

Introduction

- Exposure therapy is used to treat fear-related disorders, which often peak during adolescence¹. However, its efficacy is lower in adolescents than in adults.
- Brain regions of interest in fear regulation include the prelimbic cortex (PL), involved in fear consolidation, and the infralimbic cortex (IL), which mediates extinction learning.
- Evaluating differential impacts of massed extinction, where tones are aggregated on one day of learning, and spaced extinction², where tones are played over four days, on fear retention and PL/IL activity may have clinical significance through informing the development of novel behavioral treatments for adolescents.

Aim

This experiment aims to elucidate whether having experienced massed or spaced extinction learning is more effective in attenuating fear responses in male and female adolescent mice.

Methods

Subjects:

- Adolescent (PND30) C57BL/6J mice

Behavioral Data:

- Massed: n = 16 Male, n = 17 Female
- Spaced: n = 19 Male, n = 20 Female

Histological Data:

- Massed PL: n = 7, Spaced PL: n = 5
- Massed IL: n = 4, Spaced IL: n = 5

Apparatus:

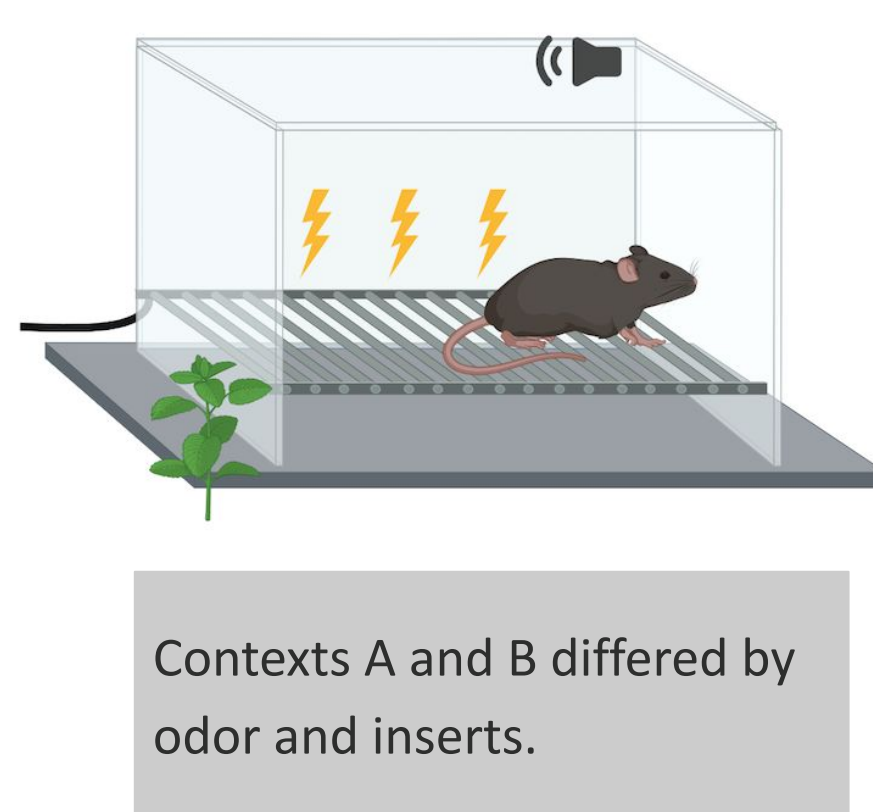
- Med Associates Conditioning Chambers (Contexts A and B)

Behavioral Protocols:

Fear conditioning:

Day 1, Context A

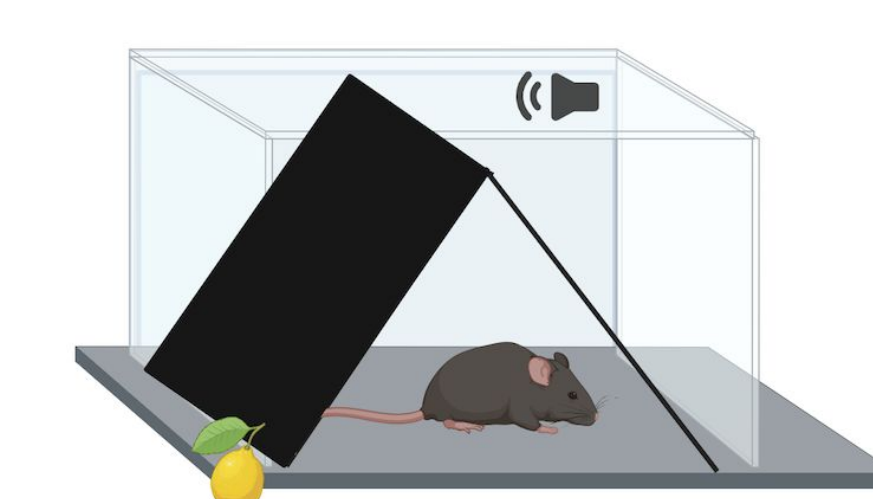
- 5 x CS+
- 20s tones, 5kHz
- 1s 0.5mA footshock US



