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Ultrasonic vocalizations as a predictor of resilience to intermittent swim stress-induced anxiety: An investigation of re-exposure effects

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Ultrasonic vocalizations as a predictor of resilience to intermittent swim stress-induced anxiety: An investigation of re-exposure effects

Introduction:

Anxiety disorders, such as post-traumatic stress disorder (PTSD), are estimated to impact 6.8% of the U.S. population (Kessler et al., 2005 & Price et al., 2015). Exposure to stress is a common risk factor in the etiology of mental illness with the experience of an acute traumatic stressor as a significant cause of PTSD (Price et al., 2015). Various forms of traumatic events can directly contribute to PTSD, including military combat, violent personal assault, severe car accidents, natural or man-made disasters, terrorist attacks and being kidnapped (Graham et al., 2016). According to U.S Department Veterans Affairs (VA), there are four major symptoms of PTSD, including re-living the trauma, avoidance of trauma-related situations, hyperarousal, and negative alternations in believes and feelings (U.S. Department Veteran Affairs, 2015). The re-living symptoms usually express in the forms of intrusive recollections, nightmares, and flashbacks (VA, 2015). The psychic numbing and dissociations are two classical forms of avoidance behaviors of PTSD patients (VA, 2015). It is not uncommon to see patients who are in hyperarousal states have insomnia, irritability and hypervigilance (VA, 2015). Due to the complexity of PTSD’s symptoms, the treatments are also rather complicated.

There are majorly two forms of treatments, including the pharmacological therapies and the psychological therapies. DeMartino et al (1995) finds that Monoamine oxidase inhibitors (MAOIs) are an effective medication to treat re-living symptoms. In terms of avoidance behaviors, such as dissociations and emotional numbness, selective serotonin reuptake inhibitors (SSRIs) are found to have therapeutic effects (MacNamara, 2016). For hyperarousal symptoms, which are related to the alternation of sympathetic nervous systems, anti-adrenergics are found to
be useful (Ravindran & Stein, 2009). In addition, psychological therapies, particularly the exposure therapies and cognitive behavioral therapies, are rather effective for PTSD patients (Castillo et al, 2016). The major purpose of aforementioned psychological therapies is breaking the relationship between particular cues related to the initial traumatic events and bad believes or feelings (Shnaider et al, 2015). Currently, there are many unknowns concerning PTSD, and better understanding of the neurobiology and treatment of PTSD is desired.

Substantial advances in understanding the neurobiology of PTSD symptomology has been made through rigorous experimentation using animal models. There are various forms of animal models for analogizing different kinds of PTSD-like symptoms, including behavioral sensitization, time-dependent sensitization, kindling and learned helplessness. Models using behavioral sensitization and kindling induce prolonged response by exposing subjects to traumatic stimulus repeatedly (Toledano, 2013, Post, 1997). Time-dependent sensitization models produce PTSD-like symptoms in subjects with one single exposure (Zhang, 2015). Furthermore, learned helplessness paradigms that employ either escapable or yoked inescapable shock or swim are widely used (Daskalakis et al, 2013). These paradigms result in anxiety-like behaviors which is a correlate of PTSD (Daskalakis et al, 2013, Maier, 2001 & Stafford et al, 2015). However, few studies have examined the “flashback” symptom of PTSD (Daskalakis et al, 2013 & Maier, 2001).

The re-living is a hallmark symptom of PTSD, which is the experience of a “flashback,” referred to the feelings of re-experiencing the trauma. The triggers of recall include perceptual stimuli (i.e. visual, body sensation, etc.), and emotional response (Kleim et al., 2013). Ehlers & Clark (2000) indicate that the flashback, or re-experiencing, contributes to elevate not only the emotional response (i.e. anger or fear), but also shame and sadness, which influences the quality
of life of PTSD patients. Since flashbacks are one of the characteristic symptoms, studying the core feature of PTSD can facilitate scientific understanding of the disorder and potentially improve the quality of life of PTSD patients. Typically, empirically modeling the re-living component of PTSD symptomology involves a stress context re-exposure, which prolongs PTSD-like behaviors in the rat (Maier, 2001).

Our laboratory employs an intermittent swim stress (ISS) paradigm, which we have found to model the social anxiety component of PTSD (Stafford et al, 2015). This paradigm is a hybrid of learned helplessness and behavioral despair models. However, we have yet to examine the effects of ISS on other symptomology, such as the flashback experience. We have also reported ultrasonic vocalizations (USVs) to predict resilience to ISS-induced anxiety. Therefore, the current study was designed to examine the effects of re-exposure to ISS and the predictive nature of USVs during the re-exposure.

**Method:**

*Animals*

Twenty adult male Sprague-Dawley rats were used, randomly assigned to ISS (n=10) and confined control (CC, n=10). Ten juvenile male Sprague-Dawley rats were used for social exploration stimuli.

*ISS Apparatus*

The ISS was administered in Plexiglas cylinders lowered intermittently (variable 60s intervals) into 15°C water for 5s. Space heaters blow warm air onto the rats during inter-trial intervals.

*Ultrasonic Detection and Analysis*
USVs emitted by ISS rats were recorded via high frequency microphone positioned ~10cm away from the ISS cylinder. The signal was refined via band-pass filter (18-32kHz), and quantified with custom LabView software.

*Social Exploration Test*

The social exploration was conducted in plastic tub cages with a layer of bedding. Adults acclimated to individual cages for 1hr, after which juvenile was placed in the cage, and the adults’ exploratory behaviors (sniffing, pinning or grooming) directed at the juvenile were recorded.

