Sensitivity to High Frequency Communication Sounds in the Inner Ear Enhanced by Selective Attention: Preliminary Findings in Mongolian Gerbils

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Audible range for Mongolian gerbils overlaps well with one for humans. This is one of the reasons why they have been used for auditory studies. So, most of studies on gerbils have been in human audible frequency range. They live in a group and use a variety of vocalizations for their conspecific communication. Roughly 80% of the fundamental frequencies of their communication calls are found in the frequency range above 20 kHz. However, thresholds for frequencies above 20 kHz have been reported higher than those for low frequencies (from 1 kHz to 16 kHz) by as much as 20 dB. We examined how gerbils utilize ultrasonic frequency range for their important vocal communication. Findings from alert animals with paired condition showed the cochlear microphonics (CM) were amplified by 2–4 dB with ultrasonic communication calls of low sound pressure levels (from 45 dB to 60 dB p.e. SPL), while no enhancement was observed with the same calls higher than 65 dB p.e. SPL. Findings also suggested that CM responses to tone burst higher than 25 kHz demonstrated magnified amplitude under the paired condition. Data strongly imply that attention to conspecifics would increase sensitivity to ultrasonic frequency range.

Keywords: Mongolian gerbil; communication call; hearing sensitivity; cochlear nonlinearity; hair cells.

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1. Introduction

1.1. Hearing sensitivity of Mongolian gerbils

Mongolian gerbil, *Meriones unguiculatus*, has its audible frequency range in common with humans and has served a model animal for the auditory system. Gerbils produce a various types of communication calls with different frequency and temporal characteristics¹ (Fig. 1). In addition, a previous study showed that gerbils changed rate of call emission in response to changes in visual and auditory information.² Although they have a good overlap in audible frequency range with humans, about 80% of their communication calls are ultrasonic (higher than 20 kHz). For instance, upward sinusoidal frequency modulated (uSFM) calls covered frequency range between 25 kHz and 50 kHz (Fig. 1(a)). This type was most often observed under conditions of mating. Short bent upward frequency modulated (bUFM) calls were most often observed while two or more animals were in contact with each other in a nonconflict context (Fig. 1(b)). This type has been called “Greeting call”. The fundamental frequency of bUFM calls ranged between ~ 30 kHz and ~ 35 kHz. The latter communication sound was called “Greeting call”, the most commonly observed, which had short duration (around 34 ms) and typically the first harmonic was only observed. These communication calls can be regarded as biologically important sounds for gerbils.

However, a previous study showed that thresholds at 25 kHz and higher frequencies were much higher than that at 4 kHz (the lowest threshold) by 13.8–63.1 dB SPL.³ The averaged threshold was 4.6 dB SPL for frequencies between 1.0 kHz and 16.0 kHz. Between 16.0 kHz

Fig. 1. Communication calls of gerbils. Top: Envelope of vocalizations, Bottom: corresponding spectrograms. (a) uSFM call, (b) short bUFMs call, Greeting call, (c) upward FM (UFM) call, (d) arched FM (AFM) call, (e) long downward FM (DFMl) call, (f) downward FM (DFM) call, (g) quasi-constant frequency (QCF) call and (h) noise-burst (NB) call.¹

and 40.0 kHz, threshold increases at a rate of 13 dB/oct., and above 40.0 kHz at a rate of 66 dB/oct. Somehow, gerbils are utilizing a frequency zone, where hearing is not so sensitive, for important vocal communication.

1.2. Cochlear microphonics and cochlear nonlinearity

Cochlear microphonics (CM) are summated receptor potentials produced by hair cells. The alternating pressure change across the cochlear partition (created by sound stimulation) is transformed by the hair cells into alternating current (AC) that, across the resistance of the cochlear fluids, appears as an AC voltage.⁴

Previous studies showed that the vibration of the basilar membrane nonlinearly amplified for the characteristic frequencies. The lower the sound intensity, the larger the amplification of deflection of the basilar membrane.^{5,6} However, nonlinear amplification disappears for high intensity sound.⁷

