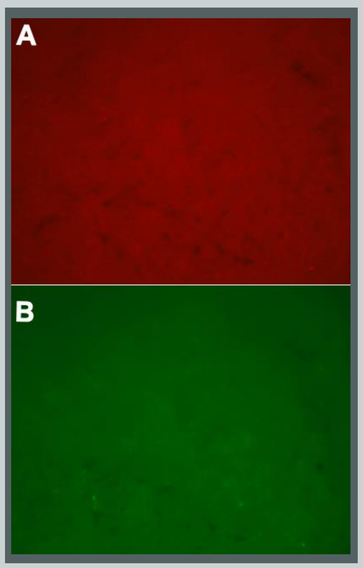


Figure 3. Image of the PMC stained in red using IBA-1 (A). Image of the PMC stained in green using NF-kB (B).

Results

- Insignificant differences in microglial soma areas and process lengths between LPS & saline groups (Fig. 1)
- Insignificant differences in IBA-1 & NF-kB stains between LPS & saline groups (Fig. 2)
- Insignificant differences in colocalization of IBA-1 & NF-kB between LPS & saline groups (Fig. 2)

Future Implications

- Microglial cells in the PMC of genetically unmodified rats did not undergo changes in morphology & NF-kB activity in PMC following LPS treatment (Migliarini et al. (2021))
- Further studies could benefit from using ALS phenotypic rats to investigate the effect of ALS genetic mutations on LPS response from microglia
- Euthanasia of rats at different time points could also be beneficial in future studies to investigate the impact of time on inflammatory microglial response after LPS treatment in PMC
- Sample size could be increased & male rats could be incorporated for broader applicability of the results
- Varying doses of LPS impact on microglial morphology & NF-kB activity

Acknowledgements & References

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