Animal Behaviour 126 (2017) 163-175

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

What's in a squeak? Female vocal signals predict the sexual behaviour of male house mice during courtship



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ARTICLE INFO

Article history: Received 5 August 2016 Initial acceptance 21 November 2016 Final acceptance 11 January 2017 Available online 8 March 2017 MS. number: A16-00704R

Keywords: broadband vocalization communication courtship house mouse intersexual communication low-frequency harmonic oestrus sexual behaviour squeak ultrasonic vocalization

Vocal production can be a two-way channel for the exchange of information between males and females during courtship. Although the ultrasonic vocalizations (USVs) of male house mice, Mus musculus, during interactions with females have been a focus of communication research, the vocalizations of females in this context remain poorly understood. During interactions with males, female mice produce audible vocalizations with a broadband harmonic structure ('broadband vocalizations', or BBVs) that are often described by human listeners as 'squeaks'. We tested the hypothesis that the production of BBVs corresponds to male and female behaviours, as well as to contextual variables like oestrous phase, by measuring BBVs, USVs and nonvocal behaviours during 39 unique male-female pairings. We found that the relationship of BBVs to other behaviours depends on the phase of courtship. A high incidence of BBVs accompanied by male-directed kicks and lunges early in interactions predicted a lack of later male mounting and male-produced '50 kHz harmonic' USVs. In contrast, there was significant temporal overlap between BBVs and 50 kHz harmonic USVs at later stages of courtship, potentially driven by mounting events. The duration of acoustically nonlinear segments of BBVs varied significantly among females, even across interactions with different males, but also varied across oestrous phase within females. These findings suggest that vocalizations could play a role in signalling acute female motivational state, identity or oestrous state during opposite-sex interactions. Since the information-bearing features of BBVs are relatively easily measured, they are potentially a useful readout of negative motivational state suitable for many research and educational applications.

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Vocalizations produced by house mice, *Mus musculus*, are an increasingly useful model for studying both the context dependence of vocal communication signals, and the bases of communication disorders (Chabout et al., 2012; Fischer & Hammerschmidt, 2011; Grimsley, Hazlett, & Wenstrup, 2013; Hanson & Hurley, 2012; Portfors & Perkel, 2014; Seagraves, Arthur, & Egnor, 2016). Mouse vocalizations have been categorized based on converging criteria including their spectrotemporal structure, the context in which they are produced, the identity of vocalizing mice and their association with nonvocal behaviours. Two major types of vocalizations that meet these criteria are ultrasonic isolation calls produced by pups and ultrasonic vocalizations produced during social interactions among adults (Egnor & Seagraves, 2016; Ehret & Haack, 1981; Heckman, McGuinness, Celikel, & Englitz, 2016; Lahvis, Alleva, & Scattoni, 2011; Liu, Miller, Merzenich, & Schreiner,

2003; Portfors & Perkel, 2014; Scattoni, Crawley, & Ricceri, 2009). Approaching vocalizations from this structural-functional perspective has allowed productive experimentation on the mechanistic bases of vocal communication in mice at behavioural, neural and genetic levels of analysis (Fischer & Hammerschmidt, 2011; Holfoth, Neilans, & Dent, 2014; Liu et al., 2003; Neilans, Holfoth, Radziwon, Portfors, & Dent, 2014; Portfors & Perkel, 2014; Roy, Watkins, & Heck, 2012; Scattoni, Ricceri, & Crawley, 2011). A third category of vocalizations about which much less is understood consists of human-audible broadband vocalizations, or 'BBVs' (Lupanova & Egorova, 2015).

Audible mouse vocalizations have been variously named 'squeaks', 'low-frequency harmonic' calls (LFHs), 'squeals', 'clicks' or 'broadband vocalizations' (Grimsley et al., 2013; Hanson & Hurley, 2016; Irwin, Kinoi, Van Sloten, & Workman, 1971; Nyby, 2001; Sugimoto et al., 2011). These vocalizations are characterized by a harmonic structure resembling that seen in the vocalizations of many other vertebrate species, including other rodents, other mammals and nonmammalian vertebrates, and that are even a feature of human speech (Bednářová, Hrouzková-Knotková, Burda,

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http://dx.doi.org/10.1016/j.anbehav.2017.01.021

Sedláček, & Šumbera, 2013; Keesom, Rendon, Demas, & Hurley, 2015; Micheyl & Oxenham, 2010; Riede, Mitchell, Tokuda, & Owren, 2005, Riede, Arcadi, & Owren, 2007; Ryan & Guerra, 2014; Suta, Kvasnak, Popelar, & Syka, 2003; Suthers & Zollinger, 2004). BBVs are produced by both female and male mice in distress, as well as during conspecific interaction (Irwin et al., 1971; Lupanova & Egorova, 2015; Matthews et al., 2008; Wang, Liang, Burgdorf, Wess, & Yeomans, 2008: White, Prasad, Barfield, & Nyby, 1998). During opposite-sex interactions, BBVs are predominantly produced by females, as demonstrated by selectively preventing either male or female social partners from vocalizing (Wang et al., 2008; White et al., 1998). In physically and socially complex environments, female mice may also produce ultrasonic vocalizations (USVs) during pursuit by males, with males and females producing USVs in close proximity (Neunuebel, Taylor, Arthur, & Egnor, 2015). Female-produced BBVs during oppositesex interactions are often tightly coupled in number and time with other female behaviours such as kicking or lunging at males, or darting away from males (Keesom & Hurley, 2016; Sugimoto et al., 2011). In contrast to the prosocial function of USVs, BBVs are therefore postulated to be acute signals of female rejection that may slow the progression of a sexual interaction (Johansen, Clemens, & Nunez, 2008). Reflecting the usage of these vocalizations in multiple contexts, male responses to playbacks of BBVs are context dependent. In the presence of olfactory cues of a predator, male mice avoid playbacks of BBVs more than when BBVs are paired with olfactory cues of females (Grimsley et al., 2013).

