Sacred Heart UNIVERSITY

Sacred Heart University
DigitalCommons@SHU

Biology Faculty Publications

Biology Department

2011

A Comparison of Prairie Vole Audible and Ultrasonic Pup Calls and Attraction to Them by Adults of Each Sex

Thomas A. Terleph
Sacred Heart University, terlepht@sacredheart.edu

Follow this and additional works at: http://digitalcommons.sacredheart.edu/bio_fac

Part of the Population Biology Commons, and the Zoology Commons

A comparison of prairie vole audible and ultrasonic pup calls and attraction to them by adults of each sex

Thomas A. Terleph[1]
(Department of Biology, Sacred Heart University, 5151 Park Avenue, Fairfield, CT 06825, USA)

Summary

Rodent pups of many species emit both ultrasonic vocalizations (USVs) and calls spanning into a lower frequency range, audible to humans (AUDs), yet there has been little systematic comparison of these different call types, or analyses of how they might differ in signal function. Here the spectral and temporal characteristics of USV and AUD pup calls are described for the prairie vole (*Microtus ochrogaster*), a model used in studies of monogamous mating and biparental care, and a species with an unusually large functional and anatomical representation of auditory cortex. Findings provide a detailed description of each call type, including changes to a number of USV characteristics over pup development. Adult attraction to the playback of these two call types was tested in groups that differed in regards to sex and reproductive status. Separate groups included virgins as well as paired animals of each sex, tested at time points either prior to the birth of pups (associated with either mid or late gestation), or at postpartum time points (parents of 2–3 day olds and parents of 8–9 day olds). Adults were attracted to both USVs and AUDs, but group differences were found only in the attraction to USVs. Males were more attracted to USVs than females, and there were interactions of sex and reproductive status. Results suggest that pup vocal communication is quite sophisticated, and that USVs and AUDs may have different communicative functions.

Keywords: Ultrasonic vocalization, monogamy, parental behaviour, prairie vole, reproduction.

1. Introduction

Juveniles of many rodent species emit ultrasonic vocalizations (USVs) when under conditions of isolation and temperature stress. These calls are

---

>20 kHz, entirely outside of the range of human hearing, and may function as a signal to parents to locate and retrieve a pup (Allin & Banks, 1971, 1972; Colvin, 1973; Hofer & Shair, 1978, 1987; Jans & Leon, 1983; Brunelli et al., 1994; Rabon et al., 2001; Mandelli & Sales, 2004). USVs may additionally be an acoustic by-product of laryngeal braking, a respiratory manoeuvre to increase oxygen delivery during cold exposure (Blumberg & Alberts, 1990).

Mouse and rat pups also produce calls with much of their power in a lower frequency range that is audible to humans (AUDs). Such calls occur when a pup struggles in the nest or pushes for its mother's nipples or in the context of rough handling by either the mother or an experimenter, possibly serving to signal a parent to cease its current behaviour (Okon, 1970, 1972; Noirot, 1972; Elwood & McCauley, 1983; Haack et al., 1983; Ehret & Bernecker, 1986; White et al., 1992; Ihnat et al., 1995). Some AUDs also include frequencies that span into ultrasound (Okon, 1970, 1972; Noirot, 1972; Haack et al., 1983; Ihnat et al., 1995). The AUDs that include ultrasonic frequencies have been associated with pain, such as when a pup is bitten or attacked by an adult, and as such may serve as a signal to deter such attacks (Colvin, 1973; Haack et al., 1983; Ehret & Bernecker, 1986). Less is known about the structure and possible functions of rodent pup AUDs relative to USVs, and neither call type has been extensively studied in socially monogamous, biparental species such as the prairie vole (*Microtus ochrogaster*).

Prairie vole pups produce both AUDs and USVs, and both sexes respond to USVs by retrieving pups (Shapiro & Insel, 1990; Rabon et al., 2001; Blake, 2002). Males often carry and retrieve pups more than females (Solomon, 1993; Lonstein & De Vries, 1999), although the opposite pattern has also been found (Hartung & Dewsbury, 1979). In contrast, in the majority of other rodent species retrieval is provided exclusively by the mother, and is controlled by sex hormones and maternal experience (Ehret & Koch, 1989). Male prairie voles otherwise display a similar parental repertoire to the females' (Solomon, 1993; Lonstein & De Vries, 1999; Young et al., 2008). However, the time course of parental expression differs between the sexes over mate cohabitation and subsequent pup development (Lonstein & De Vries, 2000; McGuire et al., 2007). There is a general increase in parental behaviour by males just prior to the birth of pups (Bamshad et al., 1994; Terleph et al., 2004; Jean-Baptiste et al., 2008), while the onset of female parenting is more closely associated with parturition (Hayes & DeVries, 2007).

It is not known if such differences extend to the adults' responses to pup USVs and/or AUDs.

Anatomical and behavioral evidence suggests that vocal signalling may be an important part of prairie vole parental care. The auditory cortex of prairie voles is disproportionally large, about twice the size of its homolog in mice, a species with approximately the same sized neocortex (Campi et al., 2007). In addition, relative to other sensory systems, the prairie vole neocortex is functionally dominated by the auditory system, with a preponderance of neurons responsive to auditory stimulation in both the primary and multimodal cortical regions (Campi et al., 2007). There is also extensive cortical and thalamic connectivity between the auditory and visual cortex in this species (Campi et al., 2010). In addition, behavioural studies have shown that prairie vole pup USVs are produced at a higher rate than those of other members of their genus (Shapiro & Insel, 1990; Rabon et al., 2001; Blake, 2002), and that prairie vole pups are more prone to social contact than the pups of other vole species, with both littermates (Wilson, 1982a) and parents (Wilson, 1982b).

