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Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse

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Most studies examining rodent ultrasonic vocalizations (USVs) have investigated pericopulatory vocal behaviour in polygynous rodents, while vocalizations related to pair bond maintenance in monogamous rodents remain unexplored. In the monogamous California mouse, *Peromyscus californicus*, we used ultrasonic playbacks and post-playback social interactions to assess possible functions of USVs. We found that females responded with approach towards USVs of an unfamiliar male (bonded male from another pair) compared to a noise control, but displayed no difference in response to calls of their partner versus noise. Responsiveness to unfamiliar males does not appear to reflect an interest in extrapair copulations because during post-playback social interactions, females displayed more agonistic behaviours and fewer affiliative behaviours towards unfamiliar, sexually naïve 'stranger' males than towards their partners. We speculate that approach to unfamiliar male USVs instead may be related to territorial defence. We further explored associations within the data set. Interestingly, female affiliation with her partner was predicted by USV output, particularly a higher number and proportion of complex call types, produced during the male partner USV elicitation phase. Female approach towards USVs was related to syllable duration of one call type in partner USVs (not in unfamiliar USVs) but no other features, and sufficient variation exists in syllable duration to allow females to theoretically distinguish between individuals based on this measure. Similarly, while pregnancy state did not influence female social behaviour, it decreased approach to playback of partner USVs but not to that of unfamiliar USVs. Overall, our results illuminate concepts about vocal communication in monogamous rodent species with strong pair bonds and suggest that functions of USVs in rodents can extend beyond mate choice.

Among socially monogamous species, intersexual vocal communication plays a key role in pair bond maintenance among mates in a variety of taxa, most notably birds (Hall, 2009), although examples in mammalian species have emerged (Geissman & Orgeldinger, 2000; Ham, Hedwig, Lappan, & Choe, 2016; Rukstalis & French, 2005). In socially monogamous birds, vocal signals may maintain pair bonds by sustaining contact when physically separated (Robertson, 1996; Silcox & Evans, 1982) and quality of vocal communication in response to an intruder can be positively related to paternity assurance (Baldassarre, Greig, & Webster, 2016). Individual recognition of pair mate vocalizations also occurs in monogamous birds (Blumenrath, Dabelsteen, & Pedersen, 2007; Robertson, 1996). However, there is a surprising dearth of studies on the role of acoustic communication in pair bonding and maintaining social bonds in rodents.

Ultrasonic vocalizations (USVs) of adult rodents are high-frequency, quickly attenuating sonic signals studied primarily in mice and rats in the context of sexual motivation (Brudzynski, 2009; Holy & Guo, 2005; Matsumoto & Okanoya, 2016; Portfors, 2007). Males produce USVs in mating contexts (Sales, 1972) and in response to ovulatory chemosignals in female urine (Nyby, Bigelow, Kerchner, & Barbehenn, 1983; White, Colona, & Barfield, 1991; Whitney & Nyby, 1979), and male USVs facilitate proximity association and subsequent female mating behaviour (Pomerantz, Nunez, & Bean, 1983; White & Barfield, 1989). Ultrasonic playbacks have added significantly to this knowledge base (*Rattus norvegicus*: Pultorak, Kelm-Nelson et al., 2015; Willadsen, Seffer, Schwarting, & Wöhr, 2014; *Mus musculus*: Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2009; Musolf, Hoffmann, &

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Penn, 2010). Acoustic communication in monogamous rodent species likely has additional functions related to challenges associated with long-term social bonds such as pair bond maintenance (Tomaszycki & Adkins-Regan, 2005) and mate coordination related to territory defence and rearing offspring (Kleiman, 1977), but this remains to be tested. To help fill this gap, we examined female approach behaviour during playback of USVs in a monogamous species, the California mouse, *Peromyscus californicus*.

The monogamous (Gubernick & Nordby, 1993; Ribble, 1991), biparental (Bester-Meredith & Marler, 2003; Cantoni & Brown, 1997), territorial and aggressive (Bester-Meredith & Marler, 2007; Davis & Marler, 2004; Marler, Bester-Meredith, & Trainor, 2003; Trainor, Bird, & Marler, 2004) California mouse is ideal for studying pair mate social behaviour (Gleason, Holschbach, & Marler, 2012; Gleason & Marler, 2010) and vocal behaviour. Both members of a pair maintain overlapping territories (Ribble & Salvioni, 1990) and produce several distinct USV types in field and laboratory settings (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Metheny, & Vonhof, 2006; Kalcounis-Rueppell et al., 2010), and the functions of calls continue to be identified. When presented with unfamiliar females, but without physical contact, sexually naïve males produce 'simple sweep' USVs at higher rates than do paired males, and these rates are rapidly modulated by pulses of testosterone (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015), suggesting that sweeps are used in courtship. Moreover, field research indicates that temporal features of lower-frequency, further-travelling syllable vocalization (SV) type USVs are altered based on whether mates are present (Briggs & Kalcounis-Rueppell, 2011). Here we examine aspects of USV function by assessing whether females differentially respond to their mates' (henceforth 'partner') USVs compared to noise versus the USVs of an unfamiliar bonded male and noise, and by exploring how aspects of USVs relate to approach to playback as well as intrapair and extrapair behaviour.

