



INTRODUCTION

- Multiple mechanisms have been proposed for **learning** and **memory** (Abraham et al., 2019). One that is under-researched is the role of **microglia**.
- TNF- α** is regulated by microglia and mediates **stress** response in the **hippocampus**, which is linked to learning and memory (Liu et al., 2019).
- Elevated TNF- α levels are associated with decreased **working memory** (Ohgidani et al., 2016).
- Prior research has focused on male rodents' stress responses in relation to memory, neglecting to study **sex as a biological variable** (Bras et al., 2022; Will et al., 2017). Our research will focus on female rodents.

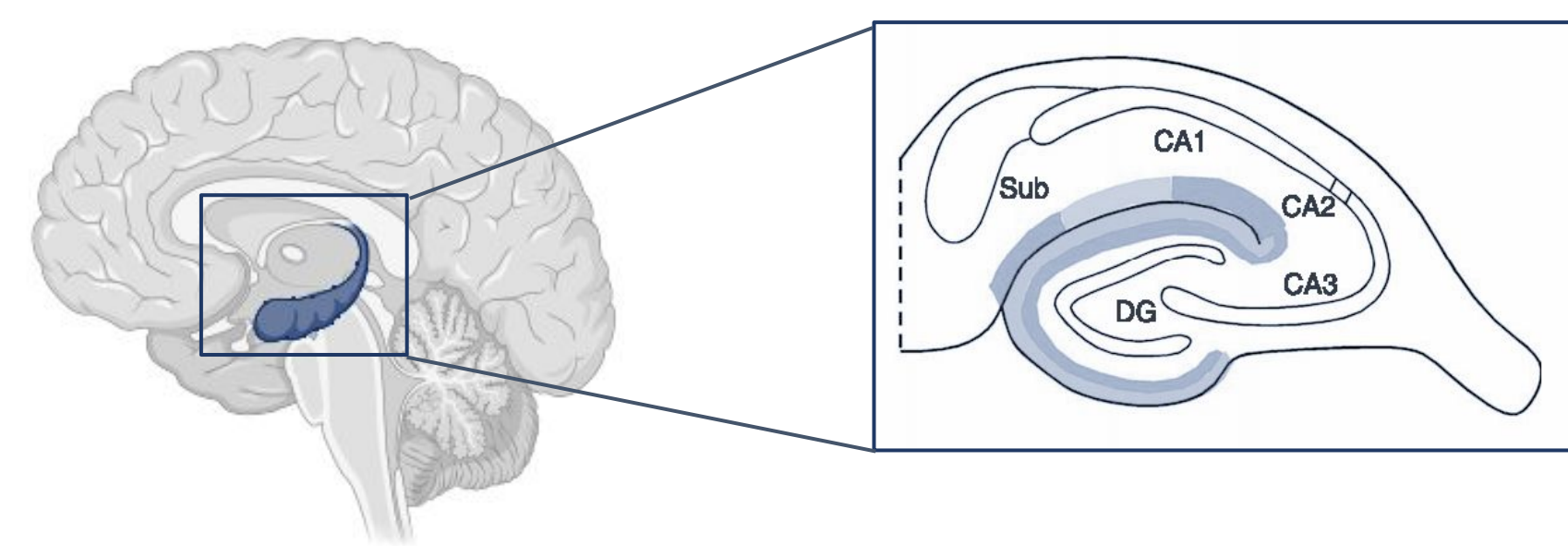


Figure 1. The dentate gyrus (DG) hippocampal region.

HYPOTHESIS

Microglia morphological activation (as measured by soma area and process length) and **TNF- α expression** in the dentate gyrus of female rats will be **greater following acute stress** in comparison to female saline rats.