1.3. Hypotheses and purposes

In this present study, we made two hypotheses and investigated hearing sensitivity of gerbils by using behavioral and physiological methods. First, we made a hypothesis that thresholds at 20 kHz and higher might be lower than those previously reported³ by increasing testing frequencies (narrow frequency intervals). Ryan³ measured only 32, 40, 50 and 60 kHz above 20 kHz. He used a shock avoidance paradigm, while we adopted a positive reinforcement with food reward in lower thresholds. While Ryan used long tone bursts of 3.8 s, we employed shorter tone bursts of 200 ms to reduce thresholds. The first purpose of our study was to behaviorally measure threshold of hearing in gerbils by increasing the number of testing frequency above 20 kHz and changing training paradigms to compare results with those reported before by Ryan. Second, we made another hypothesis that hearing sensitivity above 20 kHz might increase when a gerbil communicates with other gerbil. We recorded CM as an index for hearing sensitivity in inner ear.

2. Materials and Methods

2.1. Subjects

We used six male Mongolian gerbils in the present study. Food and water were freely available, and nesting materials made of cellulose were provided. The animal room was under a 13-h light and 11-h dark cycle with controlled temperature (22–24°C) and relative humidity (45–55%). Three males were used for operant experiments (16–18 months old, 61–64 g). A different group of three males as used for measurements of CM (7–8 months old, 73.5–85 g). All animal husbandry and experimental procedures were performed in accordance with the National Institutes of Health Guidelines for Experiments Involving Vertebrate Animals and were approved by the Animal Experimental Committee of Doshisha University.

Fig. 2. Schematized stimulus train for operant experiments. Each train consisted of three tone bursts with inter-stimulus interval of 100 ms and duration of 800 ms. Duration of each stimulus was 200 ms including 5 ms rise/fall times.

2.2. Sound stimuli

We used tone bursts for sound stimuli in the operant experiment. Stimuli were trains of repetitive three tone bursts (Fig. 2). Each tone burst had 200 ms duration including 5 ms rise/fall times. The train had 800 ms duration with inter-stimulus interval of 100 ms. Frequencies of tone bursts were 100, 200, 400, 1000, 1414, 2000, 2828, 4000, 5657, 8000, 11,314, 16,000, 22,627, 32,000, 35,777, 40,000, 44,721, 50,000, 55,901 and 62,500 Hz. Schematic temporal pattern of stimuli fed into the loud speaker is shown in Fig. 2. Actual waveforms should be different from what is shown in Fig. 2. The sound pressure level was calibrated through a microphone system.

For recordings of CM, communication calls and tone bursts were used. Call stimuli were trains of three different communication calls in the similar frequency range (Fig. 3). Each call had about 30 ms duration and covered frequency range between ~ 25 kHz and ~ 30 kHz. Inter-stimulus interval was 100 ms. Intensities of stimuli were 55–75 dB peak equivalent (p.e.) SPL. Peak equivalent sound pressure level is the root-mean-square sound pressure level of a long tone burst having the same peak-to-peak amplitude. Tone stimuli consisted

Fig. 3. Waveforms and spectrograms of a train of call stimuli for CM recordings. Stimuli were repetitive trains. Each train consisted of three communication calls of 30 ms duration covering frequency range between ~ 25 kHz and ~ 30 kHz. Inter-stimulus interval was 100 ms. Intensities of stimuli were 55–75 dB peak equivalent (p.e.) SPL.

Fig. 4. Schematized stimuli train for CM recordings. Each train consisted of three tone bursts with inter-stimulus interval of 100 ms and duration of 290 ms. Duration of each stimulus was 30 ms including 1 ms rise/fall times.

of trains of identical three tone bursts, each tone burst had 30 ms duration including 1 ms rise/fall times (Fig. 4). One train had 290 ms duration with inter-train interval of 100 ms. Frequencies of tone bursts were 4000, 8000, 16,000, 25,000 and 31,250 Hz. Intensities of stimuli were 45–65 dB p.e. SPL.

The sound stimuli were generated by computer software (Cool Edit 2000, sampling rate: 192 kHz). The sound pressure level of the stimuli was measured by a calibrated 1/4 inch condenser microphone (Type 7016, Aco) at the position of gerbil's head.

2.3. Operant experiment

We carried out training and experiment tests at an experimental cage in a sound attenuated box (inside dimension $50 \times 50 \times 51.5$ cm, Bio-Medica) with an LED light. Operant tasks were controlled from PC by using a PCI board (PCI-2725A, Interface). Sound stimuli were presented through a digital-to-analog converter (QUAD-CAPTURE, Roland), an amplifier (INTEGRATED AMPLIFIER MODEL A-10, Pioneer), and a loud speaker (DDDS5 II, ALPINE or FT28D, FOSTEX). The experimental cage had a platform in which subjects stepped on. The loud speaker was located 20 cm above the platform.

