



Vocal behaviour during aggressive encounters between Siberian hamsters, *Phodopus sungorus*



Sarah M. Keesom^{a, b, *, 1}, Nikki M. Rendon^{a, b, 1}, Gregory E. Demas^{a, b, c},
Laura M. Hurley^{a, b, c}

^a Department of Biology, Indiana University, Bloomington, IN, U.S.A.

^b Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN, U.S.A.

^c Program in Neuroscience, Indiana University, Bloomington, IN, U.S.A.

ARTICLE INFO

Article history:

Received 21 October 2014

Initial acceptance 11 November 2014

Final acceptance 18 December 2014

Available online 5 February 2015

MS. number: A14-00848R

Keywords:

acoustic analysis

agonism

call structure

dominance

same-sex aggression

vocal repertoire

Vocalizations constitute an important channel of communication for many vertebrates. Classes of vocalizations may be closely associated with particular contexts or behaviours, and variation within classes may convey information on individual identity, sex or motivational state. Rodent vocal communication has largely been studied within a reproductive context, but rodents also utter vocalizations during aggressive encounters with same-sex conspecifics. In this work we investigated same-sex vocal behaviour of Siberian hamsters, a mammalian model species for studying aggression. Males and females produced two main classes of vocalizations: high-frequency (>20 kHz), narrowband vocalizations (ultrasonic vocalizations; USVs) and lower-frequency, broadband vocalizations (broadband calls; BBCs). USVs and BBCs were further classified into subtypes based on spectrotemporal characteristics. With these classifications, we made the predictions that hamsters would utter distinct subcategories of calls, that there would be sex differences in call usage, and that BBCs would be more closely associated with aggressive behaviours than USVs. While there were no sex differences in the total number of USVs or BBCs produced by a pair, the use of vocalization subcategories varied by sex, with females uttering more variable USVs and more 'rattle' BBCs than males. In conjunction with these differences in vocal behaviour, the sexes also differed in aggression. Across both sexes, variation in aggressive behaviour correlated with variation in the number of 'squeak' BBCs and 'rattle' BBCs, whereas USVs were not related to aggression. Thus, BBCs constitute a distinct vocalization type with an important role in aggressive communication for hamsters.

© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Vocalizations are an important facet of multimodal communication for many vertebrates, since relatively rapid vocal transmission provides a complement to slower signalling modes such as chemical communication. Across vertebrate species, spectral and temporal variations in vocal signals can carry considerable information about the physical traits or motivational state of the sender (August & Anderson, 1987; Nowicki & Searcy, 2004; Pasch, George, Campbell, & Phelps, 2011; Scheuber, Jacot, & Brinkhof, 2004; Wells & Schwartz, 2006). Variation in vocal structure may also correspond to behavioural context, sex, or even to specific social behaviours. For example, males of some passerine bird species produce calls with different characteristics when females are in

close proximity compared to calls produced for long-range advertisement (Anderson, Searcy, Peters, & Nowicki, 2008; Reichard & Welkin, 2014; Titus, 1998). When in same-sex pairs, male and female house mice produce indistinguishable vocalizations; however, in opposite-sex pairs, males and females produce distinct calls, some of which are linked to specific courtship behaviours (Nyby, 2001; Wang, Liang, Burgdorf, Wess, & Yeomans, 2008). In aggressive contexts, vocalizations can also signal crucial information. For instance, male grey treefrogs, *Hyla versicolor*, alter the frequency of their calls depending on the intensity of the aggressive encounter (Reichert & Gerhardt, 2013). A conceptual framework relating variation in vocalization structure to behaviour is Morton's (1977) motivational-structural rules hypothesis. This hypothesis postulates that, between conspecifics in close proximity, submissive or affiliative motivation is signalled by high-frequency or tonal vocalizations, while aggressive motivation is signalled by low-frequency or spectrally dense vocalizations. Here, we investigate

* Correspondence: S. M. Keesom, Department of Biology, Indiana University, 1001 E. 3rd Street, Bloomington, IN 47405, U.S.A.

E-mail address: skeesom@indiana.edu (S. M. Keesom).

¹ Equal contributors.

the correspondence between same-sex aggression and vocalization structure in a rodent model for aggressive behaviour, Siberian hamsters, *Phodopus sungorus*.

Siberian hamsters inhabit the short-grass steppes of central and eastern Asia, a relatively extreme habitat with dry summers and frigid winters (Ross, 1998). Resource scarcity in this habitat is accompanied by high levels of aggression (Ross, 1998). In the wild, Siberian hamsters occur at a relatively low population density (1–6 hamsters/km²), where same-sex hamsters occupy adjacent, nonoverlapping home ranges (Wynne-Edwards, 1995, 2003). Agonistic behaviours may be important in maintaining these home ranges, as male and female hamsters exhibit high levels of aggression during same-sex encounters staged in the laboratory (Jasnow, Huhman, Bartness, & Demas, 2000; Scotti, Place, & Demas, 2007). Collectively, field observations and laboratory studies therefore depict aggression as a prominent feature of Siberian hamster social interactions.

The degree to which vocalizations complement these aggressive behaviours has not been explored. Siberian hamsters produce vocalizations spanning a wide range of frequencies (Sales, 2010). Ultrasonic vocalizations (USVs) are high-frequency (>20 kHz), narrowband calls uttered during heterosexual and same-sex agonistic encounters (Sales, 2010). Like most rodents, Siberian hamsters produce another class of vocalizations in addition to USVs: broadband calls (BBCs; ranging from 2 to 100 kHz). Neither USVs nor BBCs uttered during agonistic interactions have been studied in detail for this species. Furthermore, as these hamsters exhibit a sex difference in aggression during same-sex encounters (Wynne-Edwards & Lisk, 1987), the use of vocal communication within this context may differ between sexes.

Thus, in this study, we investigated the broadband and ultrasonic vocalizations produced by Siberian hamsters during agonistic encounters. We had three major objectives, with corresponding predictions related to work in other rodent species or derived from broad hypotheses on the relationship between spectrotemporal structure and call function. The first objective was to describe ultrasonic and broadband vocalizations in terms of their spectrotemporal characteristics, which have not previously been reported. We predicted that, as for other rodent species, we would find structurally distinct subcategories of vocalizations (Holy & Guo, 2005). Second, we determined whether males and females differed in vocal behaviour, with the prediction that males and females would differ in the usage of particular types of vocalizations, as do some other rodent species (Floody & Pfaff, 1977a; Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2012). Finally, we determined whether specific types of vocalizations were related to displays of aggression, and whether the two broad categories of vocalizations, USVs and BBCs, differed in their relationships to agonistic behaviour. Consistent with Morton's motivational-structural rules hypothesis, our prediction was that BBCs would be associated with aggressive acts.