Although BBVs in general have been associated with specific behavioural contexts, the spectrotemporal structure of BBVs within even single contexts is highly variable (Lupanova & Egorova, 2015), raising the possibility that structural variation could carry behaviourally salient information. A prominent structural characteristic of female BBVs produced in opposite-sex interactions is the presence of spectral nonlinearity. Nonlinearities are commonly encountered features of vertebrate vocalizations that are thought to be caused by abrupt transition of vocal cords into irregular modes of vibration (Fee, Shraiman, Pesaran, & Mitra, 1998; Fitch, Neubauer, & Herzel, 2002; Fuamenya, Robb, & Wermke, 2015; Titze, Riede, & Popolo, 2008; Tokuda, Riede, Neubauer, Owren, & Herzel, 2002; Wilden, Herzel, Peters, & Tembrock, 1998). There are multiple categories of nonlinearity including subharmonics, in which a doubling or tripling of harmonics occurs, and deterministic chaos, or structured noise. In a range of species, nonlinearities convey important information. Nonlinearities may attract attention from conspecifics with high efficacy, reduce habituation to alarm calls, correspond to the physical quality of vocally advertising males or signal individual identity (Blumstein & Récapet, 2009; Blumstein, Richardson, Cooley, Winternitz, & Daniel, 2008; Fitch et al., 2002; Hauser, 1993; Karp, Manser, Wiley, & Townsend, 2014; Riede et al., 2007; Wilden et al., 1998). Nonlinearities and other structural characteristics of female mouse BBVs therefore have the potential to not only serve as a general signal of motivational state, but also to signal specific information to male partners of female mice.

To assess this potential, we characterized multiple characteristics of BBVs, including vocal nonlinearities, during interactions of female mice with males. We allowed females to freely cycle through oestrous phases to further determine whether vocal structure relates to reproductive state. Structural variation in female BBVs was compared to variation in vocal and nonvocal behaviours of both partners. We predicted that (1) BBVs would be coproduced with nonvocal female behaviours, as previously reported (Sugimoto et al., 2011), (2) structural variation in BBVs would correspond to behavioural variation by females or males and (3) the characteristics of BBV structure would be influenced by female reproductive state. All three of these predictions were confirmed. We additionally discovered that the relationships between BBVs and reproductive behaviours varied across different phases of social interactions, and that BBVs in the early phase of an interaction predicted sexual behaviour by males in a later phase.

METHODS

Animals

Focal subjects consisted of 13 female CBA/I mice (the Jackson Laboratory, Bar Harbor, ME, U.S.A.) paired with males. An additional 13 male mice of the same strain served as social partners for females. All female mice were aged 7–8 weeks at the time of the focal interactions, and were previously housed individually for 2 weeks on a 14:10 h light:dark cycle, with food and water provided ad libitum. Mice were housed in standard plastic caging for laboratory mice $(28.5 \times 17.5 \text{ cm} \text{ and} 12.5 \text{ cm} \text{ tall})$, with pine bedding and supplemental nesting material. One week before behavioural recordings, females were given opposite-sex experience through three 10 min interactions with unfamiliar males on three consecutive days. Likewise, males used as social partners in experiments were given opposite-sex experience through three 10 min interactions with unfamiliar females on three consecutive days. Male and female mice used to give experience to experimental subjects were not used in experiments after these interactions.

Ethical Note

Care was taken to ensure compliance with guidelines on animal welfare in reducing the number of mice used in these studies and minimizing their pain or suffering. Mice were monitored during interactions to ensure that aggressive behaviour did not result in injury. Interactions resulting in injury would have been halted, but no interactions resulted in any injury. The balanced design, in which females were used in multiple interactions, allowed us to assess individual differences while collecting data from the same females across oestrous phase and across interactions with different males, resulting in efficient use of females. Finally, mice in the study were later used in different studies in our laboratory, as permitted by our animal welfare protocol. At the conclusion of all studies, mice were euthanized according to standard veterinary guidelines for the euthanization of small animals. All procedures were approved by the Bloomington Institutional Animal Care and Use Committee (protocol 15-021).

Experimental Design

Each female subject and male partner mouse participated in three unique social interactions, with one interaction per day and a novel partner for each interaction. Mice were transported to the recording room between 0800 and 0900 hours to allow habituation to the testing room. Interactions began between 1100 and 1400 hours. For 10 female mice, the 20 min interaction took place in the female's home cage (28.5×17.5 cm and 12.5 cm tall) inside a sound-attenuated recording chamber. Before the interaction, females in their home cages were placed in the recording chamber for 5 min. After habituation, a male of 7–8 weeks of age was added to the female's home cage and the interaction began. At the end of a 20 min interaction, the male was removed from the female's cage and returned to his home cages. From the 10 females and 10 males interacting in the home cages of females, a total of 30 unique male–female pairings were recorded.

For three separate female subjects, 20 min recordings took place in a mesh butterfly cage (~30.5 cm³), which was empty except for bedding on the flexible plastic bottom side. Mesh cages allowed assessment of whether echoes from the rigid sides of a typical plastic mouse cage could substantially alter the properties of the BBVs we observed. Mesh cages also allowed females to temporarily escape courting males by climbing the cage sides, altering the dynamics of the social interactions relative to those in the plastic cages. Prior to recorded social interactions, each female was given 20 min of habituation to the mesh cage and recording chamber, as well as 10 min of interaction with a sexually experienced male, on each of 3 days. The three male social partners of females were between 10 and 11 weeks of age. Females and males each participated in one interaction per day with a novel social partner, on three consecutive days, resulting in a total of nine unique male-female pairings. Mounting by males was never observed in the interactions that took place in mesh cages. Mesh cages were cleaned with soap and water followed by 70% ethanol, and bedding was replaced prior to the next recording.

Video and Audio Recordings of Behaviour

Both audio and video recordings were collected via a microphone and a video camera positioned above the cage, in the case of females in their home cages. Vocalizations were recorded with 16bit resolution with a condenser microphone (CM16/CMPA; Avisoft Bioacoustics, Berlin, Germany; 200 kHz maximum range) and sound card (250 kHz sample rate, UltraSoundGate 116 Hb, Avisoft Bioacoustics). Nonvocal behaviours were recorded with a CCD video camera (30 frames/s), Q-see 4-channel DVR PCI video capture card and SuperDVR software (Q-see, Digital Peripheral Solutions, Inc., Anaheim, CA, U.S.A.). Audio and video recordings were binned in 1 min periods and synchronized through the use of a timer. When the interaction began, the experimenter pushed the start button on a timer that was held up in front of the camera, signalling the start of the interaction. This timer beep could then be found visually in the spectrograms of audio data.

Oestrous State Determination

Five minutes after behavioural interactions ended, we collected vaginal epithelial cells from females via vaginal lavage. We also regularly collected samples at the same time $(\pm 1 h)$ for 1 week before and 4 days after the final behavioural interaction in order to monitor oestrous cycling and increase the accuracy of oestrous phase determination. Oestrous state was determined by cytology of Giemsa-stained vaginal epithelial cells (Goldman, Murr, & Cooper, 2007; Hanson & Hurley, 2012). Dioestrus was determined by the presence of leukocytes, pro-oestrus was determined by the presence of nucleated epithelial cells, and oestrus was determined by the presence of cornified epithelial cells.