Fear extinction:

Days 2-5, Context B

- Massed (1 day, 40 x CS)
- Spaced (4 days, 10 x CS)



Histology:

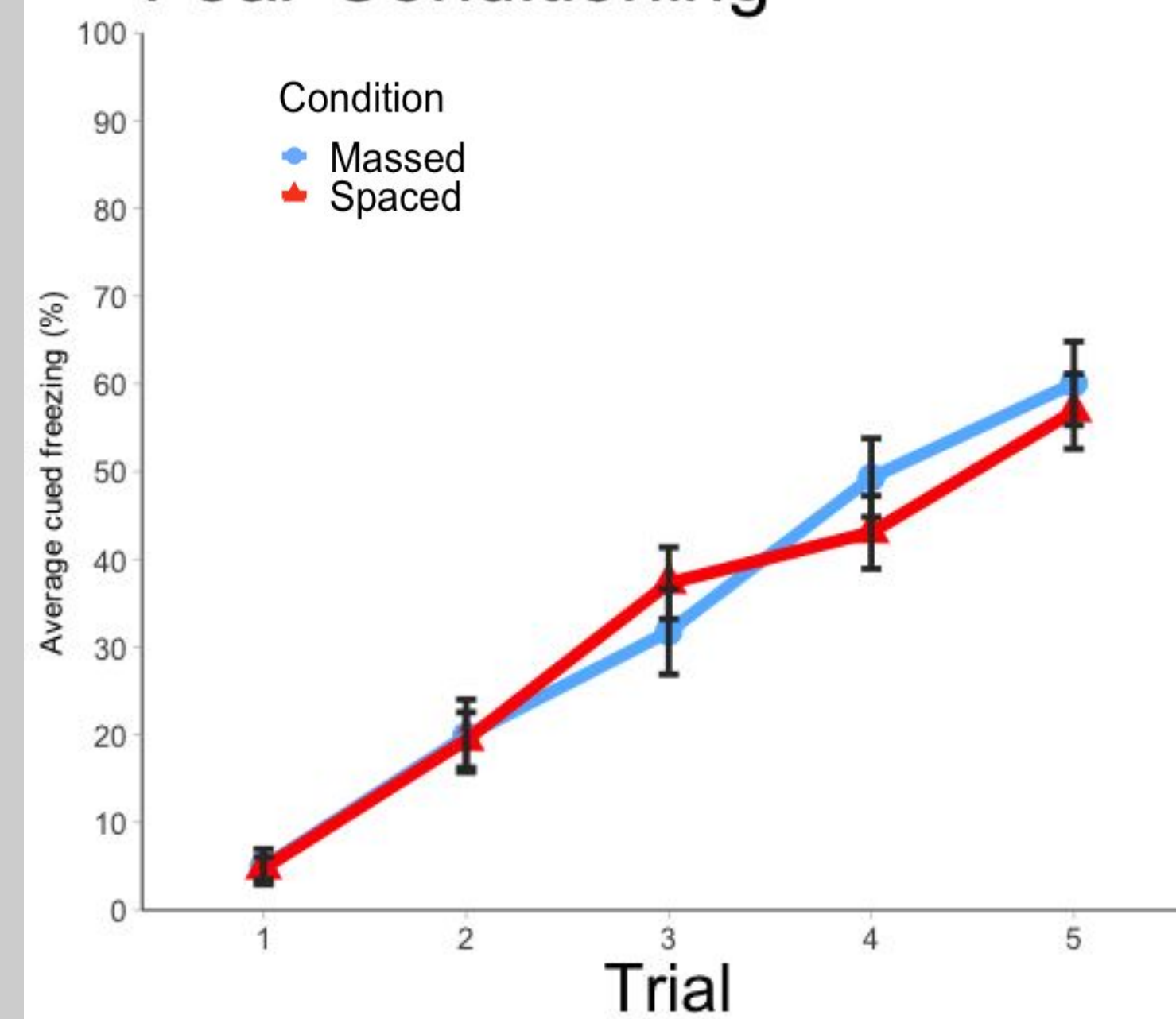
- Immunohistological assays: DAPI staining for nuclei, green fluorescent protein (GFP) staining for c-Fos (marker of neuronal activity)