METHODS

Animal Use

Adult (>60 days of age) Siberian hamsters were obtained from a breeding colony at Indiana University. Hamsters were bred and maintained on a 16:8 h light:dark cycle (lights on at 0400 hours Eastern Standard Time, EST) and group-housed at weaning (post-natal day 18). Upon entering the study, resident hamsters (females, $N = 14$; males, $N = 14$) were singly housed, and intruder hamsters (females, $N = 6$; males, $N = 6$) were pair-housed in solid-bottom polypropylene cages (27.5 × 17.5 × 13.0 cm) with Sani-chip bedding material, under the same light:dark conditions (long

days) for 10 weeks. We used separate sets of male ($N = 6$) and female ($N = 6$) hamsters for control experiments in which we made audio recordings from individuals alone in a cage. Ambient temperature was maintained at $20 \pm 2^\circ\text{C}$, and relative humidity was maintained at $55 \pm 5\%$. Hamsters were given ad libitum access to tap water and standard laboratory rodent chow (Lab Diet 5001, PMI Nutrition) throughout the experiment. Female oestrous cycles were monitored via vaginal cytology (Moffatt-Blue, Sury, & Young, 2006; Scotti et al., 2007). All females were cycling normally at the time of testing. However, we did not account for oestrous phase when pairing our female hamsters or in our analysis, as oestrous phase does not influence agonistic behaviour in female Siberian hamsters (Scotti et al., 2007). After collection of behavioural data, animals were given a lethal dose of a ketamine/xylazine cocktail, followed by a physical means of assurance, and reproductive tissues were collected to confirm competent reproductive state. All procedures were approved by the Indiana University-Bloomington Institutional Animal Care and Use Committee.

Audio/video Recording

We placed the resident hamster's home cage in a sound-attenuation chamber and positioned a microphone above the cage. We then recorded hamster vocalizations with a condenser microphone (CM16/CPMA, Avisoft Bioacoustics, Berlin, Germany) and sound card (UltraSoundGate 116 Hb, Avisoft Bioacoustics) with a sample rate of 250 kHz, allowing us to record vocalizations with frequencies of up to 125 kHz. Spectrograms were generated from audio files for subsequent analysis. We conducted all video-recording under low-illumination, red lights, with a video camera (Sony Handycam HD R-SR7) positioned in front of the cage. Trained observers used ODLog™ (Macropod Software, Eden Prairie, MN, U.S.A.) to score videos for nonvocal behaviours.

Same-Sex Social Encounters

We staged 5 min resident–intruder encounters (male: $N = 14$ pairs; female: $N = 14$ pairs) between hamsters of the same sex, same mass (within 10%), and different parents within the first 2 h of the dark phase (Jasnow et al., 2000; Scotti et al., 2007). The intruder hamster was introduced to the resident's home cage, which had not been changed for 7 days to allow the hamster to establish residency (Brain, 1975; Brain & Poole, 1974). We used each intruder no more than two times per testing day. If aggressive behaviours were to escalate to the point of physical injury, we were prepared to halt the behavioural test immediately. Interventions were never necessary, as none of the staged social encounters resulted in injury.

In addition, we conducted a set of control experiments to determine whether resident or intruder hamsters of either sex vocalize when alone in a cage. Audio and video were recorded from a resident hamster (male: $N = 3$; female: $N = 3$) alone in its home cage for 5 min. Following this period, we removed the resident hamster from its cage and introduced a same-sex intruder hamster (male: $N = 3$; female: $N = 3$). Five minutes of audio and video were then recorded of the intruder hamster, which was alone in the resident's cage.

Vocalization Analysis

Spectrograms of hamster vocalizations were generated using Avisoft SASLab Pro software (FFT-length of 512 and Hamming style window with 50% overlap; Avisoft Bioacoustics). We classified vocalizations into two main types based on spectral content: ultrasonic vocalizations (USVs) and broadband calls (BBCs). We separated vocalizations into these categories, as this classification is behaviourally

relevant for other rodent species (e.g. mice: Sugimoto et al., 2011). USVs were characterized as high-frequency (>20 kHz), narrowband calls. We measured several spectrotemporal parameters of USVs, including duration (ms), start frequency (kHz), end frequency (kHz), and mean frequency (kHz) per call. We also calculated the frequency modulation coefficient ((end frequency – start frequency)/duration of call)) for each call to assess the rate of frequency modulation. We additionally classified USVs into four subtypes based on the presence or absence of frequency jumps and harmonic segments, features which are perceptually discriminable by rodents (Neilans, Holfoth, Radziwon, Portfors, & Dent, 2014). A trained observer visually identified calls with harmonic components, and we confirmed this classification via quantitative analysis demonstrating that calls with harmonic components have a lower fundamental frequency at the centre of the call than calls without an identifiable upper harmonic (harmonic: 47.7 ± 0.7 kHz; nonharmonic, 61.5 ± 0.7 kHz; two-sample *t* test: $t_{89} = 15.15$, $P < 0.001$). (1) 'Plain' calls were characterized by an absence of frequency jumps and no discernible harmonic frequencies. (2) 'Jump' calls were characterized by the presence of at least one abrupt break in frequency, without a harmonic segment. We counted the number of jumps in frequency as an additional parameter for this call type. (3) 'Harmonic' calls were characterized by the presence of harmonics and the absence of abrupt jumps in frequency. (4) 'Complex' calls were characterized by the presence of harmonic segments and at least one frequency jump. We counted the number of jumps in frequency as an additional parameter for this call type.

We defined BBCs as vocalizations spanning a wide range of frequencies (2–100 kHz). We counted the number of BBCs and measured the duration (ms) of each call. A trained observer visually classified BBCs into three subtypes based on spectral character: (1) 'squeaks' were characterized as calls with discrete stacks of harmonic frequencies; (2) 'rattles' were characterized as calls with rapidly occurring, pulsatile bursts of noise and (3) 'mixed BBCs' were characterized as calls that featured distinct harmonically structured segments and pulsatile, noisy segments.

Nonvocal Behaviour Analysis

We measured aggression, submission and social investigation for all hamster pairs. For aggressive behaviour, we defined attacks, chases and latency to initial attack according to previous studies focused on same-sex aggression in both male (Jasnow et al., 2000) and female (Scotti et al., 2007) Siberian hamsters. We used the numbers and durations of attacks and chases, as well as latency to initial attack, to generate an aggression score for each dyad using a principal components analysis (PCA). The PCA extracted one component that explained 78.77% of the total variance in aggressive behaviour for both resident and intruder (Table 1). All measures of aggression loaded strongly onto PC1; therefore, we used PC1 as a pair's aggression score for subsequent analyses. We defined submission as being pinned down, or displaying either a supine or 'paws-up' posture. We used numbers and durations of submissive acts to calculate a submission score for each behavioural interaction using a PCA. The PCA extracted one component that explained 76.67% of the total variance in submissions for both resident and intruder (Table 1). Both measures loaded strongly onto PC1; therefore, we used PC1 as a pair's submission score for subsequent analyses. As a measure of nonagonistic social behaviour, we also scored interactions for social investigation, which we defined as the nose of one hamster in contact with the facial or anogenital region of the other. We used numbers and durations of social investigation to calculate an investigation score for each dyad using a PCA. The PCA extracted one component that explained 90.9% of the total variance in social investigation (Table 1). Both measures loaded

Table 1

Principal components (PC) loadings and eigenvalues for summed resident and intruder aggression, submission and social investigation behaviours of Siberian hamsters

Behaviour	PC1
Aggression	
Attack number	0.96
Attack duration	0.88
Chase count	0.94
Chase duration	0.95
Latency to initial attack (s)	−0.69
Eigenvalue	3.94
% Variance explained	78.77
Submission	
Submission count	0.88
Submission duration	0.88
Eigenvalue	1.53
% Variance explained	76.67
Social investigation	
Investigation count	0.95
Investigation duration	0.95
Eigenvalue	1.82
% Variance explained	90.9

Bold values indicate variables that loaded strongly within the component (≤ -0.5 or ≥ 0.5).

strongly onto PC1; therefore, we used PC1 as a dyad's social investigation score for subsequent analyses.