EXPERIMENTAL DESIGN

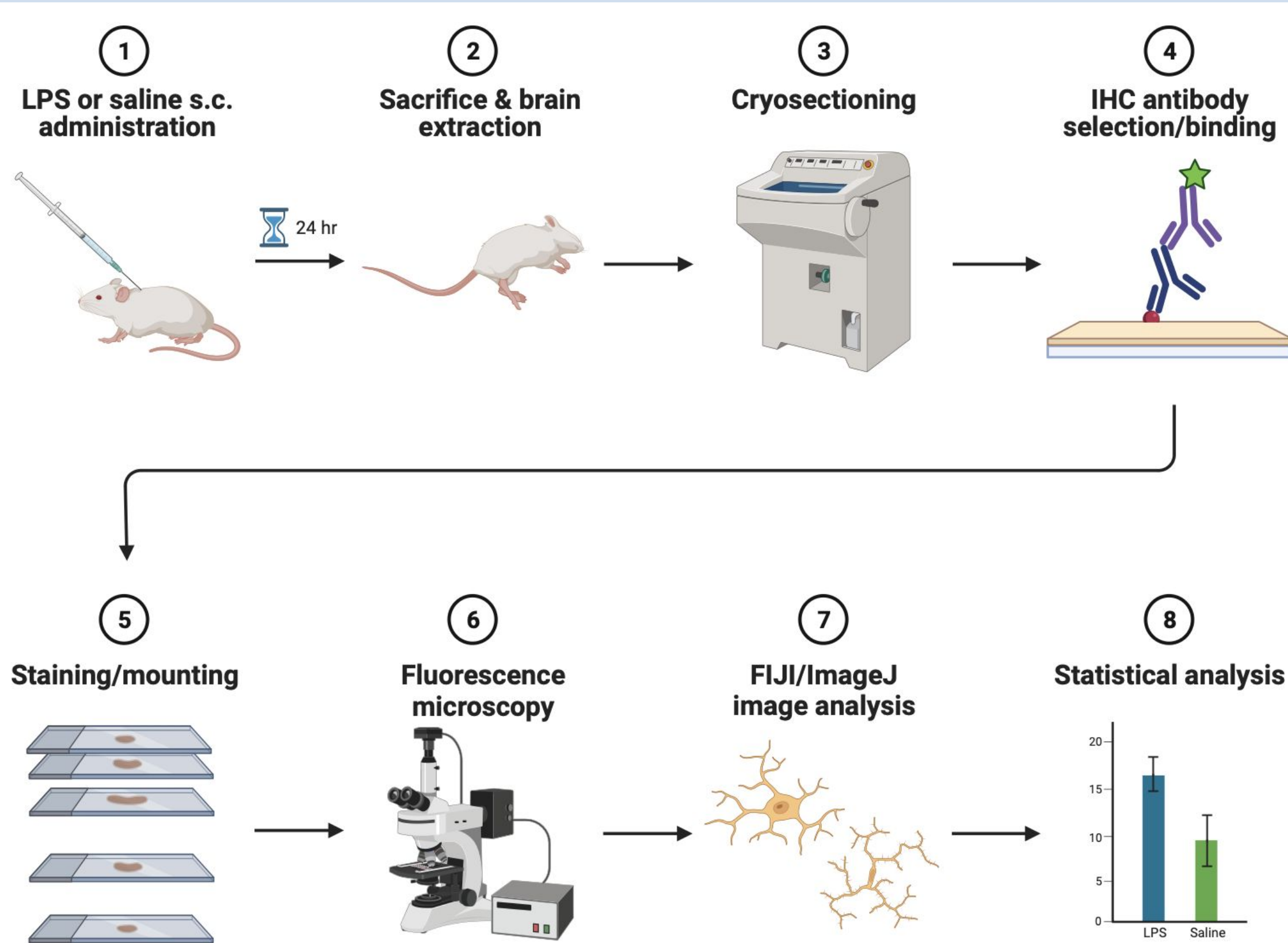


Figure 2. Experimental paradigm. Executed on 16 adult female rats. LPS injection was used to initiate acute stress.