Imaging:

- THUNDER Imager Tissue microscope (Leica Microsystems) used to visualize the PL/IL at 20x magnification
- AIVIA (machine learning model for cell counting) used to quantify neuronal activity in regions of interest during extinction

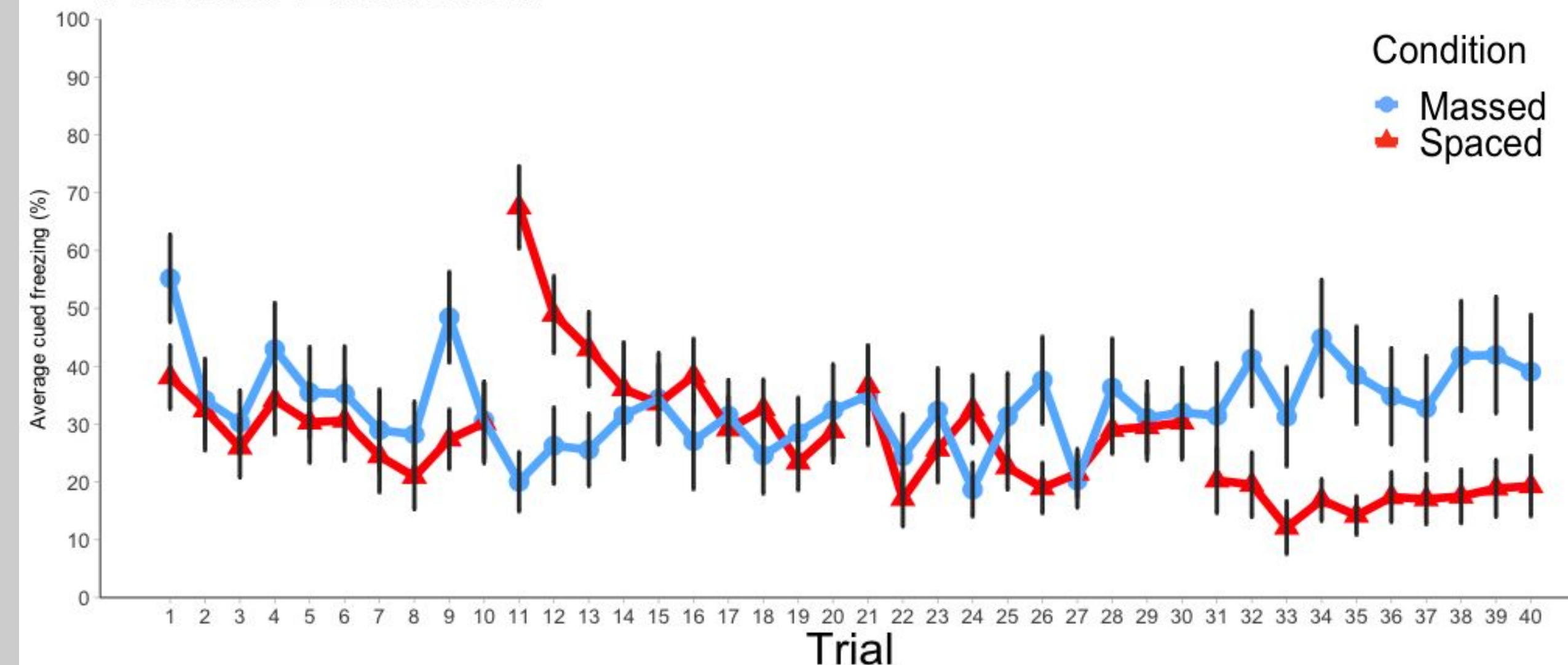