Statistical Analysis

All statistical analyses were performed in JMP v. 10.4 (SAS Institute, Inc., Cary, NC, U.S.A.) or SPSS v. 20.0 (IBM Corp., Armonk, NY, U.S.A.). Because of non-normality of the data, we used Mann–Whitney *U* tests to compare numbers of USVs and BBCs produced by males and females. To compare proportional use of USV and BBC subtypes between sexes, we conducted Pearson's chi-square tests of independence on the summed data across interactions because USVs are produced rarely, followed by Bonferroni-corrected *z* tests for pairwise comparisons between uses of particular vocalization subtypes. We conducted two-tailed, two-sample *t* tests to test for sex differences in aggression, submission and investigation behavioural scores of male and female hamster pairs. To explore relationships among the vocal subtypes, we used a PCA and extracted components with eigenvalues greater than 1. Variables that loaded strongly (≤ -0.5 or ≥ 0.5) within a component confirmed relationships between those call types. Because the behavioural data did not conform to normality, even after transformation, we used Spearman rank correlations to quantitatively assess relationships within vocal classes and between vocal and nonvocal behaviours. Reported *P* values were adjusted to control for the false discovery rate when making multiple comparisons (Verhoeven, Simonsen, & McIntyre, 2005).

RESULTS

Characterization of Siberian Hamster Vocalizations during Same-Sex Encounters

Our first prediction was that Siberian hamsters would produce distinct subcategories of vocalizations within the broader categories of USVs and BBCs. To address this, we recorded vocalizations from same-sex resident–intruder pairs of Siberian hamsters during an aggressive encounter. We used spectrographic analysis to characterize the vocal repertoires of male and female Siberian hamsters. Both female and male hamsters produced USVs and BBCs during

same-sex encounters (Fig. 1). Thirteen of 14 female pairs uttered vocalizations of some type. Within vocalizing female pairs, one pair produced only USVs, five pairs produced only BBCs and seven pairs produced both types. Ten of 14 male pairs uttered vocalizations of some type. Within vocalizing male pairs, one pair produced only USVs, four pairs produced only BBCs and five pairs produced both types. Neither sex produced any vocalizations (USVs or BBCs) as residents alone in a cage (male: $N = 3$; female: $N = 3$), or as intruders (male: $N = 3$; female: $N = 3$) alone in a same-sex resident's cage.

During 5 min same-sex encounters, hamsters produced USVs less frequently than BBCs. Hamsters produced a total of 162 USVs: females produced 114 USVs, whereas males produced 48 USVs. Of pairs that produced USVs ($N = 14$), hamsters produced a mean \pm SE of 13.35 ± 3.57 USVs. Female pairs ($N = 8$) produced 14.25 ± 5.30 USVs and male pairs ($N = 6$) produced 8.00 ± 4.49 USVs. In contrast, hamsters produced a total of 817 BBCs during same-sex pairings: females produced 588 BBCs and males produced 229 BBCs. Within pairs that produced BBCs ($N = 21$), hamsters produced 38.90 ± 8.80 BBCs. Of these, female pairs ($N = 12$) produced 49.00 ± 14.30 BBCs and males ($N = 9$) produced 25.44 ± 6.11 BBCs.

We measured three spectrotemporal variables of the hamsters' vocalizations: frequency (kHz), frequency modulation coefficient (kHz/s) and duration (ms) of USVs. USVs ranged from 33.60 kHz to 91.00 kHz, with a mean frequency of 59.838 ± 7.12 kHz, and mean frequency modulation coefficient of -6.42 ± 0.922 kHz/s ($N = 162$). USVs had a mean duration of 92.88 ± 5.81 ms ($N = 162$). We further classified USVs into four subtypes based on spectrographic structure (Fig. 1a): 57.41% were plain calls, 25.31% were jump calls, 8.02% were harmonic calls and 9.26% were complex calls. In addition, we

measured the duration of BBCs. On average (\pm SE), BBCs were 198.29 ± 5.43 ms in duration. We further classified BBCs into three subtypes, based on spectrographic appearance (Fig. 1b): 33.42% were squeaks, 64.99% were rattles and 1.59% were mixed calls.

Sex differences in BBC Duration, Proportional Use of Call Subtypes and Aggression

To assess our prediction that male and female hamsters would differ in vocal behaviour, we compared spectrographic parameters and subtype usage of USVs and BBCs between male and female same-sex pairs (Tables 2, 3). While there was no sex difference in the mean duration of USVs (females: 99.71 ± 7.28 ms; males: 76.79 ± 9.18 ms; Mann–Whitney U test: $U = 2271.5$, $N_1 = 114$, $N_2 = 48$, $P = 0.104$), frequency of USVs (females: 59.47 ± 0.90 kHz; males: 59.698 ± 1.11 kHz; $U = 2526.0$, $N_1 = 114$, $N_2 = 48$, $P = 0.492$), or frequency modulation coefficients of USVs (females: -15.74 ± 17.07 kHz/s; males: 7.24 ± 17.295 kHz/s; $U = 8930.0$, $N_1 = 114$, $N_2 = 48$, $P = 0.411$; Table 2), the sexes did differ in the duration of BBCs. Female BBCs were 206.58 ± 6.4 ms and male BBCs were 177.00 ± 10.20 ms ($U = 58283.0$, $N_1 = 588$, $N_2 = 229$, $P = 0.003$; Table 3).

While there was no sex difference in the number of USVs produced by hamster pairs (Mann–Whitney U test: $U = 38.5$, $N_1 = 8$, $N_2 = 6$, $P = 0.43$) or in the number of BBCs produced by pairs ($U = 102.0$, $N_1 = 12$, $N_2 = 9$, $P = 0.07$), the sexes did differ in proportional use of subtypes of both main vocalizations classes. Males and females differed in the proportional use of USV subtypes (Pearson's chi-square test of independence: $\chi^2_3 = 9.718$, $P = 0.021$; Fig. 2a). Males produced a significantly greater proportion of plain