A number of rodent behavioural studies, including at least one prairie vole study, have measured parental retrieval of pups by using live, vocalizing pups as stimuli (Rabon et al., 2001). Such experiments do not allow for ideal stimulus control, as the rates and ratio of AUD and USV calls can vary considerably both between and within individual pups. In addition, pup stimulus features other than the USV itself, such as odour and tactile cues, cannot be controlled in such tests. In order to avoid these issues, adult responses to playbacks of recorded USVs and AUDs were tested. This is the first vole study to use playback stimuli instead of live pups.

Furthermore, this study is also the first to characterize the calls of prairie vole pups (both USVs and AUDs) in detail, and to investigate the responses to pup calls by adults in different reproductive states. In order to determine whether or not adults show behavioural discrimination between the USVs and AUDs, and if attraction to each call type differs as a function of reproductive experience and sex, examples of each call were played to both males and females at different time points prior to and during cohabitation with a mate, including periods before the birth of pups, and over subsequent pup development. If AUDs function as a signal to a parent to adjust its handling or contact with a pup, rather than eliciting retrieval, then these signals should not attract adults. Alternatively, if the AUD's function extends beyond the role of mediating parental handling, and is a more general signal of pain or

distress, then AUDs may attract parents to render assistance. USVs are predicted to attract parental adults and males in particular. The developmental time-course of attraction is also predicted to differ between the sexes, mirroring the time course observed for other parental behaviours.

2. Material and methods

2.1. Subjects

The recorded pups and the adult subjects of playback tests were from a prairie vole colony maintained at Sacred Heart University and established from the offspring of field animals originally captured in east-central Illinois in 2008. Subjects were housed in clear plastic cages (48 × 27 × 20 cm); the bottom of each cage was covered with approximately 5 cm of bedding consisting of a layer of wood shavings, and then filled with a top layer of hay. Water and food, consisting of sunflower seeds, rabbit chow, and cracked corn, were made available ad libitum. All animals were housed in rooms at 20–25°C under fluorescent lights set at a 14:10 light/dark cycle (lights on at 7:00 a.m. and off at 9:00 p.m.).

Prior to pairing with a mate, playback test subjects were weaned at 21 days of age and housed with a same-sex sibling. Weaning at 21 days ensures that the first litter is weaned prior to the arrival of the next litter, as the gestation period of prairie voles is approximately 22 days and they mate postpartum (Witt et al., 1990). Test subjects therefore had no prior experience with alloparental care, which often occurs in this species (McGuire & Getz, 1981; Solomon, 1991; Wang & Novak, 1992). All adult test animals were 90–120 days of age and were sexually inexperienced at the start of the experiment. Both the male and female from each pair of mated animals were tested. Animal care and all test procedures complied with local, state and federal regulations and were approved by the Sacred Heart University Institutional Animal Care and Use Committee.

2.2. Pup recordings

For all recordings a pup was placed in a 24 × 20 × 41 cm plastic tank within a 80 × 80 × 80 cm sound attenuation chamber (Model 6249B, Med Associates, East Fairfield, VT), lit within by a 50 W infrared bulb. A condenser microphone was suspended, 20 cm above the animal (Avisoft Ultra Sound Gate

116-200 recorder, gain 2, sample rate 250 kHz), and each pup was recorded for 60 s. In order to induce calling, the pups' surroundings were cooled: animals were placed on a moist paper towel surrounded by ice shavings in a 10 cm diameter ring. It was not possible to accurately measure call amplitudes, as pups were free to move during recording, so the position of each animal's mouth relative to the speaker varied both between and within trials. Immediately following recording, each pup was returned to its home cage and observed to ensure that its parents would resume care towards it. All pups were readily accepted by their parents.

In order to measure USV development, recordings were obtained from a total of 11 pups from 3 litters, each recorded at ages 2, 6 and 10 days. Spectrograms were generated from the first 20 s of each recording, and used to measure temporal and spectral characteristics of the USVs, including duration, intercall interval, frequency and duty cycle. The duty cycle measure incorporates both call rate and call durations, and is thus a good way to quantify the total duration of signalling within a bout (Bradbury & Vehrencamp, 1998). It was calculated as the call duration/(call duration + median inter call interval), and was only measured for the USVs, because unlike AUDs, USVs are delivered in lengthy bouts. Measures of the total number of USVs were also obtained from the entire 60 s recording period. For the analyses of USVs at days 2, 6 and 10, measures were averaged for each animal and the groups were compared across days by one-way, repeated measures ANOVAs for each USV measure, and further analyzed with Tukey's Honestly Significant Difference (HSD) post hoc tests.

AUD calls were less common, so it was not possible to obtain a developmental sequence of these calls. AUDs were recorded from a total of 5 pups from 3 litters, each of which was 2–4 days old. In order to compare USVs and AUDs, USVs were also obtained from the same 5 pups that produced the AUDs, and during the same recording sessions.

AUD calls were defined as any call with a peak power spectral frequency (PPSF) below 20 kHz. A few recorded calls (9, from 3 animals) contained frequencies within the audible range, but were not considered to be AUD calls and were excluded from analyses because their PPSFs were above, 20 kHz (mean 32.1 ± 0.9 kHz). Only one of the animals from the group that was recorded at ages 2, 6 and 10 days emitted AUD vocalizations ($N = 8$ AUDs), and only at the age of 2 days. The small number of AUD calls relative to USVs may have been due to the fact that pups were in isolation

and were thus not being handled during recordings. In order to obtain more AUD recordings, 24 additional pups were recorded at ages 2–4 days. From this group a total of 12 AUD calls were obtained from 4 additional animals (each was 4 days old, and they were from 3 different litters). Thus, all AUDs were obtained from 5 individuals (ages 2–4 days). Call measures (described below) were taken from every AUD that an animal produced, and all iterations of each measure were then averaged for that animal. The averaged AUD measures from each of the 5 animals were then compared to matched averages of USV measures from the same animals, obtained during the same recording session.

The measures used for comparison of AUDs and USVs were call duration, as well as two spectral measures: average peak power spectrum frequency (PPSF) and bandwidth. In order to analyze call spectra, fast Fourier transforms were made of each call (Hanning window, 512 points), and the average PPSF and bandwidth were calculated for both the USVs and AUDs of each animal. Bandwidth was calculated as the difference between the minimum and maximum frequencies falling within 20 dB (V) of the PPSF. Mean durations, PPSFs and bandwidths of USVs and AUDs were then each compared by means of two-tailed, paired t-tests.

