Ultrasonic vocalizations of female Norway rats (*Rattus norvegicus*) in response to social partners

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Abstract

In many species of animals, male vocalizations function to attract mating partners and coordinate sexual interactions. While male vocalizations have been well studied in several species, the function of female vocalizations in mating contexts is not fully understood. In Norway rats (*Rattus norvegicus*), both males and females produce ultrasonic vocalizations (USVs) during sexual encounters with opposite-sex partners. The aim of this study was to test the hypothesis that female vocalizations play a role in sociosexual interactions by examining how rates of 50kHz USV production vary in relation to the sex and gonadal status of the partner, and by examining whether the proportion of frequency modulated (FM) and constant frequency calls differs between these categories of social partner. The results showed that females produced a higher total number of 50kHz USVs to intact males than castrated males, and produced similar numbers of calls to both categories of females. Females also produced a higher proportion of FM calls to male partners than to female partners, and spent more time in the vicinity of male than female partners, regardless of the partners' gonadal status. Female USVs therefore potentially provide a measure of sexual motivation and may function to promote female mate choice in this species with multi-male mating and a high risk of infanticide.

Keywords Communication, rodents, 50kHz, frequency modulated calls

Introduction

In many animal species, male vocalizations are important in mate attraction and courtship (Bradbury & Vehrencamp, 2011), and sexual selection theory has provided convincing explanations for the evolution of male vocal traits (Andersson, 1994). In contrast, questions regarding female vocalizations have been somewhat neglected, despite growing evidence that females produce vocalizations in mating contexts in several taxonomic groups, including reptiles (Young, Mathevon, & Tang, 2014), birds (Odom et al., 2014) and mammals (Neunuebel, Taylor, Arthur, & Egnor, 2015; Pradhan, Engelhardt, van Schaik, & Maestripieri, 2006). Some studies have shown that female vocalization rates vary according to the stage of the reproductive cycle (Langmore & Davies, 1997; Matochik, White, & Barfield, 1992a; Schön et al., 2007), which raises the possibility that selection will have favoured males that allocate mating effort on the basis of female vocal characteristics. Both male and female mating partners are likely to benefit from using USVs to co-ordinate mating encounters during the fertile period. However, in situations where conflicts of interest occur over matings, females could also benefit from vocalizing by promoting male-male competition, gaining matings with multiple partners and encouraging sperm competition (Pradhan et al., 2006). Thus, a greater understanding of female vocalizations could shed light on how sexual selection has acted on between-sex communication.

The ultrasonic vocalizations (USVs) produced by rodents provide opportunities to study vocalizations in a laboratory setting. Rodent USVs are elicited in a range of social situations (Wöhr & Schwarting, 2013), and production of USVs provides an indicator of affective state (Brudzynski, 2013). Male rodents produce USVs around 50kHz in frequency during courtship (McIntosh & Barfield, 1980), and females respond to playbacks of these calls with approach behavior (Willadsen, Seffer, Schwarting, & Wöhr, 2014). Different sub-types of 50kHz USVs have been recorded: *frequency modulated* (FM) 50kHz USVs are the most commonly produced calls during mating interactions (Burgdorf et al., 2008), while *constant frequency* 50kHz calls are

more often given during aggressive encounters (Burgdorf et al., 2008). Female Norway rats (*Rattus norvegicus*) have also been reported to produce 50kHz USVs during sexual interactions (Thomas & Barfield, 1985; White & Barfield, 1989; White, Colona, & Barfield, 1991). However, whether female vocalizations function to attract mating partners remains unclear (Snoeren & Ågmo, 2013), and the sub-types of 50kHz USVs given by female rats in mating contexts have yet to be fully investigated.