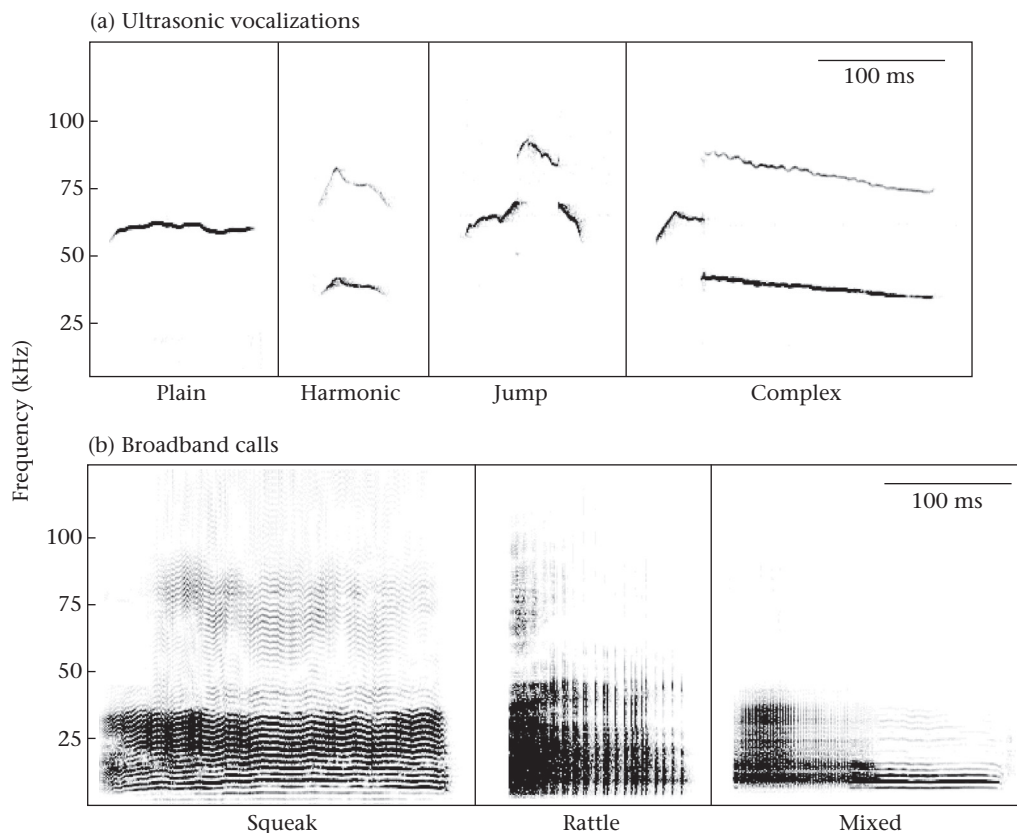


Figure 1. Spectrograms of representative vocalizations produced by Siberian hamsters during same-sex social encounters. (a) Ultrasonic vocalizations were classified based on the presence of harmonic segments or jumps in frequency, yielding four subtypes: 'plain', 'harmonic', 'jump' and 'complex'. (b) Broadband calls were divided into three subtypes based on spectral character: 'squeak', 'rattle' and 'mixed'. Additional details regarding characterization of vocalization subtypes are included in the Methods.

Table 2

Spectrotemporal parameters of ultrasonic vocalization (USV) subtypes produced by female and male Siberian hamsters

USV subtype	N	Mean frequency \pm SE (kHz)	FM coefficient \pm SE (kHz/s)	Duration \pm SE (ms)	% 1FJ	% >1FJ
Female						
Plain	58	58.44 \pm 1.37	–15.16 \pm 29.63	69.34 \pm 8.81	—	—
Jump	29	59.71 \pm 1.26	–0.10 \pm 0.04	123.45 \pm 13.74	48.28	51.72
Harmonic	13	65.28 \pm 3.09	–68.92 \pm 68.79	119.85 \pm 20.66	—	—
Complex	13	57.70 \pm 2.22	–0.06 \pm 0.05	162.08 \pm 19.46	84.62	15.38
Male						
Plain	34	60.10 \pm 1.40	10.35 \pm 24.50	65.18 \pm 9.93	—	—
Jump	12	59.69 \pm 1.49	–0.03 \pm 0.11	101 \pm 18.2	83.33	16.67
Harmonic	0	—	—	—	—	—
Complex	2	52.95 \pm 0.75	–0.67 \pm 0.51	129 \pm 97	0	100

%1FJ indicates the percentage of calls within 'jump' and 'complex' call categories with only one jump in frequency. %>1FJ indicates the percentage of calls within 'jump' and 'complex' call categories with greater than one jump in frequency.

calls (71%) than females (52%) (z score analysis: $P < 0.05$), and females produced a greater proportion of harmonic calls (11%) than males (0%) (z score analysis: $P < 0.05$). Males and females did not differ in their use of frequency jump or complex calls. Proportional use of BBCs also differed between sexes (Pearson's chi-square test of independence: $\chi^2_2 = 69.57$, $P < 0.001$; Fig. 2b). Females uttered more rattles (73%) and fewer squeaks (25%) than males; males uttered rattles 44% of the time, and squeaks 55% of the time (z score analysis: $P < 0.05$). Males and females did not differ in the proportional use of mixed calls, which were rare.

In conjunction with the sex differences in duration of BBCs and proportional use of ultrasonic and broadband call types, there was a sex difference in aggression. Female pairs displayed more aggression than males (two-sample t test: $t_{20} = -2.39$, $P = 0.01$), as determined by comparing PC_{AGG} of the pair between the sexes (Fig. 3). In contrast, the sexes did not differ in displays of submission ($t_{20} = -0.93$, $P = 0.36$) or social investigation ($t_{20} = -0.63$, $P = 0.53$).

Hamster BBCs are Strongly Associated with Aggression

We explored how vocalizations uttered during same-sex encounters of male and female hamsters were related to nonvocal behaviours, with the prediction that BBCs would be related to aggressive acts. We first conducted an exploratory PCA on the seven call subtypes (four USV subtypes and three BBC subtypes) to determine how vocalization subtypes group together. We obtained two principal components that explained a total of 71.26% of the variance (Table 4). Contrary to our classification of calls as USVs or BBCs, the subtypes did not divide into principal components along these broad categories. All USV subtypes and squeak BBCs loaded strongly together onto PC1, whereas rattle BBCs loaded strongly onto PC2. Mixed BBCs, which contain squeak- and rattle-like

features, loaded onto PC1 and PC2. To further assess these relationships, we conducted Spearman rank correlations among behaviours. As suggested by our PCA, squeaks were positively related to USVs (Spearman rank correlation: $r_s = 0.49$, $N = 28$, $P = 0.007$). However, rattles were not related to squeaks ($r_s = 0.37$, $N = 28$, $P = 0.096$) or to USVs ($r_s = 0.04$, $N = 28$, $P = 0.969$). We also explored relationships between vocal and nonvocal behaviour. Rattles and squeaks were both positively related to aggression (rattles: $r_s = 0.663$, $N = 28$, $P = 0.001$; Fig. 4a; squeaks: $r_s = 0.56$, $N = 28$, $P = 0.002$; Fig. 4b). In contrast, USVs were not related to aggression ($r_s = -0.05$, $N = 28$, $P = 0.79$; Fig. 4c). Neither squeaks, rattles, nor USVs were related to submission or social investigation (squeaks and submission: $r_s = 0.35$, $N = 28$, $P = 0.143$; rattles and submission: $r_s = 0.30$, $N = 28$, $P = 0.206$; USVs and submission: $r_s = 0.04$, $N = 28$, $P = 0.930$; squeaks and investigation: $r_s = -0.42$, $N = 28$, $P = 0.0502$; rattles and investigation: $r_s = -0.32$, $N = 28$, $P = 0.141$; USVs and investigation: $r_s = 0.03$, $N = 28$, $P = 0.890$). We also found that the different classes of nonvocal behaviour were related. Aggression and submission were positively related ($r_s = 0.54$, $N = 28$, $P = 0.006$), whereas social investigation was negatively related to both aggression ($r_s = -0.50$, $N = 28$, $P = 0.005$) and submission ($r_s = -0.47$, $N = 28$, $P = 0.019$).

