An animal behavioral model for assessing integrated longitudinal defensive threat states: Implications for post-traumatic stress disorder

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Background

- PTSD is a common and debilitating mental health disorder resulting in anxiety, impaired ability to process fear, and increased risk of cardiovascular disease.
- Fear learning is often modeled in rodents using conditioned fear learning, which is highly sensitive to sensory conditions such as environmental context, threat intensity, and circadian rhythm(Turley, 2021;Swiercz, 2020)
- Therefore, it is necessary to combine traditional behavioral measures of fear (*freezing, startle, exploration*) with physiological measures of arousal (*heart rate, blood pressure, circulating hormones*) without outside stressors or investigator intervention.

Objectives

- **To develop and validate a novel behavioral model, using lab-developed software for integrated behavioral and cardioautonomic analysis to investigate the effects of chronic, unpredictable threat reminders in pair-housed mice in the home cage on**
  - **1) Circadian- and environment- dependent CS-evoked arousal and anxiety behavior**
  - **2) Cardiovascular and autonomic function**

Methods

Animals: 10–12-week-old male and female C57BL/6 mice were used.

Home Cage Testing: Mice underwent auditory fear conditioning (20 CS/US pairings), with a CS-only group as a control (n=10-16/group) and returned to homecage. The novel lab-developed software was then used to remotely trigger CS (30sec. intermittent tones) throughout the LD cycle (6 6kHz tones over a 24-hour period, 3 light, 3 dark) over 14 days.

Behavioral Analysis: The first CS during each LD cycle was recorded and percent freezing behavior was manually scored. Videos were used to train DeepLabCut™ software for automated behavioral analysis (Fig. 3).

Anxiety and Arousal Testing: After the 14-day home cage study, mice underwent a 2 CS fear expression test in a novel context, assessment of locomotion and exploratory behavior in the elevated plus maze (EPM), and two days of both uncued and fear-potentiated startle testing. Plasma was collected for corticosterone ELISA analysis.

Cardiovascular and Body Temperature Recordings: Mice were implanted with Star-Oddi micro-HRT loggers for real-time cardiovascular and body temperature data, then returned to the pair-housed home cage. Physiological recordings were taken every minute over 7 days and 18 unpredictable CS given over light/dark cycles.

Figure 1: Experimental set-up for chronic CS delivery in the home cage

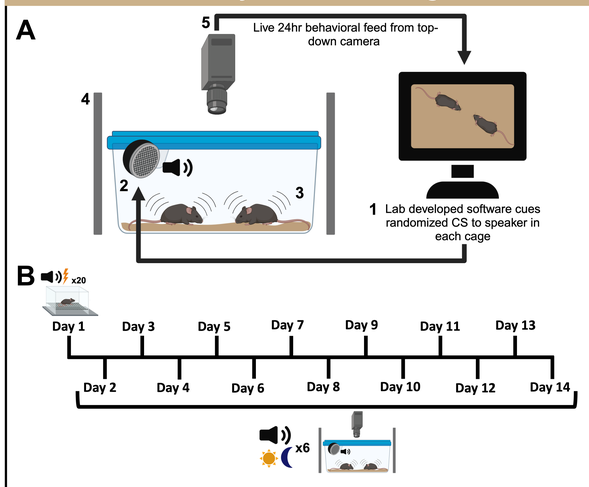

Fig 1. A novel method for assessing CS conditioned fear in the home cage. A. Integration of software and behavioral recording. *1.* The software triggers the CS. *2.* CS are played through speakers in each cage of *3.* group-housed mice. *4.* Each cage has sound-attenuating panels to control volume. *5.* Top-down cameras record throughout light and dark cycle. **C.** Behavioral protocol for fear conditioning and home cage CS delivery across day/night.

Results

Figure 2: Chronic fear exposure in the home cage reduces freezing dependent on context and circadian cycle.

