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Introduction

In humans, cognitive function remains relatively stable throughout the lifespan, but sharp declines are observed starting around age 65. While women are generally more susceptible to age-related impairments, this is confounded with women's longer life expectancy when compared to men. Despite this, rodent work typically only uses males and compares young adulthood to aged animals on the same task, rarely examining the relative contributions of age and sex to this phenomenon.

Aged animals have shown overall high levels of zif268 corresponded with their age (Trask et al., 2020), but little work has examined how changes in neural activity in more advanced age contribute to age-related memory impairment. Perineuronal nets (PNNs) have been implicated for their role in neural plasticity, and growing work has found importance for PNNs in memory expression and retention (Fawcett et al., 2022). Age-related memory deficits are associated with changes in protein degradation and function of the ubiquitin proteasome system in brain regions important for trace fear memory like the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and the dorsal hippocampus (DH; Dulka et al., 2020; Dulka et al., 2021).

Here, we assessed memory using trace fear conditioning (TFC) to characterize behavioral performance, as it shows age-related deficits (Moyer & Brown, 2006) and relies on a distributed neural network including the dorsal hippocampus (Raybuck & Lattal, 2011) and prelimbic cortex (Gilmartin & Helmstetter, 2010). Following testing, we measured changes in zif268 expression, PNN quantity, and K48 tagging in the mPFC, BLA, and DH. We hypothesized that advanced age would correspond with more memory deficits when compared to younger, but still aged, counterparts and the females would show especially pronounced effects.

Methods

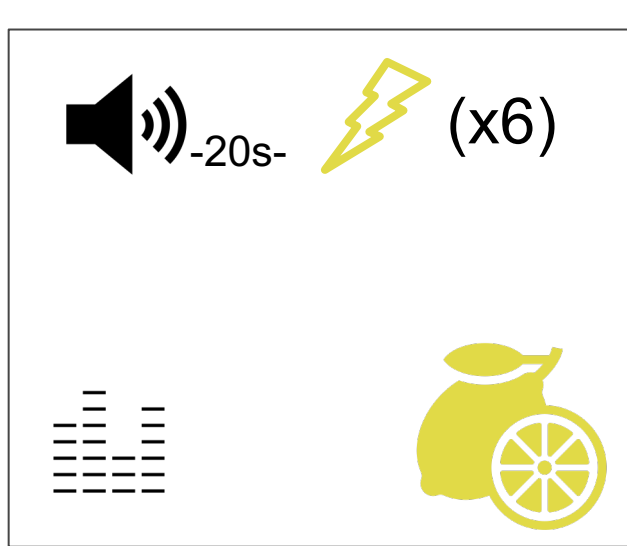
Subjects. Sprague Dawley rats (Males: n=5; Females: n=13) aged PND 590 – PND 734.

Acquisition. Animals were presented with 6 presentations of 10-s white noise CS followed by 20-s trace interval that ended with a 1 mA footshock.

Testing. The next day, animals received two test (counterbalanced). In the first, animals were placed in a novel context and given four 30-s CS-alone presentations. In the second, fear-elicited freezing was measured in the original context without the presence of any CS or footshocks.