DISCUSSION

Many animal species produce vocalizations in diverse social contexts. Here, we demonstrated that male and female Siberian hamsters produce both USVs and BBCs during same-sex agonistic encounters. In accordance with our initial prediction, we identified distinct subcategories of calls based on differences in spectrotemporal structure within each of these major classes. Consistent with our second prediction, both BBCs and USVs were sensitive to sex, with females producing longer BBCs and more variable USVs, and males producing a higher proportion of plain USVs. Finally, two distinct BBC subtypes, rattles and squeaks, were directly related to the amount of aggression displayed by a dyad, suggesting that BBCs are particularly salient cues during same-sex social interactions. In contrast, none of the subcategories of USVs that we identified, or USVs overall, were quantitatively related to any nonvocal behaviours. The following discussion further explores the relationship of different vocalization types to behaviour and sex, comparing the vocalizations that we have classified to those produced by other species.

Social Function of Siberian Hamster Vocalizations

Both male and female Siberian hamsters vocalized in the presence of a conspecific partner. Furthermore, Siberian hamsters did not vocalize when they were alone in a cage, regardless of whether they were residents or intruders. This is significant, because intruders placed into an empty resident's cage would be exposed to resident odour cues, which are an important aspect of communication for this and closely related species (Wynne-Edwards, Surov, & Telitzina, 1992). However, in the current study, same-sex odours of a resident hamster were not enough to elicit vocalizations from intruder Siberian hamsters. In contrast, other hamster species and more distantly related rodents do vocalize to conspecific odours. For example, Syrian hamsters, *Mesocricetus auratus*, and house mice, *Mus musculus domesticus*, vocalize upon exposure to opposite-sex cues (Floody, Pfaff, & Lewis, 1977; Nyby et al., 1981). The necessity of another Siberian hamster's physical presence for eliciting vocal behaviour suggests that the vocalizations we documented are likely involved in mediating direct social interactions between same-sex hamsters. This finding does not imply that olfaction is not an important modality during social interaction in

Table 3

Total numbers and durations of broadband call (BBC) subtypes produced by female and male Siberian hamsters

BBC subtype	N	Duration \pm SE (ms)
Female		
Squeak	146	230.41 \pm 11.9
Rattle	431	193.45 \pm 7.46
Mixed	11	404.45 \pm 46.3
Male		
Squeak	127	171.42 \pm 12.1
Rattle	100	181.12 \pm 17.1
Mixed	2	326.50 \pm 22.1

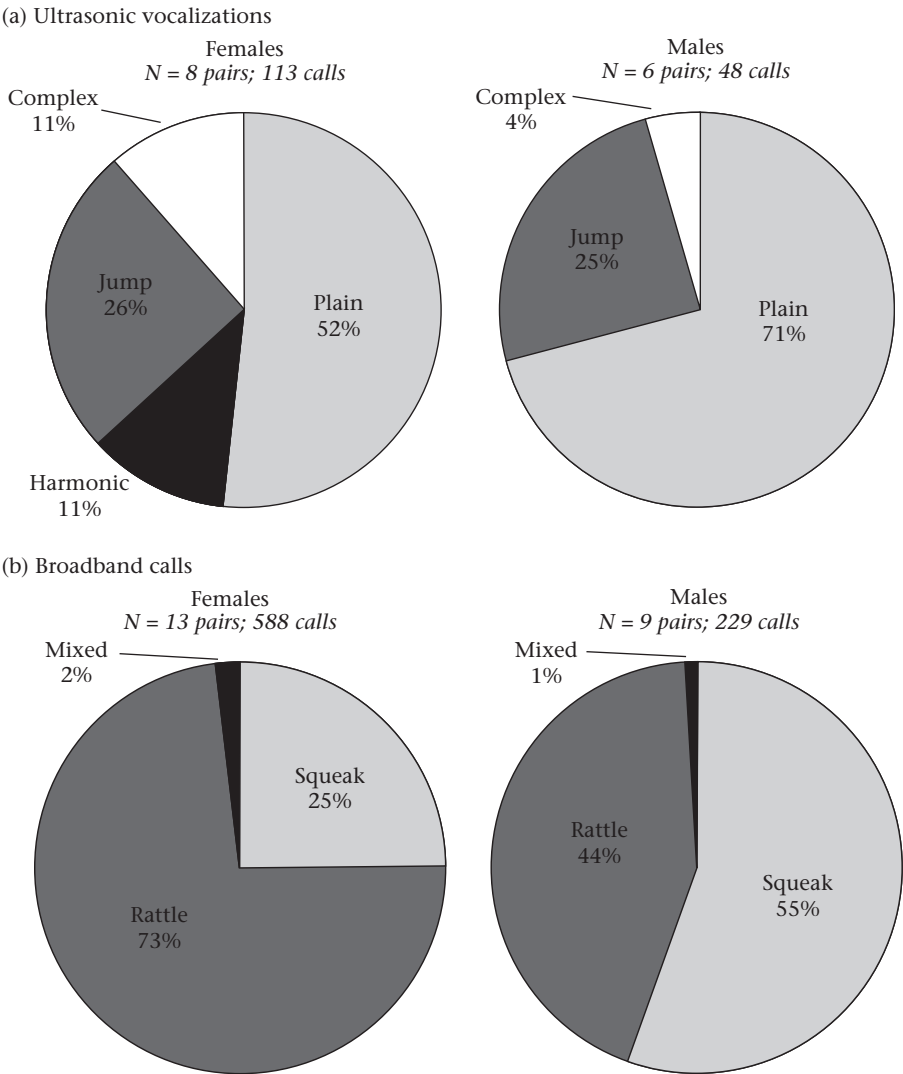


Figure 2. Proportional use of (a) ultrasonic vocalization subtypes and (b) broadband call subtypes by Siberian hamsters during 5 min resident–intruder encounters.

Siberian hamsters. Given the great importance of olfactory cues for social interaction in rodents in general (Johnston, 2003), and copious scent marking and secretion of volatile compounds by Siberian hamsters themselves (Burger et al., 2001a, 2001b; Wynne-

Edwards et al., 1992), it is highly likely that scent influences the progress of social interaction in some way other than acting as a cue that can directly trigger vocalization.

Siberian hamsters are similar to other rodent species in that they produce two general classes of vocalizations that differ in

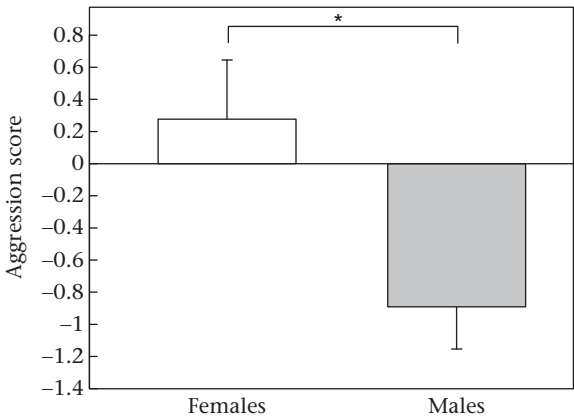


Figure 3. Sex difference in aggression score by same-sex pairs of Siberian hamsters (mean ± SE). White bar: females; grey bar: males. **P* < 0.05.