2.3. Playback tests

Adult responses to pup calls (USVs and AUDs) may be influenced not only by developmental changes to the calls themselves, but also by changes to a parent as its pups age, or to a combination of both factors. Holding the call stimuli constant in the playback tests allowed for manipulation of receiver characteristics exclusively (adult sex and reproductive status in terms of the timing of reproduction).

For playbacks tests, each adult was videotaped while both USV and AUD stimuli were played. Individuals were separated from their mate and placed in a plastic test arena (floor area 20 × 41 cm) within the same soundproof chamber that was used for pup recordings. The chamber was lit within by a 50 w infrared bulb. One wall of the test arena was composed of wire mesh (mesh squares approx 2 × 2 mm), that permitted sound to enter from an ultrasound playback speaker (Scanspeak, Avisoft Bioacoustics, Berlin, Germany) placed 15 cm from a randomly assigned corner of the test arena. A measurement grid on the floor of the test arena was used to determine

animal locations during videotaped playback trials. Each animal was allowed two minutes to habituate to the arena, after which a 10-s vocalization bout (either USV or AUD, randomly assigned) was played. The playback speaker was then repositioned to another randomly assigned corner of the arena, and after another two minutes the second stimulus type was played. Each test stimulus (USV and AUD) consisted of a bout of six calls, repeated four times over a 10-s period (Figure 1). These bouts had been recorded from a single two day old pup, and were representative examples for a pup of this age, of USV and AUD calling bouts, respectively.

Five groups of adults were tested with playbacks (Figure 2), each consisting of five males and five females: unpaired virgins (V); animals paired with a member of the opposite sex for 13 days, approximately the middle of a female's gestation (MG); animals paired for 21 days (late gestation, LG); early postpartum parents (EPP) with 2–3 day old pups; and mid postpartum parents (MPP) with 8–9 day old pups. After testing, all animals were returned to the colony and females from the MG and LG groups were monitored to confirm pregnancies.

Four two-way, between subjects ANOVAs were run on the playback results. The first ANOVA compared the mean distance that adults positioned themselves in the test arena during USV playback, relative to the playback speaker's location. The between-subject factors were sex and the timing of pairing and parental experience, hereafter referred to as reproductive status (V, MG, LG, EPP and MPP, see Figure 2). In order to ensure that there was no preexisting bias in the animals' locations within the test arena just prior to playback, a second ANOVA was run on the same groups described above, but with the dependent measure being distance during the 10 s immediately preceding stimulus playbacks, rather than the distance during playback. A third and forth ANOVA were identical to ANOVAs one and two, respectively, except that ANOVA 3 analyzed the test subjects' locations during playback of the AUD stimuli, and ANOVA 4 analyzed the test subjects' locations during the 10 s prior to playback. It was necessary to run separate ANOVAs for both AUD and USV analyses, as well as pre playback and during playback analyses, because the inclusion of an additional within subjects factor in tests would have made it impossible to interpret any between–within interactions, as there is no clear choice of an appropriate error term for interactions of this type. However, it was still of interest to demonstrate whether or not AUDs
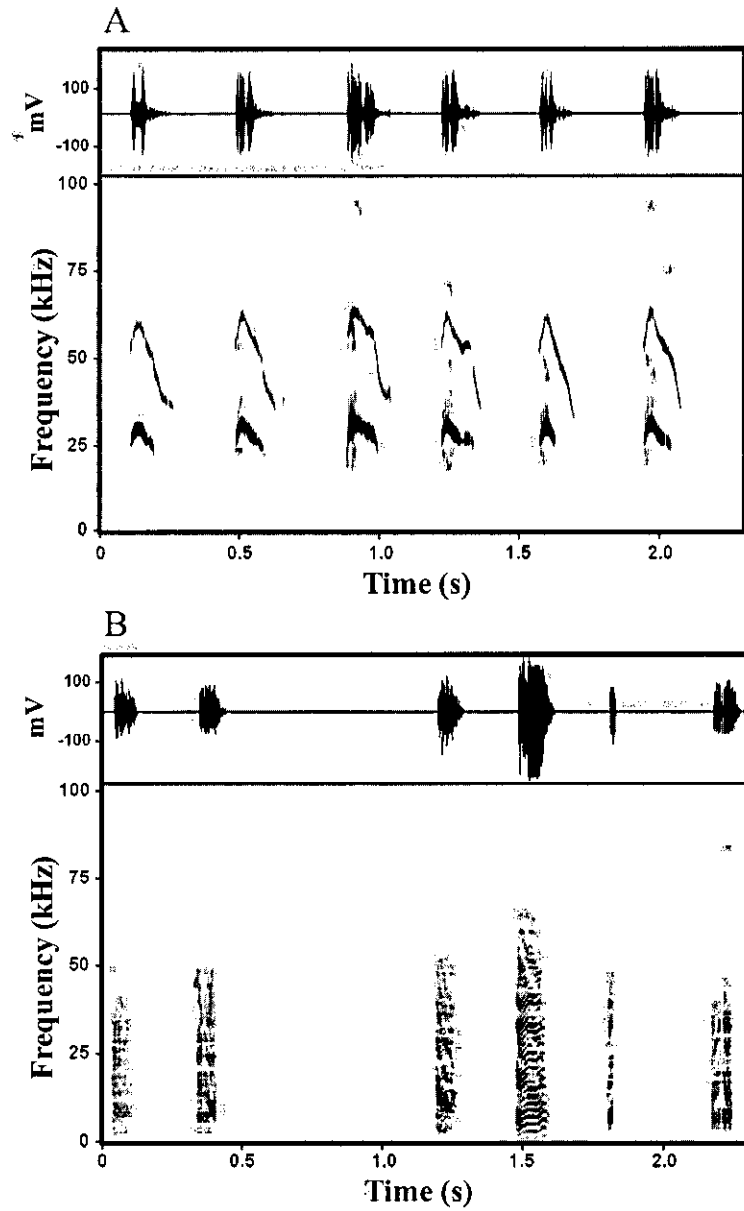
Figure 1. Waveforms and spectrograms of the calls used to generate playback stimuli. (A) Ultrasonic vocalizations (USVs) and (B) audible vocalizations (AUDs) recorded from a 2 day old pup. These stimuli are typical of a 2 day old pup's calls in terms of their spectral and temporal characteristics.
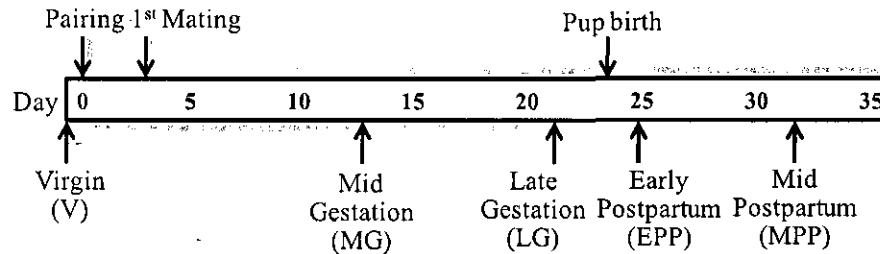
Figure 2. Typical time course of the periods in which the different groups were tested. Arrows above the timeline represent the average days at which first mating and the birth of pups occur following pairing. The five different time points during which separate groups were tested are shown below the time line. Each group consisted of five males and five females. V, unpaired virgins; mid gestation (MG), animals paired with a member of the opposite sex for 13 days, approximately the middle of a female's gestation; late gestation (LG), animals paired for 21 days, associated with the female's late gestation; early postpartum (EPP), animals with 2–3 day old pups; and mid postpartum (MPP), animals with 8–9 day old pups.