Here, we tested the hypothesis that female 50kHz USVs play a role in sociosexual interactions by examining how rates and sub-types of 50kHz USV production vary in relation to the sex and gonadal status of the partner. USVs were recorded from female rats following brief exposure to male or female partners that were either gonadally intact or had been gonadectomised. Rates of 50kHz USV production were predicted to be higher in response to male than female partners, and higher for gonadally intact than castrated males, as in previous studies (White et al., 1991; McGinnis & Vakulenko, 2003). In addition, we examined whether the proportion of FM 50kHz calls was higher for male than female partners, as previous studies have used bat detectors that do not allow discrimination of call sub-types (White et al., 1991; McGinnis & Vakulenko, 2003). USVs were recorded following removal of the partner (as in McGinnis & Vakulenko, 2003; Yang, Loureiro, Kalikhman, & Crawley, 2013) to ensure that vocalizations were recorded from the subject only. Time spent in the vicinity of the partner prior to removal was also measured and was predicted to be highest for intact males.

Methods

Subjects and stimuli animals

The subjects were eight female Lister-hooded rats, and the stimuli animals were eight Lister-hooded rats: two intact males, two castrated males, two intact females, two ovariectomized

females (all animals were supplied by Harlan, UK; gonadectomies were carried out by the supplier). All animals were housed as same-sex pairs with *ad libitum* access to food and water. Housing rooms were on a 12hr light/dark cycle (lights on 07:00) with temperature and humidity control. All appropriate guidelines regulations were observed, as set out in the Principles of Laboratory Animal Care (NIH, Publication No. 85-23, revised 1985) and the UK Home Office Animals (Scientific Procedures) Act 1986. (UK Home Office Animals (Scientific Procedures) Act 1986).

Apparatus

Tests were conducted in a rectangular arena (length=70cm, width=48cm, height=45cm; **Figure 1a**) with grey-painted wooden walls, a solid floor and transparent lid, located in a testing room with dim white lighting (15lux). The arena was divided into a larger section (length=50cm) and a smaller section (length=20cm) using a removable, transparent partition with small air holes. The lid of the larger section was marked half-way to visually distinguish the half closest to the partition. Real-time behavioral data were collected on a computer running in-house software. USVs were recorded using an UltraSoundGate Condenser Microphone CM16/CMPA (Avisoft-Bioacoustics, Germany; frequency range 10–200kHz), which was suspended above the larger section of the arena (40cm above floor level) through a hole in the lid. The analogue microphone output was digitized using an Edirol FA101 sound card (Roland Corp., Japan; 192kHz sampling rate in 24-bit format) and stored as a wave file. The sound card was operated using open source software (Pamguard, version Beta1.11.02).

104 *Experimental design*

105 Each female subject animal (henceforth 'subject') was tested eight times over a two-week
106 period, once with each stimulus animal (henceforth 'partner'), with order of exposure counter-
107 balanced across subjects. At the start of a test, a subject and partner were transported to the
108 testing room in separate boxes. The partner was placed into the smaller section of the arena, with
109 the partition lowered, before the subject was placed into the larger section, and the lid closed. For
110 the first 5 minutes, the position of the subject was recorded in real-time (i.e., subject located in
111 the half of the larger section nearest to the partition or in the half of the larger section furthest
112 from the partition). The partner was then removed from the arena and testing room and the
113 partition raised. For the next 5 minutes, the subject had access to the whole arena, and USVs
114 were recorded. The arena was cleaned after each test with 70% alcohol.

115

116 *Behavioral and USV analysis*

117 For behavioral data, we calculated the percentage of time spent in the half of the section nearest
118 to the partition during the 5 minutes when the partner was present. For USV data, we examined
119 the number and sub-type of USV produced by subjects during the 5 minutes after the partner had
120 been removed. Wave files were visualised in spectrographic displays using Audacity (version
121 2.0.1.). Spectrograms were computed using Fast Fourier Transformations with a Hanning
122 window (50% overlap frame) and an FFT size of 512. Each USV was labelled as either a 22kHz
123 call (near constant frequency of ~20-25kHz) or 50kHz call (range of ~35-75kHz, with mean
124 frequency of ~50kHz) (based on Burgdorf et al. 2008; Wright, Gourdon, & Clarke, 2010).
125 Vocalizations that did not fall into either of these two categories (<1.5%) were excluded, and
126 22kHz calls (<1% of remaining calls) were not further analysed. All 50kHz vocalizations were
127 categorised as either FM (i.e., bandwidth >8kHz) or constant frequency (i.e., bandwidth ≤8kHz),

based on visual estimation of previously published calls (Wright et al., 2010). Inter-observer reliability scores were found to be robust (intraclass correlation coefficient ≥0.95).

