The Ultra Shocking Call of Anxiety

An Evaluation of Anxiety Responses Using a Pseudo-Randomized Unsignalled Footshock Paradigm

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INTRODUCTION

Anxiety disorders are a widespread and serious health concern currently affecting approximately 18% of the adult population per year (Kessler, et al., 2005); thus, there is a strong need to develop and improve therapeutic treatments for anxiety. Moreover, because sex differences in the prevalence of affective disorders in humans are well documented, this study involves both male and female rats. Vocalizations allow for a dynamic assessment of an animal's emotional state. The ultrasonic vocalizations (USVs) of rats are produced at frequencies above the level of human hearing. USVs are often used as a tool to assess the emotional state of rats. Previous research has identified two main call types for rats: 22 kHz (related to strongly negative emotion) and 50 kHz. 50 kHz calls can then be further broken down into constant frequency (CF) and frequency modulated (FM) subtypes. FM calls are produced with a bandwidth greater than 15 kHz; these calls are related to positive emotional states. Whereas, CF calls are produced with a constant frequency and a bandwidth less than 10 kHz. Our lab hypothesizes that CF 50 kHz calls are expressions of anxiety in rats. Our lab has previously explored the vocalizations of rats across a continuum of negative affective state (i.e., from anxiety to fear) within a single testing session using a sequence of temporally consistent mild footshocks. The current experiment explores USV production in male and female rats when the temporal predictability was reduced by randomizing the time between footshocks. We utilized an unpredictable footshock paradigm with the goal of increasing or prolonging a state of anxiety as compared to our previous procedure. In this paradigm, shocks were administered across three successive days: on Day 1, mild footshocks were administered in a pseudo-randomized pattern, on Day 2, subjects were returned to the same context but did not receive footshocks, and on Day 3, a single reinstatement shock was administered. Differences in USV calling behavior across test days will be explored in male and female rats. In addition to USVs, rearing and freezing behavior were also recorded and used to assess anxiety and fear. These results will enhance our understanding of vocal expression of emotional states in rats, which improves the dominant animal model used to study anxiety disorders and potential therapeutic interventions.

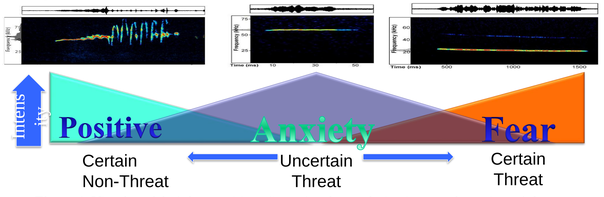

Figure 1. Visual model of the transition of emotional state during increased certainty of threat and the USVs known, or hypothesized, to elicited during these states.

METHODS

Subjects

Eighteen total young adult Long Evans rats. Ten of which were male and eight female.

Experimental Design

I. Pseudo-Randomized Footshock
- Mild footshock (0.5 mA, 0.5 s)
- 2 min pre- and post-shock
- 30-100 s inter-shock interval

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

III. Single Footshock
- 2 min pre-shock
- 6 min post-shock

Figure 2. Schematic of experimental design.

ANALYSIS

Ultrasonic Vocalizations

- Sound was recorded with an UltrasoundGate Condenser Microphone (CM 16; Avisoft Bioacoustics)
- Acoustic data were digitized and saved for later call classification (250 kHz sample rate, 16 bit, Avisoft Recorder)
- Spectograms for each session were calculated with SASLab Pro (Avisoft Bioacoustics) using 1024 FFT length, 100% frame size, FlatTop window, and 96.87% overlap

Rearing, Freezing and Call Type during Testing

I. Rearing:
- Floor contact with hind limbs only.
- Counted by blind observer per 20 sec.
- 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

III. USV Call Type
- Narrow Bandwidth – Call bandwidth < 11kHz Mean Peak Frequency > 29 kHz.
- 22 kHz Mean Peak Frequecy < 22 kHz

RESULTS

Conditioning Narrow Bandwidth vs. Rearing

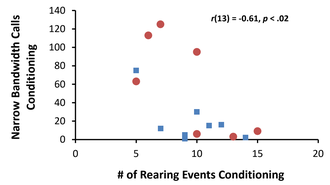

Figure 3 . In the conditioning test a moderately strong negative correlation exists between the number of rearing events and narrow bandwidth calls, unexpectedly indicating that calling decreases as rearing events increase.

Reinstatement Narrow Bandwidth vs. Rearing

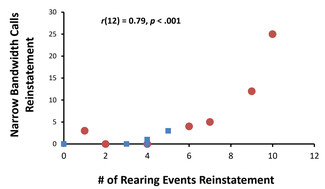

Figure 4 . In the reinstatement test a strongly positive correlation exists between rearing events and narrow bandwidth calls. This trend is as expected—calling increases as uncertainty of threat increases.