Results

Fear Conditioning

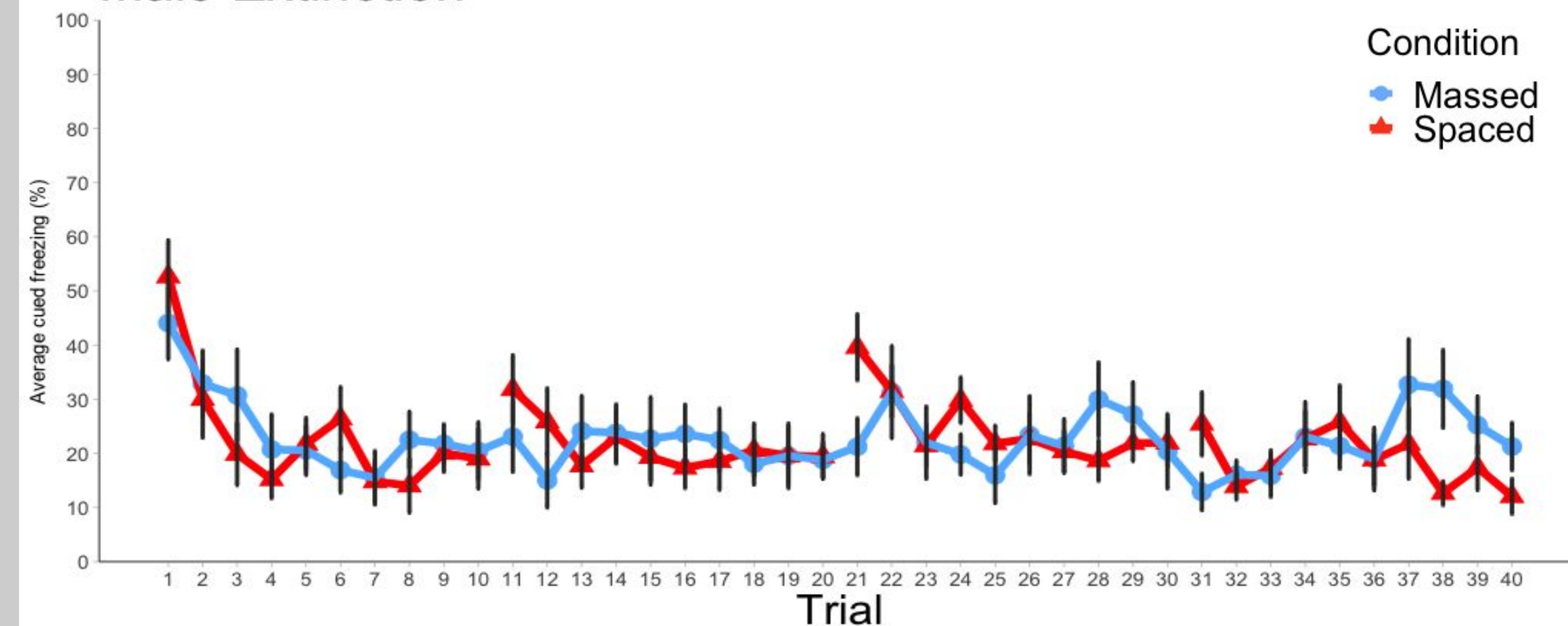


- Time spent freezing in mice is considered as a behavioral marker for fear.
- By the end of fear conditioning, there was no significant difference in cued freezing observed between the groups ($p = 0.801$)

