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ORIGINAL ARTICLE

Sex differences in the influence of social context, salient social stimulation and amphetamine on ultrasonic vocalizations in prairie voles

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Abstract

Prairie voles (*Microtus ochrogaster*) are a socially monogamous rodent species and their cooperative behaviors require extensive communication between conspecifics. Rodents use ultrasonic vocalizations (USVs) to communicate and because a prairie vole breeder pair must engage in extensive cooperation for successful reproduction, auditory communication may be critical for this species. Therefore, we sought to characterize USVs in adult male and female prairie voles, and to determine how these calls are influenced by social context, salient social stimuli and the psychostimulant drug of abuse amphetamine (AMPH). Here, we characterize prairie vole USVs by showing the range of frequencies of prairie vole USVs, the proportion of various call types, how these call types compare between males and females, and how they are influenced by social stimulation and AMPH. AMPH caused a robust increase in the number of USVs in both males and females and there was a dramatic sex difference in the complexity of call structures of AMPH-induced USVs, with males emitting more elaborate calls. Moreover, we show that novel (i.e. salient) social cues evoked differential increases in USVs across sex, with males showing a much more robust increase in USV production, both with respect to the frequency and complexity of USV production. Exposure to an estrous female in particular caused an extraordinary increase in USVs in male subjects. These data suggest that USVs may be a useful measure of social motivation in this species, including how social behaviors can be impacted by drugs of abuse.

Key words: amphetamine, dopamine, mate choice, pair bonding, ultrasonic vocalization

INTRODUCTION

Prairie voles [*Microtus ochrogaster* (Wagner, 1842)] are a socially monogamous rodent species (Getz *et al*. 1981; Carter *et al*. 1995; McGraw & Young 2010) that form pair bonds in which the breeder pair shares territory, nests and parental duties (Getz & Carter 1980; McGuire & Novak 1984; Aragona & Wang 2004; Wang &

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Aragona 2004; Curtis *et al*. 2006). This coordination of behavior and reproductive biology requires extensive communication between conspecifics. In addition to main olfactory cues and pheromones (Carter *et al*. 1980, 1989; Cushing *et al*. 1995), rodents also use auditory signals to communicate (Panksepp *et al*. 2002; Holy & Guo 2005; Barfield & Thomas 2006). Rodents emit sounds at frequencies outside of the frequency range detected by humans, called ultrasonic vocalizations (USVs) (Sales 1972; Knutson *et al*. 2002; Holy & Guo 2005; Panksepp *et al*. 2007). USVs are produced under circumstances wherein social communication is adaptive. For example, pups emit USVs when they are separated from their mother (Bell *et al*. 1974; Oswalt & Meier 1975; Insel *et al*. 1986; Shair 2007), a behavior that has been demonstrated in prairie voles (Shapiro & Insel 1990). USVs are also common during mating as well as in anticipation of copulatory behavior (Barfield & Geyer 1972; McIntosh *et al*. 1984; Holy & Guo 2005; Barfield & Thomas 2006). This behavior appears to be important for attracting a mate and coordinating behavior necessary for reproduction. Because a prairie vole breeder pair must engage in extensive cooperation to successfully reproduce, auditory communication may be critical for this species. Therefore, we sought to characterize USVs in adult male and female prairie voles.

Studies using rats and mice have demonstrated that the functional significance of USVs is related to their frequency and call structure (Guo & Holy 2007; Wright *et al*. 2010). In rats, the frequency and structure of USVs expressed during adaptive motivated behavior (especially social behaviors) are associated with motivational and hedonic valence (Knutson *et al*. 2002; Panksepp *et al*. 2002; Burgdorf & Panksepp 2006; Harmon *et al*. 2008). USVs emitted during appetitive motivation are frequency modulated calls between 50 and 70 kHz, whereas calls associated with aversion are often very simple calls often (but not always) emitted at approximately 20 kHz (Knutson *et al*. 2002; Panksepp *et al*. 2002; Burgdorf & Panksepp 2006; Harmon *et al*. 2008). In anticipation of mating, male mice emit elaborate songs consisting of highly complex USVs (Holy & Guo 2005). In additional, administration of the psychostimulant drug of abuse amphetamine (AMPH) increases USVs that are primarily frequency-modulated calls at approximately 50 kHz (Knutson *et al*. 1999; Ahrens *et al*. 2009; Mu *et al*. 2009; Ma *et al*. 2010; Wright *et al*. 2010). Importantly, recent studies have demonstrated that AMPH reward in prairie voles is similar to other rodent species and there are robust and reciprocal interactions between AMPH and social reward in this species (Aragona *et al*. 2007; Curtis & Wang 2007; Liu *et al*. 2010, 2011; Young *et al*. 2011a,b). Thus, to characterize USVs in prairie voles, we measured the frequency and call structure of adult prairie vole USVs following manipulation of social context, exposure to salient social stimulation and administration of AMPH.

This study provides a detailed characterization of prairie vole USVs by showing the range of frequencies of prairie vole USVs, the relative proportion of the various call types, how call types compare between males and females, and how calls are influenced by social stimulation and AMPH. We show that AMPH caused a robust increase in the number of USVs in both males and females. However, there was a dramatic sex difference in the complexity of call structures of AMPH-induced USVs, with males emitting calls that were much more elaborate than those produced by females. Finally, we show that visual and main olfactory exposure to an unfamiliar female, especially females in behavioral estrous, evoked an extraordinary increase in the number and complexity of USVs in male prairie voles, whereas females (whether in estrous or not) showed very few USVs when exposed to novel social stimulation. We discuss the adaptive significance of USVs in this species and suggest that USVs may prove to be a useful measure for future studies of social communication and motivation, including how prairie vole social behavior is impacted by drugs of abuse.

