Physiology & Behavior 106 (2012) 224–228

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Close relationship between the frequency of 22-kHz calls and vocal tract length in male rats

Hideaki Inagaki *, Yukari Takeuchi, Yuji Mori

Laboratory of Veterinary Ethology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 25 January 2012
Accepted 26 January 2012

Keywords:
22-kHz calls
Air puff-induced
Bioacoustics
Growth change
Rat
Vocal frequency

ABSTRACT

It is known that the size of the components of the sound production apparatus in mammals may affect the acoustic structure of vocalizations. Therefore, some acoustic variables such as voice frequency may change with age in association with body size and body weight increases. However, whether this relationship also applies to ultrasonic vocalizations emitted by laboratory rats has not been investigated. Thus, in the present study, we first recorded changes in three acoustic variables (mean frequency, duration, and bandwidth) of air puff-induced 22-kHz calls in male rats during their growth period and assessed the relationship between these changes and body weight gain (Experiment 1). Then we directly recorded several body size measures including components of the sound production apparatus in 6- and 12-week-old male rats and examined the correlation between these values and the acoustic variables of 22-kHz calls (Experiment 2). In Experiment 1, the mean frequency of 22-kHz calls in male rats during the growth period showed negative correlations with body weight gain, while the duration of 22-kHz calls showed positive correlations. In Experiment 2, only a close negative correlation between the mean frequency of 22-kHz calls and vocal tract length in male rats was found. These results suggest that the age-related decrease in the mean frequency of 22-kHz calls may be ascribed to anatomical elongation of the vocal tract length in association with the growth of male rats. These acoustic differences could inform the receivers about the age of the signaler.

1. Introduction

The sound production systems of all mammals exhibit a number of fundamental anatomical and acoustic similarities; mammal vocalizations result from a source signal, generated by vibrations of the vocal folds in the larynx using expiratory airflow from the lungs; this is then filtered by vocal tract resonance [1]. The sizes of components of the sound production apparatus have important effects on acoustic output, for example, voice frequency and call duration. Voice frequency is characterized by two components, fundamental frequency and formant frequencies; the former correlates with the length of the vocal folds [2] and the latter is closely influenced by the length of the vocal tract [3]. Because large animals have longer vocal folds and longer vocal tracts than smaller ones they generally produce acoustic signals with lower fundamental frequencies, with less dispersed formant frequencies, and with sound energy clustered in the lower frequencies [4]. In addition, larger animals should emit longer calls than smaller ones, because they possess larger lungs and have a greater air volume available for calling [4]. Supporting these ideas, the voice frequency of animals generally decreases with age while the call duration increases in association with body size increase and body weight gain [5–10].

Although rodents produce vocalizations through the larynx, their larynxes have two modes of function. The first is, as described above, common to most other mammalian species. In the case of rats, sound of 2–4 kHz is produced [11] and emitted under situations related to pain [12] or direct warning against predators [13]. The second mode of larynx function is used to make ultrasounds, which are pure sounds of a constant or variable frequency with few or no overtones caused by stabilization of the larynx such that it is used as a whistle with a very small orifice created by the vocal folds, that are tightly constricted and cannot vibrate [14,15]. It is commonly reported that laboratory rats emit long (0.3–3.0 s) ultrasonic bouts of 20–30 kHz with a narrow bandwidth of 1–4 kHz, referred to as "22-kHz calls." These ultrasonic calls are observed when rats are faced with potentially harmful or life-threatening danger or expect a known unpleasant stimulus without exact information about when it will happen, for example, the presence of a predator [16], confrontation with a dominant and aggressive rat [17], and even a light but unpredictable and sudden air puff [18] or tactile stimulus [19].

In contrast to audible vocalization in mammals, little is known about the relationship between this type of ultrasonic vocalization and body size in rats, although such information could be very useful in rodent models for disorders to disentangle in some models the effect of body size and the effect of abnormal vocal production due to a

---

* Corresponding author at: Laboratory of Veterinary Ethology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Tel.: +81 3 5841 7577; fax: +81 3 5841 8190.
E-mail address: ahin@mail.ecc.u-tokyo.ac.jp (H. Inagaki).

0031-9384/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2012.01.018

genetic mutation. However, as it is postulated that 22-kHz calls should be produced by a whistle sound generated in the larynx using expiratory airflow from the lungs and filtration through the vocal tract [20], we hypothesized that the acoustic variables of 22-kHz calls would be closely correlated with body size including the sizes of components of the sound production apparatus, as observed in the relationship between sonic vocalization and body size in other mammals [4–10]. To test this hypothesis, we first recorded changes in three acoustic variables, i.e., mean frequency, duration, and bandwidth, of 22-kHz calls in male rats during their growth period and assessed the relationship between these changes and body weight gain (Experiment 1). Then we directly measured the length of the vocal tract, the lung weight, and other major body size variables (body weight, body length, etc.) in 6- and 12-week-old male rats and examined the correlation between these values and the acoustic variables of 22-kHz calls (Experiment 2).

2. Materials and methods

2.1. Animals

A total of 32 male Wistar rats (Clea Japan, Tokyo, Japan) were used in this study. All animals were housed in pairs in wire-topped transparent cages (410×250×180 mm) with wood shavings for bedding, were provided with water and food ad libitum, and were kept on a 12-h light–dark cycle (lights turned off at 20:00). The vivarium was maintained at a constant temperature (24±1 °C) and humidity (40–45%).