Subjects were trained for 30 min/day between 1 PM and 5 PM with food reinforcement on Go–Nogo paradigm. Animals had to step off the platform if they detected sound stimuli. On the other hand, they were trained to stay on the platform if they did not detect stimuli.

Training started after subjects stepped on the platform. The sound stimulus was presented after subject stayed on the platform for 1.0–2.4 s. We defined Hit if subject stepped off the platform within 1.5 s from offset of the stimulus. Subjects were reinforced with a pellet of food in the case of Hit. We defined Miss if subject did not step off the platform after 1.5 s from offset of the stimulus. We also defined False alarm if subject stepped off the platform before offset of the stimuli. Subjects were penalized by 10 s timeout for False alarm. The room light was turned off during timeout. We began test sessions when Hit rate was higher than 85% and False alarm rate was lower than 5% for three consecutive training days. Thresholds were calculated, corresponding to 50% Hit rate.

Fig. 5. Chronic implantation of a silver electrode in middle ear. The silver electrode was placed on the bone adjacent to the round window of the cochlea for recording CM. IC socket was fixed on the vertex.

2.4. Electrode implantation for chronic CM recordings

Gerbils were anesthetized with a mixture of ketamine (35 mg/kg, i.m.) and xylazine (14 mg/kg, i.m.) after an injection of atropine sulfate (0.7 mg, i.m.). The skin over the vertex was removed and the top of cranium was exposed. After piercing cranium on the left olfactory bulb with dental drill, a reference electrode was placed around the left olfactory bulb. The auditory bulla was exposed and a small hole (about 2 mm diameter) was made. A Teflon-coated silver electrode was placed on the bone near the round window of the cochlea of the right ear for chronic CM recordings. Two electrodes were connected to an integrated circuit (IC) socket, which was placed on the vertex (Fig. 5). The body temperature was maintained during surgery using a thermostatic heating pad. After the surgery, we confirmed CM through the IC socket. After a recovery period of seven days, the experiment was conducted.

2.5. Conditions for CM recordings

We recorded CM in an experimental cage (15 × 10 × 25 cm) inside a sound proof room with light. The experimental cage was made of metal mesh and separated into two sections by a fence. Sound stimuli were presented by a loud speaker (FT28D, FOSTEX) through a digital-to-analog converter (QUAD-CAPTURE, Roland) and an amplifier (HP 465A Amplifier, HP). The loud speaker was located 35 cm above the floor. The CM data were stored in a PC after recording through an amplifier (MEG-1200, Nihonkohden) and digitized by an analog-to-digital converter (UA-101, Roland) with sampling rate of 192 kHz.

We recorded CM from behaving or anesthetized gerbils under a single condition or under a paired condition (Fig. 6). We defined the single condition when we recorded CM if the subject was alone in the experimental cage. In contrast, the paired condition consisted of a subject with an anesthetized companion gerbil for an imitating vocal producer. We placed the subject and the companion gerbil in different sections in the same experimental cage.

Fig. 6. Single (a) versus paired conditions (b) for CM recordings. The cage was separated into two sections by a fence. The paired case consisted of a subject with an anesthetized companion where sound was delivered from the loud speaker above the animal.

3. Results

3.1. Behavioral thresholds of hearing in gerbils

Lowest threshold was 2.5 dB SPL at 2 kHz and the highest threshold was 56 dB SPL at 62.5 kHz (Fig. 7). Results showed that thresholds for below 1 kHz decreased about 10 dB compared to the previous report.³ Threshold at 22.7 kHz was 4.5 dB SPL which was similar to the lowest value at 2 kHz. Thresholds increased at 32 kHz and higher. The same trends were confirmed for all three subjects.

3.2. CM generated by communication calls

Amplitudes of CM attenuated when intensities of stimuli got lowered. We could not find a difference in durations and amplitudes of CM waveforms between the single case and

Fig. 7. (Color online) Behaviorally obtained gerbil audiograms Red: present study with short tone bursts of 200 ms tone bursts and a positive reinforcement. Blue: Ryan's study³ with long tones of 3.8 s and shock avoidance.