Nonvocal Behavioural Analysis

We analysed three nonvocal behaviours (rejection, mounting, escape) from video recordings using ODLog software (Macropod Software, http://www.macropodsoftware.com/). (1) Rejection was defined as females kicking at males, darting away from males, or lunging at males with open mouths. These behaviours were grouped together because they often co-occurred, and because individual components were sometimes obscured. For example, kicks could be obscured when mice were in close contact. (2) Mounting of females by males was defined as males positioned on top of the back half of females with pelvic thrusts occurring. The total duration and number of occurrences of each of these behaviours were measured. (3) Escape by females in mesh cage

interactions was defined as females abruptly climbing up the sides of the cage walls.

Analysis of Vocalizations

Spectrograms of audio recordings were generated with an FFT length of 512 and a Hamming style window with 50% overlap (SasLab Pro software, Avisoft Bioacoustics), Both USVs and BBVs were measured. Although female mice may produce USVs during interactions with males in complex social and physical environments (Neunuebel et al., 2015), we attributed USVs to males and BBVs to females in our interactions for three reasons. First, previous studies in which one social partner had been prevented from vocalizing indicate that females are the main producers of lowfrequency BBVs during opposite-sex interactions of mice, while males predominantly produce USVs (Wang et al., 2008; White et al., 1998). Second, in the current study, BBVs were coupled tightly in time with nonvocal female rejection behaviours such as lunging or kicking at males (Supplementary Video S1). Finally, although overlaps in time between USVs and BBVs were not unusual, overlapping BBVs or overlapping USVs were never observed.

In addition to counting BBV number over time, multiple characteristics were measured from each BBV, including duration, harmonic-to-noise ratio (HNR) (Riede, Herzel, Hammerschmidt, Brunnberg, & Tembrock, 2001; Riede et al., 2005) and fundamental frequency. Spectral nonlinearities were also measured. The main types of nonlinearities encountered in female BBVs were subharmonics characterized by an abrupt doubling or tripling of the number of harmonics, or deterministic chaos (Fig. 1). Both of these types of nonlinearities add a 'creaky' or 'growly' sound quality to BBVs for a human listener during a slowed playback (Supplementary Video S2). Nonlinearities typically occurred with abrupt onsets or offsets, and were easily distinguished from portions of BBVs without nonlinearities, which were termed 'linear' segments. We quantified 'percentage nonlinearity' by dividing the summed duration of nonlinear portions of a single BBV by the total duration of the BBV and multiplying by 100.

USVs were counted and sorted into two categories: '50 kHz harmonic' calls and 'other' calls. This simple division was used over more complex schemes for categorizing male mouse USVs that we and others have used in the past (Hanson & Hurley, 2012; Scattoni et al., 2011) because of the distinct structure and function of the 50 kHz harmonic call. The 50 kHz harmonic USV contains segments with two distinct harmonically related frequency bands, with a fundamental frequency near 50 kHz, which may be preceded or followed by frequency jumps to segments with no visible harmonics (see Fig. 7a, inset). This fundamental frequency contrasts with other USVs, in which the fundamental frequency occurred at around 75 kHz. These other calls may have had upper harmonics at frequencies beyond our range of measurement, however. In addition to the pattern of frequency change over time, absolute frequency is an important criterion for distinguishing categories of calls in multiple rodent species including house mice (e.g. '70 kHz' and '40 kHz' calls of mice; '50 kHz' and '22 kHz' calls of rats), and these categories correspond to functional differences in call usage (White et al., 1998; Wöhr, Houx, Schwarting, & Spruijt, 2015). We have previously reported that 50 kHz harmonic calls are closely associated in time with mounting behaviour (Hanson & Hurley, 2012), and other authors have also demonstrated that calls with ~50 kHz harmonics are uniquely associated with mounting phases of opposite-sex interactions (Matsumoto & Okanoya, 2016). The distinction between 50 kHz harmonic calls and other call types therefore represents an important functional difference.

Because interaction involved a good deal of movement by males and females, portions of BBVs and USVs could be obscured by



Figure 1. Oscillograms (top) and spectrograms (bottom) of sample broadband vocalizations (BBVs) illustrating (a) a linear BBV, without evidence of vocal nonlinearities, (b) a segment with subharmonics at the end of the BBV (arrow), (c) extensive segments with deterministic chaos (structured noise; arrows) and (d) a BBV produced by a female in mesh caging, showing nonlinear structure.

rustling of bedding produced by kicks or other movement cooccurring with vocalization. Rustling sounds were easily distinguished from BBVs by their more extensive frequency spectrum, much shorter duration and lack of harmonic structure. Measurements of spectral characteristics of BBVs were placed with care to avoid this transient rustling.

To maintain consistency of scoring for BBV characteristics and nonvocal behaviours, the same trained observer, blind to oestrous state, scored all interactions. Multiple observers counted and classified USVs, but were all trained by an experienced observer until they reached proficiency. All observers were blind to the oestrous states of females in the interactions.

Statistical Analysis

All statistical analyses were conducted in SPSS v.23.0 (IBM Corp., Armonk, NY, U.S.A.). We first investigated how variation in vocalizations of females was related to nonvocal behaviours exhibited by females. We used a linear mixed model, allowing us to control for female ID, to investigate the relationship between number of BBVs emitted during an encounter and rejection (Garamszegi and Herczeg, 2012). Correlation coefficients were calculated from the mixed model output using Equation 11 in Nakagawa and Cuthill (2007). We measured female vocalizing relative to female escape behaviour in mesh cage encounters as the average number of BBVs in four 10 s time bins preceding females' escape up the mesh walls for all escapes for which escape was preceded by 40 s on the cage floor (N = 40). Different time bins were compared with an ANOVA. Across individual females, we compared variation in BBV rate and other BBV characteristics (total duration, percentage of nonlinear duration, fundamental frequency and HNR) with one-way ANOVAs, with Bonferroni corrections for multiple comparisons (Wright, 1992). Nonlinearities were further subcategorized as the duration of subharmonic and deterministically chaotic segments. We assessed relationships between female body mass and HNR with Spearman rank correlation.

