Classifying Ultrasonic Vocalization Subtypes During Oxycodone Self-Administration in Male Rats

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ABSTRACT

Background and Objective:

- Opioid abuse is a rampant problem in the United States, claiming the lives of over 47,000 individuals in 2018 alone.
- Understanding opioid abuse – how and why individuals administer substances to the point of dependence – remains critically important in the development of treatments for opioid use disorders.
- Opioids are known to produce subjectively positive feelings from humans, underscoring their abuse potential, however this has been difficult to study in animals.
- Intriguingly, rats make high-pitched vocal emissions called ultrasonic vocalizations (USVs), and previous work suggests that USVs act as both a communication tool and an emotional marker. Specifically, numerous studies support that USVs ~22-kHz in frequency are emitted in situations evoking a negative emotional state, whereas USVs ~50-kHz mark a positive emotional state.[1]
- More recently, researchers in the addiction field have come to realize that within the 50-kHz range of USVs, there are numerous subtypes with potential functional relevance.
- Many studies have just distinguished between frequency modulated USVs (more than a 3 kHz frequency modulation) and fixed frequency USVs.[2-3] Others have created up to 14 categories.[4] Most results point to significant changes in frequency of the fixed frequency and trill USV subtypes throughout drug administration.[2-5]
- Thus, the question is whether these subtypes represent more specific emotions that can then be used to further understand the forces behind addiction.

Methods:

- Using male rats in an oxycodone self-administration experiment, we studied if and how different 50-kHZ USV subtypes changed in frequency at different timepoints in the acquisition phase.
- These timepoints were before the lever (used for drug infusion) extended, after the lever extended, and 20 seconds after each of the first 10 infusions were received on Days 1, 7 and 13.
- USVs were scored offline using Raven Pro software (Cornell Laboratory of Ornithology).
- 7 subtypes of 50-kHz USVs were collected, and proportions were analyzed during each of the aforementioned timepoints.

Results:

- Our preliminary data suggests two major trends during the pre-lever timepoint:
  1) There is a decrease in the number of fixed frequency (FF) USV subtype throughout the sessions.
  2) On Days 7 and 13, the majority of the USVs belong to the Sweep with 1 modulation (SW_1) and Step with 1 modulation (ST_1) subtypes.

Experimental Setup

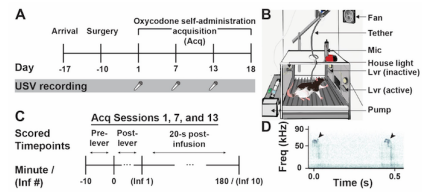

Figure 1. Experimental design for measuring USVs during the acquisition of intravenous oxycodone self-administration. **(A)** After arrival and jugular vein catheterization surgery, rats were trained to intravenously self-administer oxycodone for 18 sessions ("Acquisition" [Acq]; 3 h/d, 6 d/wk). USVs were recorded on Acq Sessions 1, 7, and 13 (grey bar, microphone [Mic] symbols). **(B)** The self-administration apparatus consisted of a fan, house light, an inactive stationary lever (Lvr), an active retractable Lvr, and an automated drug-dispensing syringe pump. Finally, the USV Mic was moved above the crevice in the ceiling of the chamber on Acq Sessions 1, 7, and 13. **(C)** During each recording session, rats were tethered into the self-administration chamber, and USVs were recorded for 10-min prior to the beginning of the self-administration session ("Pre-Lever"), during the time between the lever extension and the first drug infusion ("Post-Lever"), and 20 seconds after the first 10 infusions ("Inf 1-10"). **(D)** Spectrogram showing 50-kHz USVs (black arrowheads).