and USVs are attractive the animals in general, so separate dependent samples *t*-tests were employed to compare the distance of all subjects from the playback speaker before versus during USV playback and before versus during AUD playback.

3. Results

3.1. USV call structure and development

USVs are comprised of one or more 'syllables', hereafter referred to as elements (Figure 3A). Most USVs are a single element, although some contain 2 or 3 (rarely 4), separated by short periods of silence or rapid frequency changes (>2 kHz) (Figure 3A). USVs became simpler with age: the number of elements decreased, as did the length of the USV itself (Table 1). Similar to what has been reported previously (Rabon et al., 2001) the total number USVs peaked by day 6, but then significantly declined by day 10. These calls are generally produced in rapid bouts (Figure 3B,C), separated by pauses. The rate of USVs within bouts also decreased by day 10 (see inter USV intervals, Table 1), and duty cycle was also higher on days 2 and 6 relative to day 10. In addition to these temporal changes, spectral qualities of the USV also became simpler with age. Even though the fundamental frequency did
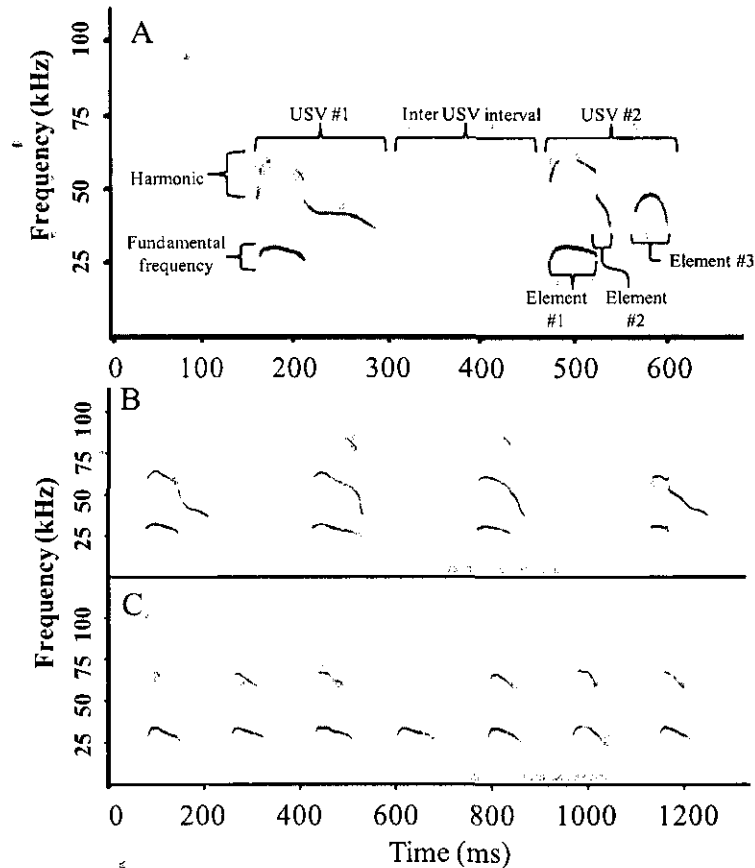
Figure 3. Spectrograms showing call measures and bout rates. (A) Spectrogram of two USVs from within a bout of calling by a 2 day old pup. Spectral and temporal measurement parameters are indicated. USVs may contain multiple elements, particularly those of young pups. No. 2 is an example of a USV that consists of three elements. (B) Spectrogram showing a representative example of a USV bout from a 2 day old pup. (C) Spectrogram showing a representative example of a USV bout from a 6 day old pup. USVs of 2 day olds are of longer duration, and have more separate elements.

not significantly change with age, on days 2 and 6 the first USV element had significantly more harmonics than on day 10 (Table 1).

3.2. AUD analyses and comparison to USVs

Most AUDs (74%) were recorded within ten seconds of the animal being placed into the recording chamber. Unlike USVs, AUDs always consist of

Table 1. Comparisons of prairie vole pup USVs at ages 2, 6 and 10 days.

