Anxiety and Fear as a Function of Threat Certainty and Sex Differences

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INTRODUCTION

Mental health illnesses are an issue of increasing prevalence in today's society. One study found that approximately 50% of the adult population in the United States meets the requirements and criteria to be diagnosed with a anxiety disorder at some point over the duration of their life. (Kessler, Chiu, Demler, & Walters, 2005) Thus, there is a strong need for the development of novel treatments for anxiety. This study focuses on refining measures of anxiety and fear in an animal model by assessing rearing and freezing in two different conditions. Moreover, because sex differences in the prevalence of affective disorders in humans are well documented, we included both male and female rats to investigate sex-related differences in behavior within this paradigm. We utilized both unpredictable and predictable footshock paradigms with the goal of increasing or prolonging a state of either anxiety of fear, respectively. Rearing behavior was used as a measure of anxiety and freezing behavior to assess fear. We hypothesized that unpredictable footshock would elevate anxiety-related behaviors compared to predictable footshocks and more so in female rats than males. These results could aid in the construction of a more efficient animal model to use in research for the study of anxiety disorders and potential therapeutic interventions.

METHODS

Subjects and Design

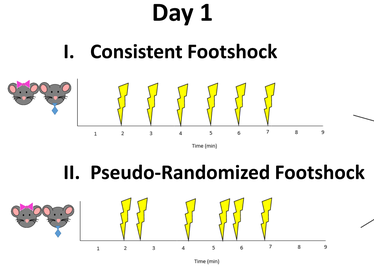
- Adult Long Evans rats (male, N=24, female, N=24)

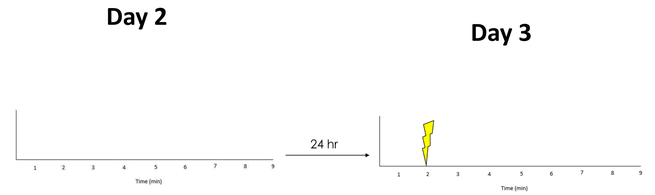
I. Unsignaled Footshock
- Mild footshock (0.5 mA, 0.5 s)
- 2 min pre- and post-shock
- 30-100 s intershock interval in the peudo-randomized condition
- 60 s intershock interval in the consistent condition

II. Context Test
- 9 min exposure to footshock environment
- No shock administration

III. Single Footshock
- 2 min pre-shock

Figure 1. Experimental design schematic.





No footshocks are administered on Day 2- the Context Test.

A single footshock is administered after the 2 min preshock on Day 3- Reinstatement of the footshocks.

Rearing and Freezing

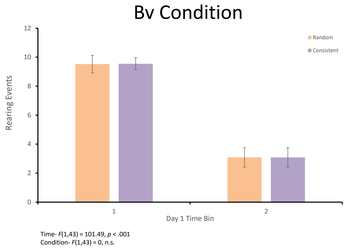
I. Rearing
- Floor contact with hind limbs only.
- Counted by blind observer per 20 sec interval.
- Used as index of risk assessment when threat is uncertain.

II. Freezing
- Lack of all but respiratory movement.
- Coded by blind observer per 8 sec interval.
- Used as an index of fear when threat is certain or imminent.

RESULTS

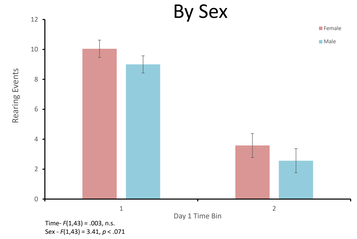
DAY 1

Rearing



Time- $F(1,43) = 101.49$, $p < .001$
Condition- $F(1,43) = 0$, n.s.

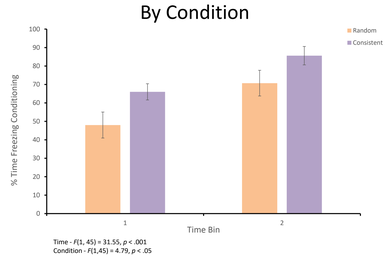
Figure 2. Bar graph showing rearing events during Day 1 Unsignaled Footshock for both the random and consistent footshock conditions. The 2 time bins are before and after the first footshock There was a significant decrease in rearing, but no difference between treatment conditions.



Time- $F(1,43) = .003$, n.s.
Sex - $F(1,43) = 3.41$, $p < .071$

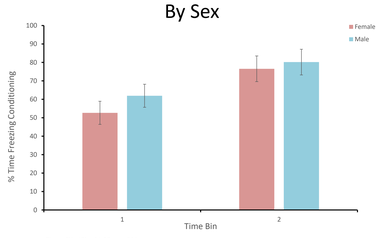
Figure 3. Bar graph showing rearing events during Day 1 Unsignaled Footshock for both male and female rats in the study. The 2 time bins are before and after the footshock. There was a significant decrease in rearing, but no difference between the sexes.

