

Affectively Valenced Conditioned Place Preference for 22-kHz and 55-kHz Ultrasonic Vocalization Groups in *Rattus Norvegicus*

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Research with *Ratticus Norvegicus* specimen is highly applicable in a variety of scientific fields, including the focus of Jennifer Honeycutt's laboratory, early life adversity, and the psychological maladjustment that results from it. Our project utilized the conditioned place preference paradigm to study affective valency of ultrasonic vocalization calls, primary communicative means of *Ratticus Norvegicus*, to establish a possible intrinsic preference that the specimen might harbor for either 55-kHz frequency calls (dubbed as "positive" and emitted during play, anticipation, contentment, etc.) and 22-kHz frequency calls (considered to be "negative" due to being produced in cases of aggression, fear, discomfort, etc.)

Methodology: We used a conditioned place preference box with three compartments (two for each stimulus tested, 55-kHz and 22-kHz recordings, respectively, and a neutral area between them). The first compartment was striped, the second was dotted, and the third (neutral) had glass walls. Recording and playback were done via specialized equipment: a camera, a speaker, and a computer with USV software, Avisoft Bioacoustics. The detailed timeline is illustrated in Figure 1 below, while the conditioning procedure is depicted in Figure 2.

Results:

- Due to the significant preference of the specimen for the striped chamber of the conditioned place preference box (Figure 3), we used a biased experimental design for statistical analysis, where the changes in the rats' baseline preferences for the chambers dependent on the condition, instead of overall duration inside the chambers, were recorded.
- The 55-kHz call recording acted as an appetitive stimulus, increasing the likelihood of rats entering their least favorite chamber, if they were conditioned inside of it, proving the common conception that the 55-kHz calls have positive affective valency.
- Alternatively, the 22-kHz reduced the probability of rats entering their most preferred chamber, acting as an aversive stimulus, supporting the idea of them having negative affective valency.
- The significance of the results became weaker or even disappeared upon the analysis of bigger time intervals (a trend for 5 minutes for Context x USV interaction and no significance for the 10-minute period.)

The study is unique and does not replicate any previously published works due to two reasons:

1. All previous inquiries in the field inferred the preference for 55-kHz calls based on drug studies, connecting 55-kHz call emission to higher production of dopamine in the brain, and 22-kHz calls to the more active basolateral amygdala. Therefore, this project is the first to investigate differences between the two call groups in a controlled environment that is not compounded by drug administration.
2. The project is also among the pioneers in the field to use the conditioned place preference box for neuroscience research without pairing it with a drug or a food to explore the fundamental ultrasonic vocalization preference of the *Ratticus Norvegicus* specimen.

Graphs/images/figures

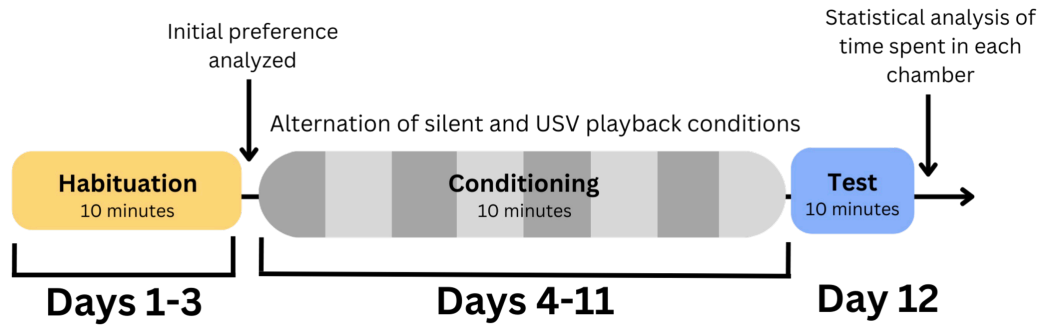


Figure 1. The experimental timeline of the study (adapted from Thiel, Okun and Neisewandar, 2008.)

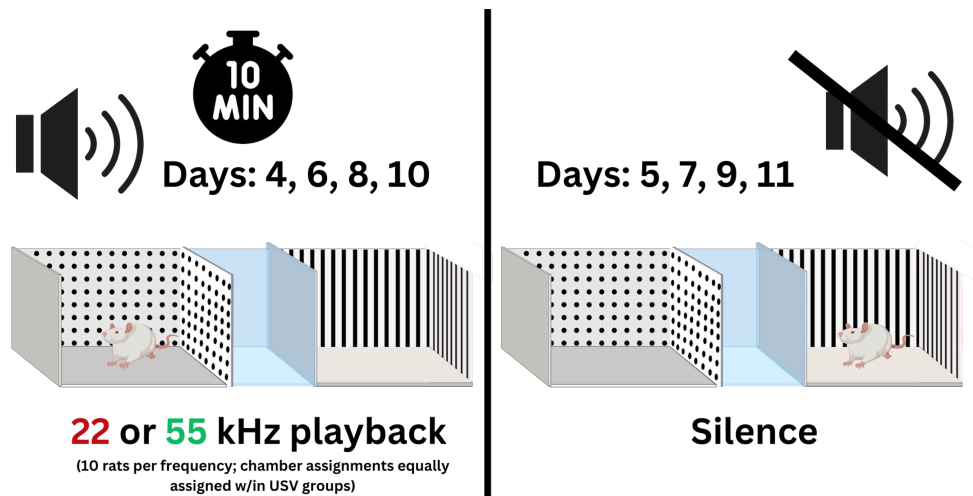


Figure 2. The conditioning procedure used in the study.

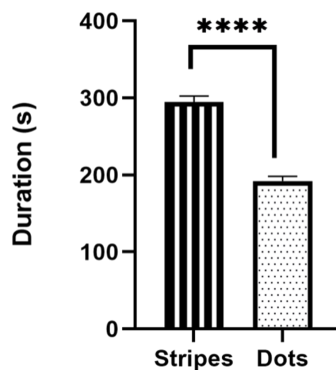


Figure 3. Length of time spent by the *Rattus Norvegicus* specimen in the two chambers of the conditioned place preference box. The striped bar on the left illustrates the number of seconds spent in the striped chamber; the dotted bar on the right shows the amount of seconds spent in the dotted chamber. The specimen showed a significant preference for the striped chamber (paired t-test, $p < 0.0001$).

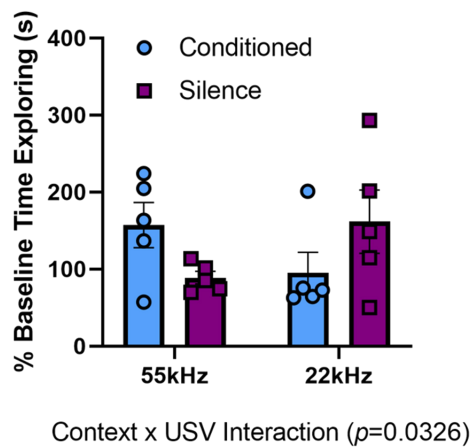


Figure 4. Significant interaction between chamber assignment and USV recording type for the 1-minute duration (three-way ANOVA, $p=0.0326$). The 55-kHz condition increased the approach behavior for the specimen's least preferred chamber, and the 22-kHz condition decreased the approach behavior for the specimen's most preferred chamber.

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