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Automating ultrasonic vocalization analyses: The WAAVES program

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HIGHLIGHTS

- Automated program to analyze ultrasonic vocalization (USV) sound files was developed.
- Highly significant correlation between program output and human assessments.
- Program completes analyses in approximately 0.3% of time compared to manual methods.
- USVs shown as useful index for detecting cocaine-associated individual differences.
- Statistical analyses reveal cocaine-associated USVs predictive of cocaine intake.

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ABSTRACT

Background: Human emotion is a crucial component of drug abuse and addiction. Ultrasonic vocalizations (USVs) elicited by rodents are a highly translational animal model of emotion in drug abuse studies. A major roadblock to comprehensive use of USV data is the overwhelming burden to attain accurate USV assessment in a timely manner. One of the most accurate methods of analyzing USVs, human auditory detection with simultaneous spectrogram inspection, requires USV sound files to be played back 4% normal speed.
New method: WAAVES (WAV-file Automated Analysis of Vocalizations Environment Specific) is an automated USV assessment program utilizing MATLAB's Signal and Image Processing Toolboxes in conjunction with a series of customized filters to separate USV calls from background noise, and accurately tabulate and categorize USVs as flat or frequency-modulated (FM) calls. In the current report, WAAVES functionality is demonstrated by USV analyses of cocaine self-administration data collected over 10 daily sessions.
Results: WAAVES counts are significantly correlated with human auditory counts ($r(48) = 0.9925$; $p < 0.001$). Statistical analyses used WAAVES output to examine individual differences in USV responses to cocaine, cocaine-associated cues and relationships between USVs, cocaine intake and locomotor activity.
Comparison with existing method: WAAVES output is highly accurate and provides tabulated data in approximately 0.3% of the time required when using human auditory detection methods.
Conclusions: The development of a customized USV analysis program, such as WAAVES streamlines USV assessment and enhances the ability to utilize USVs as a tool to advance drug abuse research and ultimately develop effective treatments.

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1. Introduction

Emotion is a crucial component of drug use, addiction and withdrawal. Exhilarating effects of cocaine invites initial drug use, cues associated with cocaine trigger emotional yearning (e.g., "cocaine craving") for the drug, even after long periods of drug abstinence (Childress et al., 1999), and pervasive negative affect aggravates dependent individuals during drug withdrawal (Koob and Le Moal, 1997; Kreek and Koob, 1998). Preclinical research to understand drug abuse disorders and devise optimal treatment strategies could be greatly facilitated by monitoring progressive changes in emotionality related to drug use and drug-associated cues. It is well established that rodents emit ultrasonic vocalizations (USVs) to communicate emotional status (Brudzynski, 2009; Burgdorf et al., 2008; Knutson et al., 1999). Recently, the utility of USVs as indices of motivational states associated with natural (Brudzynski, 2009; Burgdorf et al., 2008) and drug reinforcement (Barker et al., 2010; Ma et al., 2010; Maier et al., 2010, 2012) has expanded beyond simple affirmation of positive and negative responses to stimuli. For instance, studies from our laboratory and others have demonstrated that USVs can serve as indices of cocaine-associated learning in a drug-free environment (Ma et al., 2010; Maier et al., 2010; Meyer et al., 2012) and reveal emotional reactions that are enhanced in response to cocaine and cocaine-associated environments after brief abstinence, and decreased after chronic cocaine self-administration experience (Maier et al., 2010, 2012). Though the ability to target crucial stages during the development of drug abuse disorders is attainable by tracking cocaine-associated USVs, continuous assessment of USVs through long-term drug abuse experiments is not without drawbacks.

USV assessment has become increasingly practical with the development of sensitive, high resolution microphones that can detect and discriminate variations in sound within ultrasonic frequencies (Specht, 2004). Precise detection and quantification of characteristics unique to USVs, such as bandwidth, frequency, amplitude, duration and inter-call interval, allows for thorough analyses of individual calls and categorization of discrete call types (Wright et al., 2010). Since USV occurrence can be observed in real-time, the functional significance of unique USV call types may be revealed by further studies linking certain USVs with ongoing and impending behaviors.