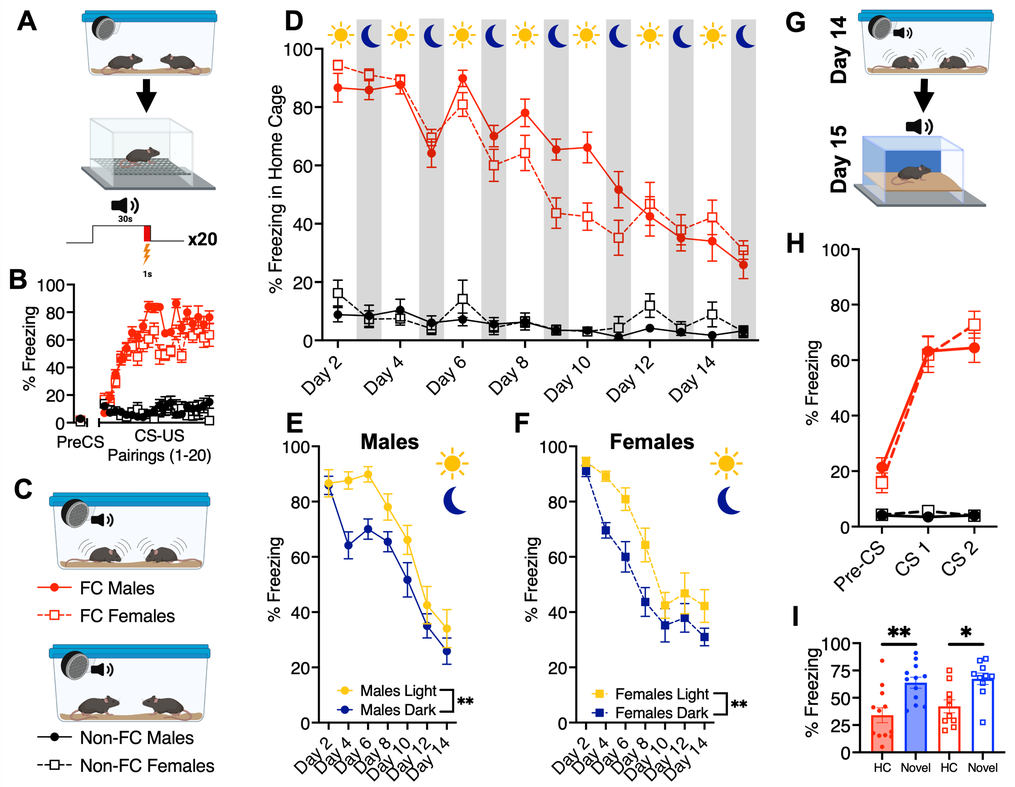

Figure 2. Fear conditioned male and female mice freeze to unpredictable conditioned tones in the home cage. A. Experimental approach for fear conditioning (20 CS/US pairings). **B**. Both males and females are successfully conditioned to the CS. **C.** Freezing analysis in the home cage of group-housed male and female mice. **D.** Manual scoring of freezing in the home cage across 14 days. **E-F.** CS-evoked freezing in the home cage is higher during the light cycle than the dark cycle in both males ($p<0.01$) and females ($p<0.01$). **G.** Experimental approach for assessing fear expression in a novel context. **H.** Both male and female fear conditioned mice have high freezing to the CS in a novel context (main effect of CS, $p<0.01$). **I.** Higher freezing in the novel context in both males ($p<0.01$) and females ($p=0.02$). n=10-16/group.

Figure 3: A DeepLabCut™ machine learning pipeline was trained to simultaneously track freezing in pair-housed mice over diurnal CS deliveries, accurate to manual analysis.

