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Effects of quinestrol on the vocal behavior of mice during courtship interactions☆

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HIGHLIGHTS

- Male mice treated by quinestrol showed alteration in vocal behavior.
- Male mice produced more courtship calls after quinestrol treatment.
- The harmonic syllables became more common in male mice treated by quinestrol.
- Female mice approached male mice treated by quinestrol more than the control.
- Sterilants could achieve "competitive reproductive interference" by affecting the ultrasonic communication of mice.

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ABSTRACT

Vocalizations are a crucial part of courtship and mating in a wide variety of species. Mating behavior, including courtship calls, is modulated by sex steroid hormones. Male mice produce courtship ultrasonic vocalizations to attract females during heterosexual encounters. However, rare is the knowledge on whether vocal behavior of mice changes under sterilant treatment which will affect gonadal hormone levels. In the present study, we treat male mice with quinestrol, which interferes with the release of the gonadotropin-releasing hormone (GnRH) and has a significant anti-fertility effect in rodents. We compared the differences in the syllable structures (including peak intensity, peak frequency, duration, and bandwidth), total number of calls, and harmonic syllable proportions between quinestrol treated and control male mice. Male mice treated with quinestrol produced more courtship calls and more harmonic syllables than control mice, whereas the parameters of call syllables showed no significant change between the two groups. The results indicate that normal male vocal behavior during sexual interactions could be retained or even reinforced after quinestrol treatment. In addition, female mice approached male mice treated with quinestrol more than control mice, suggesting that the treated male mice were more attractive to the female mice than the controls. Thus, competitive reproductive interference is enhanced. Further, findings provided behavior mechanism in vocal context of the fertility control in mice.

1. Introduction

Vocalizations, similar to visual ornaments (e.g., coloration) or weapon morphology (e.g., antlers, horns, and canines), are important components of sexual selection in many animal species, often playing a crucial role in determining the outcome of agonistic contests and female choice [1–3]. Among mice, ultrasonic vocalizations (USVs) as important factors in courtship and mating have song-like characteristics, such as different syllable types and temporal sequencing that includes emission of repeated phrases [4]. The courtship USV reflects a positive affective state [5], and serves as an important communicative function, namely mate attraction or mate choice [6]. The majority of USVs produced during male–

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female interactions in genus *Mus* are believed to be from males [5], especially in the inception phase during male–female interaction, while female mice also emit ultrasonic calls only minor differences with males' during copulation [7]. The courtship USV shows high-level sensitivity to cues indicating female proximity [8], and USVs change rapidly in response to female removal [6]. Females also demonstrate interest in USVs by approaching speakers broadcasting recorded USVs and by preferring vocalizing males to silent males [4,9,10]. Mating behavior is often causally positively related to circulating levels of sex steroid hormones produced by gonads [11]. Similar to songbirds [12,13], mouse vocal behavior is modulated by gonadal hormones during sexual interactions [14–16], where castrated male mice reduce calling, and testosterone-implanted ones restored the call [17].

Fertility control has been proposed as an alternative strategy for effective, safe, and sustainable control of pest rodents that have indeed become one of the most serious biohazards for human society (farms, forests, industries, homes) [18–20], as traditional control methods (physical machinery or rodenticide) are limited (ineffective or results in side effects for human and wildlife) [18]. Sterilants that influence the normal function of reproduction system and reproductive behavior of rodents [19,20] are useful birth control methods, and previous studies have shown mathematically that "competitive reproductive interference" could provide better controls with contraception than culling [21]. Sterile individuals whose reproductive performances are diminished continue to draw on vital resources and could participate in mating competition [21]. However, many details regarding behavior are still unclear. Quinestrol as a common sterilant disrupts normal reproduction by interfering with the activity of the hypothalamus-pituitary-ovary axis and the release of hypothalamic GnRH. This anti-fertility effect in males is manifested in disruption of spermatogenesis in the testis, decreased sperm numbers and size of the testis, and reduced testosterone levels [19,22]. Whether Sterilized individuals lose the ability to attract heterosexual individuals or some behavioral compensation mechanism emerges in this context is still unknown.