We assessed the effect of naturally varying oestrous state on BBV rate and BBV characteristics in 8 of 10 females for which repeated measures of BBVs emitted during both oestrus and dioestrus were obtained. BBVs from five females in pro-oestrus were measured; however, these BBVs varied widely in spectrotemporal characteristics (compared to oestrus and dioestrus). Therefore, we focused on the oestrus/dioestrus comparison. We used linear mixed models with oestrous state (oestrus versus dioestrus) as a fixed factor and female ID and male ID as random factors to test the effect of oestrous state on BBV rate and BBV spectrotemporal characteristics (duration, percentage of nonlinearity (subharmonics and deterministic chaos), fundamental frequency and HNR). Reported *P* values were adjusted using the Bonferroni method (Wright, 1992). We assessed whether the incidence of mounting depended on oestrous phase for eight females in which interactions occurred in both oestrous and dioestrous using a Wilcoxon signed-ranks test.

We additionally explored how both male and female vocalizations were related to male copulatory behaviour. We compared patterns of female vocalizing over time depending on whether mounting occurred during the social encounters in female home cages. Female BBVs were grouped into 1 min time bins across the 20 min encounter, and divided encounters into two types: 'mounting' and 'nonmounting'. To assess how female vocalizing varied in these two types of encounters, we used a linear mixed model with mounting (versus nonmounting) and time bin as fixed factors, and female and male ID as random factors. We used this same scheme to test whether the broad trajectory of female BBVs in the first versus second 10 min varied depending on whether mounting occurred in the encounter. We assessed whether female oestrous state influenced the timing of female BBVs by using a linear mixed model, with oestrous state (dioestrus versus oestrus) and time bin as fixed factors, and female and male ID as random factors.

We assessed the relationship between the numbers of all male USVs and only 50 kHz harmonic USVs to male mounting across interactions using Spearman rank correlations. For interactions in which mounting occurred, we used the 'song overlap null-model generator' (SONG) package in R v.3.3.1 (https://github.com/ ChristinaMasco/song; Masco, Allesina, Mennill, & Pruett-Jones, 2016) to analyse whether there was significant temporal overlap between female BBVs and male harmonic USVs during the last 10 min of interaction. We used the 'keepgaps' method of randomization to perform 100 randomizations for each interaction while maintaining the observed number of vocalizations and the duration of each vocalization as well as the gaps between vocalizations. We then combined the randomized interactions to form a null distribution of randomized overlap, which we compared to the observed overlap value using a two-tailed *t* test. To test the prediction that BBVs differed acutely around mounting events, we separated BBVs within 'mounting' interactions into two groups: perimounting BBVs and nonmounting BBVs. Perimounting BBVs were BBVs that occurred 10 s before and 10 s after a mounting event; we chose this timing in order to investigate specifically whether BBVs are influenced by a mounting occurrence. Nonmounting BBVs were BBVs occurring outside of these time zones. We used linear mixed models, with perimounting/nonmounting as a fixed factor and female and male ID as random factors, to assess how BBV rate and BBV characteristics (total duration, percentage of nonlinear duration, fundamental frequency and HNR) varied around mounting. Bonferroni corrections were applied to account for multiple comparisons.

RESULTS

Female Mouse BBVs Contain Nonlinear Structure

Ten female CBA/J mice produced a total of 6325 human-audible broadband vocalizations (BBVs) during opposite-sex social encounters with males in standard plastic mouse cages, which were the home cages of females. Each female participated in three encounters with different males, for a total of 30 unique pairings. The mean duration of all BBVs was 75.95 ± 0.324 ms, and the mean fundamental frequency was 3804.14 ± 63.03 Hz. Across all BBVs, 51.8% (3278/6325) incorporated at least one nonlinear segment, characterized by abrupt transitions into either (1) a doubling or tripling of the number of harmonics (subharmonics), or of (2) noise-like structure (deterministic chaos). Fig. 1 illustrates a BBV containing purely harmonic ('linear') structure (Fig. 1a) and BBVs containing segments with subharmonics (Fig. 1b) and deterministic chaos (Fig. 1c), which were distinct from the linear segments. We characterized the degree of nonlinearity by measuring the durations of nonlinear segments relative to the total BBV duration. Of the 3278 BBVs containing nonlinear segments, nonlinearities comprised a mean of $35.95 \pm 1.67\%$ of total duration (the mean percentage nonlinearity). Nonlinearities often occurred after an initial linear segment but could also precede linear segments by occurring at the onset of a BBV or in the middle of a BBV.

An additional three females were paired with three different males each in mesh cages, for a total of nine additional unique pairings, to assess whether the presence of nonlinearities in BBVs could be due to interference from echoes returning from the hard plastic sides of the standard mouse cages. In the mesh cage interactions, females produced a total of 784 BBVs; 339 of these (43.2%) contained at least one nonlinear segment (Fig. 1d).

BBVs Correspond to Nonvocal Behaviours by Females

BBVs produced by female mice in opposite-sex social encounters are often co-produced with male-directed kicking or lunging, or darting away from the male (Sugimoto et al., 2011; Supplementary Video S1). We therefore assessed whether BBVs corresponded to partner-directed behaviours. The number of BBVs emitted during encounters with males was significantly correlated with the number of male-directed rejection behaviours in the same encounter (linear mixed model: $F_{1,10} = 14.61$, t = 1.914, r = 0.518, P = 0.003). Fig. 2 illustrates this correlation for each of the 30 separate pairings, with distinct symbols indicating the three unique social encounters of the same individual female paired with different males. In most cases, there was a correspondence between rejection and vocalizing for single females across different encounters.

Mesh cages provided the opportunity to assess BBVs in a behavioural context in which females had the opportunity to remove themselves from males by climbing the sides of the cages.

Figure 2. Plot of the total number of broadband vocalizations (BBVs) versus the number of female rejections across 30 unique male–female pairs. Symbols indicate the three interactions of each individual female.

During interactions in mesh cages, mounting of females by males never occurred. However, BBV timing corresponded to female climbing, as demonstrated in event-triggered averages anchored to incidences of female climbing (Fig. 3). In the 40 s before females escaped up the cage sides, BBV number steadily increased, culminating in female escape ($F_{3,156} = 2.946$, P = 0.035).