Measure	Day 2	Day 6	Day 10	ANOVAs	Post hoc tests
Mean number of elements/USV	1.74 ± 0.16	1.32 ± 0.06	1.14 ± 0.03	$F_{2,20} = 12.3; p = 0.0003$	2 > 6 ($p = 0.008$) 2 > 10 ($p = 0.001$)
Mean USV duration (ms)	105 ± 7	83 ± 7	39 ± 3	$F_{2,20} = 39.2; p = 0.0001$	2 > 6 ($p = 0.021$) 2 > 10 ($p = 0.001$) 6 > 10 ($p = 0.001$)
Median inter USV interval over first, 20's (ms)	214 ± 22	160 ± 39	391 ± 76	$F_{2,20} \cong 8.9; p = 0.002$	10 > 2 ($p = 0.015$) 10 > 6 ($p = 0.002$)
Duty cycle	0.34 ± 0.02	0.39 ± 0.04	0.13 ± 0.03	$F_{2,20} = 30.7; p = 0.0001$	2 > 10 ($p = 0.001$) 6 > 10 ($p = 0.001$)
Mean fundamental frequency (first element, in kHz)	33.6 ± 2.4	28.8 ± 1.1	33.3 ± 1.6	$F_{2,20} = 2.2; p = 0.14$	No significant differences
Mean number of harmonics (first element)	1.28 ± 0.19	1.50 ± 0.14	0.61 ± 0.10	$F_{2,20} = 9.4; p = 0.002$	2 > 10 ($p = 0.014$) 6 > 10 ($p = 0.002$)
Total USVs over 60 s	130.8 ± 16.9	202.9 ± 30.7	89.8 ± 15.6	$F_{2,20} = 7.8; p = 0.003$	6 > 10 ($p = 0.003$) Days 2 and 6 do not differ ($p = 0.056$)

Values shown for each measure represent the mean ±1 SE from 11 subjects, except for inter USV interval, which is a median. The results of each of the repeated measures ANOVAs were further analyzed by post hoc tests (Tukey's Honestly Significant Difference). The duty cycle measure represents USV duration/(USV duration + Median inter USV interval). The fundamental frequency measure is the mean of the start and end of the fundamental of the first element of each call. This first element occurs, by definition, in all calls and on average it is the longest element and that with the greatest power.

a single element, and that element is always a broadband signal, extending well into the ultrasound range (compare Figure 1A and B). AUDs have a wider bandwidth (44.2 ± 8.8 kHz) relative to USVs (17.3 ± 4.2 kHz): $t_5 = 4.0$, $p < 0.05$. Seven of the 18 broadband signals had a distinct harmonic stack structure; the remaining 11 more closely resembled broadband noise (see Figure 1B, cf., 4th call with all others). Although AUDs have a wide bandwidth, much of the power is in the lower frequencies: the AUDs' PPSFs were significantly lower than those of USVs: 12.2 ± 0.6 kHz, as compared with 36.1 ± 3.8 kHz ($t_5 = 7.9$, $p < 0.01$). AUDs were also shorter in duration (62 ± 15 ms), relative to USVs (131 ± 10 ms): $t_5 = 3.7$, $p < 0.05$.

3.3. Playback experiment

The ANOVAs that compared the distances that animals positioned themselves from the test speaker in the 10 s immediately preceding USV and AUD playbacks revealed that there were no differences between any group and thus no bias in the animals' locations just prior to playback (mean distances are listed below, and shown as dotted lines in Figure 4).

A dependent samples t-test found that overall, subjects positioned themselves nearer to the speaker during USV playback (18.55 ± 1.48 cm) than just prior to playback (23.06 ± 1.19 cm) ($t_{49} = 2.77$, $p < 0.01$). Similarly, subjects positioned themselves nearer to the speaker during AUD playback (19.32 ± 1.45 cm) than just prior to playback (24.06 ± 1.19 cm) ($t_{49} = 3.10$, $p < 0.01$).

An ANOVA that compared the mean distances from the speaker during USV playback found that males were more attracted to pup USVs than females: $F_{1,40} = 4.64$, $p = 0.037$, and reproductive status significantly influenced attraction to USVs: $F_{4,40} = 6.72$, $p = 0.001$. Post hoc tests (Tukey's HSD) revealed that EPP males were more attracted to USVs than virgin females ($p = 0.025$), and more attracted to USVs than MG animals of either sex (males: $p = 0.032$, females: $p = 0.013$). There were no differences between the sexes in response to AUDs ($F_{4,40} = 0.77$, $p = 0.385$), and reproductive status did not influence attraction to AUDs ($F_{4,40} = 2.34$, $p = 0.071$). Mean distances during playbacks are summarized in Figure 4.
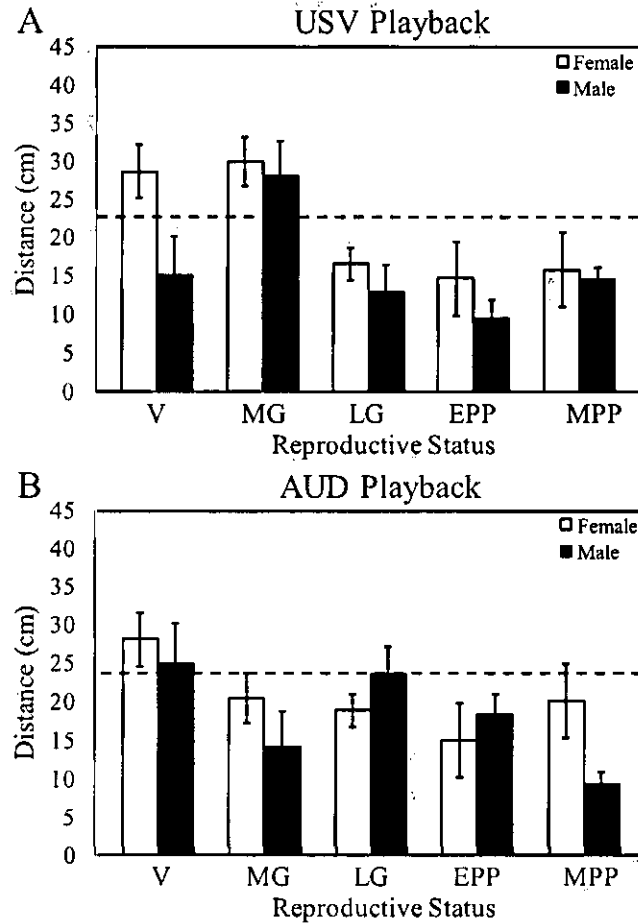
Figure 4. Results of the playback experiment. (A) Mean distance that adult females (white bars) and males (black bars) were located from the corner of the test arena nearest playback during a 10 s ultrasonic call (US). The dotted line represents the overall mean distance during the 10 s immediately preceding playback (23.1 cm). Test groups include unpaired virgins (V), animals paired for 13 days, $\approx$ mid female gestation (MG), paired for 21 days (late gestation, LG), early postpartum parents (EPP) with 2–3 day old pups and mid postpartum parents (MPP) with 8–9 day old pups. (B) The same group's responses to 10 s of audible calls (AUD). The dotted line in B represents the overall mean distance during the 10 s immediately preceding AUD playbacks (24.1 cm). Error bars represent ±1 SE. Males were significantly more attracted to calls than females, and post hocs and additional tests revealed more specific interactions (see Results).

