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## Emotional Significance and Measurement of Ultrasonic Vocalizations in Laboratory Rats

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### Abstract

Rats are extensively utilized in experimental research across biology, medicine, psychology, and related disciplines, many of which necessitate the measurement of experimental subjects' emotional states. Rat ultrasonic vocalizations serve functions of emotional expression and communication: FM-50kHz vocalizations convey pleasurable positive emotions; flat-50kHz vocalizations facilitate social communication; and vocalizations within the 22kHz range express negative emotions such as anxiety and aversion. Through the collection and analysis of rat ultrasonic vocalizations, the emotional states of rats during experimental manipulations can be quantified. This article discusses the emotional significance of rat ultrasonic vocalizations and offers recommendations regarding their measurement and analysis.

### Full Text

## The Emotional Meaning and Measurement of Ultrasonic Vocalizations in Laboratory Rats

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### Abstract

Rats are widely used in experimental research across biology, medicine, and psychology, with many studies requiring the measurement of emotional states. Ultrasonic vocalizations (USVs) in rats serve as both emotional expression and communication: FM-50 kHz USVs convey positive emotions such as pleasure, flat-50 kHz USVs facilitate social communication, and 22 kHz USVs express negative emotions including anxiety and aversion. By collecting and analyzing these vocalizations, researchers can quantify the emotional states of rats during experimental procedures. This paper discusses the emotional significance of rat USVs and provides recommendations for their measurement and analysis.

**Keywords:** rats; emotion measurement; ultrasonic vocalizations

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## 1 Classification and Emotional Significance of Rat Ultrasonic Vocalizations

The rat (*Rattus norvegicus*) is a commonly used laboratory animal across biology, medicine, and psychology. In rat experiments, ultrasonic vocalization recordings provide important behavioral evidence for assessing emotional states. USVs emitted by adolescent and adult rats can be divided into two primary categories: 22 kHz range calls and 50 kHz range calls [?]. The 22 kHz USVs are characterized by frequencies between 20–35 kHz, long durations exceeding 300 ms, and minimal frequency modulation. The 50 kHz USVs can be further subdivided into flat and frequency-modulated (FM) subtypes based on acoustic features: flat-50 kHz calls last 10–100 ms with nearly constant frequency between 35–50 kHz, whereas FM-50 kHz calls last 20–150 ms with frequencies varying between 40–80 kHz and exhibit substantial frequency fluctuations.

Experimental evidence demonstrates that different USV frequencies reflect specific behavioral and emotional states in rats. The 22 kHz USVs are associated with negative emotional states such as fear and aversion. Rats emit these calls when confronted with predators or aggressive conspecifics [?, ?], and playback of 22 kHz USVs during anxiety-related behavioral tests (e.g., open field, elevated plus maze) increases defensive behaviors and elevates c-Fos expression in brain regions involved in anxiety and fear regulation, including the amygdala and bed nucleus of the stria terminalis [?, ?]. In contrast, 50 kHz USVs are produced during rewarding experiences and social interactions, such as following amphetamine administration or during playful fighting with peers [?, ?]. Emission of these calls is neurophysiologically linked to increased activity of mesolimbic dopaminergic neurons and elevated dopamine levels in the ventral striatum nucleus accumbens [?, ?]. Regarding the behavioral significance of 50 kHz subtypes, researchers propose that FM-50 kHz USVs represent a general positive emotional state, while flat-50 kHz USVs specifically signal social contact [?]. Playback of 50 kHz USVs promotes exploratory behavior in open field and elevated plus maze tests, induces dopamine release, and increases c-Fos-positive neurons in the nucleus accumbens [?, ?]. These playback effects further validate the emotional functions of USVs. Consequently, 22 kHz USVs can serve as an index of negative emotion, while FM-50 kHz USVs can index positive emotion during experiments, enabling quantitative assessment of emotional states through acoustic signal coding.