Table 4
Principal components (PC) loadings and eigenvalues for resident and intruder vocalizations of Siberian hamsters

Call subtype	PC1	PC2
USV		
Plain	0.85	0.06
Harmonic	0.69	−0.39
Jump	0.91	−0.32
Complex	0.93	0.07
BBC		
Squeak	0.58	−0.01
Rattle	0.09	0.82
Mixed	0.50	0.74
Eigenvalue	3.49	1.50
% Variance explained	49.86	21.40

USV: ultrasonic vocalization; BBC: broadband call. Bold values indicate variables that loaded strongly within that component (≤ -0.5 or ≥ 0.5).

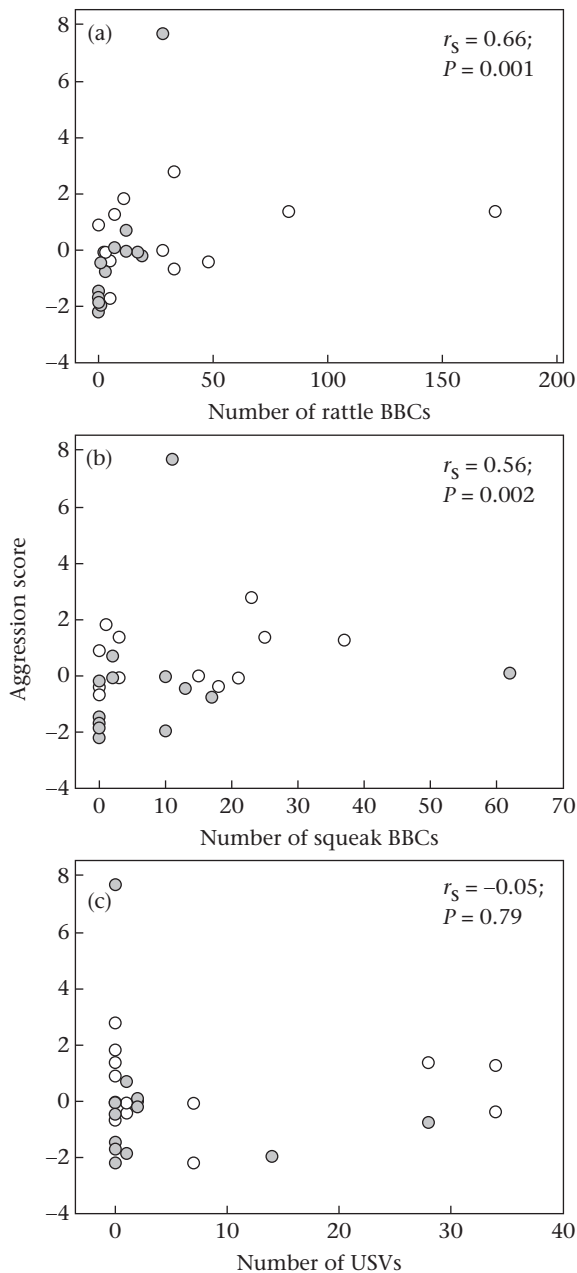


Figure 4. Relation between aggression and the number of (a) rattle broadband calls (rattle BBCs), (b) squeak broadband calls (squeak BBCs) and (c) ultrasonic vocalizations (USVs) produced by hamsters during same-sex interactions. White circles: females; grey circles: males.

structure: spectrally dense BBCs and narrowband USVs, which have been documented across rodent species (Brooks & Banks, 1973; Floody & Pfaff, 1977a, 1977b; Floody et al., 1977; reviewed in: Sales, 1972; Sewell, 1970). These extreme differences in the spectral range of USVs and BBCs raises the question of whether these two broad categories also differ in function. In the current study, both subtypes of BBCs produced by same-sex Siberian hamster dyads were significantly correlated with aggressive behaviour across dyadic interactions. These findings are interesting when considered in light of the 'motivation-structural' rules hypothesis described by Morton (1977), suggesting that animals produce structurally different sounds in distinct contexts, loosely lumped into 'hostile' and 'friendly' contexts. According to these rules, calls made during

aggressive contexts are more likely to be harsh sounding and relatively low in frequency, whereas calls are more likely to be higher in frequency and more tone-like in nonaggressive contexts. This concept has received some support in analyses of species groups (August & Anderson, 1987; Gouzoules & Gouzoules, 2000; Hauser, 1993). Manipulating hormonal physiology may further change vocalizations in conjunction with changes in aggression. For example, castrated Alston's singing mice, *Scotinomys teguina*, show higher levels of subordination accompanied by songs that have higher dominant frequencies (Pasch et al., 2011). However, across a wide range of mammal and bird species that use agonistic vocalizations, many call qualities other than tonality and frequency are associated with increased aggression; these qualities include bandwidth, intensity and call rate or number (Apfelbeck, Kiefer, Mortega, Goymann, & Kipper, 2012; DuBois, Nowicki, & Searcy, 2008; Harding, Walters, Collado, & Sheridan, 1988; Pasch et al., 2011). Moreover, exceptions to the motivational-structural rules hypothesis exist in species such as ocellated antbirds, *Phaenostictus mcleannani*, which produce higher-pitched syllables that may signal better condition or greater genetic diversity during male–male aggression (Araya-Ajoy, Chaves-Campos, Kalko, & DeWooty, 2009). Vocalizations produced by Siberian hamsters in the current study generally fit into the motivational-structural framework, with lower-frequency, spectrally noisier BBCs related to aggressive behaviours and USVs unrelated to these behaviours.

Our analyses further identified variation in the behavioural usage of two structurally distinct types of BBCs. Squeak BBCs, characterized by a continuous harmonic structure, were significantly correlated with total USVs across dyadic interactions and grouped with USVs in a principal components analysis of all the vocalization subtypes. However, 'rattle' BBCs, defined by spectral noisiness and temporal choppiness, did not group with USVs in this way and showed a higher correlation coefficient with aggression than squeaks did. Furthermore, females, the sex using more aggression in our study, produced a greater proportion of rattles than squeaks compared to males. These differences between rattle and squeak BBCs suggest that there are subtle variations in their behavioural usage. In other species, call characteristics may grade with increasing levels of aggression. For example, big brown bats, *Eptesicus fuscus*, produce a higher proportion of call types that are noisy during high-aggression interactions as opposed to low- and medium-aggression interactions (Gadziola, Grimsley, Faure, & Wenstrup, 2012). Male grey treefrogs produce aggressive calls that are lower in frequency than their advertisement calls, and furthermore show a larger frequency drop for more escalated aggression (Reichert & Gerhardt, 2013).

In contrast to BBCs, USVs were neither positively nor negatively related to aggression in our study, although both males and females produced USVs during same-sex interactions. Other rodent species also produce ultrasonic calls during same-sex encounters (Chabout et al., 2012; Kapusta, Szentgyorgi, Surov, & Ryurikov, 2006; Moles, Costantini, Garbugino, Zanettini, & D'Amato, 2007; Sales, 1972). For instance, resident female house mice produce USVs upon encountering a novel female intruder, and the number of USVs produced is strongly related to investigatory behaviours during social investigation of same-sex partners by both male and female mice (Chabout et al., 2012; Moles et al., 2007). Collectively, these studies suggest that USVs are involved in mediating nonaggressive social engagements. The fact that USVs were not related to specific nonaggressive social behaviours including social investigation in our study, does not rule out that Siberian hamster USVs could communicate important information about a vocalizer, regarding factors such as sex, reproductive state or social history. Features of our experimental design, such as the resident–intruder encounters, were intended to highlight aggressive behaviours. In the

future, addressing the issue of potential affiliative functions of vocalization could be facilitated by experimental design elements such as the use of a neutral arena for staged encounters, or prolonged co-housing of social partners before target encounters.