Statistical analyses

Statistical analyses were conducted in SPSS (version 22). After checking the assumptions of normality and sphericity (using Kolmogorov-Smirnoff and Mauchly's tests), all data were analysed using parametric statistics. Percentage of time spent in the half of the section nearest to the partition was compared to chance (50%) across all subjects using a one-sample t-test. All other data were analysed using two-way within-subject, repeated measures ANOVAs, with partner's sex, gonadal status and the interaction term as categorical predictor variables. Significant interactions were further analysed using simple effects *post hoc* tests. Effect sizes were calculated as partial eta squared (η_p^2) for main effects and interactions, and as Cohen's *d* for pair-wise comparisons. Data are presented as means and 95% confidence intervals (CI).

Results

Time spent near to partner

Across all partner categories, subjects spent more time in the half of the section nearest to the partition than expected by chance (75.2%, CI [72.5, 77.9]; t_{31}=18.99, p<0.001). Subjects also spent a significantly higher percentage of time next to the partition when the partner was male rather than female, although the difference was relatively small ($F_{1,7}$=8.54, p=0.022, η_p^2=0.55; **Figure 1b**). The main effect of the partner's gonadal status was not significant ($F_{1,7}$=0.96, p=0.360, η_p^2=0.12), and the interaction between sex and gonadal status was also not significant

($F_{1,7}$=0.51, p=0.497, η_p^2=0.07; intact male = 79.3%, CI [75.1, 83.5]; castrated male = 75.4%, CI [70.34, 80.5]; intact female = 73.2%, CI [64.2, 82.1]; ovariectomized female = 73.0%, CI [67.4, 78.6]).

Total number of USVs

The total number of USVs made by the subjects differed significantly according to the sex of the partner ($F_{1,7}$=6.93, p=0.034, η_p^2=0.50), and the interaction term between sex and gonadal status was also significant ($F_{1,7}$=7.53, p=0.029, η_p^2=0.52; **Figure 2a**). Simple effects tests revealed that females gave significantly more USVs to intact males than castrated males (p=0.039, *d*=1.14),while the total number of calls given to intact and ovariectomized female did not differ (p=0.323, *d*=0.50). The main effect of gonadal status was not significant ($F_{1,7}$=2.32, p=0.172, η_p^2 =0.25).

Proportion of 50kHz USVs that were FM

The proportion of 50kHz calls that were FM, rather than constant frequency, was significantly higher when the partner was male (0.62, CI [0.56, 0.69]) than when the partner was female (0.51, CI [0.39, 0.62]; main effect of sex: $F_{1,7}$=8.88, p=0.021, η_p^2=0.56; **Figure 2b**). The main effect of gonadal status was not significant ($F_{1,7}$=0.81, p=0.397, η_p^2=0.10), and the interaction between sex and gonadal status was also not significant ($F_{1,7}$=0.07, p=0.801, η_p^2=0.01; intact male = 0.59, CI [0.48, 0.70]; castrated male = 0.66, CI [0.55, 0.76]; intact female = 0.49, CI [0.34, 0.64]; ovariectomized female = 0.52, CI [0.31, 0.74]).

