

## Video Article

## Assessment of Ultrasonic Vocalizations During Drug Self-administration in Rats

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## Abstract

Drug self-administration procedures are commonly used to study behavioral and neurochemical changes associated with human drug abuse, addiction and relapse. Various types of behavioral activity are commonly utilized as measures of drug motivation in animals. However, a crucial component of drug abuse relapse in abstinent cocaine users is "drug craving", which is difficult to model in animals, as it often occurs in the absence of overt behaviors. Yet, it is possible that a class of ultrasonic vocalizations (USVs) in rats may be a useful marker for affective responses to drug administration, drug anticipation and even drug craving. Rats vocalize in ultrasonic frequencies that serve as a communicatory function and express subjective emotional states. Several studies have shown that different call frequency ranges are associated with negative and positive emotional states. For instance, high frequency calls ("50-kHz") are associated with positive affect, whereas low frequency calls ("22-kHz") represent a negative emotional state. This article describes a procedure to assess rat USVs associated with daily cocaine self-administration. For this procedure, we utilized standard single-lever operant chambers housed within sound-attenuating boxes for cocaine self-administration sessions and utilized ultrasonic microphones, multi-channel recording hardware and specialized software programs to detect and analyze USVs. USV measurements reflect emotionality of rats before, during and after drug availability and can be correlated with commonly assessed drug self-administration behavioral data such as lever responses, inter-response intervals and locomotor activity. Since USVs can be assessed during intervals prior to drug availability (e.g., anticipatory USVs) and during drug extinction trials, changes in affect associated with drug anticipation and drug abstinence can also be determined. In addition, determining USV changes over the course of short- and long-term drug exposure can provide a more detailed interpretation of drug exposure effects on affective functioning.

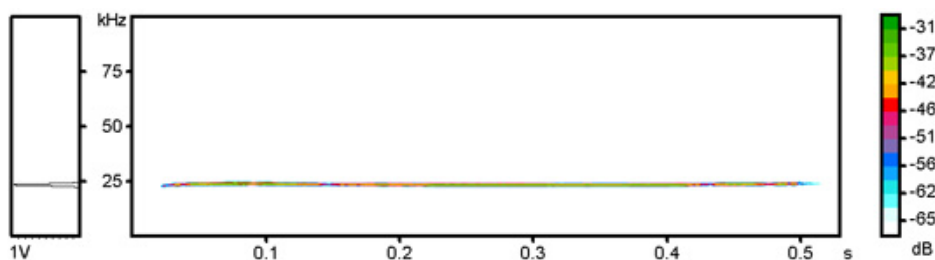
## Protocol

\* = important points

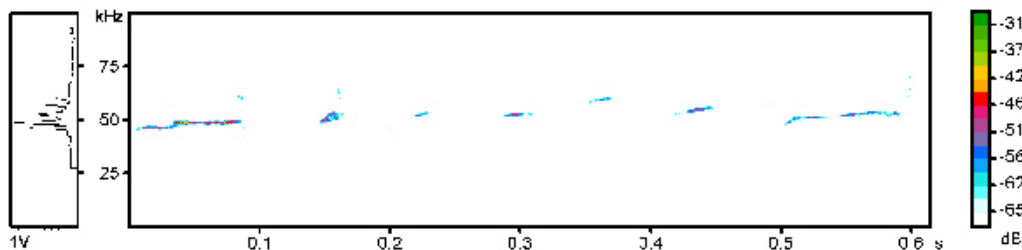
1. Well-handled male Sprague-Dawley rats are trained to lever press with food reward (45 mg sucrose pellets; Bio-Serv, Frenchtown, NJ).
2. \*Intravenous catheters are constructed out of stainless steel cannula (Plastics One, VA) and Silastic tubing. Rats undergo a jugular catheterization surgical procedure to allow drug self-administration. After surgery, catheter patency is maintained by daily flushing with 0.1 ml of a 0.9% saline, heparin and Timentin solution.
3. Cocaine (NIDA Drug Inventory and Supply and Control Program; RTI International, Research Triangle Park, NC) used in this experiment was dissolved in isotonic saline solution (0.9%) in the appropriate dose concentrations according to animal weights (0.75 mg/kg/injection) so that each injection was 0.1 ml. Control animals received saline solution in the same volume/injection.
4. \*The apparatus used for drug self-administration consists of a single-lever operant chamber (28 x 22 x 21 cm) equipped with house and stimulus lights and 3 sets of photobeams. The operant chamber is housed within a larger sound-attenuating chamber (Med Associated, St. Albans, VT) to limit outside noise and light. During the administration sessions, the animals were intravenously connected via tubing to a syringe mounted on a motorized pump (Razel Scientific Instruments, Model A, St. Albans, VT) containing either a cocaine solution (0.75 mg/kg/injection) or sterile saline. After each lever press, a stimulus light above the lever illuminates and cocaine or saline is administered over a 6-sec infusion time. Locomotor activity is assessed as the number of photobeam interruptions. The drug self-administration program is controlled, and behavioral responses (e.g., lever responses and locomotor activity) are recorded using a Med Pentium computer equipped with Med PC software (Med Associates).
5. \*To detect and record USVs, ultrasonic microphones, data acquisition hardware, recording software (Avisoft Bioacoustics, Berlin, Germany) and PC computer systems are needed. For the current experiment, ultrasonic microphones (frequency response range =10-100-kHz) are securely mounted in the center of the operant chambers. Microphones are connected to data acquisition hardware (Avisoft-Ultrasoundgate) located outside of the operant chamber and recordings are collected on a PC computer system (Dell Optiplex GX270, Windows XP Professional, Intel(R) Pentium(R) 4 CPU, 2.60 GHz, 1 GB RAM) using RECORDER multi-channel recording software (Avisoft Bioacoustics).
6. \*Each drug self-administration/USV recording session proceeds as follows:
  - a. The animals are gently taken out of their home cage and their indwelling intravenous catheter is flushed with 0.05 ml of a heparinized saline solution to detect possible catheter malfunctions.
  - b. \*Rats are connected to a swivel mounted on the operant chamber that allows either drug or vehicle administration during the self-administration sessions. Animals are gently placed into the operant chamber and the doors are quietly closed. The drug self-administration program (Med PC) and the USV recording software (RECORDER) are on two separate computer systems. Therefore, in order to be able to link behaviors within the operant chamber with USV detection, both programs need to be started simultaneously (e.g., may need two people depending on location of computers).
  - c. \*The individual USVs are recorded and time-stamped continuously throughout the session. The MedPC program controlling and recording events within the operant chamber can be written to any specification. The MedPC program automatically closes at the end of the session. The USV recording needs to be stopped manually on the USV recording-dedicated computer system.

