

ACOUSTICAL LETTER

Vocalizations in a Japanese wild-derived laboratory mouse KOR1: Development, behavioral contexts, and sound characteristics

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1. Introduction

Mice (*Mus musculus domesticus*) produce ultrasonic vocalizations in several behavioral contexts. Pups emit an ultrasonic isolation call when they are separated from their mother and littermates [1,2] and adult males produce ultrasonic vocalizations with sequential complexity comparable to birdsong when they encounter females [3–6].

In the genus *Mus*, several subspecies have been used to create laboratory mouse strains. Previously, one of the authors (Y.M.) established laboratory mice derived from *M. m. molossinus*, Japanese wild-derived inbred mice, which are named KOR1, KOR5, KOR7, AIZ2, and MAE [7]. Although mice seldom emit vocalizations in an audible range, approximately 10% of male KOR1 mice and about 2% of female KOR1 mice produce successive, songlike audible vocalizations after 3 months of age [8]. Previous studies revealed that *M. m. domesticus* produce audible vocalizations when they feel pain or disgust [6,9]. However, these audible vocalizations by *M. m. domesticus* are not emitted in a successive train as in KOR1 [6,9]. Therefore, it is not clear whether the successive audible vocalizations by KOR1 and by *M. m. domesticus* are produced in the same behavioral contexts. In addition, if the vocalization is produced in the same contexts by both subspecies, it is possible that KOR1 will also produce ultrasonic vocalizations during adult courtship and when a pup is isolated.

In this study, we 1) sought behavioral contexts that induce successive audible vocalizations in KOR1 and 2) examined whether KOR1 emits ultrasonic vocalizations in the contexts in which they have been observed in other subspecies. Specifically, we tested the vocal responses in both audible and ultrasonic ranges using adult and infant KOR1 mice in several contexts that arouse distress, anxiety, and sexual motivation.

2. Materials and methods

2.1. Animals

KOR1 mice were derived from Japanese wild mice (*M. m. molossinus*) [10] and maintained at the Saitama Cancer Center. Food and water were given ad libitum, and all the animals were housed at a constant temperature ($22 \pm 2^{\circ}$C) and humidity ($50\% \pm 10\%$) under a 12 h light/dark cycle. All of the animal experiments were approved by the Committee for Guidelines and Regulation of Animal Experiments of the Saitama Cancer Center.

2.2. Recording conditions

For the recording test, we tested five 14-week-old male KOR1 mice that emitted audible songlike vocalizations (KOR1-peep) in their home breeding cage and five male KOR1 mice of the same age that never emitted audible songlike vocalizations (KOR1-silent) in the home cage. When pups were born, we determined which individuals produced audible vocalizations each day by making careful observations when changing the cage bedding.

Adult male mice were tested under three different conditions: novel context, female urine context, and nonodorized swab context (control condition). Each adult male mouse was tested once under each condition in one day. In all contexts, the test cage was cleaned after each trial.

(1) *Novel context*: The 10 mice (5 KOR1-peep and 5 KOR1-silent) were individually put into a novel test cage ($18 \times 17.5 \times 25$ cm) in a soundproof box ($69 \times 87 \times 77$ cm) for 3 min. The novel cage had a different color and texture from the home cage.

(2) *Female urine context*: The same 10 mice were habituated to a new test cage for 15 min per day for 5 successive days before the test trial. The next day, these animals were tested with a 3 min exposure to a cotton swab odorized with urine from a female in estrus.

(3) *Nonodorized swab context (control condition)*: After the female urine test, the same male mice were presented with a nonodorized cotton swab for 3 min in the same test cage.

For mouse pups, we used the isolation test. We used eight pups obtained from two litters.

Isolation test for pups: We recorded the vocalizations of eight KOR1 pups on postnatal days 4, 7, 10, and 14. After P7

