Dorsal Dentate Gyrus Engrams During Fear Learning and Generalization: Implications for Post-Traumatic Stress Disorder

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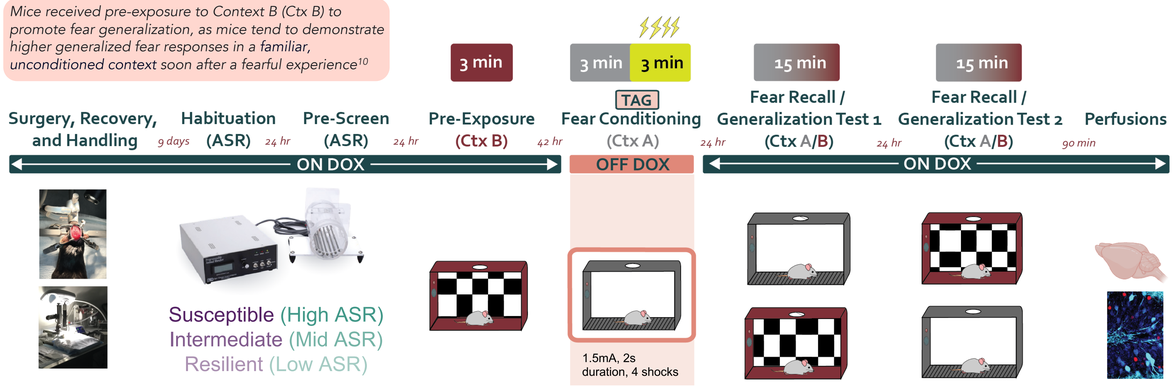
INTRODUCTION

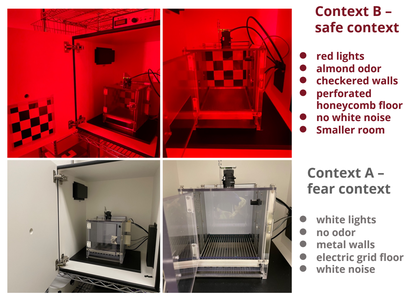
- Lifetime prevalence of Post-Traumatic Stress Disorder (PTSD) is 7.8% in the U.S.[1]
- Currently, unknown why specific subsets of individuals are more vulnerable to developing PTSD e.g., women more susceptible[1].
- Hallmark symptom of PTSD is fear generalization[2-3], where acquired fear responses to particular stimuli or contexts are transferred to other stimuli and contexts. May stem from memory-updating impairments involving a failure to remap trauma-related memory traces in the presence of new info (e.g., safety signals), and the persistent recall of these traces in the presence of non-trauma-related contexts / stimuli[4-5].
- Memories are stored as hippocampal engrams[6]. Here, we assessed these remapping deficits at the engram level in wildtype male and female c57BL/6 mice. The stability and flexibility of fear-related memory traces in the dentate gyrus (DG) were examined using a viral-based neuronal tagging strategy (Tet Tag system)[7] combined with immunohistochemistry and fluorescent confocal microscopy.
- Mice were pre-exposed to a safe context (B) and then fear conditioned in context A, where the fear memory was tagged. After 24 hours, they were placed in either context A or B, followed by the alternate context the next day, to assess fear memory and generalization. Mice were then perfused to examine memory overlap for the tested context with the tagged fear context.
- We also examined whether fear generalization or remapping deficits could be predicted using a behavioral pre-screening method associated with the acoustic startle reflex (ASR) where mice were parsed into susceptible and resilient populations based on their response to a startle stimulus delivered acoustically[8]. In mammals, the startle response is an innate reflex marked by swift contractions of facial and skeletal muscles triggered by a sudden and intense stimulus[9].