4. Discussion

4.1. USV vs. AUD call structures

The frequency range of AUDs suggests that, like USVs, they may facilitate parental localization of pups. Most AUDs closely resemble broadband noise, which is readily localized (Brown et al., 1979). In addition, AUDs include frequencies that span into the ultrasound range (see Figure 1B), short enough to be attenuated by the animal's head, thus permitting binaural intensity comparison (Heffner & Heffner, 1988). Similarly, by definition the USVs are high frequency, and usually have descending frequency modulation (see Figure 3), a feature that is also likely to facilitate localization (Brown et al., 1979). The presence of these frequency modulations in USVs actually resulted in an overestimation of the bandwidths within each element that were reported in Table 1. The bandwidth measure was across entire elements, but frequencies within portions of each element are of a narrower range (see Figure 2). Finally, the overall adult attraction to the AUDs reported here suggests that they, like USVs, are readily localizable when emitted near a receiver.

However, the fact that USVs and AUDs are spectrally and temporally different, and thus differ in transmission characteristics, suggests that they may not serve identical communicative functions. For one, AUDs may be more costly to produce than USVs, as they are both broadband and high amplitude. This is supported by the observations that AUDs were shorter than USVs, and were not produced in lengthy bouts. High frequencies also attenuate rapidly when transmitted near the ground and through the vegetation that is typical of a voles' environment (Smith, 1979; Blake, 2002). For this reason, USVs are only useful for short-range communication. In contrast, the lower frequency components of AUDs should transmit further, potentially serving as a long-distance signal. Such a signal would be an adaptive trait for older pups, as they increasingly venture from the natal nest following eye opening at approximately 10 days of age (McGuire & Novak, 1984), and are thus more likely to encounter dangers such as unfamiliar adults or predators. Previous work in fact suggests that AUD output does increase with age, with a proportional decrease in USVs, although the relationship was not quantified (Colvin, 1973). The current study confirms a reduction in USV production with age, but it was not possible to examine a developmental sequence of AUD rate, spectral and/or temporal changes, because AUDs were

only obtained from young animals. Although AUDs can potentially transmit further than USVs, such long distance transmission would make them less localizable, as higher frequency components rapidly attenuate. As such AUDs might not serve to elicit parental approach, but could still serve as a warning that is detectable by distant parents and siblings.

The AUDs described here share some of the characteristics of audible vocalizations reported for other rodents, and that have been associated with functions that do not include the elicitation of retrieval. However, it is difficult to compare audible calls across species, as superficially similar calls may have different origins, functions and/or structure. For example, audible calls of rat pups are approximately 800 ms in duration (Ihnat et al., 1995), about 10-times longer than what was found here for prairie voles (81 $\pm$ 8 ms). The audible calls of mouse pups also take different forms: one type contains ultrasonic components up to 80 kHz (Ehret & Bernecker, 1986), but has not been extensively described. A second type has the majority of its energy below 10 kHz, but frequencies rarely exceed 20 kHz (Ehret & Bernecker, 1986). In contrast, the prairie vole AUDs described here had peak power above 12 kHz and often contained multiple harmonics.

4.2. USV notes and production rate change with age

As the pups aged, their USVs became more spectrally simple, shorter in duration, and the number of elements decreased. There were also decreases in total vocal output with age: the total number of USVs, and the associated duty cycle, peaked by day 6 but then significantly declined by day 10. A similar age-related reduction in USV output has been reported previously (Rabon et al., 2001). One or more of these changing USV characteristics could influence parental attraction. In fact, adult prairie voles are known to be slower to approach or contact newborns (0-2 days, Rabon et al., 2001), and to preferentially retrieve older pups (Solomon, 1993; Lonstein & De Vries, 1999; Rabon et al., 2001). Because adult prairie voles do not retrieve pups as readily as some other rodent species (Lonstein & De Vries, 2000), one might predict that adults would only respond to a persistent signal, and that an older, more developed pup would be more capable of such increased output. In fact, prairie vole pups produce USVs up to 15 times more frequently than the closely related montane vole (Shapiro & Insel, 1990; Rabon et al., 2001). Yet the data reported here, showing an age-dependent decrease

in USV production, counter intuitively suggest that parents increase their retrieval of older pups despite decreased USV output.

The increased retrieval of older pups may simply be due to the fact that they are leaving the natal nest more often, rather than due to a change in USV output. Similarly, 10 day olds may call less than younger pups because they require less assistance in general. An older pup might in fact increase its inclusive fitness by decreasing demands on its parents, as females are often pregnant with the next litter by the time their current litter is 10 days old (Getz et al., 1981). As the USV recordings at ages 2, 6 and 10 days were obtained from the same subjects, it is also possible that the decreased rate of calling was due to increasing familiarity with the recording conditions.