We considered predictions for response to playback arising from the following two alternative hypotheses. First, if vocalizations in this context function in pair bond maintenance, analogous to observations in some socially monogamous birds (Blumenrath et al., 2007; Robertson, 1996), we predicted that females would approach their partner's USVs more compared to unfamiliar male USVs. Alternatively, if females investigate USVs for seeking extrapair mating opportunities, as suggested by findings in polygynous house mice (Asaba et al., 2014; Musolf et al., 2010) or to contribute to the combined territorial defence exhibited in this monogamous species (Ribble & Salvioni, 1990), we predicted that females would show comparatively greater approach towards unfamiliar male USVs. Following playbacks, we predicted that if females' interest in unfamiliar bonded male USVs is a result of seeking extrapair copulations, they would show affiliative and not aggressive behaviour towards unfamiliar, sexually naïve 'stranger' males, but the reverse result if they are expressing territorial defence. Given the dearth of studies of USVs in a monogamous species, we also explored several relationships between USVs and behavioural responses: (1) whether male partner USV production during elicitation (reunion after a period of pair separation) was predictive of subsequent behaviour during the social interaction; (2) whether females could theoretically distinguish males based on SV syllable duration since this call characteristic emerged as an important feature in playback; and (3) whether pregnancy would influence approach to playback or social behaviour since approximately half of the females were pregnant at the time of the trial. Overall, we used variation in male vocal production, female approach to playback stimuli and female behavioural responses during a post-playback social interaction to examine possible roles of ultrasonic communication in California mouse pairs.

METHODS

Animals

We used 68 adult female and 111 adult male California mice (mean ± SE age = 183 ± 40.9 days) reared in our laboratory colony at the University of Wisconsin-Madison. Animals were maintained in accordance with the National Institutes of Health *Guide for the care and use of laboratory animals*. The University of Wisconsin-Madison Institutional Animal Care and Use Committee (IACUC) approved animal treatment and research protocols (IACUC number L0054470-A01). Mice were housed in standard cages (48.3 × 26.7 × 15.6 cm) with one to three same-sex conspecifics after weaning (postnatal day 30) and given water and food (Purina 5015 mouse chow, Purina Mills®, St Louis, MO, U.S.A.) ad libitum. Colony rooms and testing rooms were maintained at 20–23 °C under a 14:10 h light:dark cycle. All behavioural testing was conducted under red light within 3 h of the onset of the dark cycle. Animals used in male–female dyads did not share common ancestry for a minimum of two prior generations.

USV Elicitation for Playback

Because pilot data showed that vocalizations are most reliably produced upon reunion after mate separation, we paired male–female dyads on day 0 for 12 days, separated pairs for 24 h on day 12, and made recordings upon reintroduction on day 13. Specifically, on day 12, the male partner was removed from the cage and was housed in a separate room in a Plexiglas 'elicitation arena' (90.0 × 30.0 × 30.0 cm), with food, water and bedding. The arena was divided into two chambers separated by a translucent wall containing two symmetrically located circular openings (3.8 cm diameter, centre of opening 7 cm from the side wall) covered by mesh to allow for auditory and olfactory communication but not physical contact. Two microphones sensitive to ultrasound (Emkay/Knowles FG series, frequency range 10–120 kHz, Avisoft Bioacoustics, Berlin, Germany) were positioned in opposite corners of the arena, 90 cm apart and 30 cm from the arena floor, with one corresponding to each chamber. The microphones were calibrated to equal gain (−60 dB noise floor) and recordings were collected at a 250 kHz sampling rate with 16-bit resolution using AvisoftRECORDER software (Avisoft Bioacoustics). On day 13, the female was removed from her home cage and placed into the chamber opposite the male. The recording was initiated immediately after the first male vocalization was observed on the real-time spectrogram (produced using a 512 fast Fourier transform, 50% frame overlap, high-pass filter at 10 kHz) for the channel pertaining to the male's chamber. A trained observer watched the real-time spectrogram and noted female vocalizations during the elicitation session (scored as a 'yes'/'no'), as indicated by any vocalization uniquely present in the channel pertaining to the female chamber or any vocalization with higher amplitude (i.e. darker) than that same vocalization in the channel pertaining to the male chamber. After 2 min, the recording was stopped and the female was removed. If no vocalizations were identified within 5 min, no recording was initiated and the session was stopped. We determined whether each female had mated by the time of the elicitation trial using birth latency data (number of days from pairing to birth of offspring), based on a gestation period of approximately 32 days (Gleason & Marler, 2010).

USV Playback Set-up and Approach Behaviour

After the elicitation session, the male was returned to the home cage and the female was housed in a separate room in a different