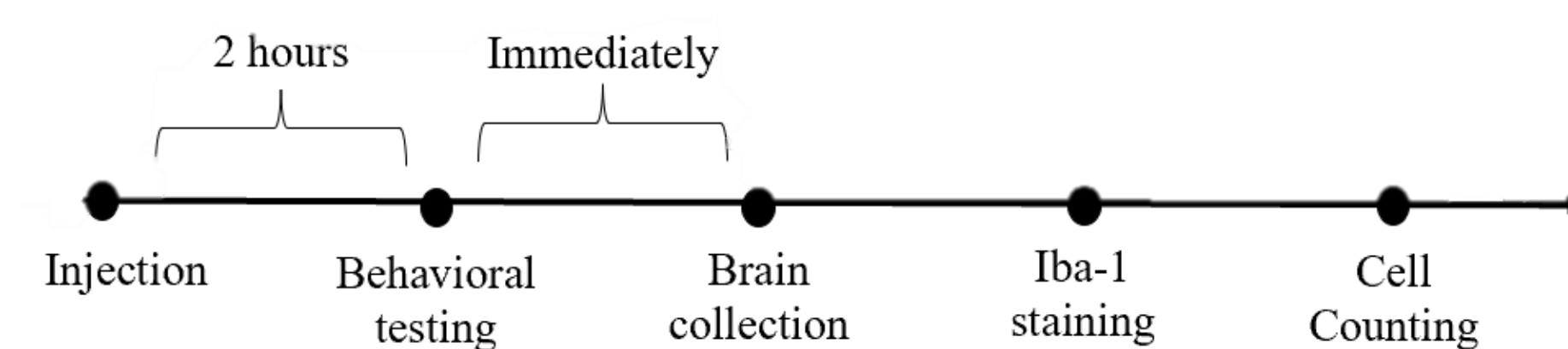


Introduction

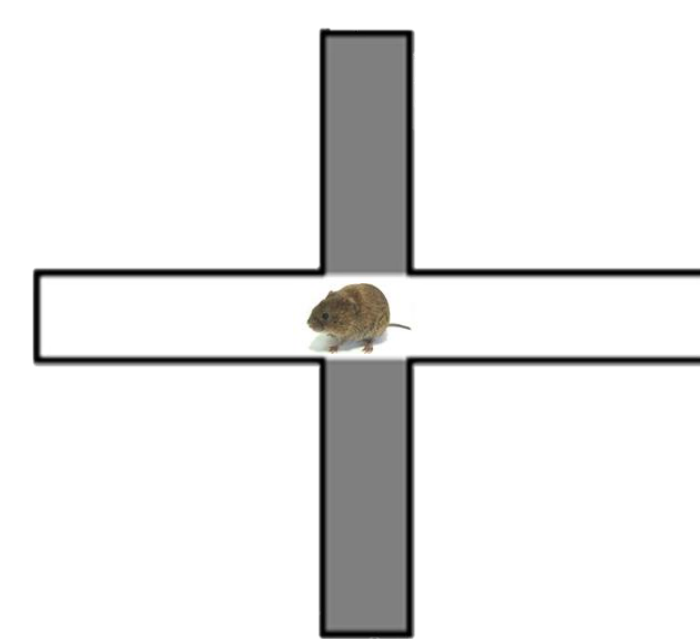
The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent species that provides an excellent opportunity to study the impact of social environments on the brain and behavior². In a recent study from the Wang lab, chronic social isolation increased anxiety-like behaviors, decreased social affiliative behaviors, and elevated neuroinflammation in selected brain regions in adult male prairie voles¹. Because chronic neuroinflammation has been associated with anxiety-like and social affiliative behaviors^{2,3}, we predict that altered neuroinflammation in selected brain regions may play a role in mediating changed behaviors associated with social isolation. As lipopolysaccharides (LPS) are large molecules that primarily activate the immune system⁴, in the present study, we treated male prairie voles with or without LPS to examine its effects on anxiety-like and social affiliative behaviors. We also immunoreactively stained the vole brain sections with Iba-1 (ionized calcium-binding adaptor molecule 1, the most commonly used protein marker of microglia activation) to measure the effects of LPS on neuroinflammation in the brain. **We hypothesized that LPS treatment will alter anxiety-like and social affiliative behaviors and increase neuroinflammation in a brain region-specific manner in male prairie voles.**