RESULTS

Microglial Soma Size

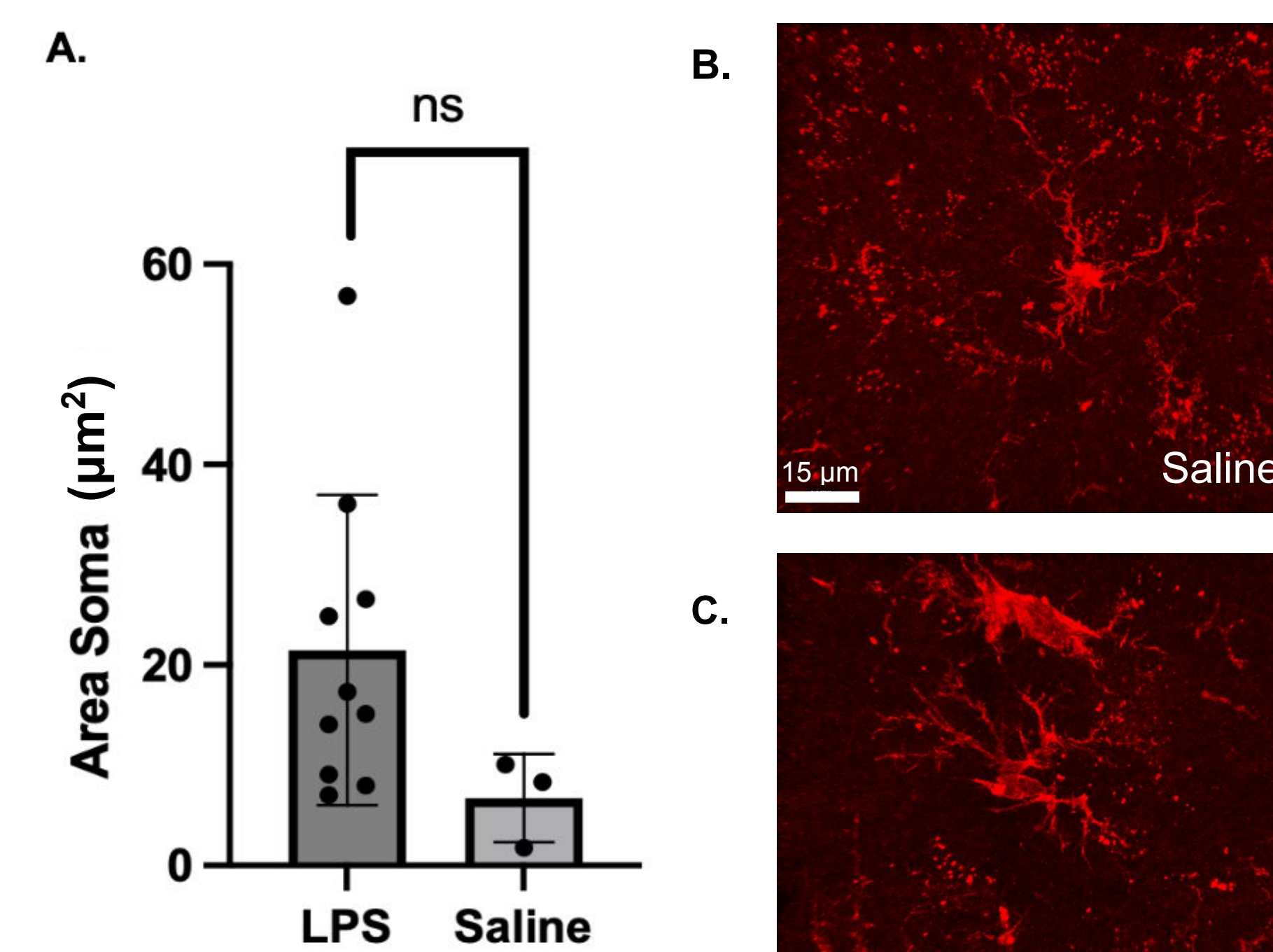


Figure 3. Microglial soma size (μm^2) is not significantly different between the saline control group and the LPS injection group. A) Graph depicting the soma size in saline vs LPS group (independent sample t-test; $\alpha=0.05$, $t=1.438$, $df=11$, $p=0.1784$). Mean soma area in LPS group was $49.13 \mu\text{m}^2$ ($SD=27.44 \mu\text{m}^2$) and saline $7.29 \mu\text{m}^2$ ($SD=9.332 \mu\text{m}^2$). B) Representative image of microglia in the dentate gyrus of the saline rat and C) the LPS rat. Images captured at 63x on a confocal microscope. Error bars indicate standard deviation.