MATERIALS AND METHODS

Subjects

Subjects were laboratory bred prairie voles. Voles were weaned at 21 days of age and housed with same sex siblings until experimental testing, which occurred between 90 and 120 days of age. Male and female sibling pairs were housed in separate animal rooms with a 14:10 h light:dark cycle with food and water provided *ad libitum*. Subjects received USV testing during the light phase, between 1000 and 1400 hours. The University of Michigan Animal Care and Use Committee approved all experiments (protocol number 10040).

Characterization of ultrasonic vocalizations in prairie voles

There is a high degree of variability across prairie voles (Phelps & Young 2003) and there is also a large degree of variation in USV call types expressed across

individual rodents of other species (Wright *et al*. 2010). Therefore, we used a large sample size, with 15 male sibling pairs (n = 30 males total) and 17 female sibling pairs (n = 34 females total), to characterize USVs in prairie voles. On recording day, individual voles were first separated from same sex cage-mates and placed inside a 25 × 25 cm plexiglass chamber for USV recording. An ultrasonic microphone (PCB Peizotronics, Depew, NY, USA), with a flat frequency range from 20 to 100 kHz, was mounted 5 cm above the chamber center. A sound absorption box enclosed the chamber and this completely prevented 'cross-talk' between chambers. The absence of cross-talk was confirmed by a pilot study in which 8-day-old vole pups were placed in 1 chamber; they emitted a very high number of loud USVs, and these were not detected in the other chamber (data not shown).

Ultrasonic vocalization recordings were sampled through a high-speed data acquisition card (National Instruments, Austin, TX, USA) at 200 kHz sampling rate with 16-bit resolution and digitally stored as .WAV files. Sonograms for USVs were generated and analyzed with Saslab Pro (Avisoft, Berlin, Germany), using a 512-point Fast Fourier Transform (FFT) and 75% overlap frame spectrogram setup. This provided a frequency resolution of 391 Hz and a time resolution of 0.64 ms. A semi-auto call labeling procedure, based on a previous study using rats (Wright *et al*. 2010), was used to label USVs. First, an automated detection algorithm of trigger threshold at −64 dB was applied to the analyzed spectrogram. All USV signals and noise artifacts were automatically labelled by the program. The recorded .WAV file was then compressed by the labelled signal and noise sections, while removing the unlabelled silence, thus reducing the file size for later manual labeling of USVs. Three trained observers, blind to treatment conditions, manually labelled the USV signals in the compressed .WAV files based on 3 spectrographic criteria: (i) temporal continuity (max discontinuity 20 ms); (ii) fundamental frequency above 20 kHz and below 80 kHz; and (iii) a signal to noise difference that revealed call structure. Labelled USV signals were then classified into 14 categories based on the distinct spectrographic shape of individual syllables. Between the 3 trained observers, a 95% inter-rater reliability was reached from a random sampled subset prior from classifying the overall 19 000 calls. When possible, we used the same categories and definitions that have been established for rats (Wright *et al*. 2010). USV categories were as follows (see Fig. 1):

1. Flat: short (approximately 30 ms) monotonic call with frequency above 25 kHz.
2. Upward ramp: short monotonic call with increasing frequency slope of 0.5 kHz/ms.
3. Downward ramp: short monotonic call with decreasing frequency slope of 0.5 kHz/ms.
4. Harmonic: constant frequency call with double frequency component stacked above.
5. Step up: discontinuous frequency jump to a higher frequency.
6. Step down: discontinuous frequency jump to a lower frequency.
7. Step in left: a discontinuous frequency jump at the beginning side of a harmonic structure.
8. Step in right: a discontinuous frequency jump at the ending side of a harmonic structure.
9. Step in both: 2 discontinuous frequency jump at the both sides of a harmonic structure.
10. U shape: a monotonic call with increasing frequencies (at least 5 kHz) at both ends.
11. Inverted U: a monotonic call with decreasing frequencies (at least 5 kHz) at both ends.
12. Miscellaneous: miscellaneous calls that bear resemblance of categories above but does not fall into classification.
13. Complex: complex structured calls during male/estrous female interaction.
14. Step composite: combination of steps and harmonics that occurs during male/estrous female interaction.

Individual voles (i.e. 1 of the 2 siblings or cagemates) were initially placed into the separate recording chambers and USVs were recorded from both individual voles for 30 min in a manner (as described above) that permitted no 'cross talk' between the 2 recordings. Siblings were then reunited and USVs emitted from both members of the sibling pair were recorded once they were reunited for another 30 min and the total number of USVs was divided by 2 in order to estimate the number of USVs per subject when 2 callers contributed to the recording (Wright *et al*. 2010).

Effect of amphetamine on prairie vole ultrasonic vocalizations

As in the initial characterization experiment, USVs were first measured when voles were isolated for 30 min and then for another 30 min after voles were reunited. Separate groups of voles received a 0.2 mL i.p. injection of saline (males n = 16; females n = 18), or saline con-