Day 1

Acquisition

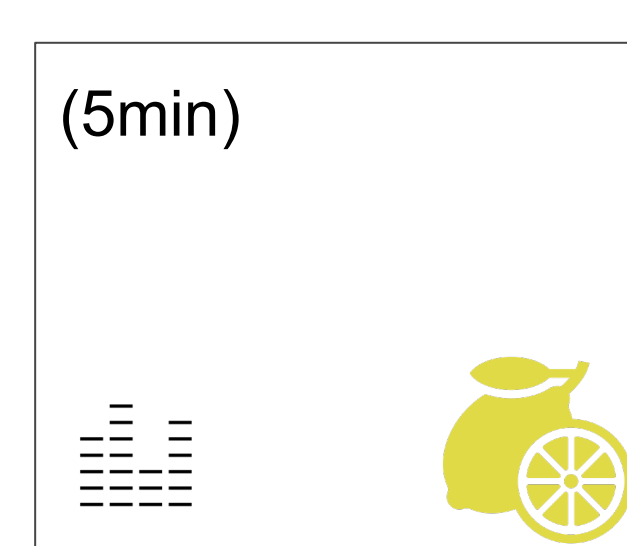


Day 2

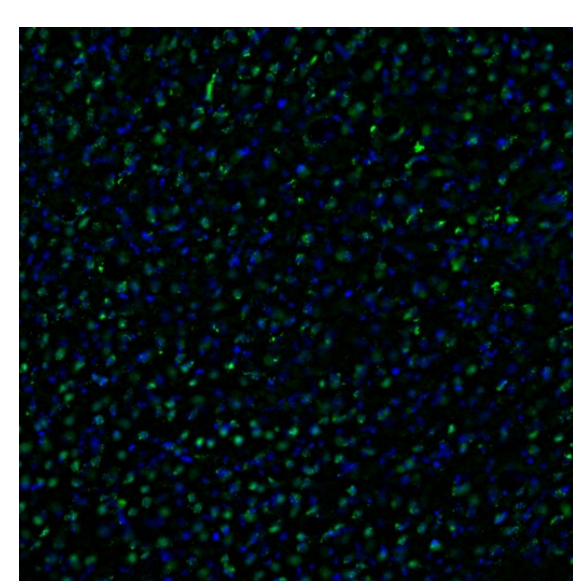
CS Test



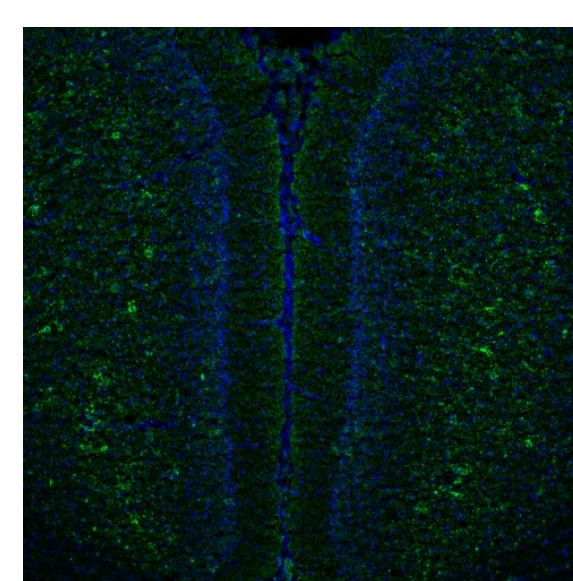
Context Test



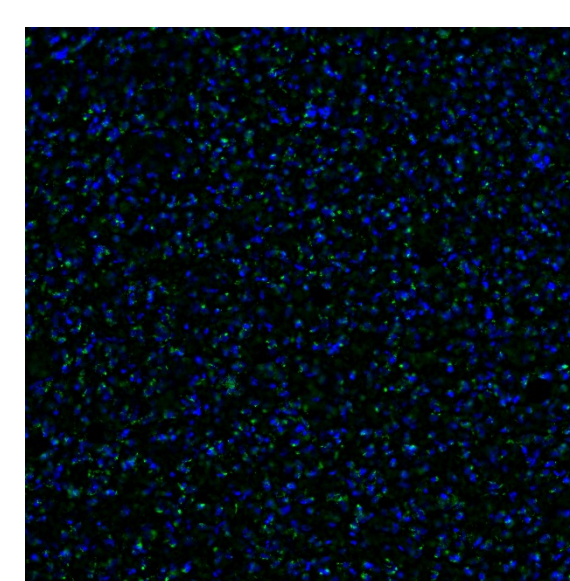
Tissue Processing. Animals were sacrificed 30 minutes following the final test and their brains were removed and immediately flash frozen. Tissue was later sliced and dissected for zif268, PNN, and K48 immunofluorescence.