BBV Structure Varies with Female Identity

1.8

Vocal nonlinearities may in part result from individually variable asymmetry between vocal cords (Fitch et al., 2002; Wilden et al., 1998). We reasoned that if this were the case for our female mice, we might observe significant variation among females in the degree of nonlinear vocal production. We therefore compared the durations of linear and nonlinear segments among females by averaging these values for each female across all three pairings. Both linear and nonlinear segments of BBVs differed significantly among females (Fig. 4). The duration of BBVs overall was different among females ($F_{9,20} = 5.173$, P = 0.0011; Fig. 4b). This was attributable to variation in both linear segments, which ranged from $43.45 \pm 2.16 \text{ ms}$ to $72.57 \pm 5.67 \text{ ms}$ ($F_{9,20} = 6.035$, P = 0.00041; Fig. 4c), and nonlinear segments, ranging from 3.23 ± 0.44 ms to $34.74 \pm 11.54 \text{ ms}$ ($F_{9,20} = 2.935$, P = 0.023; Fig. 4d). This resulted in significant variation in the proportion of nonlinearity per BBV among females ($F_{9,20} = 4.894$, P = 0.0015). This was mostly attributable to variation in the duration of deterministically chaotic segments of vocalizations, since the duration of subharmonic



Figure 3. Event-triggered average number of broadband vocalizations (BBVs) in 10 s bins preceding female escape up the sides of mesh cages (N = 40).





Figure 4. (a) Spectrogram of a female broadband vocalization (BBV). (b–f) Duration of linear and nonlinear components of BBVs across 10 females: (b) total BBV duration, (c) nonlinear segment duration, (d) linear segment duration, (d) subharmonic duration and (e) deterministic chaos segment duration. Colour code: light green = all call components; white = linear segments; dark green = all nonlinear segments; blue = subharmonic segments; yellow = deterministic chaos segments.

segments did not vary significantly among females ($F_{9,20} = 1.837$, P = 0.246; Fig. 4e), but the duration of deterministic chaos did significantly vary among females ($F_{9,20} = 4.699$, P = 0.004; Fig. 4f). Linear segments of BBVs emitted by different females also differed significantly in a measurement of spectral noisiness, the harmonic-to-noise ratio (HNR) ($F_{9,20} = 15.649$, P < 0.0000001), with heavier females emitting BBVs with lower HNRs (Spearman correlation: $r_{\rm S} = -0.649$, N = 10, P = 0.042). There were no significant differences among females in BBV rate ($F_{9,20} = 1.123$, P = 0.391) or fundamental frequency ($F_{9,20} = 1.035$, P = 0.447).

BBV Structure Varies with Oestrous State

We assessed the influence of oestrous phase on female vocalization by monitoring naturally cycling female mice over multiple social encounters. BBVs from 8 of 10 females were measured during both oestrus and dioestrus, allowing a direct comparison of vocal production in these phases, while controlling for individual variation in BBV characteristics among females. Since fewer females were measured in pro-oestrus (N = 5) and the characteristics for pro-oestrous females exhibited large variation, we focused on comparing females when they were in dioestrus versus oestrus. There was no significant effect of oestrous state on BBV rate ($F_{1,17} = 3.515$, P = 0.078) or fundamental frequency ($F_{1,17} = 0.325$, P = 1.000). Females produced BBVs of longer duration during oestrous ($84.46 \pm 3.60 \text{ ms}$) than during dioestrus ($70.60 \pm 5.43 \text{ ms}$), although this difference was not significant after Bonferroni correction ($F_{1,17} = 6.330$, P = 0.088). The absolute duration of linear segments of BBVs also did not differ significantly between oestrus and dioestrus ($F_{1,17} = 0.468$, P = 1.000; Fig. 5a). In contrast, females



Figure 5. Duration of (a) linear and (b) nonlinear segments of female broadband vocalizations (BBVs) during dioestrus and oestrus. For each plot, BBVs were measured in different phases in the same females (N = 8). *P < 0.05.

produced BBVs with nonlinear segments of significantly longer duration during oestrus $(28.43 \pm 4.47 \text{ ms})$ than during dioestrus $(14.65 \pm 2.01 \text{ ms})$ ($F_{1,17} = 7.454$, P = 0.028; Fig. 5b). Thus, females' production of vocal nonlinearities increased during oestrus compared to dioestrus.

Relationship of Male and Female Vocalization to Sexual Behaviours

Patterns of female BBVs over time varied with a key event. whether mounting of females by males occurred. There were significant main effects of both mounting ($F_{1,27} = 7.209, P = 0.012$) and time ($F_{19,533} = 1.629$, P = 0.045), with more BBVs emitted by females that were not mounted, and more BBVs emitted at early time points. The number of BBVs over 20 one-minute bins significantly diverged depending on whether mounting occurred in the social encounter or not (mounting * time interaction: $F_{19,533} = 2.252$, P = 0.002). Fig. 6a illustrates the number of BBVs in 1 min time bins, separated into two groups based on whether BBVs were produced during interactions in which mounting occurred (N = 11) or did not occur (N = 19). Background shading in Fig. 6a illustrates the average number of mounts in 2 min time bins; note that mounting predominantly occurred at later time points. During minutes 2-9, BBV rate was significantly elevated in nonmounting encounters compared to encounters in which mounting did occur (asterisks in Fig. 6a). Furthermore, the trajectories of BBVs between the first and second 10 min time periods significantly differed depending on whether mounting occurred within an interaction (mounting * time interaction: $F_{1,28} = 14.031$, P = 0.001; Fig. 6b). For interactions in which mounting occurred, BBVs increased in the second 10 min relative to the first 10 min (P = 0.004), and in interactions in which mounting did not occur. BBV number decreased from the first 10 min to the second 10 min (P = 0.026). Thus, whether a male mouse mounted the female at later stages of courtship was predicted by earlier female vocalizing, with increased vocalizing at the onset of an encounter negatively associated with mounting. To assess whether this pattern of vocalizing corresponded to female oestrous state, we separated interactions into those in which females were in oestrus versus dioestrus, considering only the females for which data were matched in these two states (N = 8). The time course of BBVs did not significantly differ between oestrous and dioestrous females, however (mounting*time interaction: $F_{19,437} = 0.876$, P = 0.614). The incidence of mounting also did not depend on whether females were in oestrus or dioestrus; mounting was not more likely to occur in either phase (Wilcoxon signedranks test: Z = -0.105, N = 24, P = 0.917).

Because BBV rate did not significantly differ among females, we further explored whether there was a correlation between BBV rate and mounting within the nine interactions in which mounting occurred. There was not a significant correlation between the number of BBVs and the total number of mounts in either the first or second 10 min of the interaction (Spearman rank correlation: first 10 min: $r_{\rm S} = -0.135$, N = 9, P = 0.692; second 10 min: $r_{\rm S} = -0.037$, N = 9, P = 0.915).