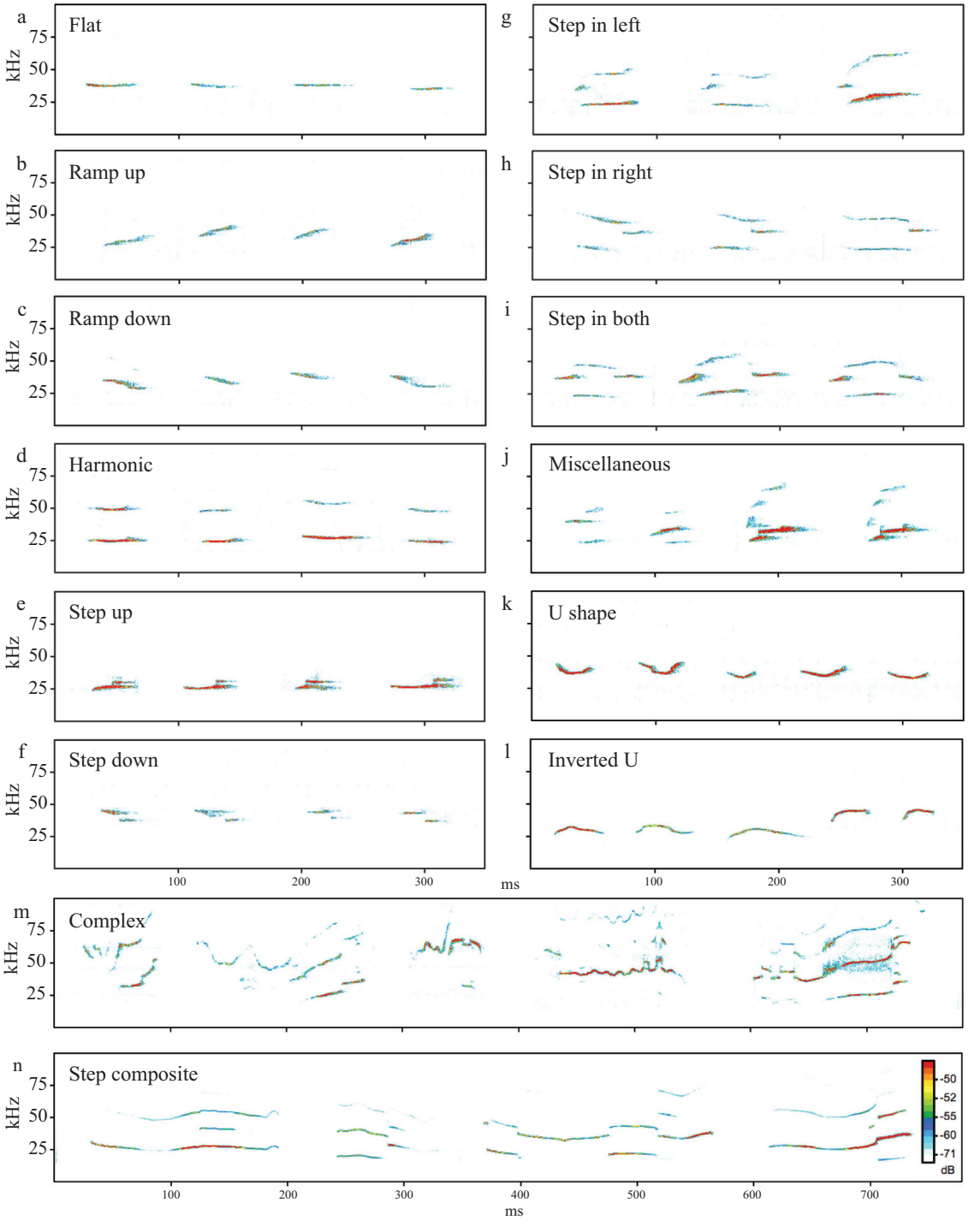

Figure 1 Representative sonograms of ultrasonic vocalizations (USVs) call types from prairie voles. A total of 14 major types of USV calls were categorized based on the structure of the vocalization. The majority of the emitted call types were types (a)–(j). During socio-sexual interaction, additional call types (k)–(n) were vocalized. The *y*-axis indicates the frequency change of the USVs in kilohertz range, whereas the *x*-axis indicates time in seconds. Color depths in the sonograms represent relative intensity strength in dB.

taining 2 mg/kg AMPH (males $n = 10$; females $n = 10$). Doses were selected based on previous research on different rodent species that elicited USVs (Ahrens *et al*. 2009; Mu *et al*. 2009).

Effect of novel social stimulation on prairie vole ultrasonic vocalizations

To investigate how different socio-sexual stimulation effects USVs in male and female prairie voles, a specialized recording chamber was designed that allowed for visual and main olfactory cues to be sensed but allowed USVs from only the subject to be detected (see also Ciucci *et al*. 2007). The recording chamber was built with transparent plexiglass with dimensions of 10 × 9 × 37.5 cm. A transparent divider divided the space into 2 chambers: 1 side for the subject and the other side for the stimulus animal. Within the subject's chamber side, a motorized fan pulled odor from the stimulus animal's chamber into the subject's chamber. The USV microphone was located in the subject's chamber to record vocalizations from the subject and did not detect USVs emitted from the chamber of the stimulus animal. USVs were recorded from subjects for 5 min after the stimulus animal was placed into the adjacent chamber. Both male ($n = 6$) and female ($n = 6$) subjects received exposure to 3 different categories of unfamiliar stimulus animals: non-estrogen-primed females, estrogen-primed females or males. Stimulus females were ovariectomized (OVX) and estrogen priming was achieved by s.c. injections of estradiol benzoate (EB) 1 μg for 3 consecutive days (Smith *et al*. 2001). Consistent with previous studies (Holy & Guo 2005), subjects and stimulus animals were allowed to interact for 2 min prior to the USV recording experiment. When males were exposed to estrogen-primed females, mating occurred in all but 1 pairing. In this case, the subject was a male prairie vole and data from this subject was not excluded from the analysis because it showed similar USVs when exposed to the estrogen-primed female in the USV recording chamber.

Data analysis

Results from Experiment 1 and Experiment 2 were analyzed with 2-way ANOVA in SPSS (IBM, New York, USA). Because the identity of the caller could not be identified during joined cage-mate recordings in Experiments 1 and 2, the number of USVs recorded from an isolated vole was pooled with those of its cage-mate for the isolated condition. This was then used to analyze the difference between isolated *versus* joined conditions on the number of USVs. Main effects of different drug treatments (control, saline, cocaine and AMPH) and sex (male or female) and their interaction effects on the number of USVs were then determined by ANOVA. Multiple comparisons of USV number under different social conditions (isolated or joined) were compared with different drug treatments and different sexes using the Wilcoxon signed-rank test as assumptions of normality were voided. In Experiment 3, the Student's *t*-test was applied to compare between different socio-sexual groups. Significant levels were considered when $P < 0.05$ for all experiments.