Methods

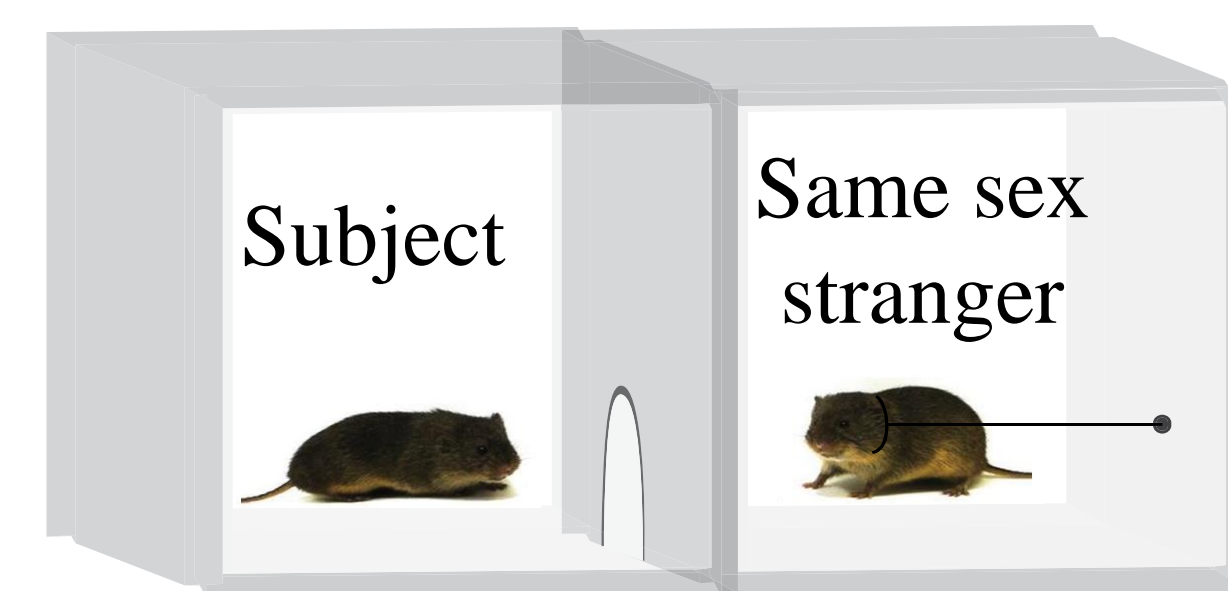
Subjects: Subjects were adult male prairie voles. They were randomly assigned to two treatment groups and received peripheral injections of either 0.9% saline (control) or 1.0 mg/kg LPS. Two hours later, each group was further assigned to sub-subgroups for either EPM or SA behavioral testing.



Elevated Plus Maze (EPM): Anxiety-like behaviors were examined in an EPM test. The apparatus consists of two open arms and two closed arms that intersect in the middle. The maze is elevated 45 cm off the ground. Subjects were placed into the center of the maze and allowed to roam for 5 minutes.