Discussion

The results showed that production of 50kHz USVs by female Norway rats varied with the sex and gonadal status of the partner, with intact male partners eliciting the highest total number of calls. In comparison, the number of 50kHz USVs given to castrated males was relatively low and similar to that given to female partners, while the rate of calling to females did not differ with the partners' gonadal status. The finding that females produce higher rates of 50kHz USVs to intact than castrated males is consistent with two earlier studies that were conducted using a different strain of Norway rat (Long Evans: White et al., 1991; McGinnis & Vakulenko, 2003). Female subjects were potentially responding to multiple cues from intact males, including vocal, olfactory and visual cues, which all vary with male hormonal status (Harding & Velotta, 2011). The current study also provided novel evidence that the proportion of FM 50kHz USVs was higher for male than female partners, regardless of the partner's gonadal status. High rates of female 50kHz USV production, particularly FM calls, are potentially indicative of high female sexual motivation. Contrary to our prediction, females did not spend more time next to partition with intact male partners compared to castrated males, which suggests that 50kHz USVs could provide a better measure of female sexual motivation than proximity measures alone.

While the ovarian status of the female subjects was not investigated in the current study, rates of 50kHz calling by female rats have been shown to be highest during the fertile phase of the ovarian cycle (Matochik et al., 1992a) and to be elicited by estrogen and progesterone treatment (Matochik, Barfield, & Nyby, 1992b). Previous studies have also shown that devocalizing female rats disrupts sociosexual behavior (White & Barfield, 1987; 1989) and that playbacks of female USVs facilitate mating interactions with male partners (White & Barfield, 1989). Female vocalizations could function to signal sexual motivation in female rats and also to attract multiple mating partners and promote sperm competition, which potentially benefits females by confusing paternity and reducing the risk of male infanticide (Ebensperger &

196 Blumstein, 2007). In support of this hypothesis, female rats mate with multiple males during an
197 ovarian cycle (Solomon & Keane, 2007) and can have litters sired by several different males
198 (Miller et al., 2010). Where females mate with multiple partners during a single cycle and sperm
199 competition is therefore high, males are predicted to allocate mating effort selectively according
200 to likely reproductive payoffs (Ramm & Stockley, 2014). Female USVs could thus be used in
201 male mate choice. Rather than focusing on the mutual benefits that both sexes are likely to gain
202 from co-ordinating mating activities, this alternative perspective highlights the potential role that
203 USVs could play in situations where conflicts of interest occur over matings.

204 Future studies could examine whether male rats preferentially attend to 50kHz FM USVs
205 and whether female traits that are correlated with fertility status, such as 50kHz USVs, influence
206 male mating strategies. A recent study reported that female USV playbacks do not evoke more
207 approach behavior by male rats than background noise (Snoeren & Ågmo, 2013). However, this
208 negative result could have been influenced by the open shape of the testing arena (c.f., Willadsen
209 et al., 2014), which may have prevented the playback stimulus from having clear directionality.
210 The role of 50kHz USVs in female-female interactions in rats could also be investigated further.
211 In the current study, the rates of calling did not differ for intact versus ovariectomized female
212 partners, in contrast to a previous study reporting that female rats called more to females that had
213 been primed with estrogen and progesterone than to ovariectomized partners (McGinnis &
214 Vakulenko, 2003). The difference in results between the two studies could reflect the fact that
215 the hormone-primed stimulus females in the study by McGinnis and Vakulenko (2003) produced
216 a different set of vocal, olfactory and visual cues than the intact females in the current study. In
217 summary, the current evidence indicates that 50kHz USVs provide a valuable insight into
218 hormone-related vocal communication patterns in rats.

219

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Figure legends

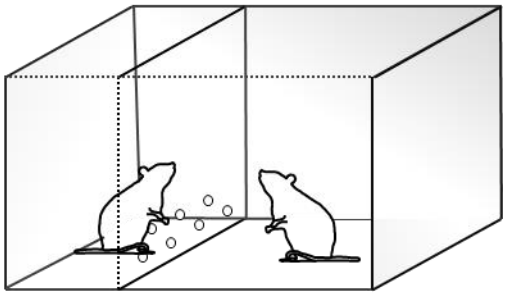
Figure 1a) Testing arena showing the partner animals in smaller section and subject in larger section, separated by a transparent partition with holes along the lower edge. b) Percentage of time spent by the subject next to the partition when the partner was male or female, where the dashed line represents the 50% chance level (means±SEMs; * $p<0.05$).