As previously described, male mice in the current study produced USVs during their interactions with females (Hanson & Hurley, 2012; Holy & Guo, 2005; Nyby, Wysocki, Whitney, & Dizinno, 1977). When all classes of USVs were considered together, there was no correlation between the number of USVs and the number of mounts across interactions (Spearman rank correlation: $r_{\rm S} = 0.012$, N = 30, P = 0.949; Fig. 7a). When we considered one particular class of USVs (50 kHz harmonic USVs) separately, the number of this USV type did correlate significantly with the number of mounts ($r_S = 0.710$, N = 30, P < 0.001; Fig. 7b). This is expected, since harmonic USVs are associated in time with mounting (Hanson & Hurley, 2012). For interactions in which mounting occurred, there was a close correspondence in time between BBVs and 50 kHz harmonic USVs. An example is illustrated in Fig. 8a, which plots the normalized number of total USVs (dashed grey line), 50 kHz harmonic USVs alone (blue line) and BBVs (red line) per 1 min bins during one interaction. As seen in Fig. 8a, although the male began producing USVs early in the interaction, 50 kHz harmonic USVs did not occur until later, in close correspondence with the incidence of mounting. To establish the extent to which USVs and BBVs overlap when mounting behaviour is prominent (e.g. Fig. 8b, Supplementary Video S2), we used the 'song overlap null model generator' (SONG) tool to analyse the temporal overlap of 50 kHz harmonic USVs and BBVs relative to random distributions of these calls in time (Masco et al., 2016). Analysis was performed on the last 10 min of interactions that resulted in mounting and that had greater than six 50 kHz harmonic USVs and BBVs. Of the nine interactions that met the analysis criteria, eight had significant $(P \le 0.05)$ levels of BBV and 50 kHz harmonic USV overlap when compared to a random distribution. As a contrast, Fig. 8c shows an interaction in which no mounting ever occurred. In this interaction, the male produced USVs, including 50 kHz harmonic USVs, early in the interaction, but these rapidly declined. BBVs occurred at a high rate throughout the interaction, but particularly in the first 11 min, so that BBVs and 50 kHz harmonic USVs were not well synchronized.

The temporal correspondence of BBVs and 50 kHz harmonic USVs could have been driven by the relationship of each to mounting. We have previously reported that 50 kHz harmonic USVs are temporally related to male mounting (Hanson & Hurley, 2012). This was also true for BBVs, as anecdotally reported in a



Figure 6. Time course of broadband vocalizations (BBVs) relative to mounting. (a) Number of BBVs in each time bin relative to the number of mounts per 2 min (*P < 0.05). (b) Trajectory of BBVs in the first 10 min versus the second 10 min during individual interactions in which mounting occurred (left: N = 11) or did not occur (right: N = 19).

previous study (Wang et al., 2008). We quantified the temporal relationship between mounting and vocalizing by separating BBVs that occurred in interactions with mounting into two categories: 'perimounting' BBVs and 'nonmounting' BBVs. BBVs that occurred in the 10 s before and 10 s after a mounting event were designated as 'perimounting'. BBVs that occurred at all other times during the social encounter were categorized as 'nonmounting'. As shown in Fig. 9a, the BBV rate was significantly higher in the 20 s around mounting than at all other times ($F_{1.10} = 36.772$, P = 0.0006). Multiple structural characteristics of BBVs in the perimounting window were also significantly different from BBVs at other times. Perimounting BBVs exhibited an increased duration compared to nonmounting BBVs ($F_{1,1450} = 19.742$, P < 0.0001; Fig. 9b), with a longer nonlinear segment ($F_{1,1450} = 21.079$, P < 0.0001), as well as a higher percentage of nonlinearity ($F_{1,1450} = 7.176$, P = 0.035). Fundamental frequency did not vary between perimounting versus nonperimounting BBVs ($F_{1.1450} = 0.042$, P = 0.838). Thus, the behavioural event of mounting is associated with distinct differences in acoustic features of BBVs.

DISCUSSION

Audible broadband vocalizations (BBVs) are the type of mouse vocalization most commonly encountered by human listeners, but they are also the type about which arguably the least is known. Like ultrasonic vocalizations (USVs), BBVs are used by mice in multiple contexts, including when mice are in distress (Irwin et al., 1971; Matthews et al., 2008). When produced by females interacting with males, BBVs are associated with physical rejection of males (Johansen et al., 2008; Sugimoto et al., 2011). They are also highly variable, containing a large proportion of spectral discontinuities indicative of nonlinear vocal production (Lupanova & Egorova, 2015). We have extended these observations to quantitatively associate audible vocalization production and spectral structure with both female and male behaviours during opposite-sex interactions. We find that structural features of female BBVs correspond to specific events, female identity and oestrous state. Moreover, the production of large numbers of BBVs in the early phases of opposite-sex interactions predicts whether male mounting behaviour later occurs. These findings suggest that variation in female BBVs along multiple dimensions potentially contains useful information for the male social partners of female mice. Furthermore, our findings suggest that female BBVs may be an experimentally tractable readout of female motivational state during intersexual interactions.

Vocal exchanges between males and females may occur for species like birds that vocalize during intersexual interaction (Reichard & Welklin, 2015). For example, male dark-eyed juncos, *Junco hyemalis*, produce a structurally distinct 'slow short-range song' when presented with a female coupled with playback of female calls (Reichard, Rice, Schultz, & Schrock, 2013), while female canaries, *Serinus canaria*, and white-crowned sparrows, *Zonotrichia leucophrys*, produce trills in conjunction with visual copulation solicitation displays in response to playback of male song (Amy, Salvin, Naguib, & Leboucher, 2015; Maney, MacDougall-Shackleton, MacDougall-Shackleton, Ball, & Hahn, 2003). Bird species that engage in male–female duets, like plain-tailed wrens, *Pheugopedius euophrys*, coordinate rapid alternations of male and female syllables by responding to acute vocal cues from their partners (Fortune, Rodriguez, Li, Ball, & Coleman, 2011). Female



Figure 7. Total number of mounts relative to (a) the total number of all ultrasonic vocalizations (USVs) and (b) the number of 50 kHz harmonic USVs. Inset spectrograms illustrate call types measured for each plot.