zif268/DAPI



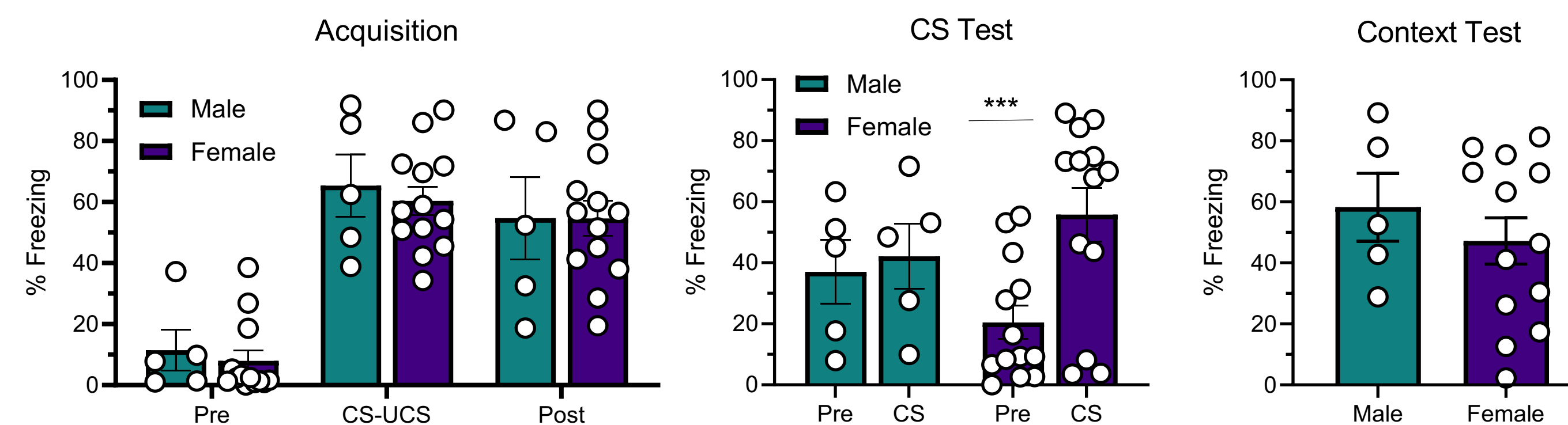
WFA/DAPI



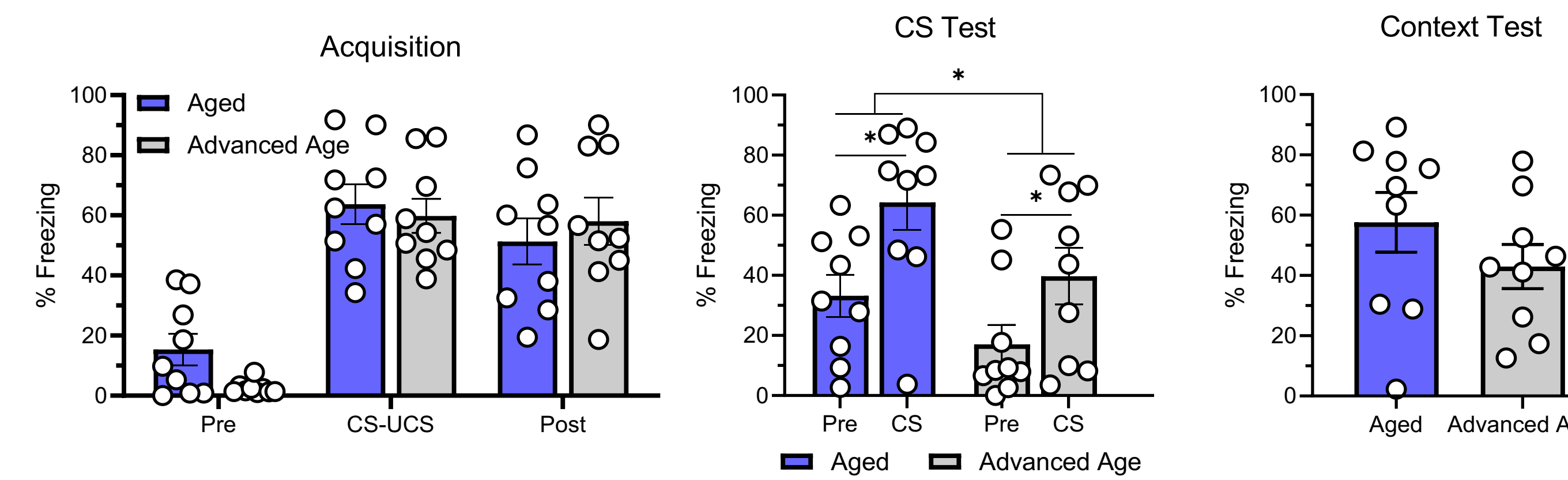
K48/DAPI

Results