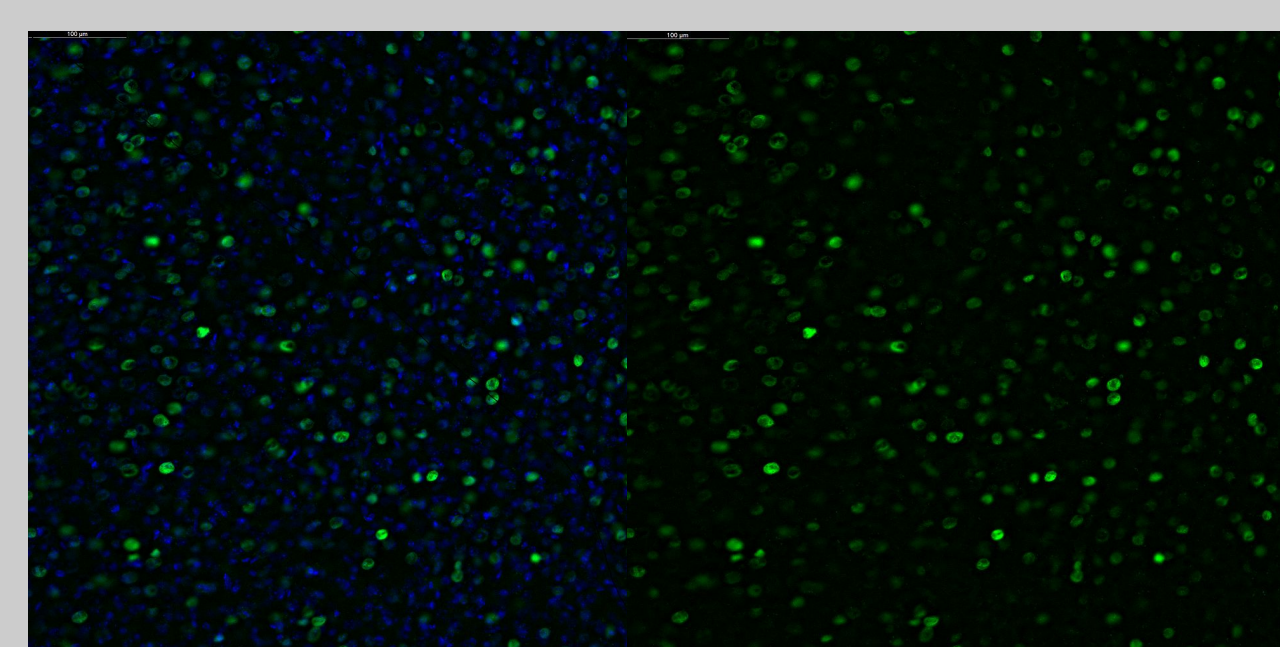
Female Extinction



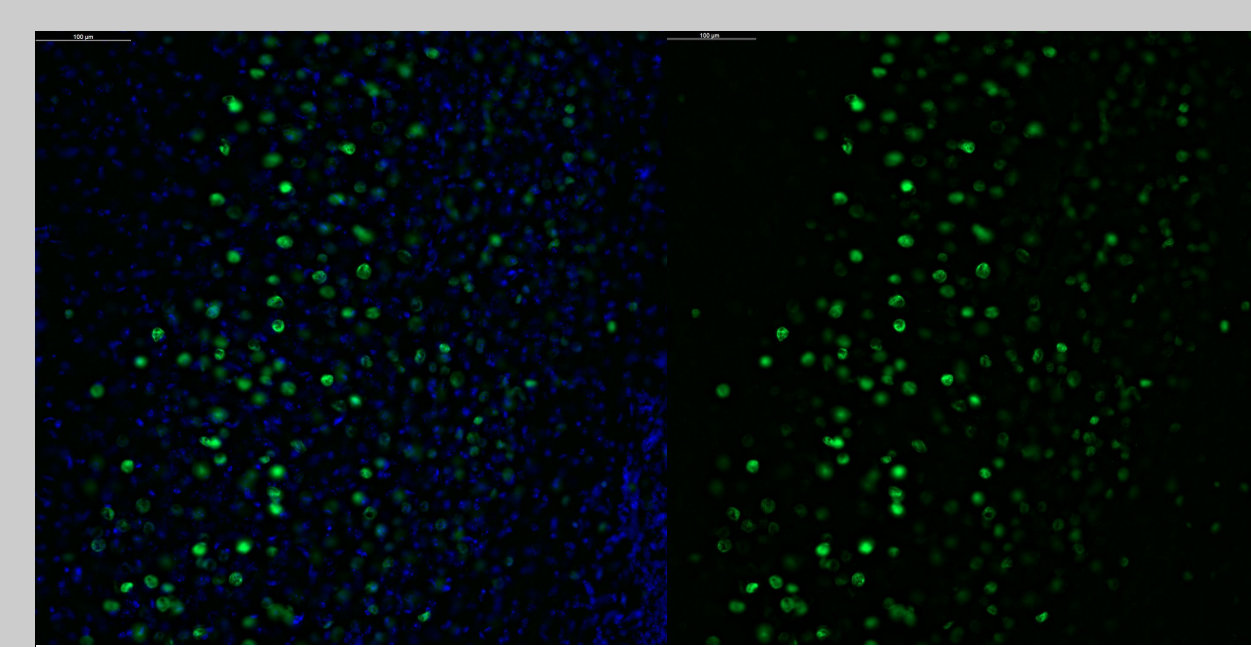
Male Extinction



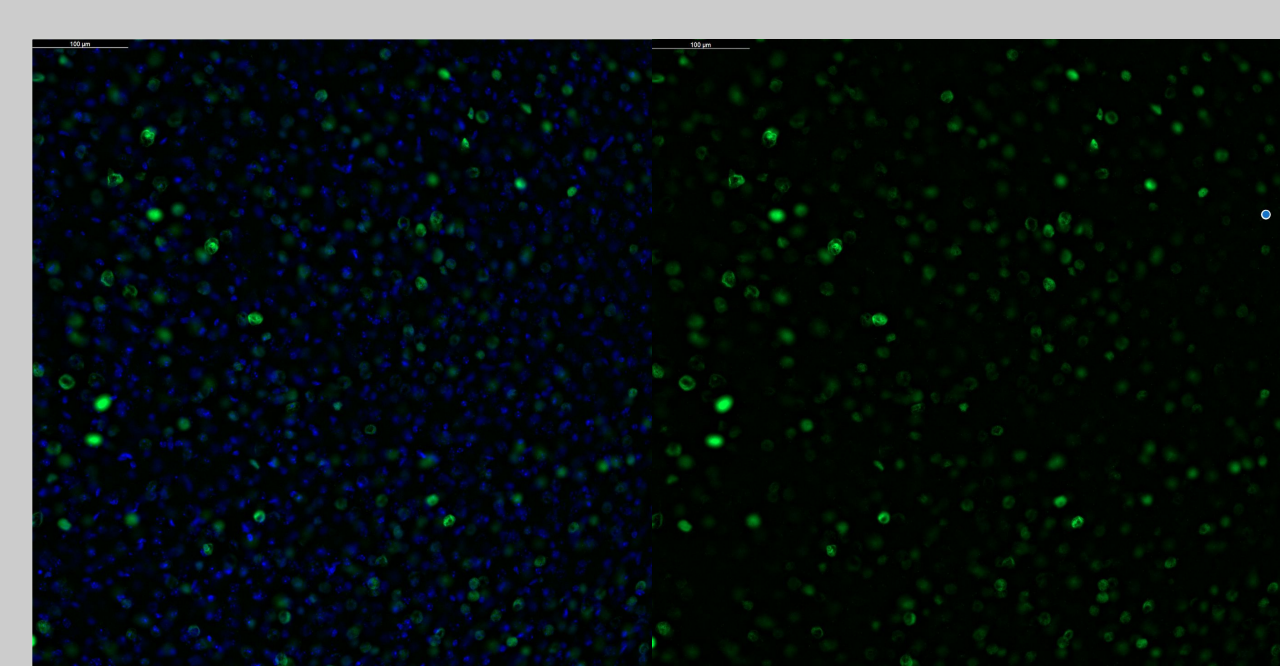
Females froze more than males ($p = 0.0016$) during