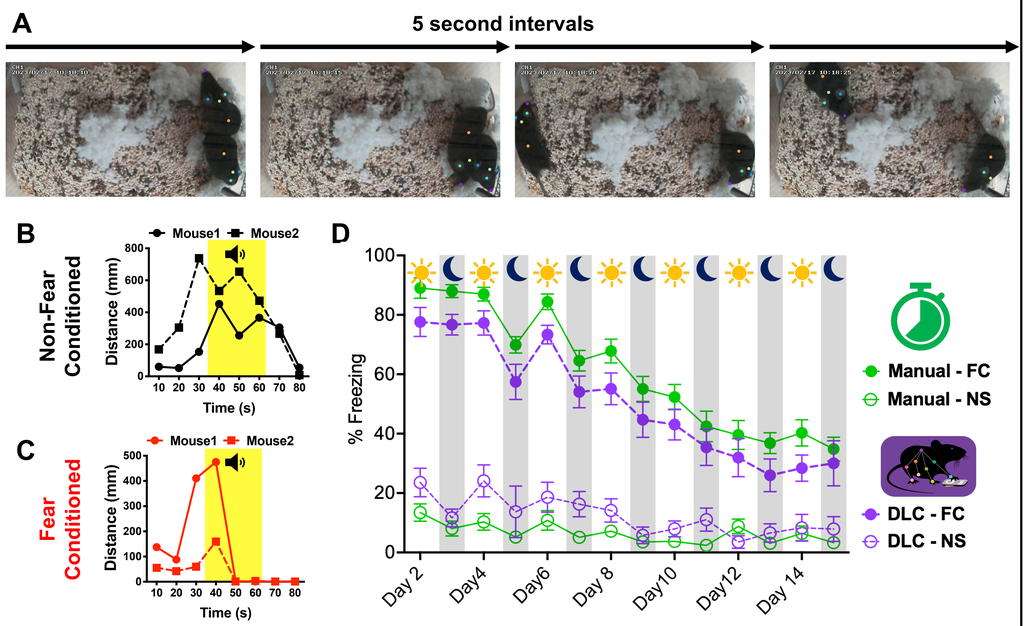

Fig 3. Successful automated tracking of pair-housed mice over 14 days using DeepLabCut™. A. Representative images of DLC tracking across 5 sec intervals in a pair-housed cage. Labels are: nose in purple; left ear in blue; right ear in green; body in yellow; tail in red. **B-C**. Automated tracking of distance traveled during CS in representative non-fear conditioned (**B**) and fear conditioned (**C**) group-housed cages. **D.** When distance traveled was converted to velocity for freezing analysis, no interaction effects were observed in automated vs. manual scoring ($p>0.05$) in group-housed mice across 14 days.

Figure 4: Chronic CS exposure in the home cage increases hyperarousal, but not anxiety, in a sex-dependent manner.