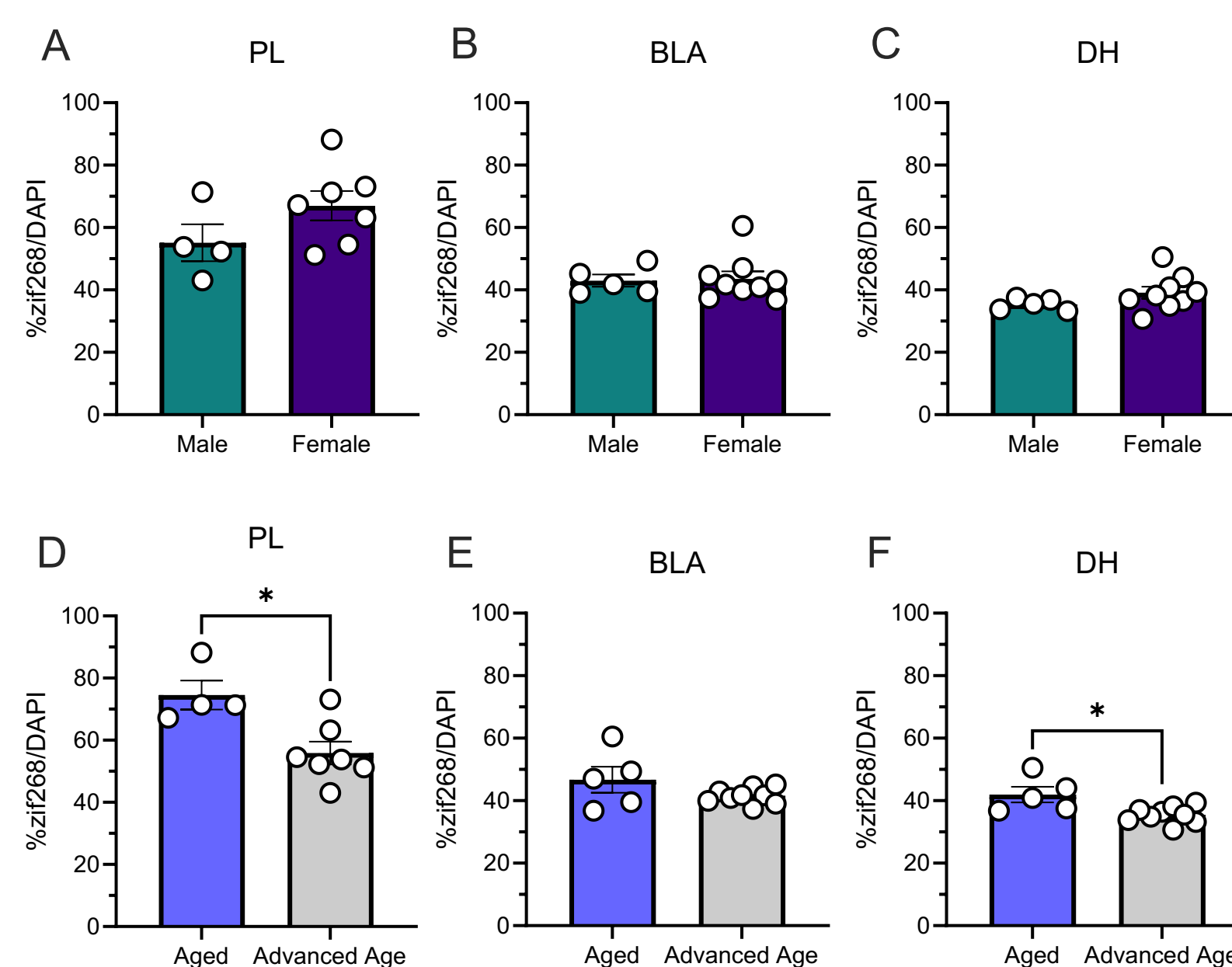
Biological sex impacts CS memory, but not acquisition or context memory in aged rats.