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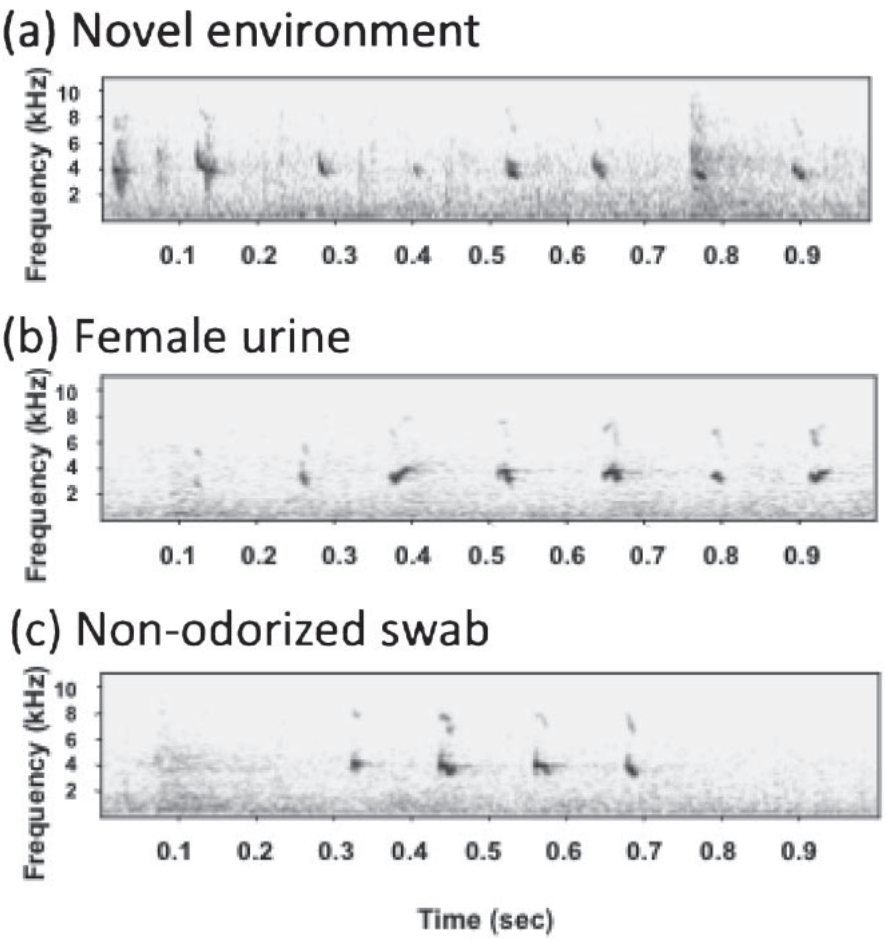

Fig. 1 Spectrograms (frequency, kHz × time, s) of audible vocalizations produced by KOR1-peep mice in the (a) novel, (b) female urine, and (c) nonodorized swab contexts.

(postnatal day 7), we used six pups because two pups had died. One pup was transported in a 500 ml plastic beaker placed in a soundproof box and recorded for 3 min. The test beaker was cleaned after each trial.

2.3. Sound recording and data analysis

In all experiments, the condenser microphones used to record ultrasonic vocalizations (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) and audible vocalizations (ECM-MS957, Sony, Tokyo, Japan) were located 10 cm above the animal. The ultrasonic microphone was connected to a preamplifier (Avisoft Ultrasound Gate 416H, Avisoft Bioacoustics), which was then connected to a personal computer (PC). The condenser microphone for recording audible vocalizations was connected directly to the microphone jack of the PC. The signals were recorded onto the hard disk via an Avisoft-Recorder USGH (Avisoft Bioacoustics) set at a 300 kHz sampling rate, and the recorded sound was stored as '.wav' files in the PC.

The recorded files were transferred to SASLab Pro (ver. 4.52, Avisoft Bioacoustics) for fast Fourier transformation (512 FFT length, 100% frame size, Hamming window, 50% time window overlap). The number of syllables, the syllable duration, and the mean peak frequency were analyzed in all contexts. The number of syllables was the number of total calls in one trial lasting 3 min. The syllable duration was the time length of each syllable. The peak frequency was the frequency at which the power was maximum in the power spectrum. In all tests, the mean values of the syllable duration and peak frequency of each syllable were calculated for each subject as the representative value in each test. In the isolated pup context, the peak frequency was analyzed on P7, when the largest number of syllables was observed. One-way analysis of variance (ANOVA) with the group (KOR1-peep or silent) as the main factor and the number of syllables, the syllable duration, or the mean peak frequency as the dependent variable followed by Tukey's honestly significant difference (HSD) test was used for statistical analysis in each test.

3. Results

3.1. Analysis of vocalizations emitted by adult KOR1 male mice

During testing, no ultrasonic vocalizations were recorded from either group of five KOR1-peep or five KOR1-silent mice under any of the three contexts. Therefore, only the audible vocalizations of five KOR1-peep mice were analyzed and are presented here.

All five KOR1-peep mice emitted audible vocalizations in each of the three contexts (Fig. 1). The number of syllables under both the novel and female urine contexts was significantly greater than that under the nonodorized swab

