Defensive Behavior of Rats in Response to 22-kHz Ultrasonic Vocalizations

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Introduction

To better understand rats as models for both communication and affective neuroscience research, the intricacies of their messaging system must be fully understood. To that effect, the rat's 22kHz ultrasonic vocalization (USV), which serves as both a conspecific warning signal and as an indicator of negative affect (Knutson, et al., 2002; Portfors, 2007), was investigated in this set of experiments. Both the duration and number of 22kHz USVs have been proposed to carry semiotic information (Brudzynski, 2005), and previous research from our laboratory has demonstrated duration effects (Niemiec & Hinderer, 2008).

In this study, we investigated the effect of number of vocalizations per call set on the defensive behavior of rats exposed to the calls. In addition, a synthetic call set was produced for comparison to the natural 22kHz USV; consistency with the natural call effects would indicate that synthetic calls are a viable option for future research involving the semiotics of rat USVs.

Methods

•Pre-recorded 22kHz USVs, obtained by exposing a separate group of adult male rats to predator odor, were arranged into sets of two and four calls.

•Subjects were 132 Long-Evans rats (69 males and 63 females), 51–54 days of age. Each rat was individually exposed to the USV set containing the number of calls assigned to its group (zero, two or four; n ≥ 32 per group), while isolated in an open field testing chamber containing Froot Loops® cereal arranged throughout as well as a plexiglass hide box and a 10 inch PVP pipe (i.e., hiding places). (See Fig. 1.) The calls were played for 5 minutes at a rate of one call set/min.

•The rat's behavioral response to the call presentation was recorded on videotape and then scored for three defensive behaviors related to negative affect: suppression of feeding, suppression of foraging, and retreat to a secure location where threat can be monitored (Apfelbach, et al., 2005)

•The second experiment (n = 33 per group) examined the effect of a synthetic USV call set (containing 2 calls) on the behaviors listed above.

Experimental Setup

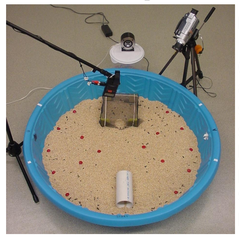

Figure 1. A plexiglass hide box, a PVP pipe, and Froot Loops® (represented by red markers) were arranged in the testing chamber as shown. An ultrasound detector was suspended over the chamber, a high-frequency speaker was positioned nearby and used to play stimuli, and a video camera was positioned to record rat behavior.

Response to 22kHz USVs (All Subjects)

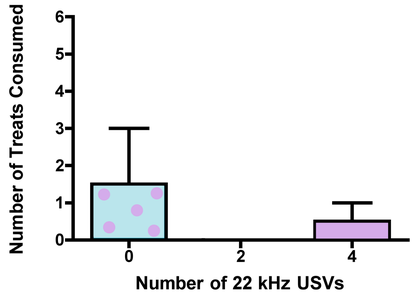

Figure 2. Comparison of treats consumed across the experimental groups. No effect of number of calls was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 1.4$, df = 2, p = 0.49).

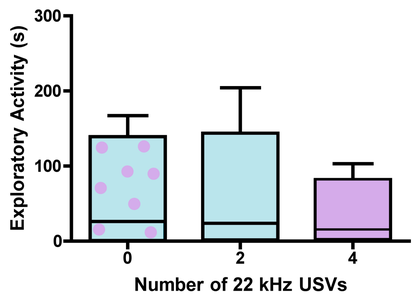

Figure 3. Comparison of exploratory activity across the experimental groups. No effect of number of calls was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 0.5$, df = 2, p = 0.78).

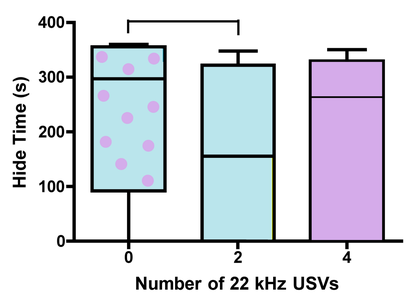

Figure 4. Comparison of time spent hiding across the experimental groups. An effect approaching significance was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 5.9$, df = 2, p = 0.052). Post-hoc analyses revealed that hide time was significantly greater in the control group than in the group exposed to 2 calls (Mann-Whitney U Test, U = 751.5, p = 0.016, Bonferroni-corrected (p_{crit} = 0.0167).

Response to Synthetic Calls (All Subjects)

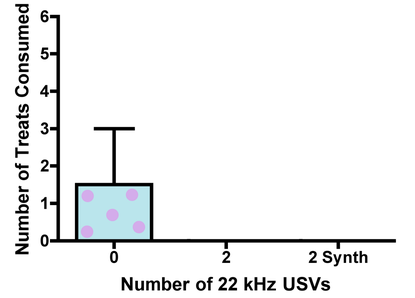

Figure 5. Comparison of treats consumed between groups exposed to natural and synthetic calls. No significant effect was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 1.6$, df = 2, p = 0.45).