4.3. Adult responses to call playbacks

The playback tests demonstrate that, even in the absence of other pup cues, both AUDs and USVs attract adult prairie voles, and that the level of attraction differs as a function of sex and reproductive status only in the responses to USVs. The different effect of reproductive status on attraction to USVs but not AUDs (cf. Figure 4A and B), suggests that the different call types may serve a different function or functions. In addition, the overall adult attraction to AUDs suggests that these calls may also elicit the retrieval of a nearby pup, a different putative function from that of the audible calls of other rodents, which are thought to signal parents to adjust their direct handling and contact (Okon, 1970, 1972; Noirot, 1971; Elwood & McCauley, 1983; Haack et al., 1983; White et al., 1992; Ihnat et al., 1995). In fact, in the current study AUD vocalizations were obtained from pups that were not being handled at the time, although most were recorded within ten seconds of the animal being placed into the recording chamber, so the pups had been recently handled by the experimenter.

It is not surprising that males were more attracted to USVs than females, given that male prairie vole parents have been reported to carry/retrieve pups more often than females (Solomon, 1993; Lonstein & De Vries, 1999), although Hartung & Dewsbury (1979) found the opposite pattern to be the case. However, the sexes did not show large differences in their overall responses to USVs across the reproductive status groups (see Figure 4A). One difference did stand out: EPP males were more attracted to USVs than V females, but did not differ from V males. This observation is similar to those

of other parental behaviors, as virgin males are highly parental and virgin females are not (Lonstein & De Vries, 2000). The V males showed no evidence of attraction to AUDs (Figure 4B). This would be unexpected if AUDs have the identical retrieval-eliciting function as USVs.

Attraction to USVs did not differ between EPP and MPP animals (see Figure 4A). This finding, coupled with the fact that the recordings of 10 day olds contained less USVs, yet parents actually prefer retrieving older pups (Solomon, 1993; Lonstein & De Vries, 1999), suggests that AUDs may also play a role in eliciting parental retrieval, as AUD production increases with age (Colvin, 1973). The hypothesis is further supported by the fact that the playback tests found parents to be responsive to both call types. Alternatively, parents may respond to one or more spectral or temporal characteristics of the calls that change with age. This study found that USVs do in fact change over the course of pup development, but it remains to be seen if something similar occurs for AUDs. Only playback studies can resolve questions such as these, by testing subjects that differ in regards to reproductive status, and employing AUD and USV playbacks from pups of different ages (the current study only used playback stimuli from a 2 day old).

The varied spectral and temporal qualities of prairie vole pup calls, as well as their developmental changes, suggest that signalling between pups and parents of this species involves an as yet underappreciated level of sophistication. The present study has demonstrated that adults of each sex are attracted to both USVs and AUDs, and that these two calls may serve different functions, as they differ in a number of spectral and temporal characteristics, and because their influence on adults differs as a function of reproductive status. Future studies will expand on this work.

Acknowledgements

References

Allin, J.T. & Banks, E.M. (1971). Effects of temperature on ultrasound production by infant albino rats. — Dev. Psychobiol. 4: 149-156.

Allin, J.T. & Banks, E.M. (1972). Functional aspects of ultrasound production by infant albino rats (*Rattus norvegicus*). — Anim. Behav. 20: 175-185.

Bamshad, M., Novak, M.A. & de Vries, G.J. (1994). Cohabitation alters vasopressin innervation and paternal behavior in prairie voles (*Microtus ochrogaster*). — Physiol. Behav. 56: 751-758.

Blake, B.H. (2002). Ultrasonic calling in isolated infant prairie voles (*Microtus ochrogaster*) and montane voles (*M. montanus*). — J. Mammal. 83: 536-545.

Blumberg, M.S. & Alberts, J.R. (1990). Ultrasonic vocalizations by rat pups in the cold: an acoustic by-product of laryngeal braking? — Behav. Neurosci. 104: 808-817.

Bradbury, J.W. & Vehrencamp, S.L. (eds) (1998). Principles of animal communication. — Sinauer Associates, Sunderland, MA, chapter 18.

Brown, C.H., Beecher, M.D., Moody, D.B. & Stebbins, W.C. (1979). Locatability of vocal signals in old world monkeys: design features for the communication of position. — J. Comp. Physiol. Psychol. 93: 806-819.

Brunelli, S.A., Shair, H.N. & Hofer, M.A. (1994). Hypothermic vocalizations of rat pups (*Rattus norvegicus*) elicit and direct maternal search behavior. — J. Comp. Psychol. 108: 298-303.

Campi, K.L., Bales, K.L. Grunewald, R. & Krubitzer, L. (2010). Connections of auditory and visual cortex in the prairie vole (*Microtus ochrogaster*): evidence for multisensory processing in primary sensory areas. — Cereb. Cortex 20: 89-108.

Campi, K.L., Karlen, S.J., Bales, K.L. & Krubitzer, L. (2007). Organization of sensory neocortex in prairie voles (*Microtus ochrogaster*). — J. Comp. Neurol. 502: 414-426.

Colvin, M.A. (1973). Analysis of acoustic structure and function in ultrasounds of neonatal *Microtus*. — Behaviour 44: 234-263.

Ehret, G. & Bernecker, C. (1986). Low-frequency sound communication by mouse pups (*Mus musculus*): wriggling calls release maternal behaviour. — Anim. Behav. 34: 821-830.

Ehret, G. & Koch, M. (1989). Ultrasound-induced parental behaviour in house mice is controlled by female sex hormones and parental experience. — Ethology 80: 81-93.

Elwood, R. & McCauley, P.J. (1983). Communication in rodents: infants to adults. — In: Parental behavior of rodents (Elwood, R.W., ed.). Wiley, Chichester, p. 127-149.