extinction. However, there was no significant difference between massed vs spaced groups ($p = 0.365$).



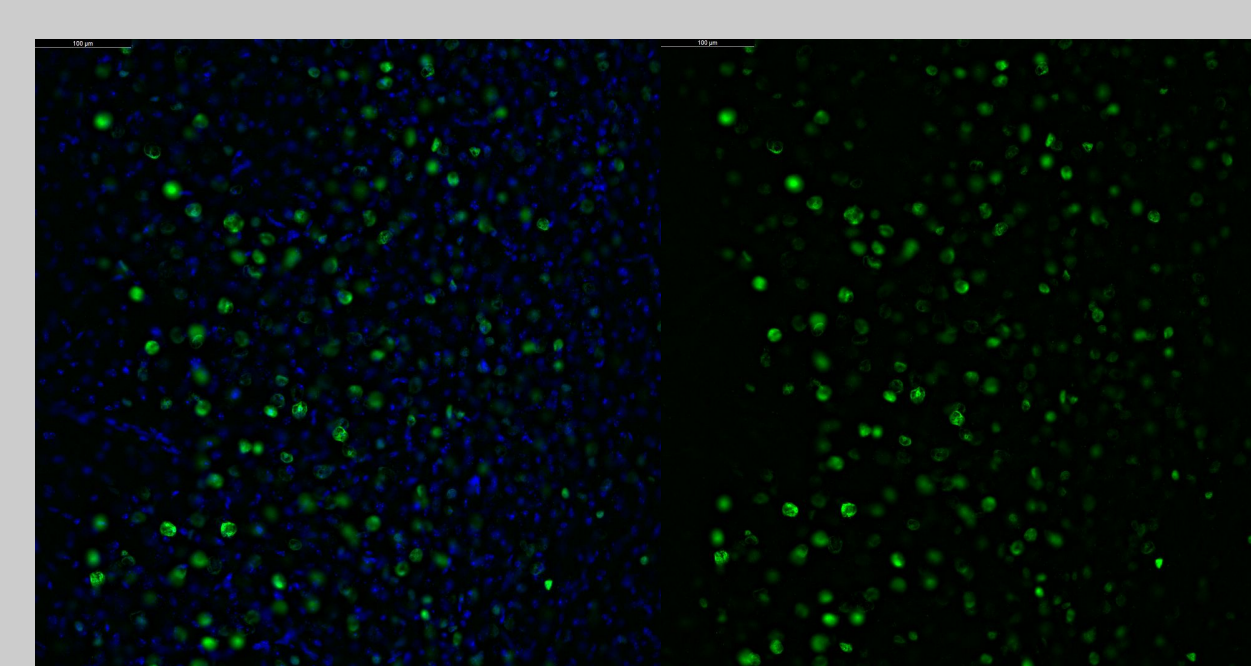
Representative image of the PL (Massed Extinction).