South African clawed frogs, *Xenopus laevis*, may either elicit male vocal responses through receptive 'rapping' calls, or suppress male vocalizations through 'ticking' calls (Elliott & Kelley, 2007; Tobias, Viswanathan, & Kelley, 1998). During mouse courtship, USVs emitted by males and BBVs emitted by females may serve opposing functions as an exchange of vocal information, with USVs eliciting female approach to the male (Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2009; Pomerantz, Nunez, & Bean, 1983; Shepard & Liu, 2011) and BBVs facilitating male withdrawal from the female (current study). Here, we discuss the attribution of BBVs to female mice, the aspects of female BBVs that carry potential information, the specific types of functions that BBVs could serve and whether the BBVs of female mice could contribute to an exchange of information with males.

The Attribution of BBVs to Females

Our assertion that BBVs are predominantly generated by females rests on several lines of evidence. First, past studies in which one social partner, either male or female, was prevented from vocalizing either by devocalization, by being anaesthetized, or by replacing live partners with olfactory stimuli, strongly support a male origin for USVs and a female origin for BBVs/squeaks, although the latter type of vocalization has been less well studied (Holy & Guo, 2005; Lupanova & Egorova, 2015; Wang et al., 2008; White et al., 1998). This generalization comes with the important caveat that in a recent study of mice in complex social environments, females produced ultrasonic vocalizations of their own in conjunction with proreceptive behaviour (Neunuebel et al., 2015). Second, in our own data, several pieces of evidence support a male



Figure 8. (a) Time course for normalized values for all ultrasonic vocalizations (USVs), 50 kHz harmonic USVs and broadband vocalizations (BBVs) during one interaction in which mounting occurred. Crosses indicate time bins in which the male mounted the female. (b) Example of overlap between harmonic USVs and BBVs. (c) Time course of all USVs, harmonic USVs and BBVs during an interaction in which no mounting occurred.

origin for USVs and a female origin for BBVs. BBVs are closely coupled in time with female behaviours, including male-directed actions (Supplementary Video S1). Additionally, if males and females were both regularly producing USVs, or were both regularly producing BBVs, these similar types of signals might overlap in time. We never observed such an overlap. Instead, we regularly observed overlap between unlike calls, USVs and BBVs (Fig. 8b), suggestive of different vocal production by different sexes. All of these arguments, however, are probabilistic. Thus, although all of our available evidence strongly supports predominantly female production of BBVs and predominantly male production of USVs in our study, we cannot claim with absolute certainty that this is the case for every individual vocalization.



Figure 9. Comparison of the (a) number and (b) rate of broadband vocalizations (BBVs) produced 10 s before or after mounting (perimounting) relative to BBVs produced at other times (nonmounting). **P < 0.001.

Nonlinearities in Female BBVs

Vocal nonlinearities were the most variable of the structural characteristics of BBVs that we measured. Across mammals, nonlinearities are common features of vocalizations. Nonlinearities are produced by often abrupt transitions of vocal cords to irregular modes of vibration, and may take the form of frequency jumps, subharmonics consisting of doubled or tripled numbers of harmonics, deterministic chaos or structured noise, or biphonation, which is the production of two nonharmonically related frequencies that may differ in trajectory (Fitch et al., 2002; Wilden et al., 1998). Abrupt vocal nonlinearities can be caused by continuous changes in input variables to the vocal tract such as airflow or vocal cord tension, so are potentially markers for affective influences on these input variables (Fee et al., 1998).

In female mice, vocal nonlinearities occur in a majority of BBVs, the most common types being deterministic chaos and subharmonics (Lupanova & Egorova, 2015). The total and relative durations of nonlinearities contained within BBVs in the current study were associated with specific characteristics of female mice. Most strikingly, there were large differences in the duration of deterministically chaotic segments among females that were consistent even when females interacted with different males, or across different oestrous phases. In terms of absolute durations, these differences outweighed interindividual differences in linear or subharmonic segments. Thus, the duration of nonlinear segments, or their ratio to linear segments, are potentially high-quality sources of information about female identity. In addition to interfemale variation, oestrous phase was associated with variation in the absolute and relative durations of nonlinearities, where females in oestrus emitted BBVs with longer-duration nonlinear segments. This places female mice within a large group of female vertebrates that alter the number or structure of vocalizations across the oestrous cycle or in response to exogenous ovarian hormones (Charlton, Keating, Rengui, Huang, & Swaisgood, 2010; Kim et al., 2010; Matochik, Barfield, & Nyby, 1992; Moles, Costantini, Garbugino, Zanettini, & D'Amato, 2007).

Whether male mice behaviourally respond to nonlinearities in female mouse BBVs is unknown. However, mice are able to discriminate among spectrally dissimilar ultrasonic vocalizations based on a different type of nonlinearity, a frequency jump (Neilans et al., 2014). Mice even demonstrate preferences among USVs based on the relatedness, familiarity and species of the vocalizer (Musolf, Hoffmann, & Penn, 2010; Musolf, Meindl, Larsen, Kalcounis-Rueppell, & Penn, 2015) as well as spectral complexity of USVs (Chabout, Sarkar, Dunson, & Jarvis, 2015). This suggests that mice could use information conveyed in vocal signals during behavioural interactions, such as the nonlinear features of female BBVs.

Many studies suggest that nonlinearities may convey important information for a range of mammalian species. In rhesus macaques, Macaca mulatta, nonlinearities may be a signal of fitness, and males that produce calls with nonlinearities are more likely to mate multiple times (Fitch et al., 2002; Hauser, 1993). In contrast, the ability to suppress nonlinearities near the frequency limits of vocal performance may signal fitness in male chimpanzees, Pan troglodytes (Riede et al., 2007). Nonlinearities are enriched in calls that signal aggression, as in big brown bats, Eptesicus fuscus (Gadziola, Grimsley, Faure, & Wenstrup, 2012). Calls with increased nonlinearity also facilitate attention to the vocalizer. For example, marmot pup vocalizations that contain nonlinearities ('screams') attract adult attention with particular efficacy (Blumstein et al., 2008) and marmot alarm calls with segments of synthetic noise mimicking deterministic chaos reduce habituation (Blumstein & Récapet, 2009). For meerkats, Suricata suricatta, vocal nonlinearities reduce the behavioural habituation of group members to alarm calls (Karp et al., 2014). Thus, nonlinearities in vocalization likely make an important contribution to communication exchanges across mammalian species.

