Comparison of Ultrasonic Vocalizations Emitted by Rodent Pups

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Abstract: Ultrasonic vocalizations (USVs) emitted by rodent pups, mouse, rat, Syrian hamster, vole, and Mongolian gerbil, were compared as a basic study for a screening test of anti-panic drugs. USVs of rodent pups, separated from their mother under a low temperature condition, were collected by Real-Time Spectrogram (RTS) apparatus, and transformed into spectrograms and power spectra by SIGNAL software. Waveforms of USVs emitted by the rodent pups showed several characteristic features, and species specificity of USVs was shown. We think that the species specificity might be due to differences of the anatomical structures in the respiratory tract and respiratory patterns in rodent pups.

Key words: power spectra, rodent pups, spectrograms, ultrasonic vocalizations

Introduction

It was reported that rodent pups emit USVs in situations of separation from the nest and low temperature [1], and the USVs were demonstrated to induce maternal retrieval in rats [1, 2, 10], mice [22] and hamsters [20]. Further, anogenital-licking behavior also was induced by USVs in rats [5]. In addition, it was shown that the USVs emitted by pups increased prolactin secretion in the mother, and maternal retrieval was induced by the increased prolactin secretion in mice and rats [4, 10, 21]. Thus, USVs are considered essential for communication between rodent pups and dam, and for reproduction. Blumberg and Alberts proposed that USVs in all these conditions may merely be an acoustic by-product of a respiratory maneuver, elicited by decreased ambient temperature, that functions physiologically [3]. Roberts showed that USVs occur during the initial phase of expiration or laryngeal braking [16]. Laryngeal braking, ubiquitous among mammalian neonates [12], improves gas exchange in the lungs by elevating end-expiratory lung volume and by preventing the collapse of the respiratory pathway [7, 8]. We thought the frequency difference could be accounted for by the sizes of the adult and pup laryngeal and tracheal apparatuses, because larger instruments produce lower frequencies, as is generally recognized. We thought the difference of lung volume originating from body size of mammals might be connected with the specific characteristics of USVs emitted by rodents, and...
in this study we compared the USVs elicited by pups of mice, rats, Syrian hamsters, voles, and gerbils.

**Materials and Methods**

**Animals**

Mice (*Mus musculus*; IVCS strain; six litters), rats (*Rattus norvegicus*; Wistar-Imamichi strain; seven litters), Syrian hamsters (*Mesocricetus auratus*; six litters), voles (*Microtus arvalis*; six litters) and Mongolian gerbils (*Meriones unguiculatus*, four litters) that had been raised in the authors’ laboratory were used for the experiment at the age of 3 days. Their body weights are shown in Table 1. From each litter, two pups, one male and one female, were selected randomly for experiments.

The lactation mothers were housed in polycarbonate cages (mice and vole: 140D × 250W × 130H mm; rat: 220D × 380W × 200H mm; Syrian hamsters and Mongolian gerbils: 210D × 370W × 150H mm), and provided with exclusive feed for laboratory animals (MB-1; produced by Funabashi Farm Co., Ltd., Chiba, Japan) and tap water *ad libitum*. The animal room was maintained at 24 ± 2°C temperature, and 55 ± 10% relative humidity, with 12-h artificial lighting from 07:00 to 19:00. This study was authorized by the Animal Committee of Nippon Veterinary and Animal Science University.

**Measurement procedure**

A pup was placed in a 500 mL glass beaker in isolation. Then, the glass beaker was put in an incubator to attenuate surrounding sounds and was placed on an ice bag (15 ± 2°C) for 5 min. The temperature of the beaker bottom was measured by a thermo-hygrometer (TRH-CZ; SHINEI). The changes of temperature on the bottom of the glass beaker at the start (0 min) and end (5 min) of the measurement are shown in Table 2.

Instruments to collect USVs of the rodent pups were set up as follows. A condenser microphone (Kunitachi Acoustic Lab., SF-12DC No.1730) was located 5 to 7 cm above the pup in the glass beaker. The microphone was connected to a computer installed with Real-Time Spectrogram software (RTS; produced by Engineering Design) which translated signals from the microphone amplifier (DIA Medical System) to eliminate noises and echoes from sampled USVs. The frequency response of the microphone is within ± 0.5 dB in the frequency range from 5 Hz to 100 kHz.

**Calculation of power spectrum**

USVs obtained from the rodent pups were transformed to power spectra by a computer installed with SIGNAL software (produced by Engineering Design).

**Results**

**Spectrograms and power spectra in mouse**

Typical murine USVs and their power spectra are shown in Fig. 1. The waveforms of USVs exhibited two patterns in spectrogram analysis. They were named M-I and M-II. Appearance rates of male and female pups in M-I were both 5/6 (83.3%), and both the sexes in M-II were 6/6 (100%).

**Spectrograms and power spectra in rat**

Typical USVs emitted by rats and their power spec-
Fig. 1. Typical USV spectrograms and power spectra of the mouse. [a] shows a typical spectrogram of the mouse USV. The horizontal axis represents time (s), and the vertical axis represents frequency (kHz). M-I and M-II in the spectrogram label the types of waveforms. [b] shows a typical power spectrum of a wave form in M-I, and [c] shows a typical power spectrum of a wave form in M-II. The horizontal axes represent frequency (kHz), and vertical axes represent intensity (dB).