Microglial Processes length

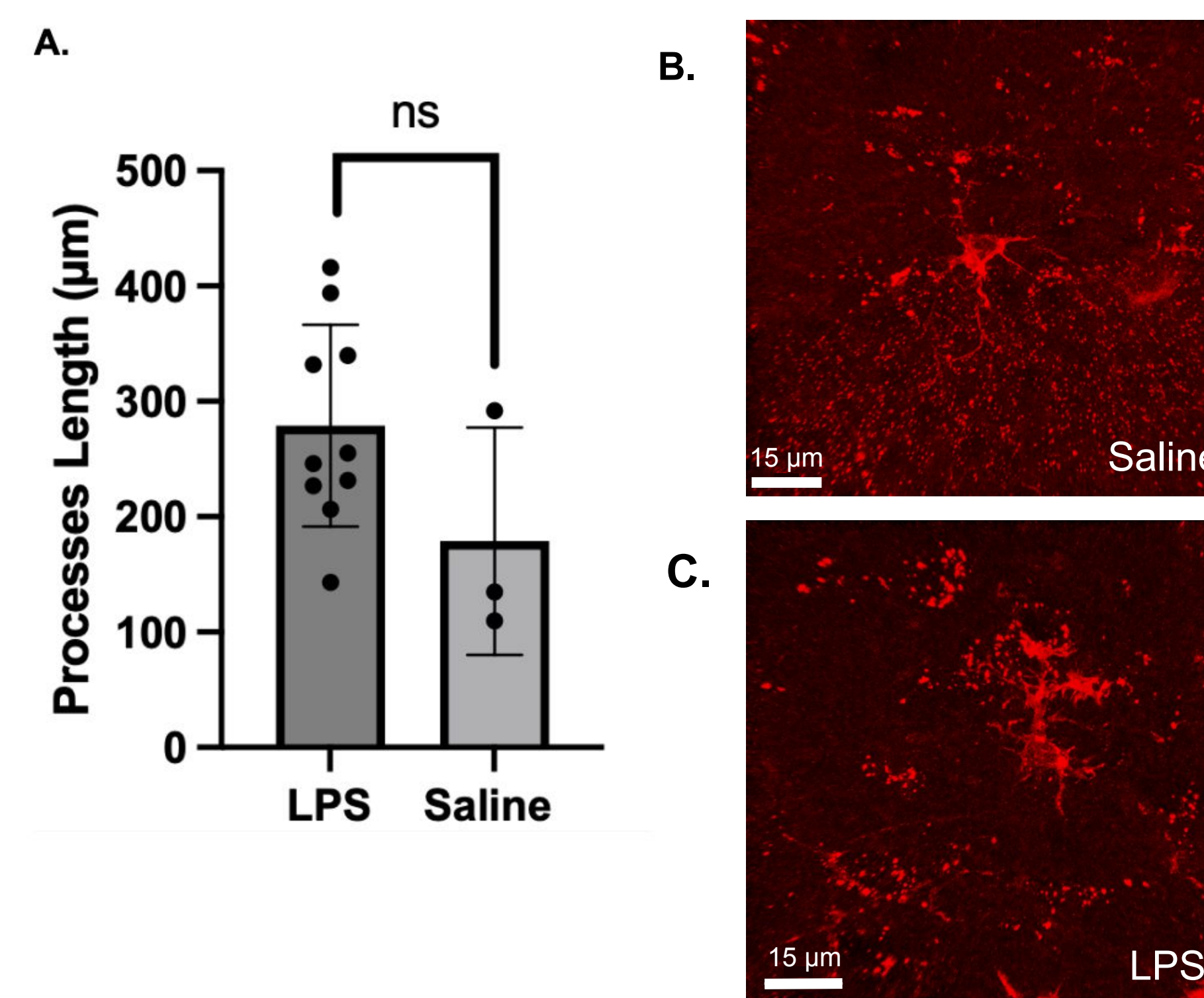


Figure 4. Microglial processes length (μm) is not significantly different between the saline control and LPS group. A) Graph depicting the processes length in saline vs LPS group (independent sample t-test; $t=1.70$, $df=11$, $p=0.1172$). Mean soma area in LPS group was $279.1 \mu\text{m}$ ($SD=87.46 \mu\text{m}$) and saline $178.8 \mu\text{m}$ ($SD=98.55 \mu\text{m}$). B) Representative image of microglia in the dentate gyrus of the saline rat and C) the LPS rat. Images captured at 63x on a confocal microscope. Error bars indicate standard deviation.