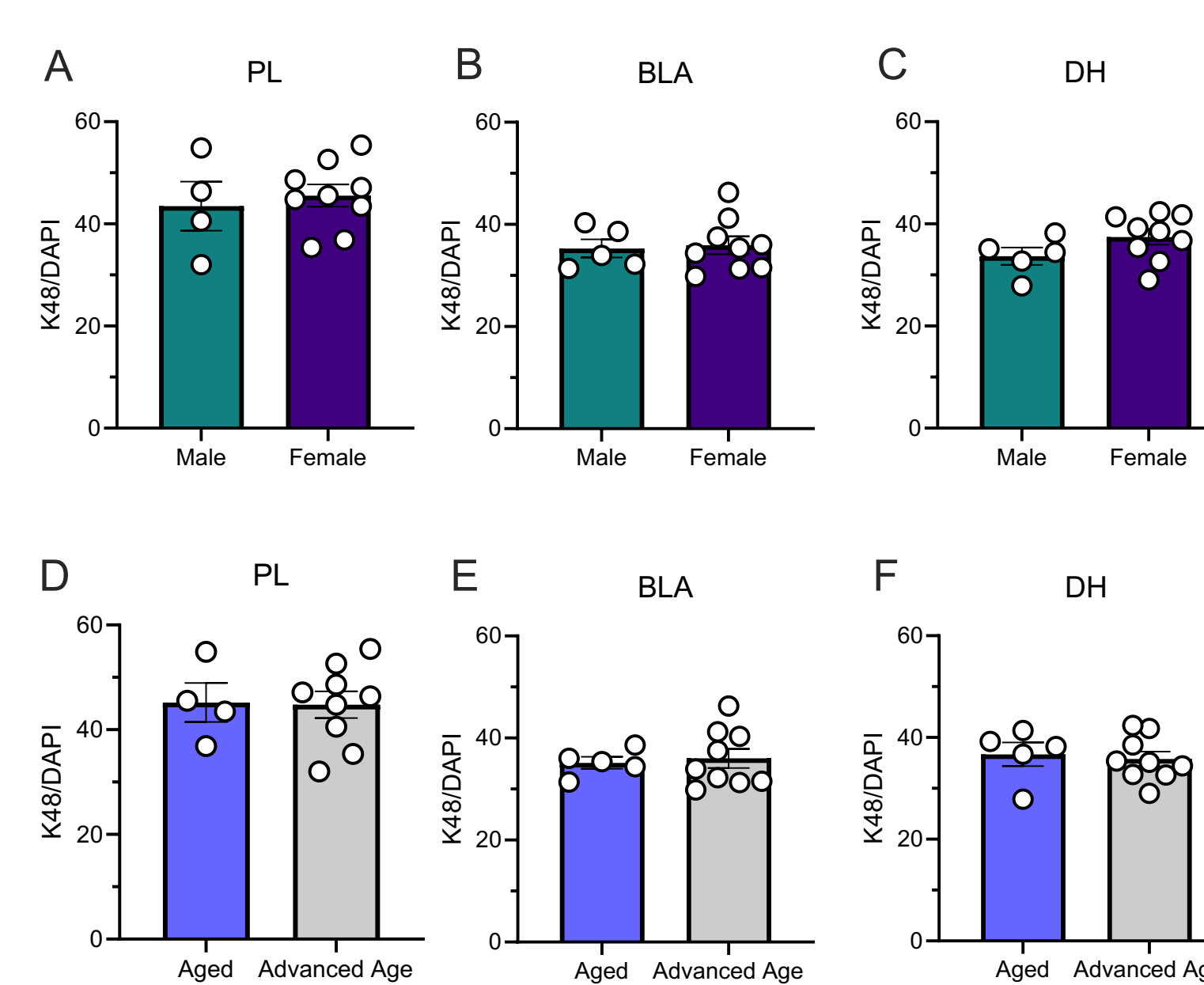
Advanced age impacts CS memory, but not acquisition or context memory. Impaired memory for the CS, but not context, is associated with a more advanced age.