Elevated Plus Maze



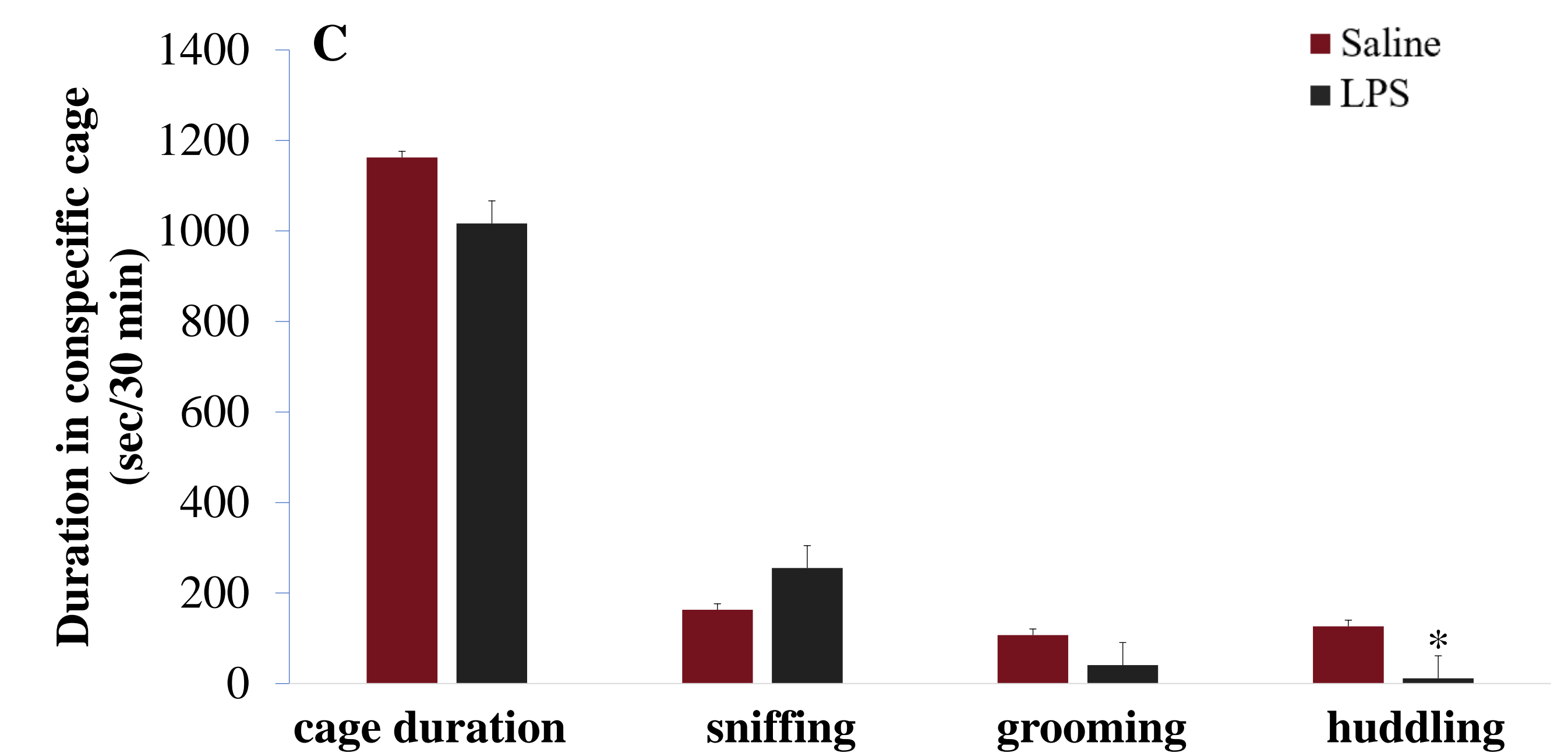