USVs can provide added insight to the study of drug abuse disorders. By monitoring USVs before, during and after drug intake, cocaine associative learning and progressive changes in affect accompanying transition to excessive drug use can be examined (Ahrens et al., 2009; Ma et al., 2010; Maier et al., 2010, 2012). In addition, individual differences in affective responses to drugs and drug-paired environments can be discerned by comparing USVs across subjects (Ahrens et al., 2009). Yet, a major roadblock limiting USV data utilization is the overwhelming burden to attain accurate USV assessment in a timely manner. For instance, a highly reliable method of USV quantification is obtained through human visual inspection of spectrogram files in conjunction with auditory confirmation. This method assures that visualized sound objects can be further distinguished as USVs or noise. For humans to be able to detect sounds produced within the range of ultrasonic frequencies of interest (e.g., usually 30–100 kHz), playback of USV sound files must be slowed to 4% of its actual speed. In other words, since one second of data would take 25 s to play back at a distinguishable speed, a 10-min file would require at least 4 h to tabulate. Considering that drug self-administration sessions are typically at least 1-h in duration, unanalyzed data sets tend to accumulate rapidly. The inevitable lag between data collection and analyses may be part of the rationale to report small subsets of USV data rather than complete drug administration sessions (e.g., first 30 min of 2 h; 3 out of 20 min) (Mahler et al., 2013; Wright et al., 2012).

There are available software programs for automated USV assessment (e.g. Avisoft's SASLAB Pro; Avisoft Bioacoustics, Berlin, Germany) that can tabulate sounds occurring within certain ultrasonic frequency ranges. However, programs designed for naturalistic settings are not specifically suited for experimental environments common to pre-clinical drug abuse studies, such as operant chambers equipped with levers, drug swivels and counter-balances. As a result, background sounds, such as those produced by animal activity, contact between any objects within the test chamber, or any noise-producing event within the ultrasonic microphone detection range may be automatically tabulated as USV calls. Thus, in creating a program to accurately assess USVs, it is imperative to identify distinguishing characteristics to separate USV calls from noise. In the process of developing an automated program, we found that background noise varies greatly between test environments and experimental conditions. Therefore, the parameters that we report here are unique to our operant chamber environment (see Section 2 below).

The automated program we developed, "WAAVES" (WAV-file Automated Analysis of Vocalizations Environment Specific) utilizes MATLAB's (The Mathworks, Inc., Natick, MA) *Signal* and *Image Processing Toolboxes* to import USV files and identify signals meeting base USV call criterion. The potential USV calls are then filtered through a series of requirements that separates USV calls from noise.

To demonstrate the functionality of WAAVES, USVs in the 50–55 kHz (flat and frequency-modulated, or FM types) and 22–28 kHz ranges were assessed in a group of rats self-administering cocaine in a self-administration and in vivo microdialysis experiment. Using the WAAVES findings, animals were categorized into High and Low USV Caller groups. Statistical analyses were then used to examine relationships between individual differences in 50–55 kHz USV responses to cocaine and cocaine-associated cues, with self-administered cocaine intake and locomotor activity data. These data have not been previously published.

2. Materials and methods

2.1. Animals and surgery

Male Sprague-Dawley rats were received at 5 weeks of age and handled for at least 2 weeks prior to the start of the experiment. Animals were trained to press a lever with sucrose pellets as reinforcement. All animals underwent jugular catheterization and stereotaxic surgery as previously described (Depoortere et al., 1993; Feduccia et al., 2010; Maier et al., 2008).

2.1.1. Apparatus, groups and experimental procedure

The apparatus consisted of a single retractable-lever operant chamber (28 cm × 22 cm × 21 cm) located within a sound-attenuating box (Med Associates, St. Albans, VT). The lever was located on the right wall in the back corner with a stimulus light above it. An ultrasonic microphone (UltraSoundGate CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) was located in the center panel of the right wall, 1 cm from the chamber ceiling and a maximum distance of 36 cm from any point in the chamber.

Within these chambers, all animals ($n = 15$) participated in 10-day (5 days/week) cocaine self-administration sessions. Daily sessions began with a 10-min pre-drug ("cocaine anticipatory") interval within the darkened operant chamber. During this time, the lever was retracted and cocaine was not available. Immediately following the pre-drug interval, chamber lights were illuminated and a one-hour cocaine self-administration session commenced.