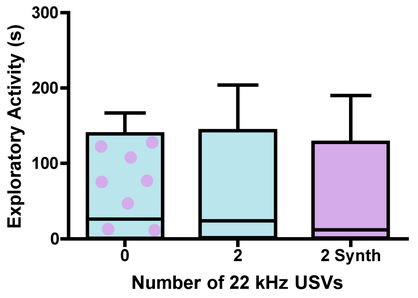

Figure 6. Comparison of exploratory activity between groups exposed to natural and synthetic calls. No significant effect was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 0.6$, df = 2, p = 0.75).

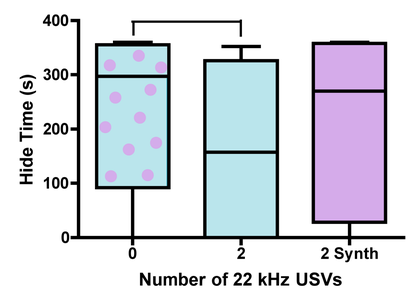

Figure 7. Comparison of time spent hiding between groups exposed to natural and synthetic calls. A significant effect was found (Kruskal-Wallis one-way ANOVA, $\chi^2 = 6.2$, df = 2, p = 0.046), however, post-hoc analyses did not show a significant difference between the synthetic call group and either the control group or the natural call group.

Control Group Comparison to Previous Study (All Subjects)

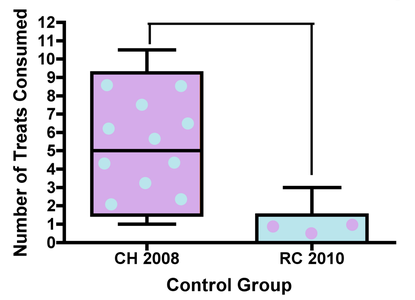

Figure 8. Comparison of treats consumed between the control group of Hinderer, 2008 and the current study's control group. Rats in the current study consumed significantly fewer treats, indicating a more negative affect (Mann-Whitney U Test, U = 1036, p = 0.0001).

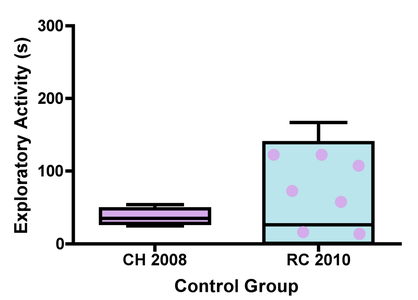

Figure 9. Comparison of exploratory activity between the control groups of Hinderer, 2008 and the current study. No significant difference was found (Mann-Whitney U Test, U = 677, p = 0.1).

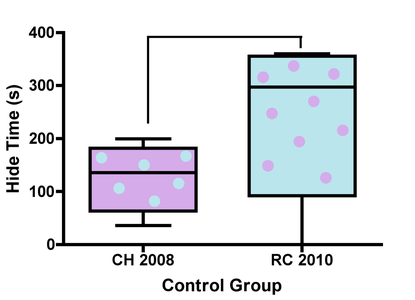

Figure 10. Comparison of time spent hiding between the control groups of Hinderer, 2008 and the current study. Rats in the current study spent significantly more time hiding, indicating a more negative affect (Mann-Whitney U Test, U = 913, p = 0.0001).

Discussion

•Our results show no systematic effect of number of real USVs or synthetic USVs on defensive behavior. Similar results were seen for both male and female rats (analyses not shown).

•Data from our control group suggested that even those animals who were not exposed to any distress calls exhibited high levels of anxiety. We subsequently discovered that the rats used in this experiment came from a different source than the rats used in previous studies.

•Comparison of our control group of rats with the control group from a previous study confirmed significant differences between the two groups. The control group from the current experiments consumed significantly fewer treats and spent significantly more time hiding, indicating that these rats were experiencing high baseline levels of negative affect. Rats in the current study were also observed to engage in more freezing behavior in the open field than rats in the previous study, resulting in increased variability in the exploratory activity measure.

•We speculate that the high baseline level of negative affect displayed by our subjects contributed to "ceiling/floor" effects in the defensive behaviors measured in response to the 22kHz USVs.

•Future work will involve replicating this experiment with rats from our original source, examining other structural aspects of 22kHz rat USVs for semiotic content, and exploring the possibility of using synthetic calls as potential stimuli.

Literature References

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