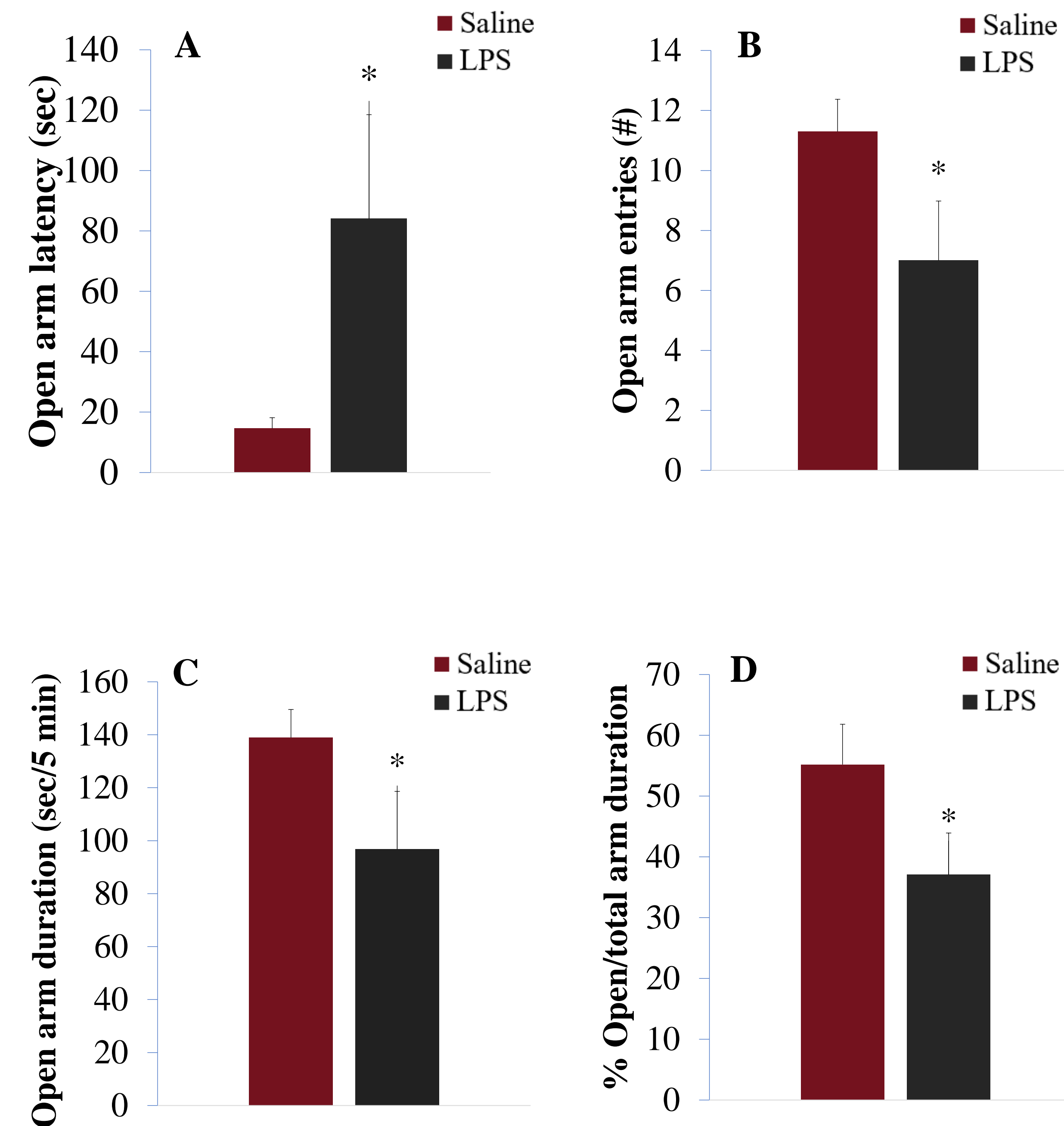
Social Affiliation