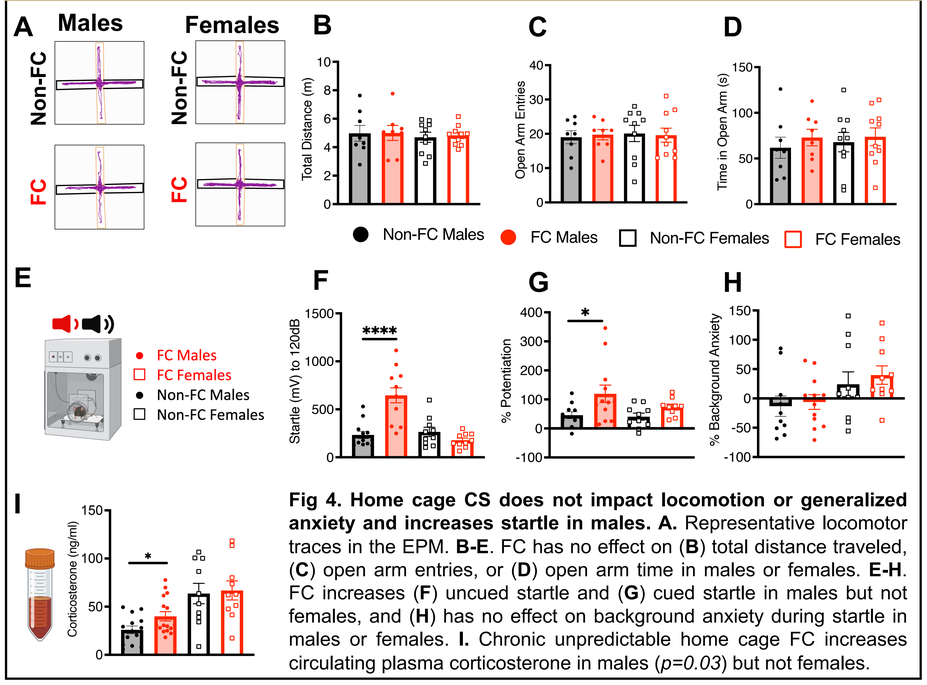

Fig 4. Home cage CS does not impact locomotion or generalized anxiety and increases startle in males. A. Representative locomotor traces in the EPM. **B-E**. FC has no effect on (**B**) total distance traveled, (**C**) open arm entries, or (**D**) open arm time in males or females. **E-H**. FC increases (**F**) uncued startle and (**G**) cued startle in males but not females, and (**H**) has no effect on background anxiety during startle in males or females. **I.** Chronic unpredictable home cage FC increases circulating plasma corticosterone in males ($p=0.03$) but not females.

Figure 5: Chronic diurnal CS exposure in the home cage causes conditioned increases in heart rate and body temperature in pair-housed mice.

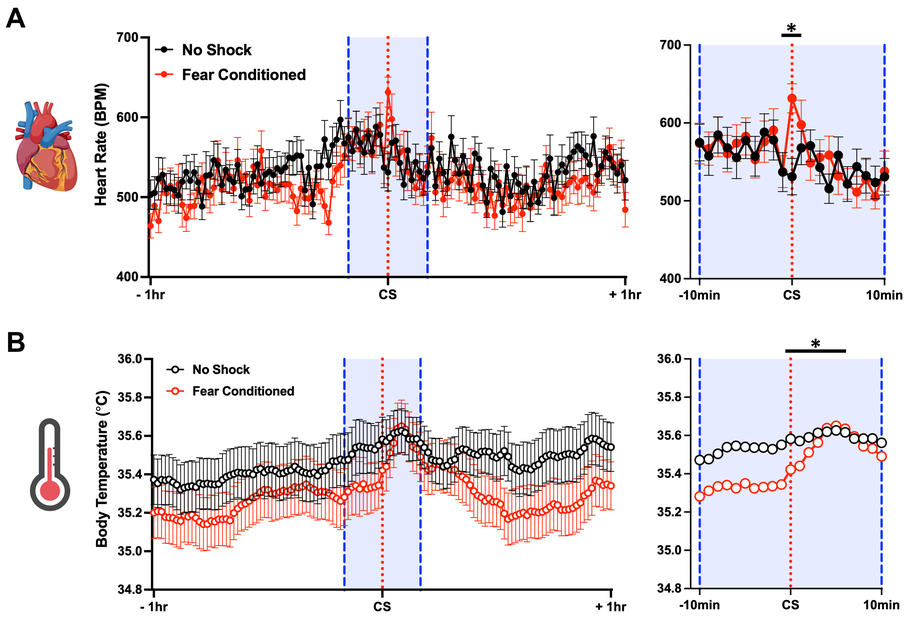

Fig 5. Diurnal heart rate and body temperature recordings from pair-housed mice during unpredictable CS delivery. A. Cardiovascular recordings from pair housed fear conditioned and non-fear conditioned mice were taken using Star-Oddi implantable micro-loggers during 18 unpredictable CS deliveries over 7 day/night cycles. FC mice had a significantly CS-evoked increase in heart rate ($p=0.02$, n=2/group). **B.** Body temperature was recorded from CS deliveries during this 7-day diurnal period. FC mice had a significantly CS-evoked increase in body temperature ($p=0.01$, n=2/group).

Summary and Conclusions

- Quantified CS-evoked freezing in pair-housed mice in the home cage across time, successfully replicated using simultaneous tracking with DLC.
- Identified an increased startle phenotype and increased stress hormones in males.
- Recorded real-time conditioned cardiovascular and body temperature responses in pair-housed mice
- **Developed and validated an integrated behavioral paradigm using principles of Pavlovian fear conditioning to improve preclinical PTSD phenotyping.**

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