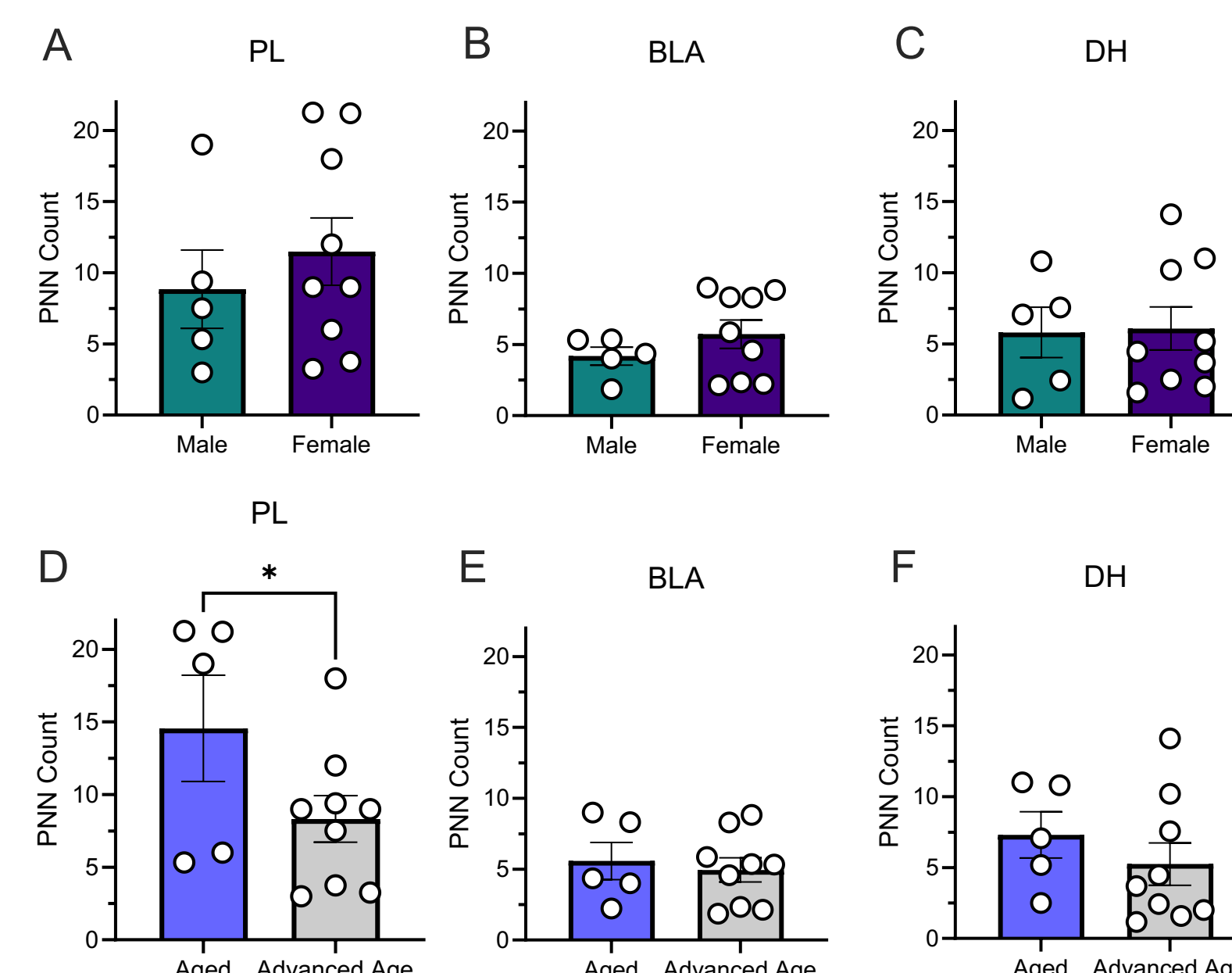
Sex does not have an influence on zif268 in the PL, BLA, or DH. Zif268 is decreased in the PL and DH in older animals.