Function of Female BBVs

Female mice, when presented with tethered males, pace sexual interactions by leaving and returning to their social partners (Johansen et al., 2008). In the absence of the opportunity to temporarily leave male partners, a suite of female behaviours that couples vocalizing with physical rejection is proposed to serve a parallel pacing function. Multiple aspects of the relationship between female BBVs and nonvocal behaviours observed in the current study support the hypothesis that vocalizations are a component of pacing behaviour. First, BBVs produced by females were closely associated in time with kicking at males, openmouthed lunging at males, or darting away from males, as previously reported (Sugimoto et al., 2011). This close relationship likely drove the significant positive correlation between the number of female BBVs and nonvocal rejection behaviours across interactions, which was evident even for single females across interactions with different males (Fig. 2). Second, our mesh cages allowed the females in our study to temporarily escape from male partners by retreating up the sides of the cage, where males did not follow, making this an effective pacing paradigm. On average, female retreats were preceded by an escalating series of BBVs, so that there was a close association between BBVs and voluntary female escape. Finally, whether male mounting occurred at later time points coincided with significant differences in the amount of female vocalizing at earlier time points, when a high incidence of vocalizing predicted a low level of later mounting. All of these findings provide strong circumstantial evidence that female vocalizing and other rejection behaviours play a role in the time course and outcome of sexual interaction.

BBVs produced in the second half of interactions did not conform to this pattern. Rodent sexual behaviour may be separated into multiple distinct phases that have been defined by male behaviour (Pierce, Sawrey, & Dewsbury, 1989). The first of these, consisting of the time period up to the first male intromission, has been called the intromission latency (IL) phase. A second phase, between the first intromission and male ejaculation, has been called the ejaculation latency (EL) phase. A third phase, termed postejaculatory, is one that we did not observe in most of our 20 min interactions, and is associated with low levels of vocalizations in male mice (Nyby, 1983). The behaviours of males in the current study reflected the first two of these stages, with an increased incidence of mounting, and of mounting-associated 50 kHz harmonic USVs, in the second half of the 20 min interactions. Female BBVs at different times showed different relationships to these male behaviours, characterized by one of two general patterns corresponding to whether mounting by males occurred. In the first pattern, described earlier, a high number of BBVs in the first half of the interaction was associated with decreased male mounting in the second half. In a second pattern, later BBVs occurred at a higher rate and showed distinctive spectrotemporal structure around mounting. These later BBVs also overlapped in time with 50 kHz harmonic USVs. The different patterns of BBVs in the early and late phases of male-female interactions raise the interesting possibility that BBVs produced in later stages of the interaction are functionally distinct from earlier BBVs.

The progression of vocal and nonvocal behaviour over the phases of courtship is paralleled by physiological changes in the auditory system. A recent study monitored changes in serotonin levels in an auditory midbrain region, the inferior colliculus (IC), of male mice interacting with females (Keesom & Hurley, 2016). Serotonin increased on a gradual timescale consistent with the ejaculation latency phase, so that serotonin was significantly increased from baseline by 12 min following the introduction of a female and remained elevated for the rest of the encounter. Interestingly, the level of serotonergic activity was not correlated with the behaviour of subject males, including the production of USVs, but was inversely correlated with the rejection behaviour of the female partner, including the production of BBVs. Thus, serotonergic activity in the male mouse IC reflects the valence of a particular interaction from the perspective of a male. Because serotonin modulates the responses of IC neurons to auditory stimuli including vocalizations (Hurley & Pollak, 1999, 2005), increases in serotonin during later but not earlier phases of courtship could selectively change how later-phase BBVs are processed in auditory regions.

How Could BBVs Influence Male Behaviour?

Because female BBVs are typically performed as one component of a suite of rejection behaviours, and because we did not independently manipulate the presentation of BBVs, we cannot distinguish whether the behavioural correlations we observed were driven by BBVs alone. For example, whether males mounted females or not could be driven by kicking and lunging. Alternatively, a signal component that we did not measure could underlie both female rejection and male mounting. Furthermore, any influence of BBVs on male behaviour could take multiple forms. One possibility is that BBVs, as high-intensity and close-range signals that are coordinated in time with kicking or lunging (Supplementary Video S1), could reinforce the message of acute rejection. BBVs could also provide specific information on relevant factors such as female oestrous state, acute motivational state or female identity.

Consistent with the hypothesis that BBVs can influence male behaviour, male mice respond to playbacks of BBVs, albeit in a context-dependent manner. Males more often approach speakers playing BBVs ('low-frequency harmonic' vocalizations) when these are paired with female odour than when the same playbacks are paired with cat odour (Grimsley et al., 2013). This finding complements other evidence that multimodal influences create context-appropriate auditory responses in mice. For example, auditory potentials of mice are influenced by behaviourally salient odours like fox scent (Halene, Talmud, Jonak, Schneider, & Siegel, 2009). In a social context, mice may use vocal as well as olfactory information to identify reproductive partners of optimal relatedness (Asaba, Hattori, Mogi, & Kikusui, 2014). Taken together, these studies suggest that multimodal synthesis is important to the interpretation of vocal signals in mice. Acoustic signals from females during interactions with males may therefore interact with other modalities such as olfaction or somatosensation. Our finding, that BBVs at different phases of a social interaction are associated in different ways with courtship behaviour, further suggests that the short-term behavioural context surrounding vocalizations could influence the salience of female vocal signals for males, even at different time points across an interaction.

Conclusions

Male mice show robust responses to olfactory cues of female urine and bedding with behaviour indicative of arousal, including the production of USVs (Holy & Guo, 2005; Nyby et al., 1977). The structure of USVs can be influenced by female cues, such as those indicating oestrous phase (Hanson & Hurley, 2012). Given our finding that BBVs produced by female mice may signal acute motivation during intersexual interaction, we propose a model in which olfactory-mediated arousal of male mice can be modified by short-term tactile and auditory signals from females. In the initial phase of an opposite-sex interaction, a high incidence of BBVs corresponds to subsequent male serotonergic neurophysiology and decreased male arousal. Nonlinear structural elements of BBVs further have the potential to provide specific information on female identity and oestrous state. The qualities of BBVs that carry potential information, including the rate of production and the duration of nonlinear segments, are relatively easily measured, making them potentially suitable for automated analysis. As human-audible signals, BBVs can be recorded without the use of specialized ultrasonic acoustic equipment, which could also facilitate their use in educational settings.

Acknowledgments

We thank Mr Patrick Ransbottom for assistance with analysis of ultrasonic vocalizations and Mr Bradley Wise for assistance with formatting figures. We also thank Ms Christina Masco for allowing us to use her 'SONG' tool and assisting us in applying it to our call data. This work was supported in part by funding from the Oak Ridge Associated Universities and by the Center for the Integrative Study of Animal Behavior at Indiana University.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav. 2017.01.021.

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