Spectrograms and power spectra in Syrian hamster
A typical USV emitted by a Syrian hamster and its power spectra are shown in Fig. 4. Only one kind of USV was observed in the Syrian hamster spectrogram. Slight signals as a noise were observed mostly in the middle of the USV. Appearance rates of typical USV were 6/6 (100%). Moreover, audible vocalizations (ADV) were observed in all Syrian hamsters (100%). Structural differences between USV and ADV were observed in the spectrograms.

Spectrograms and power spectra in vole
Typical USVs of voles and their power spectra are shown in Fig. 5. Two kinds of vole USV were observed in the spectrogram. They were named as V-I and V-II. The appearance rate of V-I was 6/6 (100%). The appearance rates of V-II were 3/6 (50%) in both males and females. All V-II, which were in the high frequency range, were observed at the end of the measurement time.

Spectrograms and power spectra in Mongolian gerbil
Typical USVs of Mongolian gerbils and their power spectra are shown in Fig. 6 and Fig. 7. Waveforms of USVs in the Mongolian gerbil mainly exhibited three patterns in spectrogram analysis. They were named as G-I, G-II, and G-III. The appearance rates of each were 100%.

Discussion
Several characteristics and the species specificity of USVs, waveforms and power spectra were shown in the present study.

The emission of USVs begins when brown adipose tissue (BAT) of the pup begins producing heat, and
Fig. 2. Typical USV spectrograms of the rat. [a], [b], [c], and [d] show typical spectrograms of the rat USV. The horizontal axes represent time (s) of USVs, and the vertical axes represent frequency (kHz). R-I, R-II, R-III, and R-IV in spectrogram label the types of waveforms.

Fig. 3. Typical USV power spectra of the rat. [a], [b], [c], and [d] show typical power spectra of each wave form of the rat USV. The horizontal axes represent frequency (kHz) of USV, and the vertical axes represent intensity (dB) of USV.

Oxygen consumption and respiratory rate also increase at this time [3]. Oxygen consumption among rodent pups does not differ clearly, but it was reported that the respiratory frequency and pattern are different among rodent species [11, 13]. Roberts showed, by monitoring air flow from the nostrils of rodent pups [16, 18],
Fig. 4. Typical USV spectrograms and power spectra of the Syrian hamster. [a] and [c] show typical spectrograms of USVs and ADVs of the Syrian hamster. The horizontal axis represents time (s), and the vertical axis represents frequency (kHz). [b] and [d] show power spectra of an USV in [a] and an ADV in [c]. The horizontal axis represents frequency (kHz), and the vertical axis represents intensity (dB).

Fig. 5. Typical USV spectrograms and power spectra of the vole. [a] shows a typical spectrogram which was produced by all voles. [c] shows a typical spectrogram which was produced by some voles. The horizontal axis represents time (s) of USVs, and the vertical axis represents frequency (kHz) of USV. [b] represents a power spectrum of the waveform shown by an arrow in [a]. [d] represents a power spectrum of the waveform shown by the arrow in [c]. The horizontal axis represents frequency (kHz), and vertical axis represents intensity (dB).
that USVs occur during the initial phase of expiration [16]. In addition, Roberts (1975) showed that the emission of USVs requires integrity of the larynx [12]. Reznik (1990) reported differences of the anatomical structures in the respiratory tract, specifically the larynx and trachea, in rodent animals [15]. Therefore, we thought the species specifics of waveforms and power spectra of USVs emitted by rodent pups might be in-

Fig. 6. Typical USV spectrograms of the Mongolian gerbil. [a], [b], and [c] show typical spectrograms of the Mongolian gerbil USV. The horizontal axis represents time (s) of USV, and the vertical axis represents frequency (kHz) of USV. G-I, G-II, and G-III in spectrograms indicate types of waveforms.

Fig. 7. Typical USV power spectra of the Mongolian gerbil. [a], [b], and [c] show typical power spectra of each wave form of the Mongolian gerbil. The horizontal axis represents frequency (kHz) of USV, and the vertical axis represents intensity (dB) of USV.
duced by the differences of the anatomical structures in the respiratory tract and the respiratory pattern [11]. In this study, rat pups emitted various distinct waveforms compared to the other species that had only similar patterns of USVs. Expiratory duration in rats is prolonged because of laryngeal constriction, air flow is reduced, and intrathoracic pressure increases [3, 16]. USV emission occurs as air passes through the constricted plate of the larynx, similar to a bird whistle [18]. As shown in Table 1, the body weight of rat pups was heavier than those of the other rodent pups, so we thought muscular movement associated with laryngeal constriction in rats might be more active than in the other rodent pups, and the four types of USV waveform emitted by rat pups might be induced by movement associated with the larynx.

The central nerve including GABA, opiate and serotonin neuron is thought to be important in the regulation of USVs. It was reported that the USVs were decreased by treatment with benzodiazepines [23], opiates [6] and serotonin receptor agonists [14, 24]. These chemicals belong to anti-panic drugs. Accordingly USV was expected to be used as a screening test for anti-panic drugs [25]. Nevertheless, the relationship between the development of the normal anxiolytic neuronal system and the attenuating effect of diazepam, a benzodiazepine receptor ligand, on the ultrasonic vocalizations of rodent pups is not obvious. Also, the mechanism is the subject of study, but it has been reported that the calming effect induced by benzodiazepines acts upon the larynx [9]. It may be possible that studies on the more detailed species specificity of USVs promote expansive researches on the central nerve which adjusts USVs and on the species specificity of the sensitivity of anti-panic drugs.

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