- d. At the end of the session, the animals are gently taken out of the operant chamber, disconnected from the swivel and flushed with 0.05 ml heparinized saline to clear the catheter from drug residue.
7. \*For logistical purposes, we used a separate computer system for USV analyses (Dell Optiplex 745, Windows XP Professional, Intel(R) Core(TM), 2 CPU, 2.40 GHz, 2 GB RAM), but the analyzing software can also be installed on the same computer system as the recording software. USV analyses must be performed carefully to ensure accuracy:
    - a. \*SASLab Pro analyzing software (Avisoft Bioacoustics, Berlin, Germany) allows the recorded USVs to be visible and audible for the human experimenters displayed in a spectrogram.
    - b. \*Using the available configuration settings, the resulting spectrogram can be modified for individual preferences. In addition, background noises in low frequencies (e.g., generated by the fan of the operant chambers) can be cut out to allow the software to automatically detect sounds above that frequency in the spectrogram. These functions assist in data analyses as by making the sound files shorter and faster to assess.
    - c. Background noises in the same frequencies as USVs are sometimes detected in the spectrogram. Since the sound files are recorded as .wav files, the researcher needs to listen to the sound files during assessment to confirm and differentiate USVs from background noise.
    - d. \*The analyzing software can create Excel files with parameters such as call frequencies, bandwidth, duration, and exact time of the emitted USV. The latter allows the correlation of USVs to the exact time before or after drug is self-administered. This allows USVs to be used as real-time measurements of the rat's emotional response at specific instances during the experimental procedure.

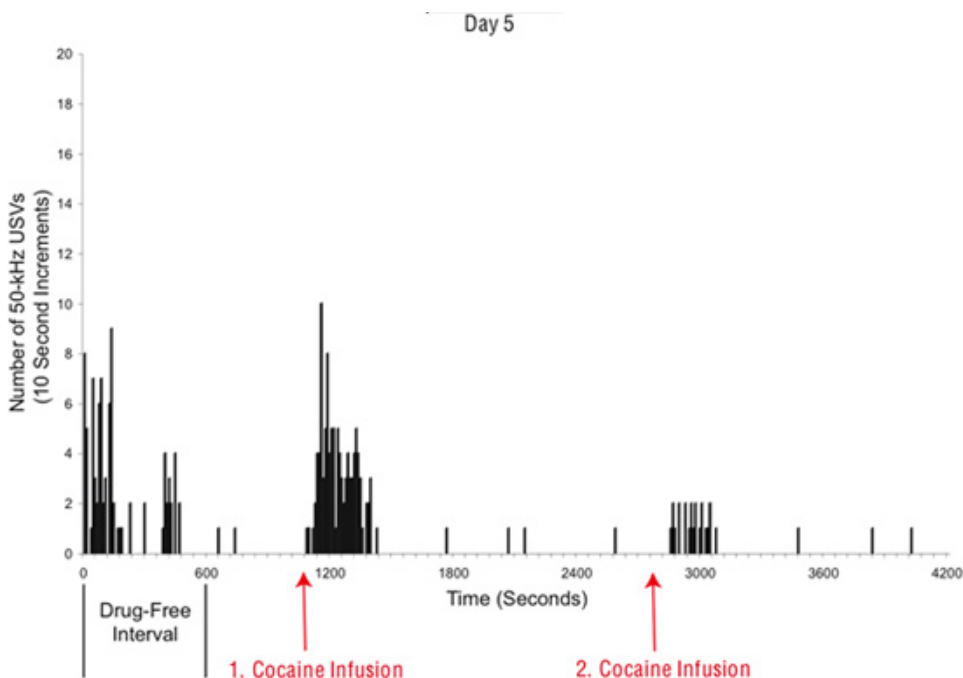
## Representative Results:



**Figure 1: Spectrogram display of a 22-kHz range USV.** Long calls in the 22-kHz range are elicited in conjunction with aversive stimuli and are thought to reflect a negative emotional state.