EXPERIMENTAL DESIGN

Mice received pre-exposure to Context B (Ctx B) to promote fear generalization, as mice tend to demonstrate higher generalized fear responses in a familiar, unconditioned context soon after a fearful experience[10]



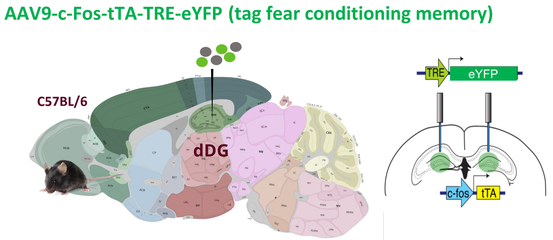
Context A and B were distinctly different along the dimensions of odor, tactile, visual, and auditory cues



NEURONAL TAGGING

Viral Tet Tag Strategy: Activity-Dependent Tagging dDG Cells Involved in Encoding a Fear Memory

AAV9-c-Fos-tTA-TRE-eYFP (tag fear conditioning memory)

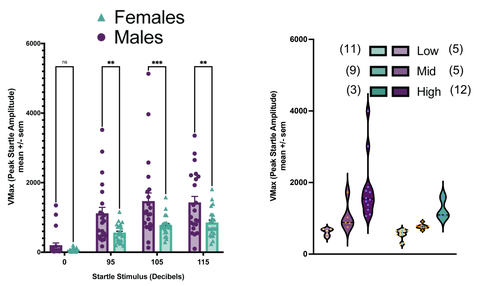


- Male and female c57BL/6 mice
- Injections of an adeno-associated virus (AAV) were targeted to the dDG such that mice would express the tetracycline transactivator (tTA) protein driven by the c-Fos promoter, encoding the light sensitive opsin, channelrhodopsin-2 (ChR2) fused to the fluorescent reporter eYFP under the control of the tetracycline response element (TRE)

RESULTS

ACOUSTIC STARTLE RESPONSE

Males (n=22) showed higher startle compared to females (n=21) for each acoustic stimulus.

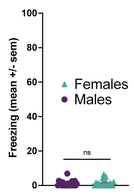


Peak startle responses (VMax) to stimuli were averaged, and mice were parsed into
Low (<700; n=16)
Mid (700-1000; n=14)
High (>1000; n=15)

We categorized the Low as Resilient and High as Susceptible

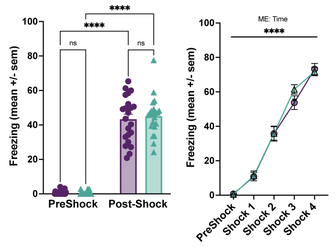
CONTEXT PRE-EXPOSURE

Mice did not freeze during pre-exposure to context B



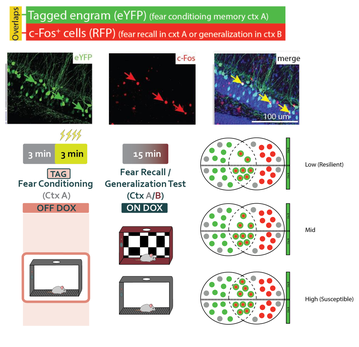
FEAR CONDITIONING

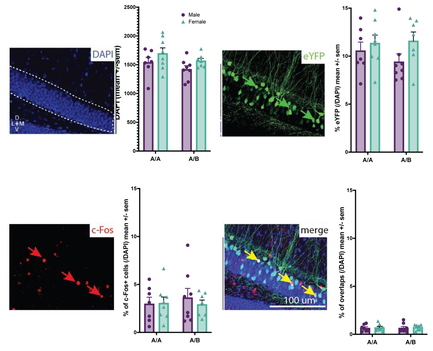
Mice froze post-shock in context A. Mice demonstrated increased freezing with each successive shock



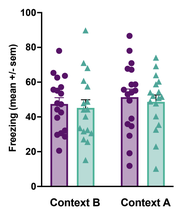
REMAPPING DEFICITS

There were no differences in across all cell counts - engrams were the same size.

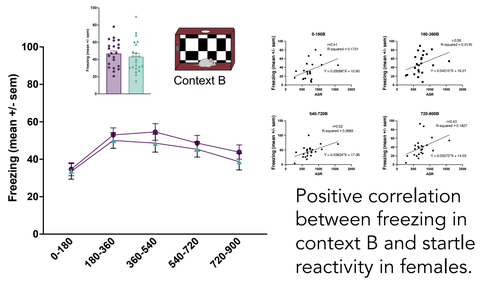




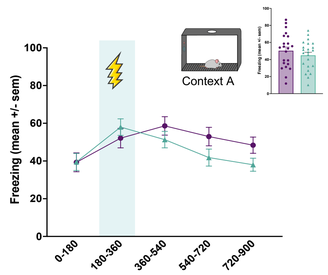
FEAR GENERALIZATION



Pre-exposure to both a safe context and then subsequent exposure to a fear-related context can modulate generalization of contextual fear[10]



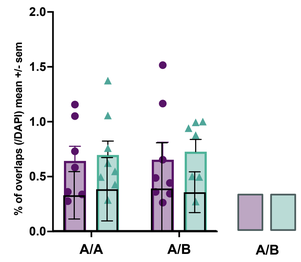
Positive correlation between freezing in context B and startle reactivity in females.



