

Ultrasonic vocalizations during intermittent swim stress as an indicator of resilience to social anxiety

Adele M. Jones, Nathaniel Stafford, & Robert C. Drugan
Psychology Department, University of New Hampshire, Durham, NH 03824, USA

Introduction

Anxiety disorders are the most prevalent mental illness in the United States, and depression is projected to soon be the second most disabling condition worldwide (1). The two disorders frequently co-occur, and those affected by both often show significantly poorer coping and reduced recovery rate (2), with less than 50% experiencing a full recovery after treatment.

Exposure to stress is considered a trigger to these illnesses in those that are vulnerable. By examining individuals who are stress resilient, previously unappreciated neural systems could be uncovered. Thus, studying the resilient brain may aid in discovering previously novel targets for drug discovery.

The intermittent swim stress (ISS) model is a valuable tool to assess the aforementioned issues as it produces signs of behavioral depression and increased social anxiety (3,4). Moreover, it produces both vulnerable and stress-resistant subjects (5) Recent work suggests that animals emitting ultrasonic vocalizations (USVs) during ISS are behaviorally resilient, indicated by reduced behavioral despair and attenuated learning deficits in subsequent water-based tests (6). However, this effect needs to be examined in non-water context tests, and it is not yet known if USVs forecast resilience to ISS-induced anxiety.

The current study tests if USV production will forecast resilient behavior in the social exploration (SE) model, a test of social anxiety.

Method

Animals

24 adult male Sprague-Dawley rats were used, randomly assigned to ISS (n=12) and confined control (CC, n=12). 12 juvenile male Sprague-Dawley rats were used for social exploration exposure.

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 the experimental rat were recorded via a high frequency microphone positioned ~10cm away from the ISS cylinder. The signal was refined via band-pass filter (18-32kHz), while number, duration, frequency and time of the call were quantified with custom Lab View software.

SE 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 behavior (sniffing, pinning or grooming) towards the juvenile was recorded.

Data Analysis

Group differences were analyzed via one-way ANOVA. Comparisons among means was conducted using Fisher's LSD.

Procedure

Day 1: SE Baseline

- A 3-min social exploration test was conducted for all rats to establish social exploration baseline score.
- One juvenile was used per four adult rat SE tests, and no adult was exposed to the same juvenile twice.
- The adult rats exploratory time towards the juvenile was manually scored by two simultaneous observers.