**Figure 2: Spectrogram display of 50-kHz USVs.** Short "flat" and "frequency modulated" 50-kHz USVs are elicited in anticipation and during cocaine self-administration. They are associated with positive affect.



**Figure 3: Cocaine-Induced 50-kHz range USVs.** Graph depicts USVs (10-sec bins) elicited before and after self-administered cocaine injections (0.75 mg/kg/inj) in a representative rat on Day 5 of cocaine self-administration sessions. Immediately after placement within the operant chamber and prior to cocaine availability, cocaine-anticipatory USVs were elicited and during self-administration, cocaine-induced USVs were emitted.

## Discussion

Rats vocalize in ultrasonic frequencies that serve a social and communicatory function and express subjective emotional states<sup>1,2</sup>. 22-kHz USVs are triggered by events that cause anxiety and distress, such as footshock cues, the presence of predators, aversive drugs and social defeat<sup>2,3</sup>. Therefore, these typically long USVs (>0.3s) are thought to represent a state of negative affect of the animal. Conversely, shorter 50-kHz USVs (>0.3s) are emitted during and in anticipation of positive encounters, such as feeding, mating and social play, and have thus been linked to a positive emotional state of the animal<sup>2,4</sup>. These calls have also been associated with reinforcing events related to the activation of the dopaminergic system<sup>5</sup>. For example, increased 50-kHz USVs have been elicited by systemic injections of cocaine<sup>6</sup> or d-amphetamine<sup>7</sup> and environments previously paired with drugs of abuse<sup>8-10</sup>.

It has been proposed that increases in locomotor activity in rodents relate to enhanced motivation and appetitive behavior<sup>11-14</sup>. However, this relationship is indirect at best. By studying the emergence and persistence of USVs of particular frequencies, it is possible to gain insight to emotional effects of various aspects of drug experience.

In this experimental setup, we collected USV recordings immediately before and during drug self-administration sessions. This allowed us to examine the development of drug expectation during the non-drug interval just prior to the start of the session and to assess the direct effects of cocaine on USVs. The drug self-administration method allows rats to directly control their own drug intake levels and develop accurate expectations of the timing and frequency of drug administration. As a result, USVs detected during these sessions reflect accurate expectations of drug delivery. In addition, USVs can be assessed during intervals prior to drug availability (e.g., anticipatory USVs), in rats receiving experimenter-administered drugs, and during drug extinction trials. Therefore, changes in affect associated with drug anticipation, non-contingent drug administration and drug abstinence can also be determined. Analyses of USV changes over the course of short- and long-term drug exposure could provide a more detailed interpretation of drug exposure effects on affective functioning.

In addition to drug self-administration experiments, USV recordings in operant chambers can also be used for a variety of other behavioral manipulations associated with reward or aversion, such as alcohol drinking, food reinforcement, and fear conditioning and extinction procedures.

## Important Methodological Details

Since the MedPC software can be used to precisely manipulate experimental conditions, such as lever availability and lighting conditions, its use in combination with USV recording and analyses allows emotional effects of drug-associated cue presentations (i.e., light presentations or other sensory stimuli) and the direct effects of drug intake to be determined. Therefore, to ensure overlapping timelines of self-administration procedures and USV recordings, it is crucial that both software programs (e.g., MedPC and RECORDER) are activated simultaneously at the start of each session.

Due to the enormous amount of digital space required to store USV data (e.g., a 10-minute sound file requires approx 250 MB), caution must be taken when multiple animals are run on a single computer system at the same time. To avoid recording errors, the RAM size of the recording computer system needs to be substantial (1-2 GB). Other ways to decrease recording errors include lowering the USV resolution during recording and/or reduce the number of animals run at one time. In addition, it is important that the researcher is vigilant to all aspects of data collection throughout the experimental procedure. For instance, the accumulation of a large amount of recording data on the hard drive may cause the USV recording program to pause. In this case, the recording program would need to be restarted immediately to avoid loss of USV data and de-synchronization of USVs with other behavioral measures.

Environmental conditions may influence USV calling. For example, the touch by an unfamiliar human can cause the animal to emit negative 22-kHz calls, which could be mistaken as an aversion to the experimental conditions. Therefore, intensive handling prior to experimental manipulations is an important aspect of behavioral experiments. In addition, since novel environments can also elicit non-specific USV calls, animals should be habituated to the test environment if possible.

## Disclosures

No conflicts of interest declared.

## Acknowledgements

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