Freezing



Time - $F(1, 45) = 31.55$, $p < .001$
Condition - $F(1,45) = 4.79$, $p < .05$

Figure 4. Bar graph shows percent time spent freezing during Day 1 Unsignaled Footshock for both the random and consistent footshock conditions. The 2 time bins are after the first footshock . There was a significant increase in freezing but no difference between the treatment conditions.

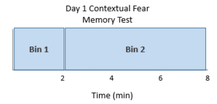


Time - $F(1, 45) = 31.55$, $p < .001$
Sex - $F(1,43) = 0.62$, n.s.

Figure 5. Bar graph shows the percent time spent freezing during Day 1 Unsignaled Footshock for both male and female rats in the study. The 2 time bins after the first footshock. There was a significant increase in freezing, but difference between the sexes.

Statistical Analysis

Rearing: A 2x2 repeated measures ANOVA was used to analyze the data for both the main effects (time, condition, and sex) and interaction effects (time x condition, and time x sex).



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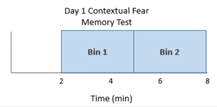
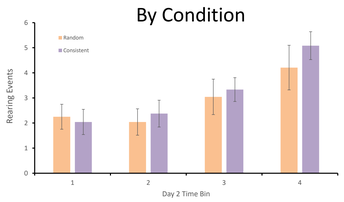


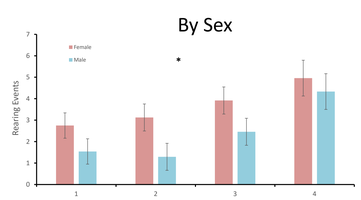
DAY 2

Rearing



Time- $F(3,132) = 13.0$, $p < .001$
Condition- $F(3,132) = 0.47$, n.s.
Time x Condition- $F(3,132) = 0.47$, n.s.

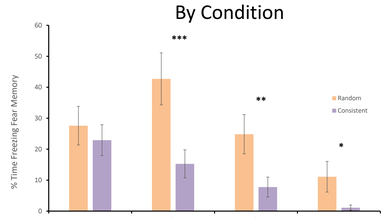
Figure 6. Bar graph showing rearing events during Day 2 Context Test for both the random and consistent footshock conditions. The time bins are separated into 2 minutes per bin in order to analyze trends in rearing over time. There is no statistically significant difference in the rearing between the two conditions over time.



Time- $F(3,132) = 13.0$, $p < .001$
Sex- $F(1,44) = 4.27$, $p < .05$
Time x Sex- $F(3,132) = 0.60$, n.s.

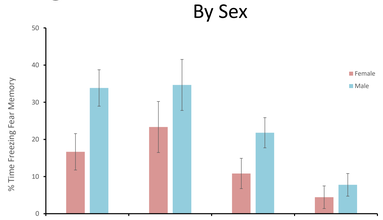
Figure 7. Bar graph showing rearing events during Day 2 Context Test for both male and female subjects in the study. The time bins are separated into 2 minutes per bin in order to analyze trends in rearing over time. Females exhibited more rearing behavior than males.

Freezing



Time - $F(2.4, 110.31) = 14.64$, $p < .001$
Condition $F(1,46) = 6.04$, $p < .02$
Condition x Time $F(2.4, 110.31 = 3.42$, $p < .029$

Figure 8. Bar graph showing percent time spent freezing during Day 2 Context Test for both the random and consistent footshock conditions. The time bins are separated into 2 minutes per time bin to analyze trends in freezing over time. The subjects in the random condition persisted longer in freezing.

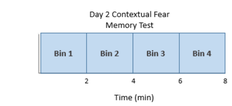


Time - $F(2.4, 107.08) = 13.96$, $p < .001$
Sex $F(1,46) = 2.97$, $p < .09$
Sex x Time $F(2.32, 107.08) = 1.08$, n.s

Figure 9. Bar graph showing percent time spent freezing during Day 2 Context Test for both male and female subjects in the study. The time bins are separated into 2 minutes per bin in order to analyze trends in freezing over time. Males trended towards exhibiting more freezing behavior and persisting longer in freezing over time.

Statistical Analysis

Rearing: A 2x4 repeated measures ANOVA was used to analyze the data for both the main effects (time, condition, and sex) and interaction effects (time x condition, and time x sex).