2.2. Experimental apparatus and procedures

Rats aged 4 weeks ($n = 8$) participated in Experiment 1, at the beginning of which they were moved to the experimental room and kept in their home cages for at least 60 min. Each animal was then transferred to a wire-topped transparent experimental cage (410×250×180 mm) and habituated to the cage for 5 min, after which the wire lid was removed and the animal received air puff stimuli that have been shown to reliably induce 22-kHz calls in rats [18,20]. A total of 30 air puffs at an interstimulus interval of 2 s were directed to the nape of the neck of the subject and delivered from a nozzle (10 mm outer diameter and 2 mm caliber) at a distance of approximately 50 mm from the subject. The pressure of the air puff was maintained at 0.3 MPa by a pressure valve according to earlier studies [18,20]. Immediately after the air puff stimuli, we placed the wire lid on the experimental cage and recorded 22-kHz calls for 10 min, using an ultrasound microphone (Condenser Microphone CM16/CMPA; Avisoft Bioacoustics, Berlin, Germany) set at a distance of 50 mm from the top of the wire lid. Data acquisition hardware (UltraSoundGate 116Hbm; Avisoft Bioacoustics) and recording software (Avisoft-RECORDER Version 4.0; Avisoft Bioacoustics) on a personal computer were also used. Settings included sampling rate at 100 kHz and a 16-bit format. The abovementioned sequence of air puff stimuli and the subsequent recording of 22-kHz calls were successively repeated a total of three times for each subject. All animals were weighed on the day of the experiment. The same experimental protocols were also used when subject rats were 6, 8, 10, and 12 weeks old.

Rats aged 6 and 12 weeks (animals before and after sexual maturation; $n = 12$ and $n = 12$, respectively) participated in Experiment 2, in which the same apparatus and procedures as in Experiment 1 were used to record 22-kHz calls and body weights. One day after Experiment 2, the subjects were sacrificed and the vocal tract length (VTL) was measured. The VTL was defined as the length between the rostral edge of the thyroid cartilage and the base of the upper incisor of the subject rats placed in the dorsal position (Fig. 1). Lung weight, body length (length between the tip of the nose and the base of the tail), head length (length between the tip of the nose and the cranial edge of the occipital condyle), cervical length (length of the cervical vertebrae), thoracic length (length of the thoracic vertebrae), and lumbar length (length of the lumbar vertebrae and sacrum) were also measured. Although the diameter of the vocal tract seems to be one of the critical factors affecting the acoustic variables of 22-kHz calls, we could not measure it because of technical reasons.

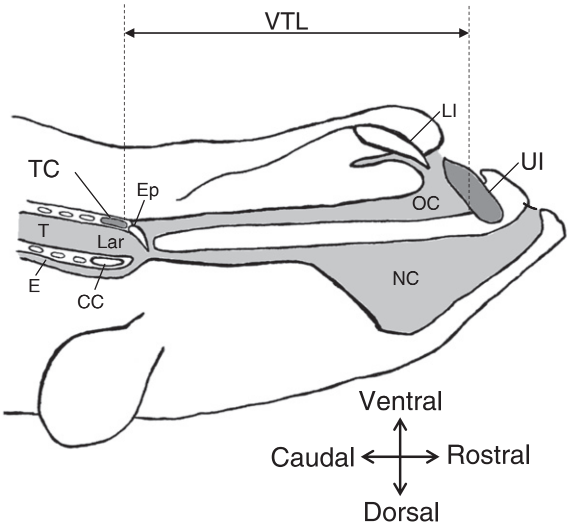

Fig. 1. Schematic drawing of the anatomical and morphometric features used in this study; CC: cricoid cartilage, E: esophagus, Ep: epiglottis, Lar: larynx, LI: lower incisor, NC: nasal cavity, OC: oral cavity, T: trachea, TC: thyroid cartilage, UI: upper incisor, VTL: vocal tract length.

This study was approved by the Animal Care and Use Committee of the Faculty of Agriculture, The University of Tokyo.

2.3. Analysis of 22-kHz calls

For spectrogram generation, recordings were transferred to Avisoft-SASLab Pro (Version 5.1; Avisoft Bioacoustics) and a fast Fourier transformation (FFT) was conducted. Spectrograms were generated with an FFT-length of 512 points and a time window overlap of 50% (100% Frame, FlatTop window). All calls (ranging from 10 to 851 calls) obtained from each subject were used to analyze the mean frequency (at which the distribution of the amplitude in the frequency spectrum reaches 50% of the total distribution), duration, and bandwidth (maximum frequency minus minimum frequency), all of which were measured automatically using Avisoft-SASLab Pro.

2.4. Data analysis

As weight should theoretically be proportional to the cube of a linear dimension, $\log_{10}$ body weight and $\log_{10}$ lung weight were used in this study, according to earlier studies [3,21,22]. In Experiment 1, we evaluated changes with age in the mean frequency, duration, and bandwidth of 22-kHz calls and calculated Pearson's correlation coefficients to examine correlations between body weight gain and changes in the three acoustic variables of 22-kHz calls. In Experiment 2, we statistically compared the mean frequency, duration, and bandwidth of 22-kHz calls between groups of rats aged 6 and 12 weeks using Student's *t*-test. The criterion for statistical significance was $p < 0.05$. In addition, the correlations between acoustic variables of 22-kHz calls and body size variables were statistically analyzed by calculations of Pearson's correlation coefficients. To avoid Type I errors from multiple statistical tests a Bonferroni correction was adopted [23], namely, we used a modified significance criterion ($p < 0.006 = 0.05/8$, where 8 is the number of statistical tests conducted on given data).