Day 2: ISS

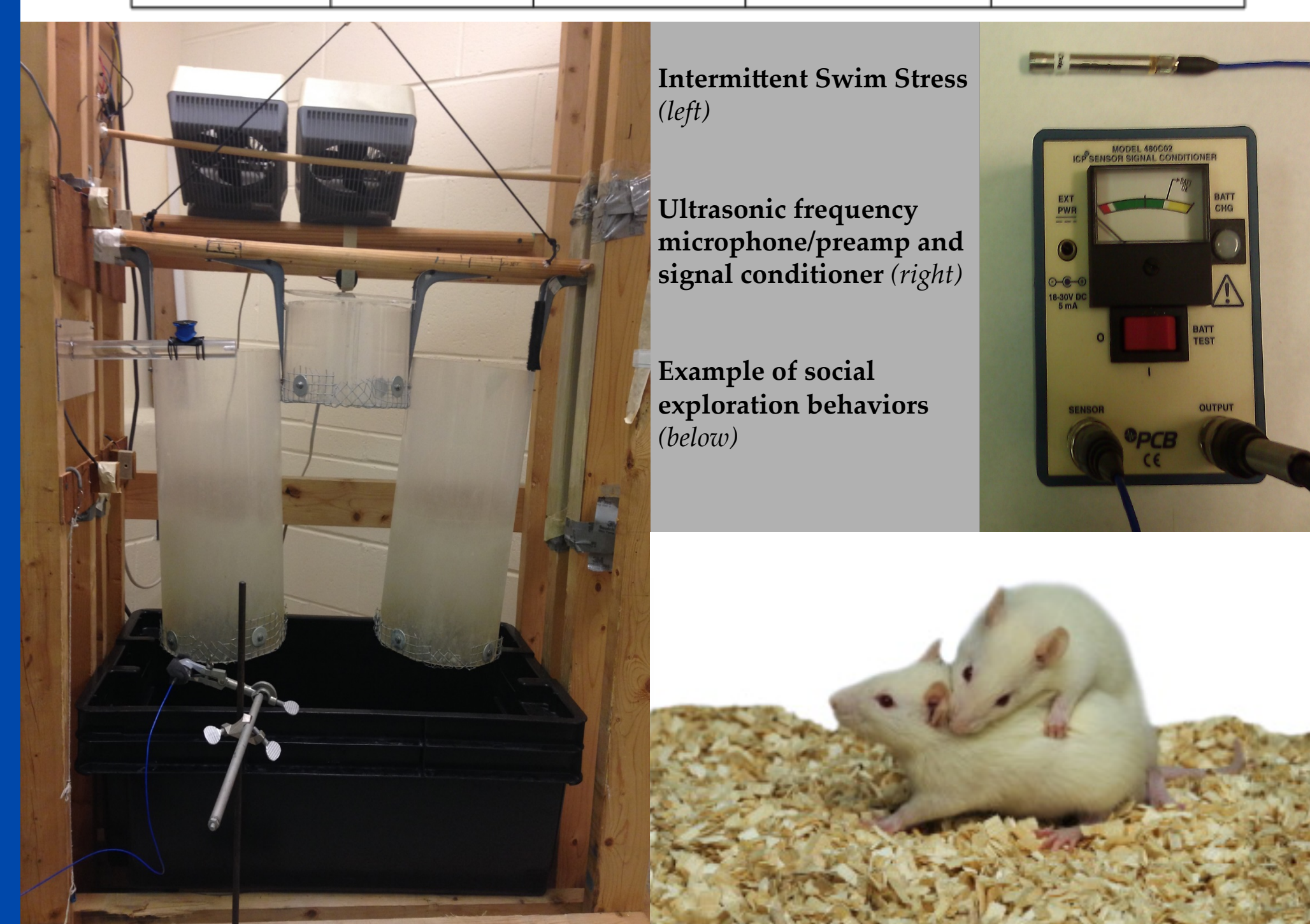
- 24hrs following SE baseline, rats were randomly assigned to ISS or CC groups. Pairs of one ISS and one CC were exposed to 80 trials of ISS.
- Experimental rats were submerged and forced to swim in cold-water for 5s, while CC rats were placed in the shorter cylinder so as to receive the same handling without exposure to the water.
- USVs emitted by the ISS rat were recorded, and rats were categorized as callers or non-callers.

Day 3: SE Test

- 24hrs after ISS, all rats again experienced the 3-min social exploration test, and exploratory behaviors were recorded by observers blind to group membership. ISS-induced decrease in exploratory behaviors indicates anxiety.

Timeline

Day 1	Day 2	Day 3
Pre-ISS Social Exploration Baseline	ISS Rats	ISS Callers
	CC Rats	ISS Non-callers
	ISS w/ Cold Water Stress	Post-ISS Social Exploration Test
	ISS Exposure w/ No Cold Water Stress	CC Rats
		Post-ISS Social Exploration Test



Results

Figure 1: Visual depiction of SE scores of the single USV emitting (Caller) rat compared to ISS non-callers and confined controls.