Neither sex nor advanced age influence K48 expression in the PL, BLA, or DH.



While sex does not influence PNN amount, advanced aged corresponds with PNN amount in the PL, but not DH or BLA.



Conclusions

The advanced aged group performed worse in the trace fear task as indicated by less freezing during the CS test. While sex didn't seem to impact TFC, male rats did not increase their freezing from the pre-CS period into the CS but females did.

Neural activity assessed through zif268 was decreased in advanced age. This suggests that even in advanced age zif268 expression can track with behavioral performance (as in Robinson et al., 2024). However, there was no impact of sex.

The lack of differences in K48 expression could be attributed to our use of total count rather than examining tagging in different compartments (e.g., synaptic, nuclear) using Western blotting as in previous studies (e.g. Farrell et al., 2023). While there may not be a difference in total K48, the amount may differ within compartments to contribute to memory impairment.

More advanced age is associated with a reduction of PNNs in the PL, corresponding with a less robust memory in that group. As before, sex did not seem to influence PNN quantity.

Altogether, these results suggest that even when comparing groups that would both be considered 'aged', more advanced age is a stronger predictor of memory impairment given less overall trace fear memory and decreased neural activity among the older animals. The present results suggest that biological sex seems to have a less direct role in memory impairment or neural activity than natural aging.

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References

- Dulka, B. N., Pullins, S. E., Cullen, P. K., Moyer Jr, J. R., & Helmstetter, F. J. (2020). Age-related memory deficits are associated with changes in protein degradation in brain regions critical for trace fear conditioning. *Neurobiology of Aging*, *91*, 160-166.
- Dulka, B. N., Trask, S., & Helmstetter, F. J. (2021). Age-related memory impairment and sex-specific alterations in phosphorylation of the Rpt6 proteasome subunit and polyubiquitination in the basolateral amygdala and medial prefrontal cortex. *Frontiers in Aging Neuroscience*, *13*, 656944.
- Farrell, K., Auerbach, A., Liu, C., Martin, K., Pareno, M., Ray, W. K., ... & Jarome, T. J. (2023). Sex-differences in proteasome-dependent K48-polyubiquitin signaling in the amygdala are developmentally regulated in rats. *Biology of sex Differences*, *14*(1), 80.
- Fawcett, J. W., Fyhn, M., Jendelova, P., Kwok, J. C., Ruzicka, J., & Sorg, B. A. (2022). The extracellular matrix and perineuronal nets in memory. *Molecular Psychiatry*, *27*, 3192-3203.
- Gilmartin, M. R., & Helmstetter, F. J. (2010). Trace and contextual fear conditioning require neural activity and NMDA receptor-dependent transmission in the medial prefrontal cortex. *Learning & Memory*, *17*, 289-296.
- Moyer Jr, J. R., & Brown, T. H. (2006). Impaired trace and contextual fear conditioning in aged rats. *Behavioral Neuroscience*, *120*, 612.
- Raybuck, J. D., & Lattal, K. M. (2011). Double dissociation of amygdala and hippocampal contributions to trace and delay fear conditioning. *PLoS one*, *6*, e15982.
- Robinson, P. K., Met Hoxha, E., Williams, D., Kinzig, K. P., & Trask, S. (2024). Fear extinction is impaired in aged rats. *GeroScience*, *46*, 2815-2825.
- Trask, S., Dulka, B. N., & Helmstetter, F. J. (2020). Age-related memory impairment is associated with increased zif268 protein accumulation and decreased Rpt6 phosphorylation. *International Journal of Molecular Sciences*, *21*, 5352.