In the present study, we aimed to reveal the effects of sterilization treatments on reproductive behavior by investigating variations in mouse courtship USVs. Thus, we tested the effect of quinestrol on vocal behavior in male mice by comparing the courtship USVs of quinestrol treated and control treated mice. We were particularly interested in whether quinestrol treatment would affect the vocal behavior of mice and how the syllable types or the spectral and temporal features of courtship USVs change following treatment. Courtship behavior is regulated by the neurosecretory hypothalamus system [23,24], and quinestrol's inhibition of the GnRH release has a significant anti-fertility effect in rodents [19,22]. Hence, we hypothesized that male mice treated with quinestrol would reduce their courtship USVs and the call structures would change during courtship, consequently reducing their sexual attraction to females. Alternatively, we hypothesized that the treated individuals would increase calling to compensate for impairment in reproductive physiology. Sterilants could promote "competitive reproductive interference" by affecting the ultrasonic communication of mice.

2. Method

2.1. Subjects

All Swiss mice (produced by Guangdong Medical Laboratory Animal Center) were housed individually in plastic cages (30 × 20 × 16 cm) in a temperature and humidity-controlled colony room (22–25 °C, 50–60%), and maintained on a 12/12 dark–light cycle (lights on at 08.00–20.00) with ad libitum access to food (rat pellets produced by Guangdong Medical Laboratory Animal Center) and water. All procedures were approved by the Ethics Committee of Guangdong Institute of Applied Biological Resources, Guangdong Academy of Sciences.

2.2. Experimental setting

Mouse vocalizations were recorded with an Avisoft Bioacoustics USG 116(e) detector (Avisoft Bioacoustics, Berlin) equipped with an Avisoft FG series microphone on a 2-m cable in a soundproofed chamber (3 × 2.3 m and 2.5 m high). The calls were recorded at a sampling rate of 250 kHz. Video was recorded with a PICO2000 series multimedia digital video monitor. The video camera and microphone were positioned 20-cm above the recording cage (50 × 35 × 20 cm).

2.3. Experimental procedure

Naive adult male mice aged 4–6 months and weighing 45–60 g were selected and randomly divided into two groups (treatment group: 15; control group: 15). Naive females aged 4–6 months and weighing 40–55 g were used as stimulus mice. All mice were not subjected to sexually experience before experiment. Thirty heterosexual encounters (treatment group: 15; control group: 15) involving 30 males and 30 females were observed in daylight. Treatment mice were administered with quinestrol in sunflower oil vehicle at dose of 50 mg/kg body weight by gavage for 4 days consecutively. The control mice received the same equal volume of sunflower oil for 4 days consecutively. The selective dose was based on previous studies [25–28] and preliminary experiment, which ensured that the reproductive system of the male mouse was affected. Heterosexual encounters were performed after one week of the final treatment. Then the courtship calls and overt behavior were recorded during this context.

Each male met one female with lower body weights singly in the recording cage during recording and video period, and no individual was used repeatedly. Each male was monitored (audio and video recording simultaneously) for 5 min prior to a female being added to the cage, so they could adapt to this environment context. The male–female interactions took place and were observed for 30 min (audio and video recording simultaneously), after which the female was removed.

2.4. Sound analysis

Vocalizations during the first 5 min of interaction were selected and analyzed. Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin) was used to analyze parameters of vocalizations. Call parameters were measured automatically from spectrograms generated with a fast Fourier transform length of 512 points and a time-window overlap of 96.87% (100% frame, Hamming window). The spectrogram was produced at a frequency resolution of 488 Hz and a time resolution of 0.064 ms. we measured the peak intensity, peak frequency (the frequency that was produced at the maximum amplitude), and bandwidth (computed as highest frequency minus lowest frequency at −30 dB) from spectra. Syllable duration (defined as the time between starting and ending frequencies) was measured from oscillograms. Additionally, syllable rate (number of call elements (syllable) produced per unit of time) and the proportion of harmonic syllable (with frequency jumps) were also measured. The harmonic syllable type contained at least one segment with one or more harmonic, possesses a longer bandwidth and duration than the tonal type. This indicates that harmonic syllables could contain more contact information, potentially encouraging receptive behavior by females. Moreover, previous studies have showed that harmonics syllables are associated with mounting behavior that is very important in copulation [6,29].

2.5. Video analysis

The observations of the overt courtship behavior were made during the 30-min interaction. Female actions, of which the occurrence frequencies were counted, were cataloged using the following definitions. "Female approach male" was recorded when the female moved close to the male. "Female reject male" consisted of a kick at the male in