RESULTS

Classification of ultrasonic vocalizations in prairie voles

Studies in other rodent species have shown that there is a tremendous variety in USV call types (Wright *et al*. 2010; Scattoni *et al*. 2011). Furthermore, USV function appears to differ according to call-type specificity (Knutson *et al*. 2002; Holy & Guo 2005). Therefore, we used spectrogram analysis to characterize call structures and frequency of USVs emitted by prairie voles. The most common call type in prairie voles (see Figs 2–4) has a 'flat' (Fig. 1a) component and occurs between 25 and 45 kHz. Most vocalizations that are comprised of a single component, such as 'ramp-up' (Fig. 1b), 'ramp-down' (Fig. 1c) and U-shapes (Fig. 1j,k) also occur within this frequency range. Calls that are comprised of multiple components occur either within a large range with a lower fundamental frequency component occurring at approximately 25 kHz and a higher harmonic component occurring at approximately 50 kHz, with other components occurring within this frequency range (Fig. 1d,g–l) or more closely associated either within the lower (Fig. 1e) or higher (Fig. 1f) frequency range. When males are exposed to estrogen-primed females, we observed USVs that occurred at expanded frequency ranges that extended to approximately 80 kHz and were either highly complex 'mating-complex' (Fig. 1m) or less complex 'mating-step composite' (Fig. 1n).

Quantitative and qualitative changes of ultrasonic vocalizations during baseline and following drug administration

Under baseline conditions, both male and female prairie voles emitted a very modest number of USVs when isolated, approximately 50 calls in 30 min, and

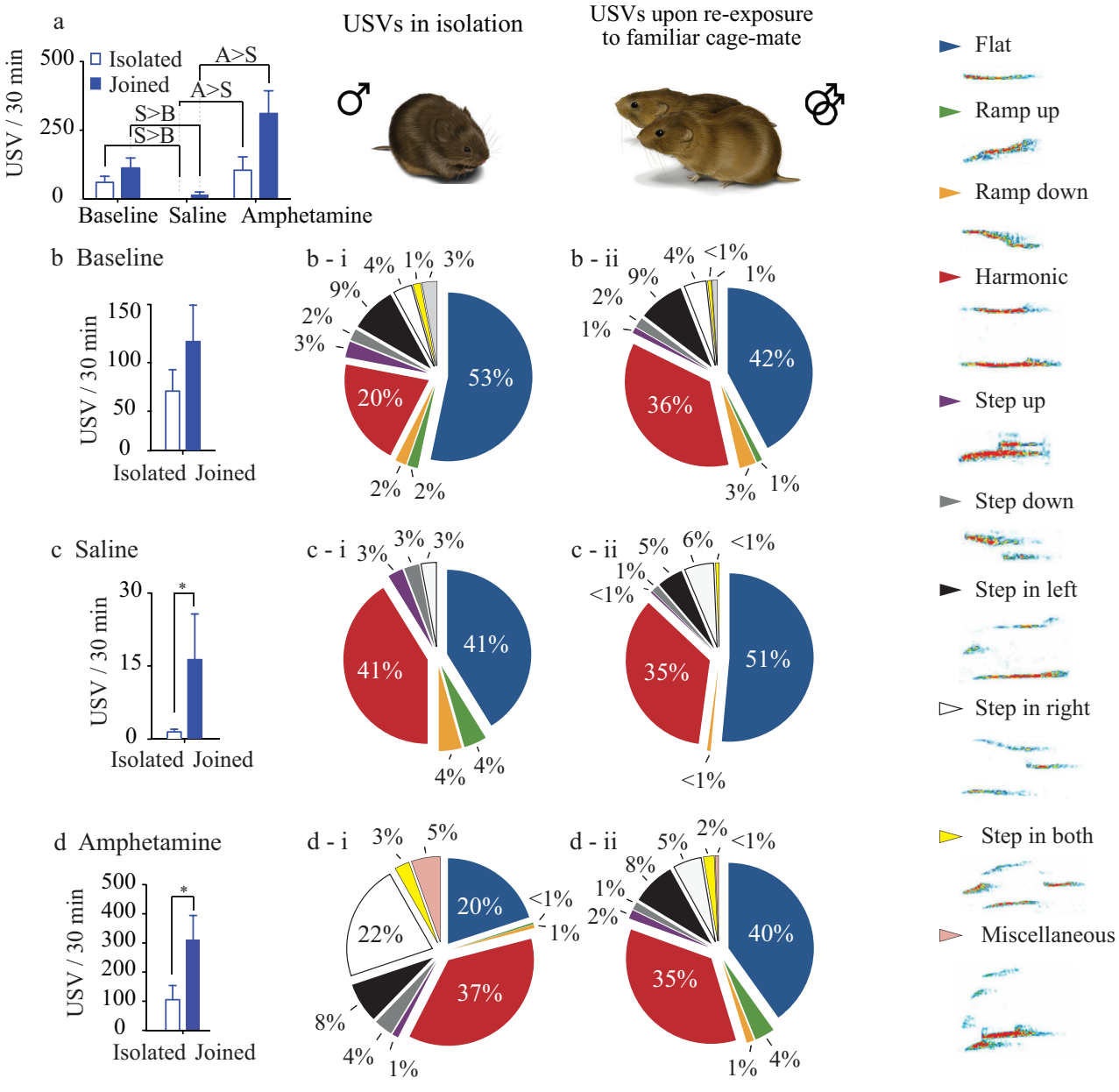

Figure 2 Mean ultrasonic vocalizations (USV) counts and call type distribution for male prairie voles under different social contexts and following amphetamine (AMPH) treatment. (a) The mean quantitative effects of saline and AMPH injections. Error bars indicate standard error from the mean. > signs indicate significantly greater than based on a 2-way ANOVA. S, saline; B, baseline; A, AMPH. (b) Baseline data (i.e. the handling control). Bar graph represents the mean number of overall USVs emitted and pie charts indicate the proportion of call types during the isolated condition (i) and once voles were rejoined with their cagemate (ii). (c) Data following control saline injections. (d) Data following AMPH injections. *Indicates overall USV frequency significantly greater in the 'joined' condition compared to the isolated condition. Pie chart sections are segregated according to color with different colors indicating a different call type; the color code is shown on the right-hand side of the figure.