Cell Count of Microglia and TNF- α

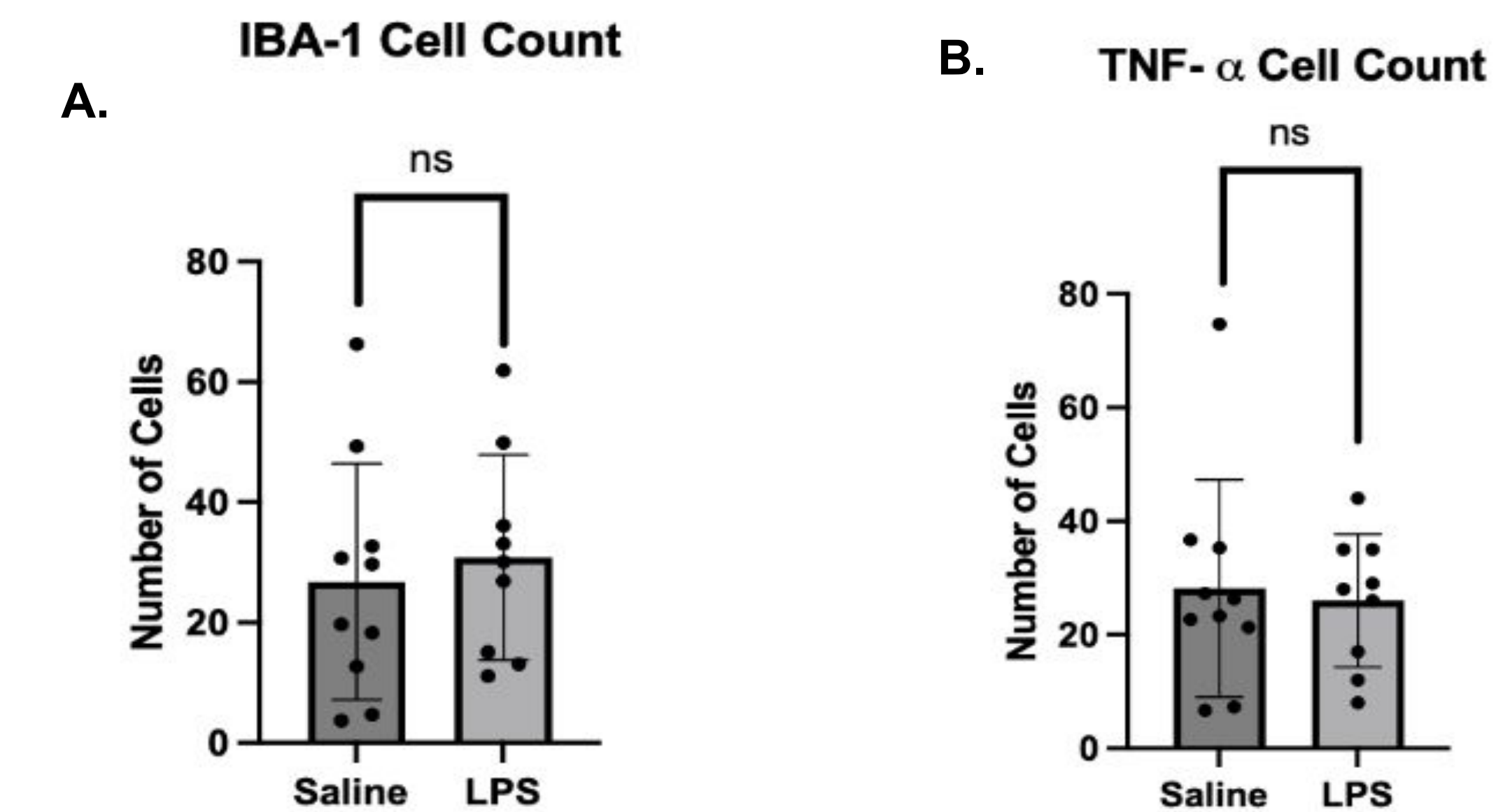


Figure 5. Neither IBA-1 cell count nor TNF- α cell count are significantly different between the saline and LPS groups. A) A graph depicting the number of microglia cells in saline rats vs the LPS group (independent sample t-test; $t=0.6414$, $df=17$, $p=0.5298$). B) A graph depicting the number of TNF- α cells in saline rats vs the LPS group (independent sample t-test; $t=0.2844$, $df=17$, $p=0.7795$). Error bars indicate standard deviation.