No sex differences in fear generalization, however, female mice reached peak freezing earlier than males during recall in context A, in a manner predictive of the shock received during conditioning (180-360s interval)

Mice that fear generalized (A/B) demonstrated just as much overlap as mice that were returned to the fear context (A/A) confirming our hypothesis.

There were no sex differences.



CONCLUSIONS & FUTURE DIRECTIONS

- Males showed higher startle responses – this is contrary to our original hypothesis. However, this has been shown in the literature previously[11].
- Mice will typically show fear generalization in a context they are familiar with[10] ; Pre-exposure to the safe context promoted generalization there.
- There were no sex differences in fear conditioning, fear memory recall, or fear generalization, however, female mice reached peak freezing earlier than males during recall in context A, in a manner predictive of the shock received during conditioning.
- There was a positive correlation between freezing in context B and startle reactivity in females. Therefore, ASR may be a viable behavioral screen of fear generalization in females.
- In the context of remapping deficits, mice placed in a safe environment (A/B) exhibited a similar degree of neural overlap as those returned to the original fear context (A/A). Given that these mice also displayed fear generalization, our findings support the hypothesis that impairments in memory updating—specifically in remapping contextual representations—may underlie fear generalization in PTSD.
- We will add a group that does not receive pre-exposure to the safe context (hypothesizing this group will show less generalization and fewer overlaps). We also plan to run a cohort using an outbred strain (e.g., Swiss Webster) assuming more genetic variability will lead to more phenotypic variability. Finally, we will try and rescue these deficits via phasic activation of the locus coeruleus.

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REFERENCES

1. Koenen KC, Ratanatharathorn A, Ng L, et al. (2017) Posttraumatic stress disorder in the World Mental Health Surveys. Psychol Med. 2017;47(13):2260–2274. 2. Morey, RA, Haswell, CC, Stjepanović, D *et al.* (2020) Neural correlates of conceptual-level fear generalization in posttraumatic stress disorder. *Neuropsychopharmacol.* **45**, 1380–1389. 3. Dunsmoor JE, Mitroff SR, LaBar KS. (2009). Generalization of conditioned fear along a dimension of increasing fear intensity. *Learn Mem.* 24;16(7):460-9. 4. Grella, SL, Neil, JM, Edison, HT, et al. (2019). Locus coeruleus phasic, but not tonic, activation initiates global remapping in a familiar environment. *J Neurosci*, 39(3), 445-455. 5. Grella SL, Gomes SM, Lackie RE, et al., (2021). Norepinephrine as a spatial memory reset signal. Behav Pharmacol. 32(7):531-548. 6. Semon, RW, The Mneme. (1921) Cornell University Library 7. Reijmers LG, Perkins BL, Matsuo N, et al. (2007). Localization of a stable neural correlate of associative memory. *Science.* 317(5842):1230-3. 8. Rasmussen DD, Crites NJ, Burke BL. (2008). Acoustic startle amplitude predicts vulnerability to develop post-traumatic stress hyper-responsivity and associated plasma corticosterone changes in rats. *Psychoneuroendocrinol.* 33(3):282-91. 9. Pantoni MM, Herrera GM, Van Alstyne KR, et al. (2020). Quantifying the Acoustic Startle Response in Mice Using Standard Digital Video. *Front Behav Neurosci.* 14:83. 10. Iordanova MD, Honey RC. (2012). Generalization of contextual fear as a function of familiarity: the role of within- and between-context associations. *J Exp Psychol Anim Behav Process.* 38(3):315-21. 11. Plappert CF, RodenBücher AM, Pilz PKD (2005). Effects of sex and estrous cycle on modulation of the acoustic startle response in mice. *Physiol Behav*, 84(4): 585-94.

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