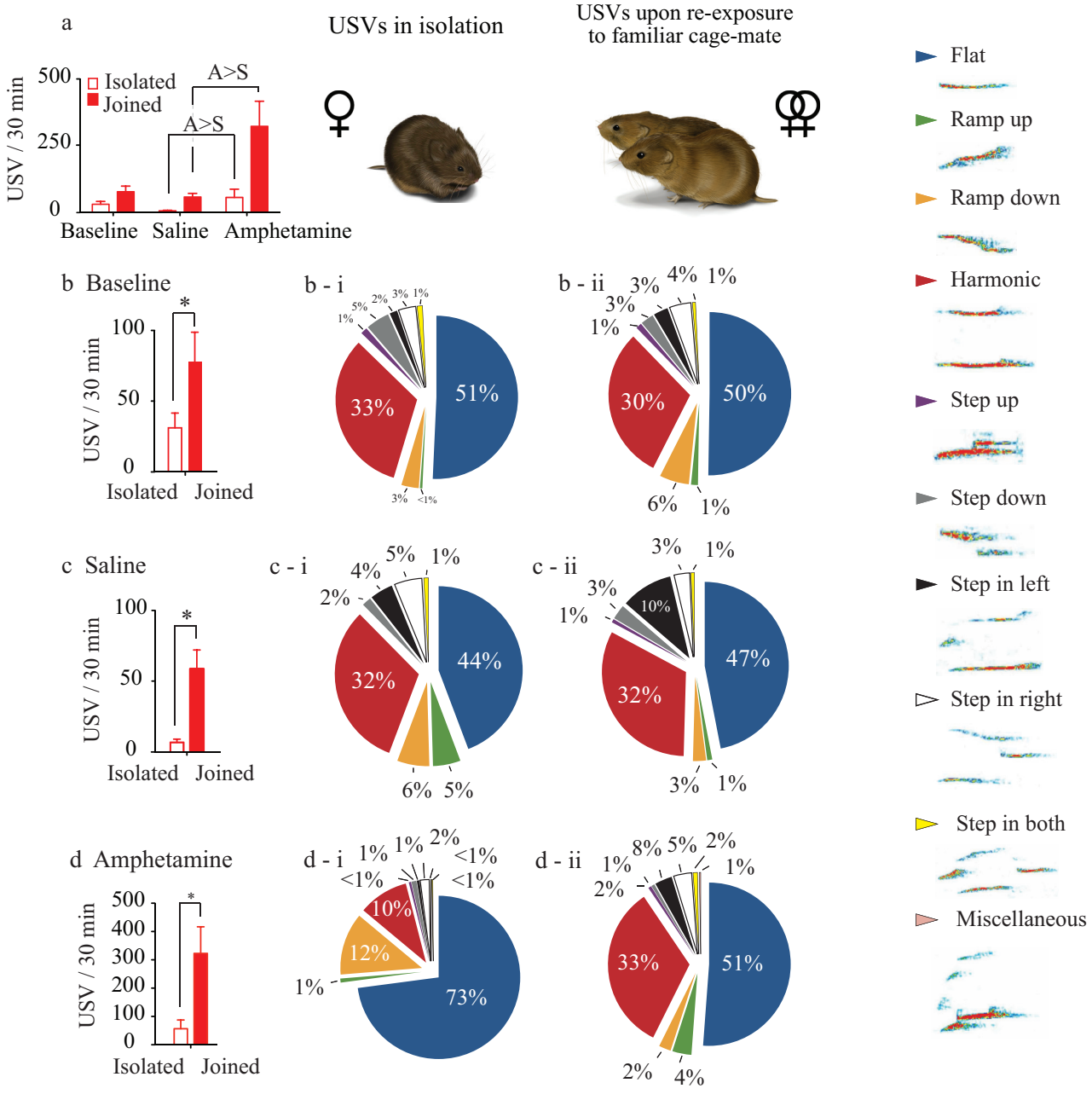

Figure 3 Mean ultrasonic vocalization (USV) counts and call type distribution for female prairie voles under different social contexts and following amphetamine (AMPH) treatment. Symbols and labels are the same as for Fig. 2.