Colocalization of IBA-1 and TNF- α

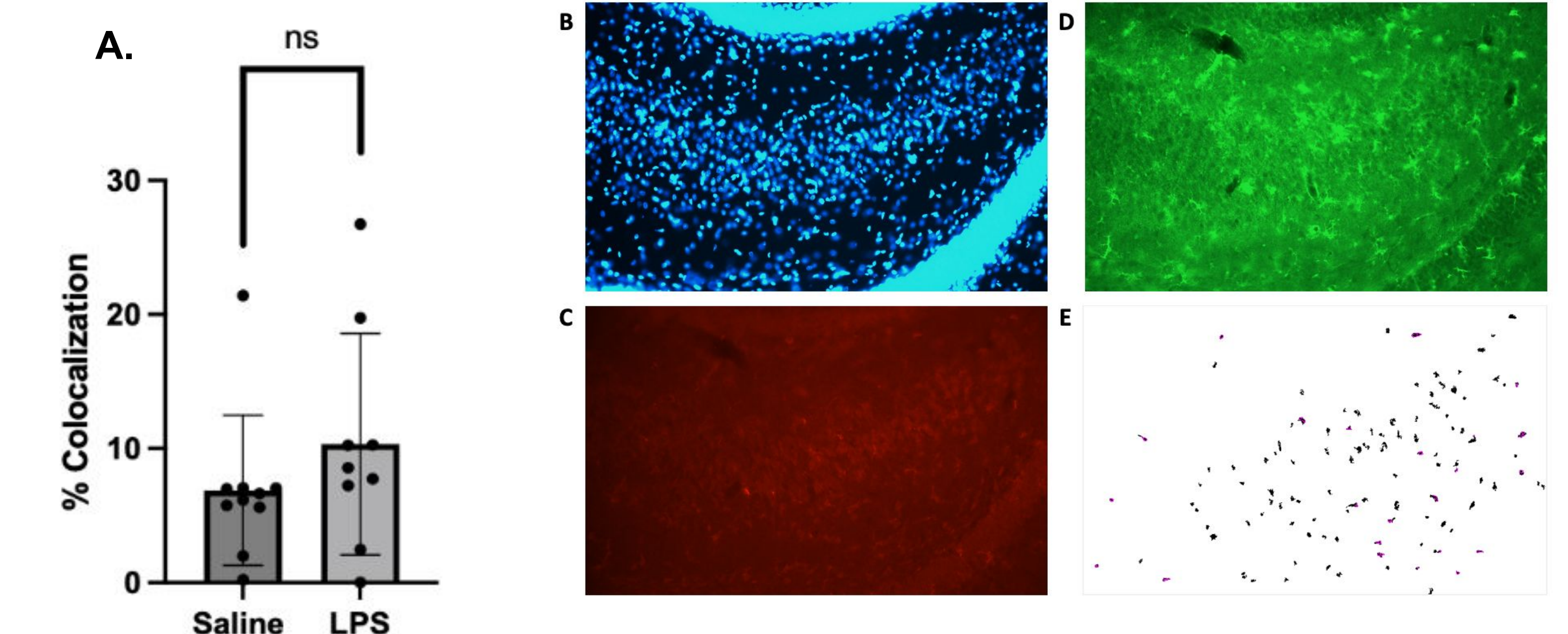


Figure 6. The percent colocalization between IBA-1 and TNF- α in the saline vs LPS groups showed no significance. A) A graph depicting the percent localization of IBA-1 and TNF- α in saline and LPS groups (independent sample t-test; $t=0.6414$, $df=17$, $p=0.5298$). Error bars indicate standard deviation. Percent colocalization was found using ImageJ and data was analyzed using GraphPad Prism. Representative images of LPS injected rat. B) DAPI staining for cell nuclei in the 358 nm channel, C) IBA-1 staining for microglia in the 568 nm channel, D) TNF- α staining in the 488 nm channel, and E) colocalization between microglia and TNF- α represented in magenta.

DISCUSSION

- Implications:**
 - The absence of significant differences in microglial morphology and TNF- α expression challenge our initial hypothesis and findings from previous studies (Bras, 2022; Liu, 2019).
 - Our results suggest that the relationship between microglial activation and acute stress is more complex than previously understood.
- Study Limitations:**
 - Our study focused on morphological changes and colocalization of IBA-1 and TNF- α , which differ from previous studies which used mRNA expression and behavior.
 - Small sample size restricts the statistical power of our results.
 - Focus exclusively on female rats limits generalizability of our findings, especially in light of the contrasting results from studies using male subjects.
- Future Directions:**
 - Increasing **sample sizes** with both **male and females** subjects.
 - Using **behavioral tasks** to target hippocampal function to understand cognitive impacts.
 - Extend TNF- α expression analysis to explore **regional differences** within the hippocampus (CA1 and CA3 areas).

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