Getz, L.L., Carter, C.S. & Gavish, L. (1981). The mating system of the prairie vole, *Microtus ochrogaster*: field and laboratory evidence for pair-bonding. — Behav. Ecol. Sociobiol. 8: 189-194.

Haack B., Markl, H. & Ehret, G. (1983). Sound communication between parents and offspring. — In: The auditory psychobiology of the mouse (Willott, J.F., ed.). Charles C. Thomas, Springfield, IL, p. 57-97.

Hartung T.G. & Dewsbury, D.A. (1979). Paternal behavior in six species of muroid rodents. — Behav. Neural Biol. 26: 466-478.

Hayes, U.L. & De Vries, G.J. (2007). Role of pregnancy and parturition in induction of maternal behavior in prairie voles (*Microtus ochrogaster*). — Horm. Behav. 51: 265-272.

Heffner, R.S. & Heffner, H.E. (1988). Sound localization in a predatory rodent, the northern grasshopper mouse (*Onychomys leucogaster*). — J. Comp. Psychol. 102: 66-71.

Hofer, M.A., & Shair, H.N. (1978). Ultrasonic vocalization during social interaction and isolation in 2-week-old rats. — Dev. Psychobiol. 11: 495-504.

Hofer, M.A., & Shair, H.N. (1987). Isolation distress in two-week old rats: influence of home cage, social companions, and prior experience with littermates. — Dev. Psychobiol. 20: 465-476.

Ihnat, R.,White, N.R. & Barfield, R.J. (1995). Pup's broadband vocalizations and maternal behavior in the rat. — Behav. Proc. 33: 257-272.

Jans, J.E., &-Leon, M. (1983). Determinants of mother–young contact in Norway rats. — Physiol. Behav. 30: 919-935.

Jean Baptiste, N., Terleph, T.A. & Bamshad, M. (2008). Changes in paternal responsiveness of prairie voles (*Microtus ochrogaster*) in response to olfactory cues and continuous physical contact with a female. — Ethology 114: 1239-1246.

Lonstein, J.S. & De Vries, G.J. (1999). Comparison of the parental behavior of pair-bonded female and male prairie voles (*Microtus ochrogaster*). — Physiol. Behav. 66: 33-40.

Lonstein, J.S. & De Vries, G.J. (2000). Sex differences in the parental behavior of rodents. — Neurosci. Biobehav. Rep. 24: 669-686.

Mandelli, M. & Sales, G. (2004). Ultrasonic vocalizations of infant short-tailed field voles, *Microtus agrestis*. — J. Mammal. 85: 282-289.

McGuire, B. & Novak, M. (1984). A comparison of maternal behaviour in the meadow vole (*Microtus pennsylvanicus*), prairie vole (*M. ochrogaster*) and pine vole (*M. pinetorum*). — Anim. Behav. 32: 1132-1141.

McGuire, B., Parker, E. & Bemis, W.E. (2007). Sex differences, effects of male presence and coordination of nest visits in prairie voles (*Microtus ochrogaster*) during the immediate postnatal period. — Am. Midl. Nat. 157: 187-201.

McGuire, M.R. & Getz, L.L. (1981). Incest taboo between sibling *Microtus ochrogaster*. — J. Mammal. 62: 213-215.

Noirot, E. (1972). Ultrasounds and maternal behavior in small rodents. — Dev. Psychobiol. 5: 371-387.

Okon, E.E. (1970). The effect of environmental temperature on the production of ultrasounds by isolated non-handled albino mouse pups. — J. Zool. 162: 71-83.

Okon, E.E. (1972). Factors affecting ultrasound production in infant rodents. — J. Zool. 168: 139-148.

Rabon, D.R., Sawrey, D.K. & Webster, W.D. (2001). Infant ultrasonic vocalizations and parental responses in two species of voles (*Microtus*). — Can. J. Zool. 79: 830-837.

Shapiro, L.E. & Insel, T.R. (1990). Infant's response to social separation reflects adult differences in affiliative behavior: a comparative developmental study in prairie and montane voles. — Dev. Psychobiol. 23: 375-393.

Smith, J.C. (1979). Factors affecting the transmission of rodent ultrasounds in natural environments. — Am. Zool. 19: 431-441.

Solomon, N.G. (1991). Current indirect fitness benefits associated with philopatry in juvenile prairie voles. — Behav. Ecol. Sociobiol. 29: 277-282.

Solomon, N.G. (1993). Comparison of parental behavior in male and female prairie voles (*Microtus ochrogaster*). — Can. J. Zool. 71: 434-437.

Terleph, T.A., Jean Baptiste, N. & Bamshad, M. (2004). Mechanisms and time course for induction of paternal behavior in prairie voles (*Microtus ochrogaster*). — J. Mammal. 85: 1124-1129.

Wang, Z.X. & Novak, M.A. (1992). Influence of the social environment on parental behavior and pup development of meadow voles (*Microtus pennsylvanicus*) and prairie voles (*M. ochrogaster*). — J. Comp. Psychol. 106: 163-171.

White N.R., Adox, R., Reddy, A. & Barfield, R.J. (1992). Regulation of rat maternal behavior by broadband pup vocalitations. — Behav. Neural Biol. 58: 131-137.

Wilson, S.C. (1982a). The development of social behaviour between siblings and non-siblings of the voles *Microtus ochrogaster* and *Microtus pennsylvanicus*. — Anim. Behav. 30: 426-437.

Wilson, S.C. (1982b). Parent–young contact in prairie and meadow voles. — J. Mammal. 63: 300-305.

Witt, D.M., Carter, C.S., Chayer, R. & Adams, K. (1990). Patterns of behaviour during postpartum oestrus in prairie voles, *Microtus ochrogaster*. — Anim. Behav. 39: 528-534.

Young, K.A., Liu, Y. & Wang, Z. (2008). The neurobiology of social attachment: a comparative approach to behavioral, neuroanatomical, and neurochemical studies. — Comp. Biochem. Physiol. 148: 401-410.