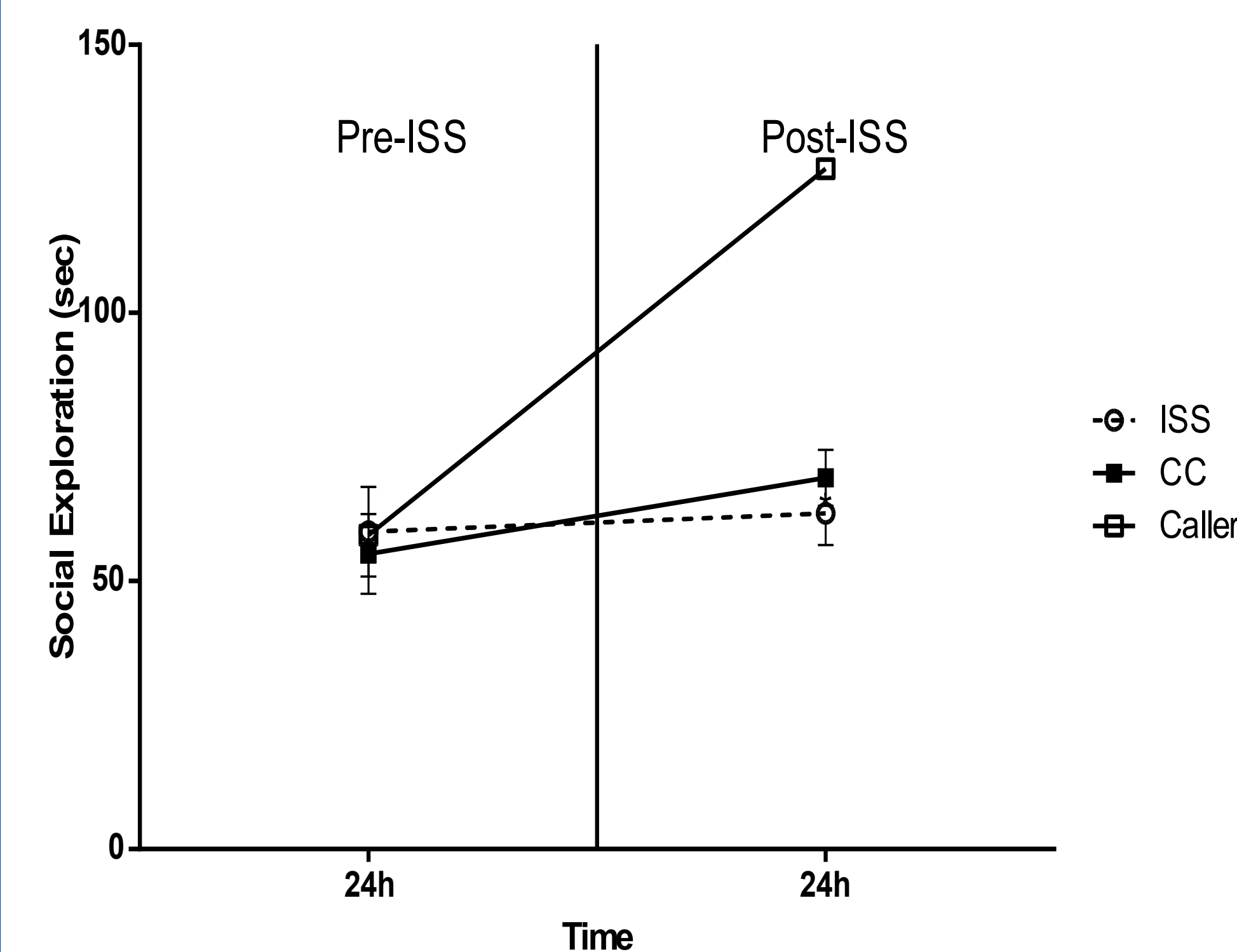
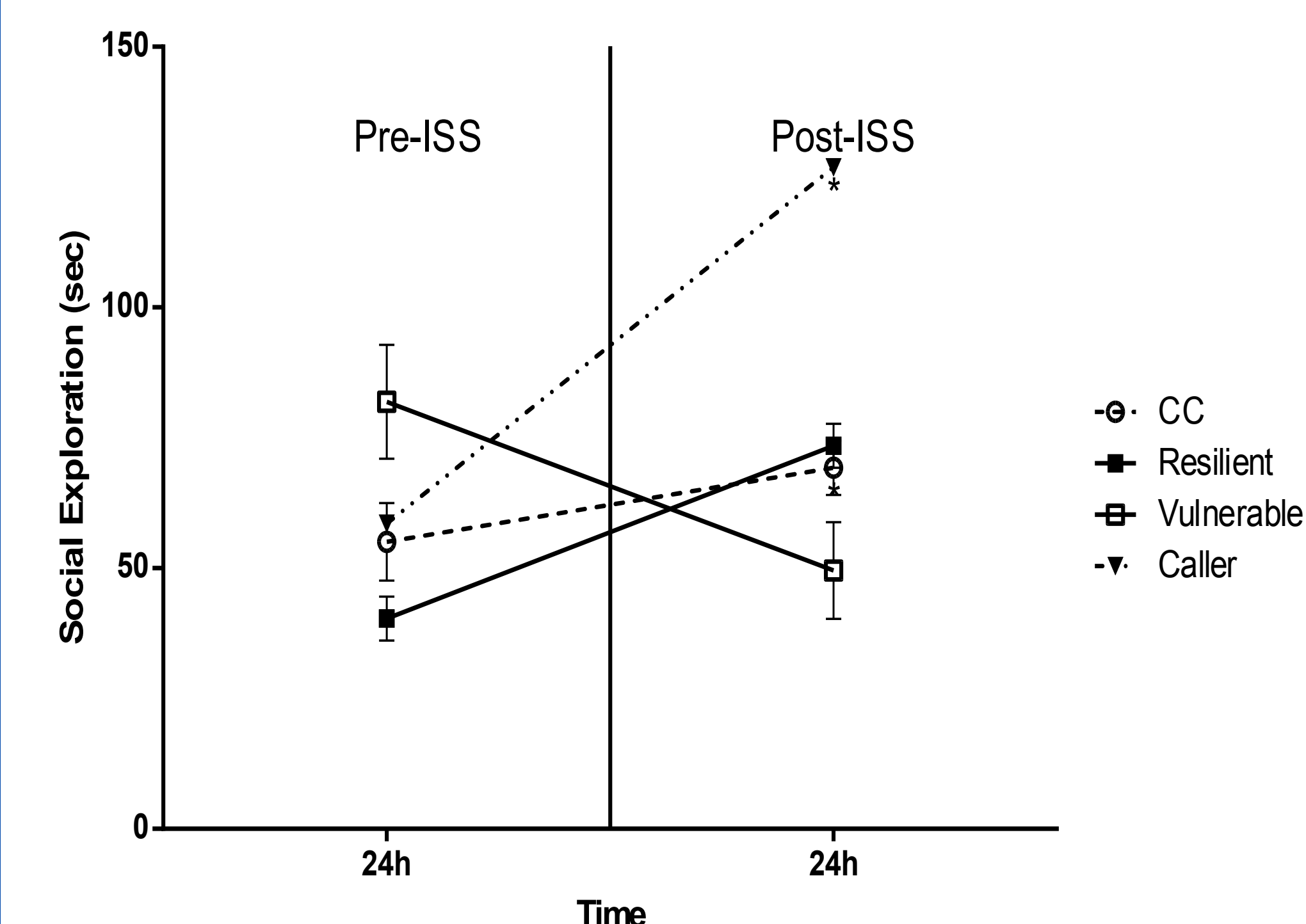


Figure 2: ISS non-caller rats were further split into vulnerable and resilient groups based on an increase or decrease in social exploration test scores from baseline. Vulnerable vs. resilient groups differed significantly at pre-ISS ($p=0.013$) and post-ISS ($p=0.031$) SE measures. ISS caller included for visual comparison.



Conclusions

□ USV emission predicted resilience to ISS-induced social anxiety in the SE test

□ Furthermore, 80 trials of ISS produced a dichotomy of resilient and vulnerable animals, characterized by an increase or decrease of SE time, respectively

□ Interestingly, the caller began in the non-caller resilient and CC cohorts at baseline, but substantially increased SE time post-ISS compared to other resilient animals

□ Future work will attempt to examine group differences between callers and non-callers, as well as activity during ISS as a potential mediator of resilience

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Acknowledgements

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