The Rat as a Potential Animal Model for Dysphonia

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Abstract

Outcome objectives
Dysphonia, or hoarseness, is the primary complaint of patients with unilateral vocal-fold paralysis due to damage to the recurrent laryngeal nerve (RLN). Animals models for RLN injury exist, but are generally limited to histological, endoscopic, and electrophysiological approaches. As a step towards using the rat as a model system for dysphonia, we have characterized ultrasonic vocalizations in healthy adult animals.

Methods
Ultrasonic vocalizations were evoked individually from adult male Sprague-Dawley rats by manual stroking on two different days one month apart. The vocalizations were visualized and recorded by using an ultrasound microphone attached to a computer. Parameters such as duration, amplitude, pitch, and entropy were measured for each vocalization as well as the preceding silent period with Sound Analysis Pro 2011 software.

Results
For a total of 980 vocalizations from five rats, the following parameter values (mean ± standard deviation) were obtained: duration, 14 ± 10 ms; amplitude, 83 ± 6 dB; pitch, 57 ± 7 kHz; and entropy, -3.9 ± 1.0. No differences were noticed among animals or days. However, the vocalizations were distinct from the background noise during the preceding silent period: amplitude, 69 ± 3 dB; pitch, 42 ± 6 kHz; and entropy, -1.2 ± 0.9. Virtually none of the vocalizations were located in the 20-kHz band associated with distress.

Conclusion
Ultrasound vocalizations can be recorded from adult rats easily and reliably. The features of the vocalizations are consistent among individuals and over time. Experiments with RLN-lesioned animals are now underway.

Introduction
Dysphonia, or hoarseness, is the primary complaint of patients with unilateral vocal-fold paralysis due to damage to the recurrent laryngeal nerve (RLN), which innervates most intrinsic muscles of the larynx (4). The loss of muscle tone leads to a bowing of the vocal fold and incomplete glottic closure, resulting in three typical complications in human patients: dysphonia, difficulty breathing (dyspnea), and a risk of aspiration. Restoration of muscle tone requires the RLN to regenerate from the site of injury to the laryngeal muscles, a process that is slow and inefficient, and functionally imprecise. Currently, no treatments to promote recovery are available besides “watchful waiting”. Severe damage, such as RLN transection, necessitates delicate surgical intervention via anastomosis of the severed segments or transfer of a nearby nerve to the distal RLN segment.

Animal models to test novel treatments for RLN injury exist, but are generally limited to histological, endoscopic, and electrophysiological approaches. Rats, like other rodents, produce vocalizations in the ultrasound range, below 25 kHz in response to aversive stimuli and above 50 kHz in response to attractive stimuli (2). However, dysphonia has not been studied in rats; for other rodents, there is only a single, inconclusive study about dysphonia in guinea pigs (1).

As a step towards using the rat as a model system for dysphonia, we have measured and characterized ultrasonic vocalizations in healthy adult animals.

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Materials and Methods

Animals
Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were maintained in an AAALAC-accredited facility with free access to water and food. The age at the time of the recordings was between two and four months. The study was approved by the Animal Care and Use Committee of SUNY Downstate Medical Center.

Recording Vocalizations
Ultrasonic vocalizations were evoked from individual rats by manual stroking (3) on different occasions between one and three weeks apart. Vocalizations up to 125 kHz were recorded with an ultrasonic microphone and visualized and recorded on a laptop computer.

Analyzing Vocalizations
Eleven sound features were measured for each vocalization as well as the preceding silent period with Sound Analysis Pro 2011 software (5; http://soundanalysispro.com):

- Duration: the length of the vocalization (ms)
- Amplitude: the “loudness” of the vocalization (dB)
- Frequency Modulation: variability with respect to frequency (*)
- Amplitude Modulation: variability with respect to amplitude (ms^-1)
- Pitch: the fundamental frequency of the vocalization (Hz)
- Mean Frequency: the average frequency of the vocalization (Hz)
- Peak Frequency: the “loudest” frequency of the vocalization (Hz)
- Goodness of Pitch: the harmonic quality of the vocalization (dB)
- Continuity over Time: average contour duration over time (ms)
- Continuity over Frequency: avg. contour duration over frequency (Hz)
- Wiener Entropy: spectral width and uniformity of the vocalization

Results

Specific features distinguish vocalizations from noise. For a total of 980 vocalizations, spectral sound features were compared with those of the preceding silent period that contained only background noise. The vocalizations were clearly distinct from background noise with respect to amplitude, amplitude modulation, frequency—pitch, mean, and peak— and entropy (marked with *). Only 1% of the total range is shown in the plot for amplitude modulation.

Vocalization features are consistent among animals. The four sound features that distinguished the rat vocalizations from background noise were compared among animals and found to be consistent. Dotted line, median of all recordings.

Vocalization features are consistent over time. The four sound features that distinguished the rat vocalizations from background noise were measured at different time points and found to be essentially identical.

Conclusions
- Ultrasound vocalizations can be recorded from adult rats easily and reliably.
- Amplitude, amplitude modulation, frequency, and entropy distinguish the rats’ vocalizations best from background noise.
- The features of the vocalizations are consistent among individuals and over time.
- It remains to be shown whether unilateral vocal-fold paralysis alters the rat’s ultrasound vocalizations.

References