*Re-exposure*

The re-exposure was performed in the ISS apparatus with 3 cm water at the bottom of the tank. The re-exposure pairs(ISS/CC) went through the one movement of ISS apparatus followed by 10-min of confinement.

*Data Analysis*

Social exploration times were analyzed via two-way repeated measures ANOVA. Re-exposure behavior was analyzed via one-way ANOVA

*Procedure*

Day one conducted social exploration (SE) baseline test. For baseline score, a 3-min SE test was conducted for each rat. One juvenile was used per 4 adult rat SE tests, and no adult was exposed to the same juvenile twice. Two observers scored the adult exploration time. Twenty-four hours later, on day two, rats were randomly assigned to ISS or CC, and pairs (ISS/CC) were exposed to 80 trials of ISS. ISS rats were forced to swim for 5s, while CC rats were placed in the shorter cylinder without exposure to the water. USVs emitted by the ISS rats were recorded. Twenty-four hours after ISS, on day three, re-exposure pairs went through one movement of ISS
apparatus followed by 10-min confinement. Sixty minutes after re-exposure, all rats went through the 3-min SE test, and exploratory behaviors were recorded by observers blind to group membership. ISS-induced decrease in exploratory behaviors indicates anxiety.

**Experimental Design:**

![Experimental Design Diagram]

**Results:**

![Results Graph]

Figure 1: Social Exploration Time before (pre-ISS) and after ISS (post-ISS)
Figure 2: Visual inspection of the SE times for ISS subjects (A) depicting callers in the re-exposure (n=1) and no re-exposure (n=1) conditions (B).

Figure 3: The percentage of time of re-exposure group spent on freezing (a) or grooming during 10 minutes of re-exposure to ISS apparatus.

Figure 3 indicates that re-exposure to the ISS apparatus resulted in a significant time spent freezing in the ISS, but not in the CC groups [F (1, 9) = 24.30, p = 0.001]. There were no differences in grooming behavior. Figure 1 shows that no significant main effect of stress [F (1,16) = 1.28, p = 0.274], re-exposure [F(1,16) =0.02, p = 0.904], or time [F(1, 16) = 1.70, p =
No interactions were significant. The right panel of Figure 2 (B) is the visual inspection of the social exploration times for ISS rats depicting callers in the re-exposure (n=1) and no re-exposure (n=1) conditions. The penal on the left of Figure 2 (A) is the individual time differences for all ISS groups (re-exposure and non re-exposure) before and after ISS. The data points spread out.

**Discussion:**

The results of this experiment did not confirm our original hypothesis that context re-exposure would magnify the effects of ISS on social interaction. Interestingly, the results indicate that re-exposure to ISS apparatus for the ISS rats resulted in a significant time spent freezing, but not the confined control (CC) groups indicating an enhanced fear response to the context. Combined with the fact that there were no differences in grooming behavior, re-exposure likely resulted in prolonged fear, but not anxiety, which may have been attenuated by exposure to the juvenile social exploration stimulus. Fear is different from anxiety. Fear is triggered by danger, and it is an adaptive response related to defensive behaviors (Rau et al, 2005). Freezing is a reliable measure of fear in rodents, particularly in rats, and it is defined as “the absence of all movement except that necessary for respiration” (Rau et al, 2005, p.7). The current study utilized time sampling to calculate the freezing percentage, in which every rat’s behavior was scored every 10 seconds. The formula of calculating the freezing percentage was that the number of observation of freezing divided the total number of observation during the 10-min re-exposure \(N_{\text{total}} = 10 \times 6 = 60\) and times 100. The percentage of grooming was determined in the same way. As to anxiety, it is more associated with uncertainty, and grooming behavior is a good indicator of anxiety (Doyle & Yule, 1959). Our results that ISS induced prolonged fear response instead of social anxiety are inconsistent with previous studies.
Stafford et al (2015) found that post-ISS social exploration time for non-callers was significantly less than the pre-ISS SE time (i.e. baseline SE time). In other words, ISS was found to induce social anxiety in rats which did not include a re-exposure condition (Stafford Jones & Drugan, 2015). However, the current study, in Figure 1, finds that there is no significant effect of stress or re-exposure on social exploration. Combining the results of Figure 3, the current study doesn’t show ISS can induce social anxiety. Nonsignificant stress and re-exposure effects on social exploration are likely due to small sample size and individual differences. There are only five rats per group, which provides small sample size and power, and we had only one caller per group (Figure 2), which could not provide enough power for statistical analysis. So, there was no further analysis of the predictive nature of ultrasonic vocalizations during the re-exposure. With a larger sample size, we would have more statistical power in our analysis, and a better idea about what the difference between callers and non-callers would be in re-exposure models. Another possible reason why significant effects didn’t show in SE test was that 80 trials of ISS were not enough to induce social anxiety. The 100 trials of ISS instead of 80 trials would produce robust effects, which could be easier to observe and analysis.

For future work, we will use a larger sample size to make sure that we can get sufficient information and data for further analysis. Also, we will use 100 trial of ISS instead of 80 trials to exaggerate the effects. In addition, in future experiment, we will focus on individual differences and re-exposure effects on ISS-induced fear.
Reference:


Maier, S. F. (2001). Exposure to the stressor environment prevents the temporal dissipation of behavioral depression/learned helplessness. Biological Psychiatry, 49(9), 763-773. doi:10.1016/S0006-3223(00)01095-7


