Shocking News: A Rat’s Tail of Uncertainty
An Evaluation of Anxiety Responses Using a Pseudo-Randomized Unsignaled Footshock Paradigm
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INTRODUCTION
Anxiety disorders are a devastating 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. This pilot study focuses on the refinement of behavioral measures of anxiety in an animal model by assessing freezing, rearing, and ultrasonic vocalizations (USVs) in an elevated state of anxiety. 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.

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 (related to play behavior) (Brudzynski & Panksepp, 2006). 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 12 kHz; these calls are related to positive emotional states. Whereas, CF calls are produced with a constant frequency and a bandwidth less than 12 kHz. Our lab hypotheses that CF 50 kHz USVs are expressions of anxiety in rats (Taylor, Urbano, & Cooper, 2017).

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 constant mild footshocks. The current pilot 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-random pattern. On Day 2, subjects were returned to the same context but did not receive footshocks, and on Day 3, a single nonaversive shock was administered. In addition to USVs, rearing and freezing behavior were also recorded and used to assess anxiety and fear. To examine sex differences, both male and female rats were tested in this paradigm. These results could add to the construction of a more comprehensive timeline to research the study of anxiety disorders and potential therapeutic interventions.

METHODS

Materials and Procedures

Experimental Design

I. Pseudo-randomized footshock
- Mild footshocks (0.5 mA, 0.5 s)
- 2 min pre- and post-shock
- 30-120 s inter-shock interval

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

III. Single Footshock
- 2 min pre-shock

USV Recording Procedure

- Sessions were continuously recorded with an Ultrasound/Condenser Microphone (CM 36, Artek Biosciences)
- Digitized and saved for later calls classification (250 kHz sample rate, 16 bit, AudioFile Recorder)
- Spectrograms for each session were generated using Pari (Pari Biosciences) using 1024 FFT length, 100% frame size, FlatTop window, and 96.875% overlap.

Subjects

Adult Long-Evans rats (male, N=4; female, N=4)

RESULTS

USVs Across Session

Figure 1: Scatterplot showing all USVs produced during footshock paradigm. Each dot represents one call produced during the session. CF 50 kHz post-shock USVs were produced more than twice as often as the CF 50 kHz pre-shock USVs.

Figure 2: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 1. E- = 0.027, p = .844. There were no differences detected.

Figure 3: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 1. E- = 0.027, p = .844. There were no differences detected.

Figure 4: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 2. E- = 0.027, p = .844. There were no differences detected.

Figure 5: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 2. E- = 0.027, p = .844. There were no differences detected.

Figure 6: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 3. E- = 0.027, p = .844. There were no differences detected.

Figure 7: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 3. E- = 0.027, p = .844. There were no differences detected.

Figure 8: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 1. Male rats produced CF 50 kHz USVs at a similar rate, but female rats produced a lower rate of CF 50 kHz USVs.

Figure 9: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 1. Male rats produced 22 kHz USVs. Female rats produced 22 kHz USVs and persisted longer in calling.

Figure 10: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 2. Male rats produced CF 50 kHz USVs at a similar rate, but female rats produced a lower rate of CF 50 kHz USVs.

Figure 11: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 2. Male rats produced 22 kHz USVs. Female rats produced 22 kHz USVs and persisted longer in calling.

Figure 12: Proportion of subjects in each group exhibiting CF 50 kHz USVs during Day 3. Male rats produced CF 50 kHz USVs at a similar rate, but female rats produced a lower rate of CF 50 kHz USVs.

Figure 13: Proportion of subjects in each group exhibiting 50 kHz USVs during Day 3. Male rats produced 22 kHz USVs. Female rats produced 22 kHz USVs and persisted longer in calling.

USV Classification

CF 50 kHz USVs
- Male: 100%
- Female: 100%

50 kHz USVs
- Male: 100%
- Female: 100%

SUMMARY AND DISCUSSION

Male rats exhibited more fear as expressed by time freezing and produced 22 kHz USVs, whereas female rats exhibited a higher rate of 50 kHz USVs and expanded longer calling. However, rearing behavior was similar between male and female rats.

Individual differences in the prevalence of affective disorders in female rats may be related to differences in the modulation of defensive behavior along a continuum related to strongly negative emotion. 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.

REFERENCES