Figure 2a) Total number of 50kHz USVs given by subjects per minute following exposure to male or female partners that were either intact (grey bars) or gonadectomised (white bars) (means±SEMs; * $p<0.05$). b) Proportion of 50kHz USVs that were FM following exposure to male or female partners (means±SEMs; * $p<0.05$).

315 **Figure 1**

316

a)



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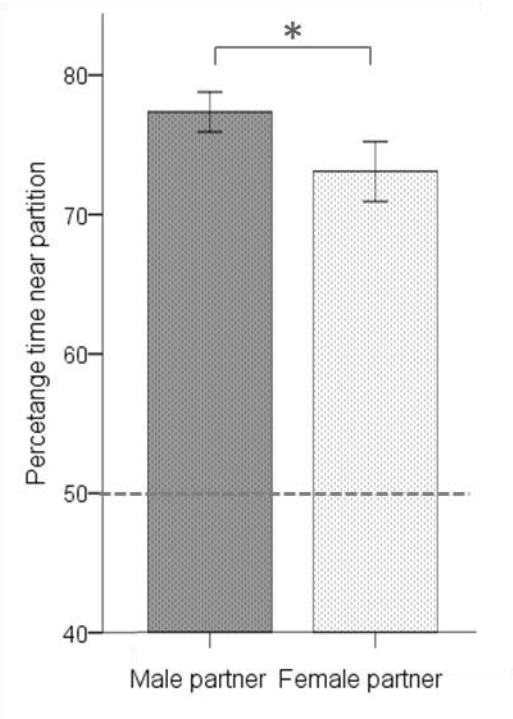

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318 **Figure 2**

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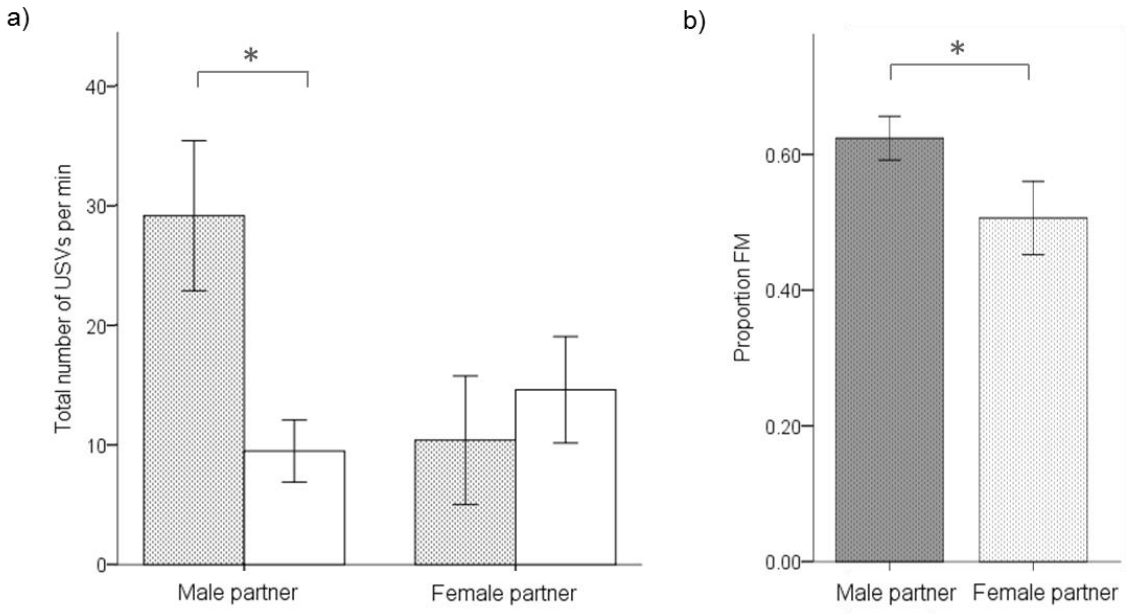

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