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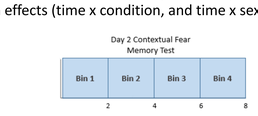
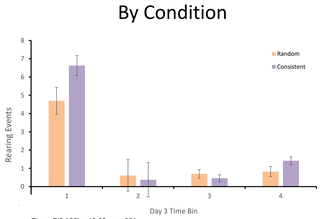


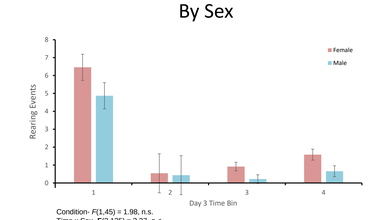
DAY 3

Rearing



Time- $F(3,135) = 48.09$, $p < .001$
Time x Condition- $F(3,135) = 2.77$, $p < .05$

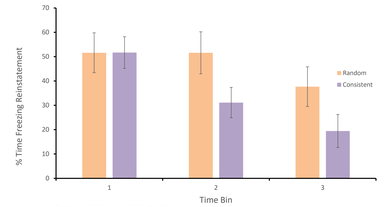
Figure 10. Bar graph showing rearing events during Day 3 Reinstatement test for both the random and consistent footshock conditions. The time is split into 4 two minute time bins in order to analyze trends in rearing over time. There is no statistically significant difference in the rearing between the two conditions over time. Rearing was eliminated by the reinstatement footshock.



Condition- $F(1,45) = 1.98$, n.s.
Time x Sex- $F(3,135) = 3.37$, $p < .02$

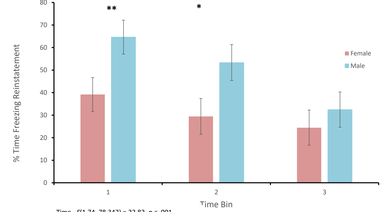
Figure 11. Bar graph showing rearing events during Day 3 Reinstatement test for both the male and female subjects. The time is split into 4 two minute time bins in order to analyze trends in rearing over time. There is no statistically significant difference in the rearing between the sexes over time.

Freezing



Time - $F(1.74, 78.342) = 22.82$, $p < .001$
Condition $F(1,45) = 1.75$, n.s.
Condition x Time $F(1.74, 78.342) = 5.42$, $p < .01$

Figure 12. Bar graph showing percent time spent freezing during Day 3 Reinstatement test for both the random and consistent footshock conditions. The time bins are separated into 2 minutes per bin in order to analyze trends in freezing over time. Subjects in the random condition trended towards exhibiting more freezing behavior and persisting longer in freezing behavior over time following the reinstatement footshock.

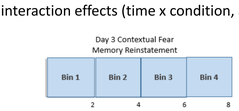


Time - $F(1.74, 78.342) = 22.82$, $p < .001$
Sex $F(1,45) 4.05$, $p < .05$
Sex x Time $F(1.74, 78.342) = 3.85$, $p < .05$

Figure 13. Bar graph showing percent time spent freezing during Day 3 Reinstatement test for both the male and female subjects. The time bins are separated into 2 minutes per bin in order to analyze trends in freezing over time. Males exhibited significantly more freezing behavior and persisted far longer in freezing over time.

Statistical Analysis

Rearing: A 2x4 repeated measures ANOVA was used to analyze the data for both the main effects (time, condition, and sex) and interaction effects (time x condition, and time x sex).



Freezing: A 2x3 repeated measures ANOVA was used to analyze the data for both main effects (time, condition, and sex) and interaction effects (time x condition, and time x sex).

SUMMARY AND DISCUSSION

Day 1
- Rearing decreased with exposure to footshocks and freezing increased.
- There were no significant differences due to experimental condition or sex.
- Subjects were driven to a fearful state by the footshock exposure on the first training day.

Day 2
- Rearing increased during the context test day.
- Freezing was increased and persisted longer in the random footshock condition.
- There was a trend towards females freezing less than males
- Animals in the random condition showed better contextual fear memory than the consistent condition, and males trended toward greater fear conditioning than females.

Day 3
- Rearing was elevated and decreased rapidly following exposure to the reinstatement footshock.
- Reinstatement persists longer in the random footshock condition than in the consistent footshock condition.
- These data are consistent with findings that unpredictable stimuli are learned more rapidly than predictable.
- Male rats exhibited extended freezing following the reinstatement footshock, consistent with a stronger fear memory trace than females.
- There are sex differences in reinstatment of the fear memory, which is likely related to amygdala-dependent fear memory and aggressive behavior in males.

Overall

This experiment demonstrates that, as according to the Rescorla-Wagner Learning Model, surprising stimuli are more readily acquired in fear-memory. Although manipulating threat certainty did not induce a state of anxiety in the animals, we demonstrated significant behavioral differences in fear-memory in both condition and sex over time. Furthermore, this study highlights the importance of utilizing both male and female subjects within animal models of emotion because sex-related differences exist in emotional responses in both human and non-human animals alike.

REFERENCES

Kessler, R. C., Chiu, W. T., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the national comorbidity survey replication (NCS-R). *Archives of General Psychiatry*, *62*(6), 617–627.

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