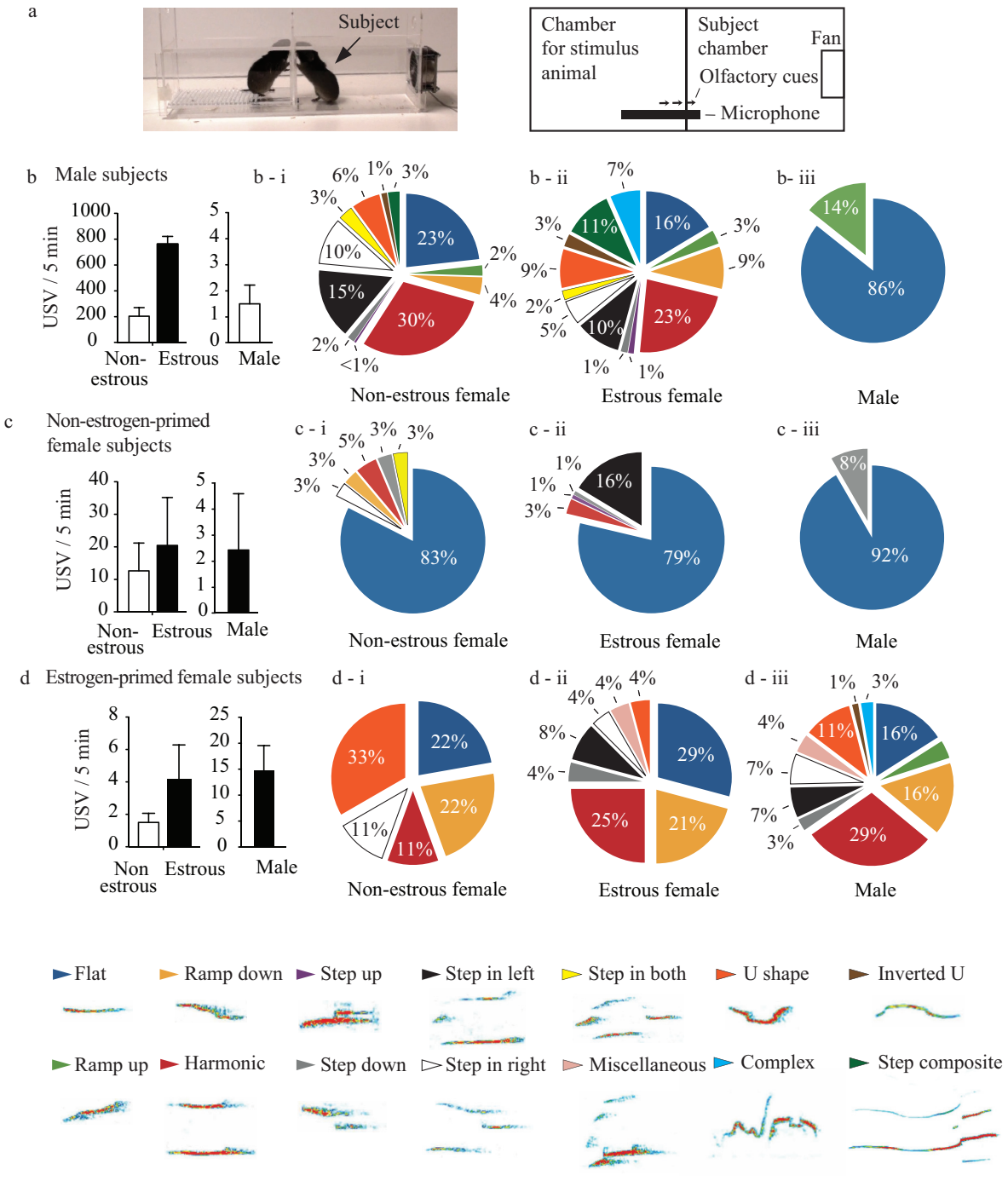

Figure 4 Mean ultrasonic vocalization (USV) counts and call type distribution for male and female prairie voles under different socio-sexual interactions. (a) A recording chamber designed to isolate the subject's USV was utilized; photo on left, diagram on right. The subject is isolated in the right recording chamber with intact visual and olfactory cues from the stimulating animal located in the left chamber. (b) Data from male subjects with quantification of total USVs emitted following the presentation of non-estrous primed female, estrous primed female and male subjects. The proportion of the different call types are shown in the pie charts for these stimulus groups, respectively, in (bi), (bii) and (biii). Color coded key for the USV call types is shown at the bottom of the figure. Data similarly presented for female subjects that were non-estrogen-primed (c) and for female subjects that were estrogen-primed (d).

this did not differ between males and females (compare Figs 2a and 3b) (males: 60.8 ± 22.1 calls; females: 31.3 ± 10.5 calls; $P = 0.205$). The majority of calls emitted by both male and female prairie voles were of the 'flat' call type, the most simplistic call type, and this occurred at a frequency of approximately 35 kHz (Fig. 1a). For males, the total number of USVs in the 'baseline' condition was not significantly altered by being reunited with their cage-mate (Fig. 2a and b) ($P = 0.094$). However, when specific call types were analyzed, we found that social reunion significantly increased 'harmonic' call types and decreased 'flat' calls (Fig. 2bi and ii) (Fisher's exact test: $P = 0.03$). For females, overall call frequency was significantly increased when female cage-mates were reunited (Fig. 3a) ($P = 0.002$) but the pattern of USVs was not altered (compare Fig. 3bi and ii) (Fisher's exact test: $P = 0.935$).

Compared to baseline values (i.e. handling controls), saline injections significantly decreased the number of USVs in males (Fig. 2a; $P = 0.046$; statistics collapsed across social conditions because USV frequency did not differ between 'isolated' and 'joined' for males in this baseline condition) but not in females (Fig. 3a; $P = 0.396$). Under saline control injection conditions, being reunited with their cage-mates significantly increased USV frequency in males (Fig. 2c; $P = 0.043$) and females (Fig. 3c; $P = 0.007$). For both male and female subjects, saline injections did not appear to alter the proportion of call types with the possible exception of an increase in 'step in left' calls following social reunion if subjects had received a control injection of saline (compare black portions of the pie charts in ci to cii for both males in Fig. 2 and females in Fig. 3).

Given that i.p. saline injections can be stressful (Bales & Carter 2003), it seems likely that the stress of the injection contributed to decreased USV frequency in males and, perhaps, the lower mean USV values in females during the isolated condition. It is well established that male and female prairie voles show very different stress responses (DeVries *et al*. 1996) and this might, in part, explain why females showed a more robust increase in USV frequency upon social reunion during the saline control condition. Finally, because USVs were sensitive to control injections of saline, for statistical comparisons, USVs following AMPH injections were compared to saline controls.

Amphetamine caused a robust increase in USVs in both males (Fig. 2a) and females (Fig. 3a) (males saline isolated: 1.3 ± 0.5; males AMPH isolated: 104.8 ± 48.4 calls; $P = 0.008$) (males saline joined: 16.2 ± 9.4; males AMPH joined: 311.1 ± 82.5 calls; $P = 0.00003$) (females saline isolated: 6.4 ± 2.3; females AMPH isolated: 56.7 ± 30.9) (females saline joined: 58.4 ± 13.5; $P = 0.0181$; females AMPH joined: 322.8 ± 93.8 calls; $P = 0.0001$).