## 2 Measurement and Analysis of Ultrasonic Vocalizations

### 2.1 Recording Parameters and Equipment

Optimal USV recording requires microphones with a sampling rate of 384 kHz. According to the sampling theorem for bandpass signals, the minimum sampling rate should be 2–4 times the signal bandwidth [?]. In practice, equipment limited to 192 kHz only achieves a frequency ceiling of approximately 60 kHz in spectrograms. While sufficient for capturing 22 kHz USVs, this falls short of covering the full frequency range of 50 kHz calls. Therefore, 384 kHz sampling rate microphones and recording software are recommended for comprehensive USV measurement. The open-source software Audacity supports recording at up to 384 kHz with 24-bit PCM precision. Microphone placement 15–60 cm from the rat's mouth is optimal; distances under 5 cm capture excessive respiration and footstep noise that interferes with analysis, while distances exceeding 150 cm yield signals too weak for reliable detection. Environmental background noise must also be minimized, as electrical equipment can generate 30 kHz electromagnetic interference that may obscure 22 kHz USV identification.

[Figure 1: see original paper] illustrates representative 50 kHz USVs recorded in our laboratory: Panel shows an FM-50 kHz call with pronounced frequency modulation and opposing peaks, while panel displays a flat-50 kHz call appearing as a short, flat line in the spectrogram. [Figure 2: see original paper] shows a representative 22 kHz USV (panel ) with nearly 500 ms duration and minimal waveform variation. Note the prominent line at 30 kHz in both figures, representing electromagnetic noise from surrounding electrical currents.

### 2.2 Analysis Methods

Recorded audio can be analyzed using MATLAB to generate spectrograms via Fast Fourier Transform (FFT) (as shown in Figures 1 and 2), followed by manual screening by multiple trained researchers with calculation of inter-rater reliability (correlation between scorers) [?]. Automated analysis software such as Avisoft, Ultravox, and the MATLAB-based DeepSqueak [?] can also identify and quantify USVs. When waveform variation is subtle, distinguishing flat from FM calls can be challenging. In such cases, we recommend first examining the waveform: calls with opposing peaks should be classified as FM. Second, assess frequency variation ( $\Delta$ frequency): changes exceeding 5 kHz indicate FM calls, while smaller variations suggest flat calls [?]. Incomplete noise elimination may cause software misidentification, necessitating manual screening of automated results. Some noise signals closely resemble rat USVs in waveform and frequency; when spectrographic distinction is impossible, professional audio software like Audacity can adjust playback speed. Pitch-shifted playback allows auditory discrimination—noise typically sounds like metal friction, whereas rat calls resemble bird chirps. Slowing 22 kHz USVs to 50–20% of original speed and 50 kHz USVs to 10–5% of original speed reduces their pitch into the human audible range and extends brief 10–20 ms calls for easier recognition.

### 2.3 Applications and Quantification

Rat USVs have been widely applied in psychiatric and pharmacological research, with 22 kHz USVs assessing anxiety or pain intensity [?] and 50 kHz USVs evaluating positive emotional states [?]. Researchers can either count all USVs throughout a recording session or sample specific time windows to calculate calls per unit time. Recent studies predominantly quantify USVs by counting call number and cumulative duration, using group differences in these metrics to assess drug effects. In behavioral research, USVs enable precise, emotion-coded differentiation of aggressive behaviors—for example, distinguishing between offensive aggression and playful aggression. Compared to alternative emotion measures such as corticosterone levels or skin conductance, USV recording is more convenient, faster to analyze, and completely non-invasive.

Real-time FFT during recording also allows experimental monitoring via spectrogram inspection. For instance, if a rat emits numerous 22 kHz USVs before a T-maze learning test, this may indicate anxiety from handling. Briefly comforting the rat or waiting until 22 kHz USVs cease can help exclude emotional confounds from learning and memory assessments. We encourage researchers to incorporate USV collection and analysis in future studies requiring rat emotion measurement.

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## Author Contributions

Yin Bin and Yu Delin: Conceptualized the research proposition and designed the study protocol.

Yu Delin and Yin Bin: Conducted the research, collected, cleaned, and analyzed the data.

Yu Delin: Drafted the manuscript.

Yin Bin: Revised the final version of the paper.

*Note: Figure translations are in progress. See original paper for figures.*

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