Siberian Hamsters Produce Different Calls Dependent on Sex

In addition to finding that BBCs were related to aggression across individual pairings of hamster dyads, there were also sex differences in vocal and nonvocal behaviour. In our study, female pairs were more aggressive than male pairs. The direction of this sex difference is opposite to one reported in a previous study, in which male pairs were more aggressive than female pairs (Wynne-Edwards & Lisk, 1987). Both this study and the current study documented substantial levels of aggression in both sexes, however, and the difference between the previous and current study could be due to different methods, including pre-experiment housing and multiple differences in the paradigm used to assess aggressive behaviours (Wynne-Edwards & Lisk, 1987). The vocal behaviour of females complements their heightened aggression compared to males in this study. Females produced quantitatively more BBCs and uttered longer duration BBCs than males. In addition, females used a significantly larger proportion of rattle calls, which were more tightly associated with aggressive acts.

Although there was no sex difference in the absolute number of USVs produced, there were differences between the sexes in their proportional use of USV subtypes. Females used fewer plain calls and a greater number of complex calls than males, and they produced more frequency jumps within the 'jump' category (50% of jump calls had more than one frequency jump, in contrast to ~15% of male calls in this category). Female Syrian hamsters (Floody & Pfaff, 1977a) and female house mice (von Merten, Hoier, Pfeifle, & Tautz, 2014) have also been reported to produce more jumps than males. As a result of these differences between the sexes in our study, female Siberian hamsters in same-sex interactions produced more variable USVs, and a greater range of USVs, than did males. Findings on sex differences in call structure and usage in other small rodents are mixed. For example, calls produced by male and female house mice in response to a female intruder differ in the proportional usage of USV syllable types, but not in the number or spectrotemporal characteristics of calls (Hammerschmidt et al., 2012). However, male and female California mice, *Peromyscus californicus*, that were observed in the wild did not differ in their vocalizations when alone and in the presence of a conspecific (Briggs & Kalcounis-Rueppell, 2011).

Future Directions

As a model of aggressive behaviour, Siberian hamsters are well suited to exploring aggressive vocal communication and how it is influenced by sex. An important aspect of the aggressive behaviour of this species is its expression across seasons, since Siberian hamsters show more aggression during the nonbreeding season when reproductive organs have regressed and gonadal hormones are relatively low (Jasnow et al., 2000; Scotti et al., 2007). Exploration of seasonal variation in the production of aggressive vocalizations could therefore be an interesting direction for future research. In addition to hamsters, song sparrows, *Melospiza melodia*, also display year-round aggression, changing the composition and characteristics of song across breeding versus nonbreeding periods (Maddison, Anderson, Prior, Taves, & Soma, 2012; Wingfield, 1994). For hamsters, we would predict that the incidence of BBCs in general, and rattle calls in particular, increase when hamsters are placed in short-day conditions that evoke nonbreeding-like suites of behaviour and increased levels of aggression. All of these considerations suggest

that Siberian hamsters are an excellent model system in which to study the physiological mechanisms by which the converging effects of context, sex and season influence the common output of aggressive and vocal production.

Acknowledgments

We thank C. Amadi for assistance in recording behaviours, oestrus staging and vocalization analysis, A. Amez for assistance in recording behaviours, and C. Athans and K. O'Malley for nonvocal behavioural analysis. This work was supported by grants from the National Science Foundation (NSF IOB 0543798 to G. E. Demas) and the National Institute on Deafness and Other Communication Disorders (DC-008963 to L. M. Hurley), and in part by a NSF graduate research fellowship to N. M. Rendon and NSF REU to C. Amadi.

References

- Anderson, R. C., Searcy, W. A., Peters, S., & Nowicki, S. (2008). Soft song in song sparrows: acoustic structure and implications for signal function. *Ethology*, 114, 662–676.
- Appelbeck, B., Kiefer, S., Mortega, K. G., Goymann, W., & Kipper, S. (2012). Testosterone affects song modulation during simulated territorial intrusions in male black redstarts (*Phoenicurus ochruros*). *PLoS One*, 7, e52009.
- Araya-Ajoy, Y.-m., Chaves-Campos, J., Kalko, E. K., & DeWoody, J. A. (2009). High-pitched notes during vocal contests signal genetic diversity in ocellated antbirds. *PLoS One*, 4, e8137.
- August, P., & Anderson, J. (1987). Mammal sounds and motivation-structural rules: a test of the hypothesis. *Journal of Mammalogy*, 68, 1–9.
- Brain, P. (1975). What does individual housing mean to a mouse? *Life Sciences*, 16, 187–200.
- Brain, P., & Poole, A. (1974). Some studies on use of standard opponents in intermale aggression testing in TT albino mice. *Behaviour*, 50, 100–110.
- Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, 82, 1263–1273.
- Brooks, R., & Banks, E. (1973). Behavioural biology of the collared lemming (*Dicrostonyx groenlandicus* (Trail)): an analysis of acoustic communication. *Animal Behaviour Monographs*, 6, 1–83.
- Burger, B., Smit, D., Spies, H., Schmidt, C., Schmidt, U., & Telitsina, A. (2001a). Mammalian exocrine secretions XVI. Constituents of secretion of supplementary sacculi of dwarf hamster, *Phodopus sungorus sungorus*. *Journal of Chemical Ecology*, 27, 1277–1288.
- Burger, B., Smit, D., Spies, H., Schmidt, C., Schmidt, U., Telitsina, A., et al. (2001b). Mammalian exocrine secretions XV. Constituents of secretion of ventral gland of male dwarf hamster, *Phodopus sungorus sungorus*. *Journal of Chemical Ecology*, 27, 1259–1276.
- Chabout, J., Serreau, P., Ey, E., Bellier, L., Aubin, T., Bourgeron, T., et al. (2012). Adult male mice emit context-specific ultrasonic vocalizations that are modulated by prior isolation or group rearing environment. *PLoS One*, 7, e29401.
- DuBois, A. L., Nowicki, S., & Searcy, W. A. (2008). Swamp sparrows modulate vocal performance in an aggressive context. *Biology Letters*, 5, 163–165.
- Floody, O. R., & Pfaff, D. W. (1977a). Communication among hamsters by high-frequency acoustic signals: I. Physical characteristics of hamster calls. *Journal of Comparative and Physiological Psychology*, 91, 794.
- Floody, O. R., & Pfaff, D. W. (1977b). Communication among hamsters by high-frequency acoustic signals: III. Response evoked by natural and synthetic ultrasounds. *Journal of Comparative and Physiological Psychology*, 91, 820.
- Floody, O. R., Pfaff, D. W., & Lewis, C. D. (1977). Communication among hamsters by high-frequency acoustic signals: II. Determinants of calling by females and males. *Journal of Comparative and Physiological Psychology*, 91, 807.
- Gadziola, M. A., Grimsley, J. M., Faure, P. A., & Wenstrup, J. J. (2012). Social vocalizations of big brown bats vary with behavioral context. *PLoS One*, 7, e44550.
- Gouzoules, H., & Gouzoules, S. (2000). Agonistic screams differ among four species of macaques: the significance of motivation-structural rules. *Animal Behaviour*, 59, 501–512.
- Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., & Fischer, J. (2012). The structure and usage of female and male mouse ultrasonic vocalizations reveal only minor differences. *PLoS One*, 7, e41133.
- Harding, C. F., Walters, M. J., Collado, D., & Sheridan, K. (1988). Hormonal specificity and activation of social behavior in male red-winged blackbirds. *Hormones and Behavior*, 22, 402–418.
- Hauser, M. D. (1993). The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *American Naturalist*, 528–542.
- Holy, T., & Guo, Z. (2005). Ultrasonic songs of male mice. *PLoS Biology*, 3, 2177–2186.
- Jasnow, A. M., Huhman, K. L., Bartness, T. J., & Demas, G. E. (2000). Short-day increases in aggression are inversely related to circulating testosterone concentrations in male Siberian hamsters (*Phodopus sungorus*). *Hormones and Behavior*, 38, 102–110.