Freezing vs. Rearing

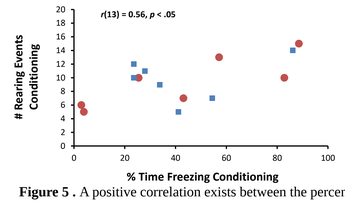

Figure 5 . A positive correlation exists between the percent time freezing in the conditioning test and the number of rearing events. This trend is unexpected since our lab hypothesizes an inverse relationship between freezing and rearing. Further investigation is needed to explain these findings.

Conditioning Narrow Bandwidth Calls vs. Freezing

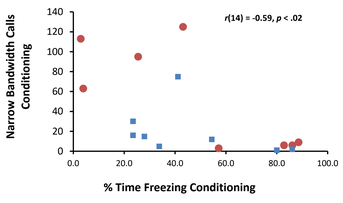

Figure 6 . In the conditioning test a negative correlation exists between percent time freezing and narrow bandwidth calls. This trend is as expected—calling decreases as threat becomes more certain.

Context Freezing vs. Conditioning Freezing

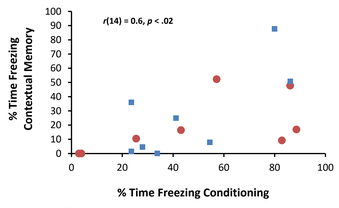

Figure 7. A moderately strong positive correlation exists between percent time freezing in the conditioning test and percent time freezing in the context test, indicating learning occurred between day one and two.

Reinstatement Freezing vs. Context Freezing

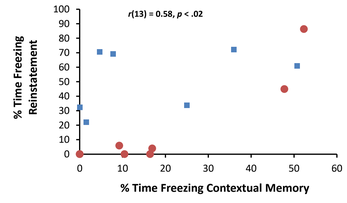

Figure 8. A positive correlation exists between percent time freezing in the context test and percent time freezing in the reinstatement test, indicating learning occurred between day one and two.

Conditioning Freezing Sex Differences

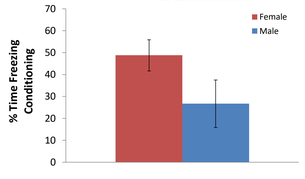

Figure 9 . In the conditioning test there was no significant difference in freezing between males and females.

Context Freezing Sex Differences

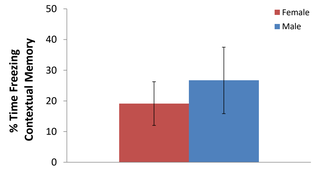

Figure 10. In the context test there was no significant difference in freezing between males and females.

Reinstatement Freezing Sex Differences

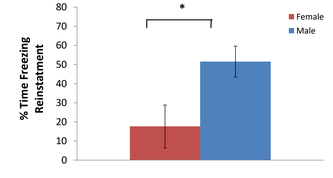

Figure 11. In the reinstatement test there was a significant difference in freezing between males and females, indicating that males have formed a stronger fear memory association with the stimulus.

Training Day 1 – Call Type Distribution

Sex	Narrow Bandwidth	22 kHz*
Male	Sum: 214 Avg: 21.4 10/10 rats produced NB calls	Sum 526 Avg 52.6 8/10 rats produced 22 kHz calls
Female	Sum: 420 Avg: 52.5 8/8 rats produced NB Calls	Sum 526 Avg: 3.13 2/8 rats produced 22 kHz calls

Reinstatement Day 3 – Call Type Distribution

Sex	Narrow Bandwidth*	22 kHz*
Male	Sum: 14 Avg: 1.4 5/10 rats produced NB calls	Sum 378 Avg 37.80 8/10 rats produced 22 kHz calls
Female	Sum: 50 Avg: 6.25 6/8 rats produced NB Calls	Sum 5 Avg: .63 2/8 rats produced 22 kHz calls

CONCLUSIONS

- We predicted that there would be a positive correlation between narrow bandwidth calls and rearing behavior because it is an anxiety-related behavior.
- During contextual fear memory conditioning, the expected positive relationship between narrow bandwidth calls and rearing was not observed. However, the predicted negative relationship between freezing behavior and narrow bandwidth calls was observed during contextual fear memory conditioning and testing.
- A strong positive correlation between rearing and the production of narrow bandwidth calls was observed during reinstatement of fear memory.
- Freezing behavior and rearing behavior were correlated during contextual fear memory training, and freezing behavior during training was a predictor of future conditioning behavior. These data indicate that active exploration is related to memory formation.
- Freezing behavior and 22 kHz calls are indicative of a fearful state in rats. There were sex differences during reinstatement testing in freezing behavior, showing increased fear memory in male rats. Similarly, there were differences in the number and frequency of 22 kHz calls in male rats compared to females.