However, despite similar increases in the number of AMPH-induced USVs in males and females, there was a notable sex difference in the distribution of call types between males and females. In males, AMPH caused a robust increase in the many non-flat call types such as, 'harmonic', 'step-in-left', and 'step-in-right' when males were isolated (Fig. 2di) and to a lesser degree when males were rejoined within their sibling (Fig. 2dii). Conversely, AMPH-induced USVs in females were largely (approximately 75%) of the 'flat' call type when females were isolated (Fig. 3di) but this returned to typical levels (approximately 50%) when they were reunited with their cage-mate (Fig. 3dii). Isolated male prairie voles were the only group to show the highly complex 'drug' call type (Fig. 2di). These call types are labelled here as 'miscellaneous' (see Fig. 1j).

Socio-sexual experience influencing ultrasonic vocalization behavior

To determine how novel social stimulation alters USVs of both male and female subjects, a specially designed recording chamber was created that detected only USVs emitted only by the subject and not the stimulus animal (Fig. 4a). Male prairie voles showed an extremely large increase in the number of USVs when they are exposed to an unfamiliar non-estrogen-primed female (Fig. 4bi), significantly more compared to when males were isolated or reunited with a familiar male cage-mate (Fig. 2b) (isolated: $P = 0.0007$; joined: $P = 0.0088$). Interestingly, the proportion of USV call-types when males were exposed to non-estrogen-primed females was similar to that that seen following AMPH injection (although the number of calls evoked by the novel social stimulus was much higher) (compare Figs 2d and 4b). Following novel non-estrogen-primed female stimulation and AMPH exposure the respective percentage of call-types were: 23% *vs* 20% 'flat' calls; 30% *vs* 37% 'harmonic' calls; and 25% *vs* 30% 'step-in' calls. These data suggest that AMPH somewhat 'mimics' USVs evoked by salient social stimulation.

When males were exposed to an estrogen-primed female, the number of USVs were remarkably increased, approximately 4× more compared to those emitted following exposure to a non-estrogen-primed female ($P = 0.004$) and approximately a 12-fold increase over that seen following AMPH (Fig. 4b). Moreover, a large per-

centage of male USVs following exposure to an estrogen-primed female were extremely complex (Fig. 4bii) and these complex features were unique to exposure to estrogen cues (as described in Fig. 1m and n). However, when males were exposed to unfamiliar males, they emitted essentially no USVs (Fig. 4b) and what little USVs that were detected were almost entirely of the 'flat' call type (Fig. 4biii).

When female prairie voles (either estrogen-primed or non estrogen-primed) were exposed to novel social cues, either unfamiliar non-estrogen-primed females, estrogen-primed females or males, they showed a relatively small number of USVs (Fig. 4c and d). For non-estrogen-primed females, the majority of the calls had a 'flat' structure (Fig. 4ci–iii). As with males, this majority proportion of calls evoked by novel social stimulation proportion have a structure that is very similar to that seen following AMPH (compare Fig. 4ci–iii to Fig. 3di). Together, these data demonstrate that, in both male and female subjects, novel/salient social stimulation evokes specific patterns of USVs that are very similar to patterns evoked by AMPH administration. This is especially interesting given that the specific patterns of socially-evoked patterns of USVs are highly sex-specific, with males showing a much more robust increase in both USV frequency as well as their complexity in structure.

DISCUSSION

Rodents utilize USVs to facilitate communication that promotes reproduction (Barfield & Geyer 1972; McIntosh *et al*. 1984; Holy & Guo 2005; Barfield & Thomas 2006). Based on the working hypothesis that prairie voles also utilize USVs to facilitate the many cooperative behaviors that underlie their pair bonding and reproductive behaviors, the present study provides a detailed characterization of prairie vole USVs and determines how these calls are altered by motivational stimulation (social and non-social) in adult males and females. This study demonstrates that prairie vole USVs are primarily emitted by males when they encounter an unfamiliar female that is in estrous. Not only do males show a high number of calls when exposed to a female, but the calls are also highly complex in their structure. Conversely, females show much fewer USVs upon exposure to an unfamiliar conspecific and these calls have a mostly simple ('flat') structure. Despite these robust sex differences, AMPH caused a similarly robust increase in the frequency of USVs emitted by both males and females. However, the complexity of AMPH-induced USV structure was altered in a sex-specific manner. AMPH increased the complexity of USVs in male voles, but the majority of AMPH-induced USVs in females once again consisted of the most simple call type (i.e. the 'flat' structure). Together, the data demonstrate that AMPH can robustly increase USVs in both male and female prairie voles and that the structure of AMPH-induced USVs mimics the sex-specific patterns of USVs evoked by salient social stimulation. The current findings suggest that USVs may be a useful measure for future studies of social motivation, mate choice, and the interactions between social and drug rewards in this species.

Rodent USVs are important for coordination of reproductive behaviors and other aspects of social motivation. For instance, mice and rats emit USVs during copulation and in anticipation of copulation (Barfield & Geyer 1972; McIntosh *et al*. 1984; Holy & Guo 2005; Barfield & Thomas 2006). The present study revealed striking sex differences in prairie vole USVs evoked by salient social stimulation (i.e. presentation of a novel conspecific that is a potential mate). Specifically, male prairie voles showed a robust increase in USVs when they were exposed to unfamiliar females, particularly if the female was in estrous. This effect was associated with anticipation of mating or motivation to attract a mate because males showed almost no USVs when exposed to a male conspecific. In contrast to male subjects, females showed fewer USVs when exposed to a salient social stimulus (i.e. novel conspecifics, including estrous females). It is possible that the evolutionary significance of this is that complex USVs are used by males to attract mates, whereas females do not emit USVs (but rather listen and decide) when engaged in mate choice. This would be similar to mate choice mechanisms suggested in other non-monogamous species (Holy & Guo 2005) and our future studies aim to test this hypothesis in prairie voles. Together, the present data suggest that the primary function of USVs emitted by adult prairie voles is for males to utilize USVs to facilitate/coordinate copulation. Future studies focused on female mate choice are needed to determine if males are using USVs to attract a mate or perhaps advertise their quality as a potential mate or partner.