Representative image of the IL (Massed Extinction).

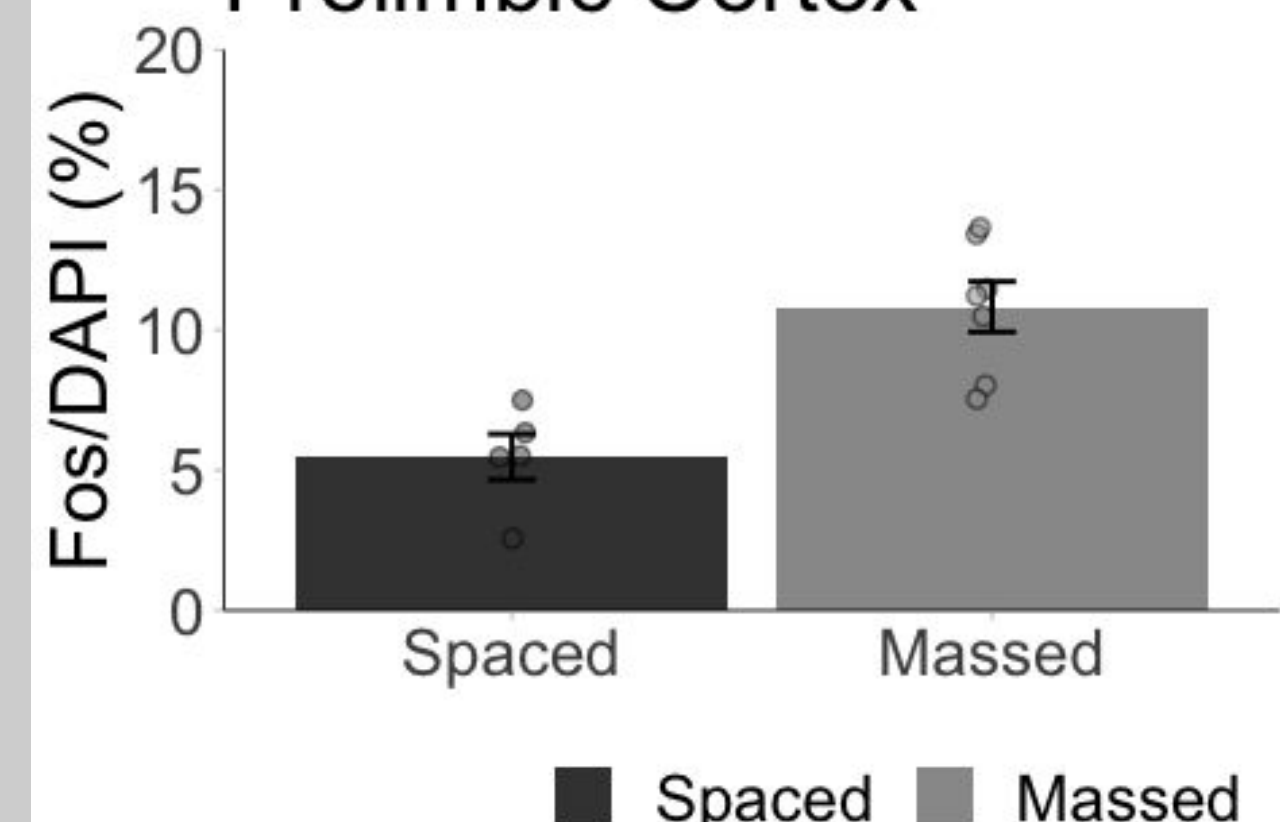


Representative image of the PL (Spaced Extinction).