Social Affiliation Test (SA): Social affiliative behaviors were examined using a SA test. The apparatus consists of two cages connected by a hollow tube to allow the subject to freely move between them. A conspecific male stimulus vole is tethered to one of the cages. The subject is released into the empty cage and the test lasted for 30 min.

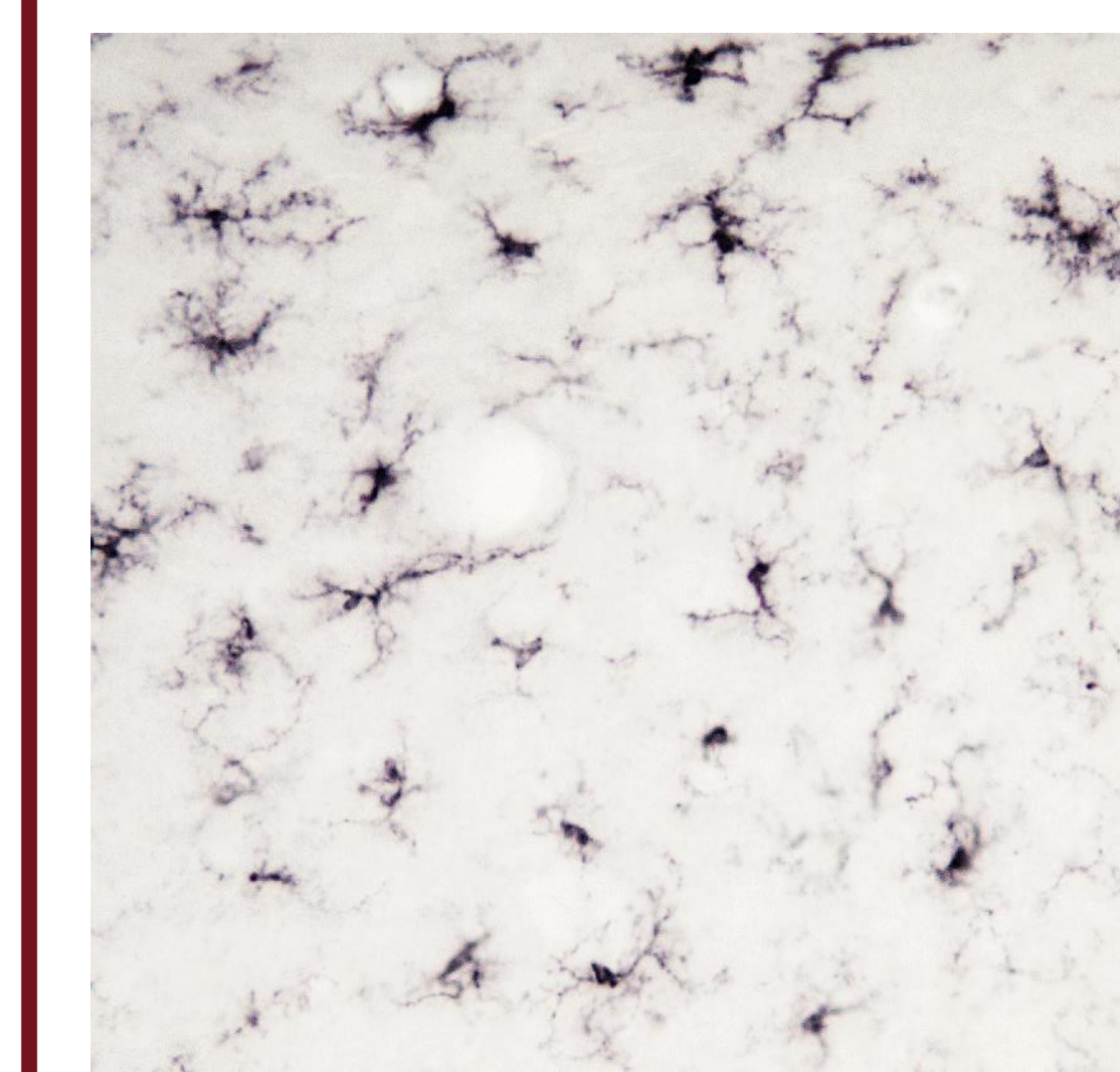
Iba-1 Immunocytochemistry: Iba-1 is a neuroinflammation marker. The set of SA sub-groups were perfused immediately after behavioral testing and their brains were extracted. Coronal sections were stained with Iba-1 antibody.

Data Analysis: Behavioral data were analyzed by *t-test*.

1 LPS treatment increases anxiety-like behaviors



3 Iba-1 staining and on-going procedure



The image on the left illustrates Iba-1 stained cells from a vole brain section. These cells will be quantified in selected brain regions involved in anxiety-like and social behaviors, including the prefrontal cortex (PFC), nucleus accumbens (NAcc), bed nucleus of the stria terminalis (BNST), paraventricular nucleus (PVN), dentate gyrus of the hippocampus (DG), amygdala (AMY), and dorsal raphe nucleus (DR). Group differences in Iba-1 cell density will be compared between the control and LPS-treated voles by *t-test*.

Conclusions

- LPS increases anxiety-like behaviors in the male prairie vole.
- LPS decreases social affiliative behaviors with a stranger in the male prairie vole.
- We also predict that LPS treatment will increase Iba-1 labeling in a brain region-specific manner.

References

1. Donovan, M., et al. (2020) *Neurobiology of Stress*, 13.
2. Getz, L. L., & Carter, C. S. (1996). *American Scientist* 84(1), 56-62.
2. Nelson, L. H. & Lenz, K. M., (2017) *Behavioral Brain Research* 316: 279-293.
3. VanRyzin, J. W., Yu, S. J., Perez-Pouchoulen, M., & McCarthy, M. M., (2016) *eNeuro* 3(6).
4. Zhao, J., et al. (2019) *Sci Rep*, 5:37.

Acknowledgement

Supported by NIH grants: NIMH 108527-R01 & NIMH 125408-R01

2 LPS treatment decreases social affiliation behaviors