Fig. 8. (Color online) Power spectrograms of CM responded to communication calls, 25–30 kHz FM sweep, from a behaving S1. (a) 55 dB, (b) 75 dB, Blue: single, Red: paired.

the paired case. The power of CM responding to communication calls stimuli was highest around 27.4 kHz. For 55 dB and 60 dB p.e. SPL in the paired case, we confirmed that power spectrograms of CM were elevated by 2–4 dB in comparison to the single case (Fig. 8). However, power spectrograms of CM in the paired case were the same as the single case if the intensity of sound stimuli was higher than 65 dB p.e. SPL.

We also recorded CM response to call stimuli from anesthetized subjects. The power of CM was highest around 27.4 kHz, the same as the behaving conditions. On the other hand, we could not find any difference in 55 dB and 60 dB p.e. SPL between the single and the paired cases when the subject was anesthetized.

3.3. CM of tone bursts

We found the increment of the CM responded to 25 kHz and 31.1 kHz tones in the paired case as compared with the single case (Fig. 9). The amplification of CM occurred if intensities of stimuli were lower. If intensities of stimuli were 45 dB p.e. SPL, CM responses to 25 kHz

(a)

(b)

Fig. 9. (Color online) Power spectrograms of CM responded to 25 kHz tone bursts from a behaving animal. (a) 45 dB, (b) 65 dB, Blue: single, Red: paired.

Fig. 10. Power spectrograms of CM responded to 8 kHz tone bursts. Sound pressure levels were 45, 55 and 65 dB p.e. SPL. No differences were shown between single and paired cases.

and 31.1 kHz were amplified by 3.6–5.4 dB. No changes in CM magnitude were observed between single and paired cases for frequencies of 4, 8 and 16 kHz (Fig. 10). The higher the frequency of tone bursts, the larger the augmentation in the CM amplitude. The lower the intensity of stimuli, the larger the amplifications in the CM magnitude.

4. Discussion

4.1. Behavioral audiograms

Two different training methods were employed for the present and the previous behavioral studies of Ryan.³ A shock-avoidance paradigm was used by Ryan³ while a positive reinforcement with food reward was used in the present study. In addition, sound stimuli used by Ryan were long tone bursts of 3.8 s with 10 ms rise-fall times, while we used short tone bursts of 200 ms with 5 ms rise-fall times as stimuli. We expected that thresholds could get lower than the previous study because of these reasons. Indeed, thresholds for frequencies below 1 kHz got lower by 5–10 dB. However, thresholds for frequencies higher than 20 kHz stayed more or less the same (Fig. 7).

4.2. CM generated by communication calls and tones

Subjects vocalized in response to the call stimuli under the paired condition. We speculated that the subject might attempt to communicate with the other gerbil. We also confirmed that the subject also vocalized in response to 25 kHz and 31 kHz short tone bursts. These findings suggest that gerbils recognize sounds, which had about 30 ms duration and ranged between 25 kHz and 35 kHz, as communication calls (probably Greeting calls).

A previous study showed that there were CAP (compound action potentials) reduction and CM increments during the attention to visual stimuli.⁸ Electrical stimulation of efferent fibers to the cochlea was reported to augment CM amplitude.⁹ Relationships between CM and CAP have to be clarified. In this study, CM increments were only observed for sounds higher than 25 kHz in frequency and lower than 65 dB p.e. SPL in sound pressure level only when a companion animal was sitting in the same cage. The findings suggest that cochlear sensitivity might increase in accordance with CM amplification. In addition, we could not find increased CM under anesthesia. The amplification of CM might occur by the attention to vocalization of a companion animal.

5. Summary

Behavioral threshold measurements for frequency with positive reinforcement could create lower threshold in the frequency range lower than 1 kHz but no improvement was found in the frequency range higher than 20 kHz compared to those with negative reinforcement. Mongolian gerbils communicate with their conspecifics using vocalization higher than 20 kHz. However, their thresholds for high frequency are NOT low compared to those for lower frequencies. The magnification in CM in the frequency range above 20 kHz was

observed only for low intensities only when the subject was alert and paying attention to a companion animal. Findings indicate that selective attention to a conspecific animal may improve sensitivity for high frequencies at the inner ear level but general attention to stimuli cannot make any difference in sensitivity.

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