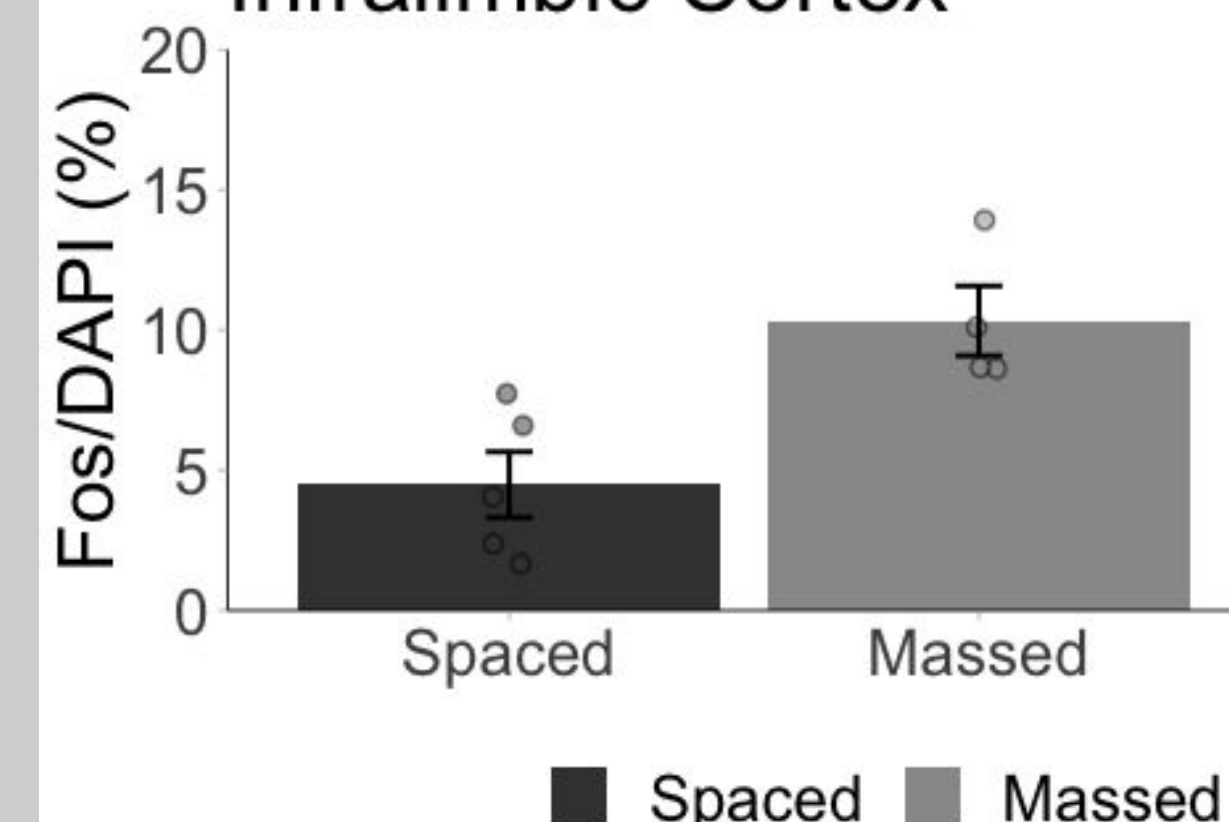
Representative image of the IL (Spaced Extinction).

Prelimbic Cortex



There are significantly less c-Fos cells in the PL of the spaced group ($p = 0.002$).

Infralimbic Cortex



There are significantly less c-Fos cells in the IL of the spaced group ($p = 0.012$).

Discussion

Behavioral Results:

- The Massed and Spaced groups were both able to extinguish their fear responses to similar levels, but females were overall found to freeze more than males.

Imaging Results:

- Higher density of c-Fos stained neurons indicated a higher level of localized activity.
- While there were no behavioral differences in the Massed and Spaced extinction data, the imaging data seems to suggest that there is increased activity in the PL and IL regions of the Massed group.
- PL/IL differences could potentially impact long-term recall of fear or extinction learning, which would need to be explored further

Future work:

- Powering sex differences in imaging data
- Contrasting specific functions of the PL/IL in extinction learning, as well as expanding into the ventral hippocampus (VH) and basolateral amygdala (BLA)

Relevance:

- Our data suggests that in adolescents, there is different underlying neural circuitry in processing the Massed vs. Spaced conditions.
- Further, our data revealed sex differences in the extinction behavior, in line with previous findings.
- Overall, this work shows the importance of including both adolescents and females into studies.

References

- Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, 62(6), 593. <https://doi.org/10.1001/archpsyc.62.6.593>
- Gerhard, D. M., & Meyer, H. C. (2021). Extinction trial spacing across days differentially impacts fear regulation in adult and adolescent male mice. *Neurobiology of Learning and Memory*, 186, 107543. <https://doi.org/10.1016/j.nlm.2021.107543>

Acknowledgments

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