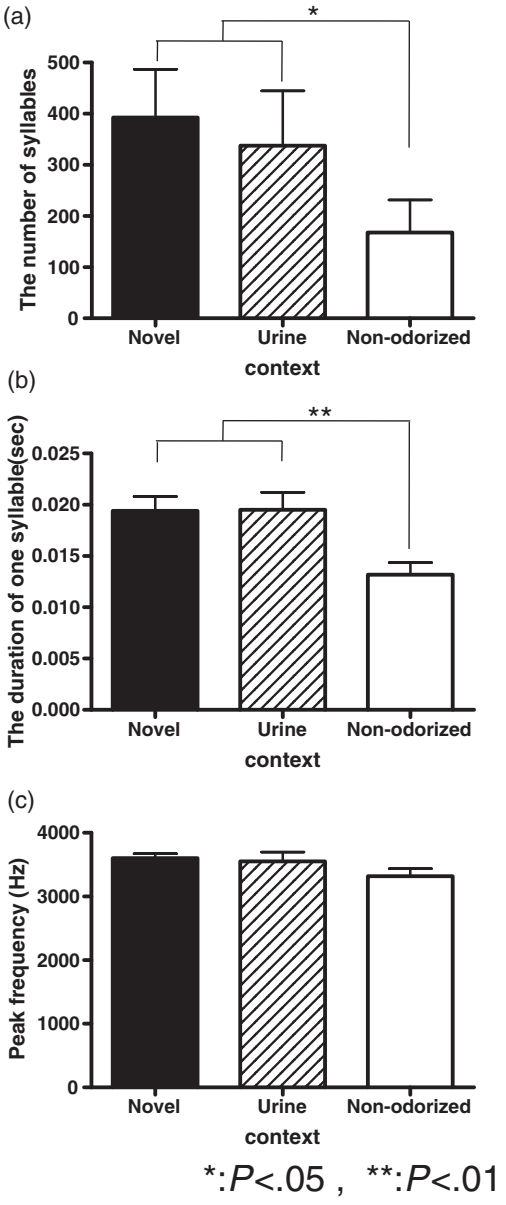

Fig. 2 Column graphs of (a) number of syllables/trial, (b) mean duration of one syllable (s), and (c) peak frequency (Hz) for the novel, female urine, and nonodorized swab contexts with KOR1-peep mice.

control context ($F(2,8) = 5.21$, $p < 0.05$; novel vs non-odorized swab, $p < 0.05$; female urine vs nonodorized swab, $p < 0.05$) (Fig. 2(a)). The duration of one syllable in both the novel and female urine contexts was significantly longer than in the nonodorized swab context ($F(2,8) = 10.73$, $p < 0.01$; novel vs nonodorized swab, $p < 0.01$; female urine vs non-odorized swab, $p < 0.05$) (Fig. 2(b)). However, the peak frequency of the audible vocalizations did not differ significantly among all contexts ($F(2,8) = 3.22$, n.s.) (Fig. 2(c)).

3.2. Analysis of isolation calls emitted by KOR1 mouse pups

Although none of the KOR1 adult mice emitted ultrasonic vocalizations, all KOR1 pups emitted isolation calls in the ultrasonic range (Fig. 3 top). The number of ultrasonic vocalizations emitted by the KOR1 pups reached a peak at

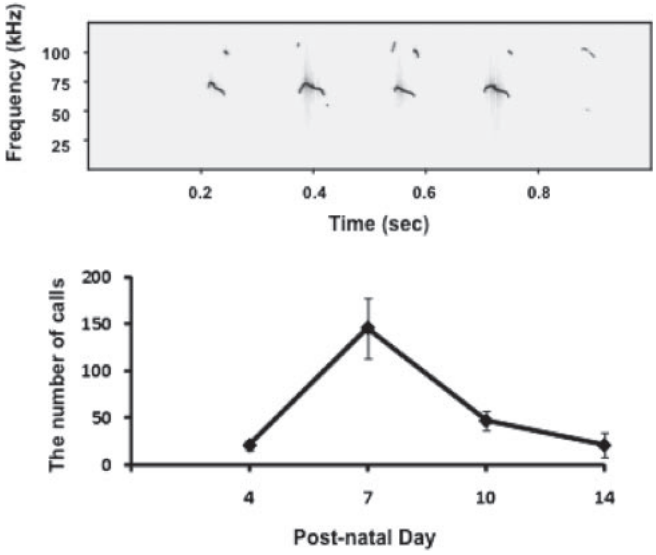

Fig. 3 Top: Spectrograms (frequency, kHz × time, s) of the ultrasonic calls produced by a KOR1 mouse pup. Bottom: Number of isolation calls in KOR1 mouse pups.

P7 and then started to decrease toward P14 (Fig. 3 bottom). This pattern is also seen in *M. m. domesticus* [2,11]. The mean peak frequency was about 75 kHz. This is similar to the isolation calls of an *M. m. domesticus* strain, C57BL/6, which produces calls at around 70 kHz [12].

4. Discussion

Our study identified arousal-dependent vocalizations that have songlike features in the audible range in KOR1 mice. The audible vocalizations increased in both courtship contexts which arouse sexual motivation and novel environments which arouse anxiety, suggesting that this increase is not controlled by context-dependent conditions but by general arousal. Therefore, the audible songlike vocalizations of KOR1 were not produced by the same mechanisms as the audible vocalizations of *M. m. domesticus*. Instead, it seems that KOR1 produce successive audible vocalizations associated with a breathing problem caused by arousal.

Similarly to the laboratory mouse *M. m. domesticus*, the KOR1 pups emitted ultrasonic vocalizations when isolated. Thus, isolation calls are well preserved as they are of fundamental importance for the survival of the pups. In addition, this indicates that KOR1 mice possess the central and peripheral mechanisms necessary to emit ultrasonic vocalizations, at least as pups, but they do not use ultrasonic vocalizations in a courtship context. Because vocalization in a courtship context is not critical for successful copulation [9,13], it is not surprising that ultrasonic song is not a common feature in these subspecies.

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