Previous studies in other rodent species support this possibility. Male rats emit USVs prior to copulation (Sales 1972) and male-evoked USVs facilitate successful mounts and intromissions (Barfield & Thomas 2006). In mice, males emit USVs when they encounter a female or female pheromones associated with behavior-

al estrous (Wysocki *et al*. 1982; Sipos *et al*. 1992; Stowers *et al*. 2002). Here, we utilized a chamber that allowed detection of visual and main olfactory stimulation of the stimulus animal but did not allow direct physical interaction during the USV recordings and thus could not be impacted by pheromones. However, in our study, the test subject and stimulus animal were allowed to interact for 2 min (which included mating) prior to USV testing and therefore we cannot rule out a potential contribution of pheromonal exposure during their social interactions prior to the USV recordings. Future studies are needed to determine which female stimuli increase male USVs associated with mating opportunities. However, it is clear from the current study that male prairie voles (which are socially monogamous) share the robust increase in USVs evoked by mating opportunities that has been described in non-monogamous rodent species (Barfield & Geyer 1972; McIntosh *et al*. 1984; Holy & Guo 2005; Barfield & Thomas 2006). This finding suggests that female cues that predict the opportunity to mate, trigger males to advertise their potential as mates, and that that there is nothing fundamentally different about this process in this rodent species that has a strong tendency toward social monogamy.

While we detected fewer USVs emitted by female subjects in this study, we did not measure USVs while animals were copulating. This is notable because in rats, non-estrous females rarely vocalize but females emit USVs during copulation (Thomas & Barfield 1985; Barfield & Thomas 2006). Thus, while it seems quite clear that auditory communication during male-female social interactions is more robustly driven by male USVs, it remains possible that females produce USVs during copulation that may be relevant for reproductive coordination in this species. Additionally, social context had a more significant influence over USV production in females compared to males. Specifically, reunion with their familiar cage-mate (typically a sibling) increased the production of USVs in female subjects to a greater extent compared to male subjects. It is possible that this is because of the stress of social isolation (Grippo *et al*. 2007b, 2008) had a more profound effect of reducing USVs production in females and social reunion had a stronger effect on evoking USVs in females because of the elimination of the stress of social isolation. It is, indeed, important to emphasize that as prairie voles are a social species, it is possible that calls emitted by voles under 'basal conditions' in which the voles were isolated may partially represent the stress response of isolation.

Intake of drugs of abuse, including AMPH, has also been shown to increase USVs in mice and rats (Knutson *et al*. 1999; Ahrens *et al*. 2009; Mu *et al*. 2009; Ma *et al*. 2010; Wright *et al*. 2010). Here, we provide the first demonstration that AMPH also increases USVs in male and female prairie voles. As in control contexts (both handling and saline controls), AMPH-induced increases in USVs were modulated by social context. Meaning, USV frequencies were higher when voles were paired with their familiar cage mate (usually their sibling) and this most likely reflects a combination of a reduction in USV production due to the isolation stress experienced by the prairie vole (a highly social species) (Grippo *et al*. 2007a, 2008) and, perhaps even more obviously, that greater USVs would be expected when animals are in social context given that the function of USVs is social communication. The enhancement of AMPH evoked USVs in social context, compared to isolated conditions, has implications for AMPH reward value in this species. Thus far, the rewarding properties of AMPH have been studied using conditioned place preference testing and animals are always socially isolated during conditioning trials (Aragona *et al*. 2007; Liu *et al*. 2010). Previous data using rats suggests that there is a positive correlation between drug evoked USVs and the drug's effectiveness as a reinforcer using place conditioning (Knutson *et al*. 1999). Therefore, these data suggest that it may be of interest to determine if AMPH is a more effective reinforcer in prairie voles if animals receive drug conditioning in a social context (i.e. in the presence of a familiar conspecific).

Despite the dramatic sex differences in the frequency of increased USVs evoked by salient social stimuli (robust increase in males; modest increase in females), the average AMPH evoked increases in USVs is extremely similar across males and females. However, the pattern of USVs induced by AMPH was quite complex in male subjects (i.e. calls shows very complex call structure, including trills and harmonics) but very simple in female subjects (i.e. mostly 'flat' call structures). The most intriguing aspect of these sex-specific patterns of drug-evoked increases in USVs, is that the patterns roughly match those seen when subjects were exposed to unfamiliar conspecifics (quite complex in males; very simple in females). This may be indicative of some physical constraint that prevents females from emitting complex USVs as adults. An alternative possibility is that USV structure is indicative of the rewarding/reinforcing nature of the AMPH exposure (Knutson *et al*. 2002; Panksepp *et al*. 2002; Burgdorf & Panksepp 2006; Harmon *et*

al. 2008). Future studies are needed to examine the possible relationship between call frequency and structure evoked by AMPH in both male and female voles and how this is related to the reinforcing or motivational impact of the drug.

Finally, prairie voles have proven to be a very effective model for the study of interactions between AMPH and social reward (Aragona *et al*. 2007; Curtis & Wang 2007; Liu *et al*. 2010, 2011; Young *et al*. 2011a,b). Specifically, AMPH promotes place conditioning in this species but the rewarding properties of AMPH are reduced if male subjects are pair bonded (i.e. a greater AMPH dose is needed to induce a conditioned place preference) (Liu *et al*. 2011). Further, pre-exposure to AMPH impairs partner preference formation in male prairie voles (Liu *et al*. 2010). Importantly, these interactions are mediated by DA transmission within the nucleus accumbens (NAc) shell (Liu *et al*. 2010, 2011). Previous studies in rats have shown that infusion of AMPH into the NAc shell increases USVs (Burgdorf *et al*. 2001) and DA regulation of pair bonding is also specific to the NAc shell (Aragona *et al*. 2003, 2006; Aragona & Wang 2007, 2009). This suggests that USVs may provide a useful measure to examine how the relative rewarding values of drug and social rewards interact in prairie voles and (while this in no way excludes the involvement of other brain systems) how this is mediated by neural processing within the NAc shell.

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