- Johnston, R. E. (2003). Chemical communication in rodents: from pheromones to individual recognition. *Journal of Mammalogy*, 84, 1141–1162.
- Kapusta, J., Szentgyorgi, H., Surov, A., & Ryurikov, G. (2006). Vocalization of two Palaearctic species of hamster: Eversmann hamster *Allocricetulus eversmanni* and grey hamster *Cricetulus migratorius*. *Bioacoustics*, 15, 315–330.
- Maddison, C. J., Anderson, R. C., Prior, N. H., Taves, M. D., & Soma, K. K. (2012). Soft song during aggressive interactions: seasonal changes and endocrine correlates in song sparrows. *Hormones and Behavior*, 62, 455–463.
- von Merten, S., Hoier, S., Pfeifle, C., & Tautz, D. (2014). A role for ultrasonic vocalisation in social communication and divergence of natural populations of the house mouse (*Mus musculus domesticus*). *PLoS One*, 9, e97244.
- Moffatt-Blue, C., Sury, J., & Young, K. A. (2006). Short photoperiod-induced ovarian regression is mediated by apoptosis in Siberian hamsters (*Phodopus sungorus*). *Reproduction*, 131, 771–782.
- Moles, A., Costantini, F., Garbugino, L., Zanettini, C., & D'Amato, F. R. (2007). Ultrasonic vocalizations emitted during dyadic interactions in female mice: a possible index of sociability? *Behavioural Brain Research*, 182, 223–230.
- Morton, E. S. (1977). On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *American Naturalist*, 111, 855–869.
- Neilans, E. G., Holfoth, D. P., Radziwon, K. E., Portfors, C. V., & Dent, M. L. (2014). Discrimination of ultrasonic vocalizations by CBA/CaJ mice (*Mus musculus*) is related to spectrottemporal dissimilarity of vocalizations. *PLoS One*, 9, e85405.
- Nowicki, S., & Searcy, W. A. (2004). Song function and the evolution of female preferences: why birds sing, why brains matter. *Annals of the New York Academy of Sciences*, 1016, 704–723.
- Nyby, J. G. (2001). Auditory communication among adults. In J. F. Willott (Ed.), *Handbook of mouse auditory research: From behavior to molecular biology* (pp. 3–18). Boca Raton, FL: CRC Press.
- Nyby, J. G., Wysocki, C. J., Whitney, G., Dizinnio, G., Schneider, J., & Nunez, A. A. (1981). Stimuli for male mouse (*Mus musculus*) ultrasonic courtship vocalizations: presence of female chemosignals and/or absence of male chemosignals. *Journal of Comparative and Physiological Psychology*, 95, 623.
- Pasch, B., George, A. S., Campbell, P., & Phelps, S. M. (2011). Androgen-dependent male vocal performance influences female preference in Neotropical singing mice. *Animal Behaviour*, 82, 177–183.
- Reichard, D. G., & Welklin, J. F. (2014). On the existence and potential functions of low-amplitude vocalizations in North American birds. *Auk*, 132, 156–166.
- Reichert, M. S., & Gerhardt, H. C. (2013). Gray tree frogs, *Hyla versicolor*, give lower-frequency aggressive calls in more escalated contests. *Behavioral Ecology and Sociobiology*, 67, 795–804.
- Ross, P. D. (1998). *Phodopus sungorus*. *Mammalian Species*, 595, 1–9.
- Sales, G. D. (1972). Ultrasound and aggressive behaviour in rats and other small mammals. *Animal Behaviour*, 20, 88–100.
- Sales, G. D. (2010). Ultrasonic calls of wild and wild-type rodents. In S. M. Brudzynski (Ed.), *Handbook of mammalian vocalization: An integrative neuroscience approach* (Vol. 19, pp. 77–88). London, U.K.: Academic Press.
- Scheuber, H., Jacot, A., & Brinkhof, M. W. (2004). Female preference for multiple condition-dependent components of a sexually selected signal. *Proceedings of the Royal Society B: Biological Sciences*, 271, 2453–2457.
- Scotti, M.-A. L., Place, N. J., & Demas, G. E. (2007). Short-day increases in aggression are independent of circulating gonadal steroids in female Siberian hamsters (*Phodopus sungorus*). *Hormones and Behavior*, 52, 183–190.
- Sewell, G. D. (1970). Ultrasonic communication in rodents. *Nature*, 227, 410.
- Sugimoto, H., Okabe, S., Kato, M., Koshida, N., Shiroishi, T., Mogi, K., et al. (2011). A role for strain differences in waveforms of ultrasonic vocalizations during male–female interaction. *PLoS One*, 6, e22093.
- Titus, R. C. (1998). Short-range and long-range songs: use of two acoustically distinct song classes by dark-eyed juncos. *Auk*, 115, 386–393.
- Verhoeven, K. J., Simonsen, K. L., & McIntyre, L. M. (2005). Implementing false discovery rate control: increasing your power. *Oikos*, 108, 643–647.
- Wang, H., Liang, S., Burgdorf, J., Wess, J., & Yeomans, J. (2008). Ultrasonic vocalizations induced by sex and amphetamine in M2, M4, M5 muscarinic and D2 dopamine receptor knockout mice. *PLoS One*, 3, e1893.
- Wells, K. D., & Schwartz, J. J. (2006). The behavioral ecology of anuran communication. In P. M. Narins, A. S. Feng, & R. Richard (Eds.), *Hearing and sound communication in amphibians* (pp. 44–86). Berlin, Germany: Springer.
- Wingfield, J. C. (1994). Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Hormones and Behavior*, 28, 1–15.
- Wynne-Edwards, K. E. (1995). Biparental care in Djungarian but not Siberian dwarf hamsters (*Phodopus*). *Animal Behaviour*, 50, 1571–1585.
- Wynne-Edwards, K. E. (2003). From dwarf hamster to daddy: the intersection of ecology, evolution, and physiology that produces paternal behavior. *Advances in the Study of Behavior*, 32, 207–262.
- Wynne-Edwards, K. E., & Lisk, R. D. (1987). Behavioral interactions differentiate Djungarian (*Phodopus campbelli*) and Siberian (*Phodopus sungorus*) hamsters. *Canadian Journal of Zoology*, 65, 2229–2235.
- Wynne-Edwards, K. E., Surov, A., & Telitzina, A. (1992). Field studies of chemical signalling: direct observations of dwarf hamsters (*Phodopus*) in Soviet Asia. *Chemical Signals in Vertebrates*, 6, 485–491.