7 Subtypes of 50-kHz USVs

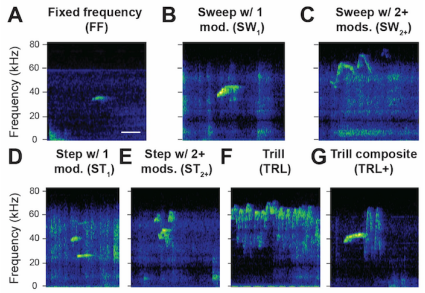

Figure 2. Spectrogram images of USV subtypes. Pictures capture 25 ms. All USVs must be ≥ 15 ms. **A)** FF USV has less than a 3 kHz frequency modulation. **B)** SW_1 USV has one frequency modulation ≥ 3 kHz. **C)** SW_{2+} USV has two or more frequency modulations ≥ 3 kHz. **D)** ST_1 USV has one gap that is < 15 ms. **E)** ST_{2+} has 2+ gaps that are < 15 ms. **F)** TRL USV has rapid oscillations in frequency. **G)** TRL+ USV has a combination of any of the prior USVs, but must contain a TRL.

Number of infusions & active lever presses increase over sessions

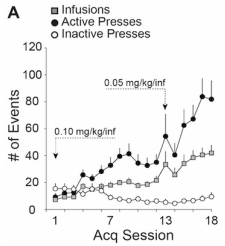

Figure 3. Intravenous oxycodone self-administration data across acquisition. Plotted are infusions earned, active and inactive presses across acquisition (mean ± S.E.M). The unit dose of oxycodone was halved from 0.10 mg/kg/inf (days 1-12) to 0.05 mg/kg/inf (days 13-18). Data are from two cohorts (final N=12). Main effects were tested using repeated-measures ANOVA with session # as the independent variable. Post-hoc contrasts for each measure were conducted against session 1 and were adjusted for using Bonferroni correction. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Post-hoc tests showed a significant increase in number of infusions from session 1 on sessions 8, 16, 17 and 18 as well as a significant increase in the number of active lever presses from session 1 on sessions 16,17 and 18. Post-hoc tests showed no significant difference between sessions for the number of inactive lever presses.

Majority of 50 kHz calls made during pre lever extension phase

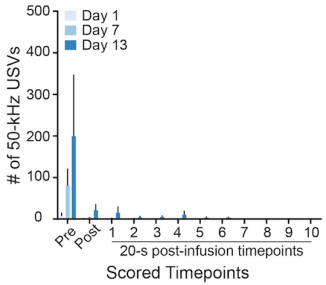

Figure 4. Average total number of 50-kHz USVs. Plotted are the average number +/- SEM of 50-kHz USVs made at each of the designated timepoints on Days 1,7, and 13. The number of 50-kHz USVs during the pre-lever timepoint was greater during Acq Day 13 [199 +/- 148.38] vs. Acq Day 7 [79.67 +/- 41.35] and Acq Day 1 [7.67 +/- 7.67]. N=3.

Pre-lever timepoint presents trends in subtype frequency changes

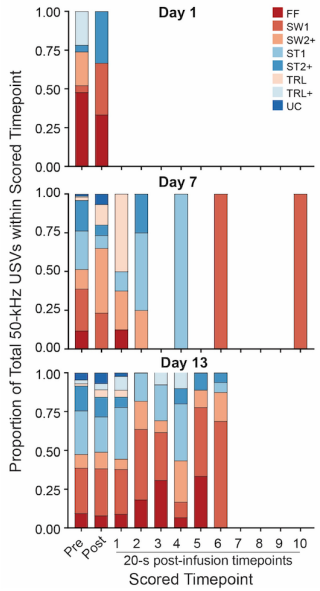

Figure 5. Percentage of total number of calls for each 50-kHz USV subtype at designated timepoints on Acq Days 1, 7 and 13. This preliminary data suggests that during the pre-lever timepoint, there is a decreasing trend in the FF subtype across sessions. Furthermore, on Acq Days 7 and 13 during that pre-lever timepoint, the majority of 50-kHz USVs appear to belong to SW1 and ST1 subtypes, but overall there is a greater variety of 50-kHz subtypes. Data shown are mean percentages. N=3.

Ongoing/Future Directions

- Future work will include scoring and classifying USV subtypes across the remaining subjects in this study to finalize the present analysis. With a greater sample size, more significant trends can be concluded.
- Additionally, if significant alterations in 50-kHz USV subtypes are observed in this model of opioid abuse, it will be useful to identify the neurobiological correlates of such behavior to better understand the underlying nature of addiction.

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