Functions of Vocalization in Sociosexual Behaviors in a Seminatural Environment

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Abstract

Both male and female rats produce vocalizations in the presence of a potential sexual partner. In this study we evaluated the role of vocalizations in sociosexual behaviors in an ecologically valid procedure. Three males and four females were housed in a seminatural environment. In each group one or two males and females were devocalized and the other subjects were sham operated. Sociosexual interactions between males and females were recorded for a period of one hour when all four females were receptive so that the males had the choice to interact either with vocalizing or with silent females. Devocalized and sham operated males displayed very similar behavioral patterns. There was no difference in any of the male sexual behavior patterns nor in male-initiated non-sexual social interaction. Female vocalizations do not contribute to the regulation of sociosexual interaction. Devocalized males received as much attention from females as sham operated males, with the exception of paracopulatory behavior with short duration which were more frequently directed towards the sham operated males than to the devocalized males. This was the case for both silent and vocalizing females. It appears, then, that devocalized males are inferior to sham males with regard to the capacity to induce female paracopulatory behaviors. However, this has no consequence for sexual interaction, since devocalized and sham male copulated equally. In sum, these data show that vocalizations play a very limited role in rat sociosexual behavior in a seminatural environment. Furthermore, this indicates that vocalizations have no evident function during copulatory interactions.

Keywords: Vocalization, seminatural environment, sexual behavior, social behavior, rat

Functions of Vocalization in Sociosexual Behaviors in a Seminatural Environment

Vocalization is one of many activities that animals perform during social and sexual interactions. Numerous species of mammals are able to generate various sounds in accordance to different contexts. The production of vocalizations in rodents is one example. There are more than 50 species of rodents known to produce vocalizations (Sales, 2010), and many are in the ultrasonic range. Rats produce ultrasonic vocalizations (USVs) when they meet intruders (Thomas, Takahashi, & Barfield, 1983), or are exposed to a predator (Blanchard, Blanchard, Agullana, & Weiss, 1991). Adult and juvenile rats also emit 50 kHz USV during rough and tumble play (Burgdorf et al., 2008; Knutson, Burgdorf, & Panksepp, 1998; Lukas & Wohr, 2015). USVs in similar frequency are emitted by juvenile mice when there is non-aggressive play interaction (Panksepp et al., 2007).

USV has been detected in mating interaction in 11 species of myomorph rodents (Sales, 1972). For instance, male rats produced USVs during copulation (Barfield & Geyer, 1972; White, Cagiano, Moises, & Barfield, 1990). They were found to call more to hormone-treated ovariectomized females than to untreated ones (Geyer & Barfield, 1978). In addition, castration and sexual fatigue reduced the male USVs in quantity (Geyer, Barfield, & McIntosh, 1978). Male mice were reported to produce complex sounds in contexts of courtship and copulation (White, Prasad, Barfield, & Nyby, 1998; Whitney, Coble, Stockton, & Tilson, 1973). However, female mice rarely call in a sexual context (Whitney et al., 1973). Golden hamster vocalizations are not as complex as rats and mice, but they also present a great number of calls in sexual encounters (Fernández-Vargas & Johnston, 2015).

It has been proposed that vocalizations have a communicative function during sexual interactions in rodents. As reviewed in Barfield, Auerbach, Geyer, & Mcintosh (1979), vocalizations produced by male rats elicit females to approach and they exhibit more

paracopulatory behavior (a series of solicitation patterns during sexual behavior, including orientation, darting and ear wiggling). This conclusion was mainly based on two observations: (1) females exhibited a shorter latency to dart and a higher rate of darting to the male partner after being primed with transmitted USVs from a copulating pair, in which female vocalization was masked out (Geyer, McIntosh, & Barfield, 1978), and (2) darting by the female was greater in tests with extensive male vocalizations (Geyer, Barfield, et al., 1978). Also female rats call during mating, but there is no evidence that their vocalizations directly affect male activity (White & Barfield, 1989). Nevertheless, it was reported that female USVs affect her own behavior. This proposal was based on results showing that devocalized females displayed more paracopulatory behaviors than intact females. Furthermore, intact females displayed more such behaviors when copulating with deafened male partners than when copulating with intact males (White & Barfield, 1987). The intriguing question of how the female could know that the male was deafened remains unanswered.

It should be noted that there are several experiments showing that USVs have no evident function in sexual interactions. For instance, rats do not approach the playback of USVs emitted by the opposite sex (Snoeren & Ågmo, 2013, 2014a), and they approach a devocalized rat of the opposite sex as much as they approach vocalizing rats. To the contrary of previous assumptions, it was found that vocalization did not affect sexual attraction, since the amount of time rats spend approaching a devocalized individual of the opposite sex was the same as the time they approached a sham operated individual of the opposite sex (Snoeren & Ågmo, 2014b; Thomas, Howard, & Barfield, 1982). Moreover, copulatory behavior is not affected by devocalization of neither males nor females (Ågmo & Snoeren, 2015). Taken together, these results indicate that the role of USVs during copulation is still far from established. A reexamination is therefore needed.

Most existing studies exploring the role of USVs in social and sexual contexts have been limited to the standard laboratory setting. Either the animals have been exposed to the playback of sounds without physical contact with conspecifics or the behavior of an opposite sex pair with one or both members being devocalized has been observed for a short period of time in a small cage. The conclusions based on such settings could lack external validity since rats naturally live in groups, where several males simultaneously interact with several females. In this study, we were interested in the role of vocalizations during sociosexual interaction in a group of rats, particularly with regard to potential effects on attractiveness and copulatory achievement (mount, intromission, ejaculation and lordosis). To that end, we observed the behavior of devocalized and vocalizing subjects in a seminatural environment. Sociosexual interactions were observed when ovariectomized females were brought into estrus by the administration of ovarian hormones. The results from this study give valuable information concerning the importance of USVs in a procedure with considerable external validity.

Methods

Subjects

Sixteen female and twelve male Wistar rats (250–300g upon arrival) were obtained from Charles River (Sulzfeld, Germany). The animals were housed in same sex pairs in Macrolon® IV cages in a room with controlled temperature (21 ± 1 °C) and humidity (55 ± 10 %) and a 12:12 h light/dark cycle (lights on 2300). Commercial rat pellets and tap water were provided ad libitum.

All females were ovariectomized under isoflurane anaesthesia. Estradiol benzoate (EB) and progesterone (P) (Sigma, St. Louis, MO, USA) were dissolved in peanut oil (Apoteksproduksjon, Oslo, Norway) and were injected subcutaneously. The females received 18 μg/kg of EB and 1 mg/rat of P approximately 48 h and 3–4 h before observation, respectively. Injection volume was 1ml/kg and 0.2 ml/rat, respectively.

Eight females and six males were devocalized under isoflurane anesthesia three weeks before the experiment. After a 2-cm incision was made on the ventral surface of the neck, we separated the sternohyoideus muscles to expose the trachea and location of the recurrent laryngeal nerves. The nerve was freed from the surrounding fascia, lifted up and a section of about 3 mm of the nerve was removed bilaterally. In addition, eight females and six males received a sham operation, in which the same procedure as the devocalization was followed, except for the section of the nerve. Buprenorphine (0.05 mg/kg) was subcutaneously administered to the rats at surgery and again every 12 hours for the following 3 days. All devocalized rats in this experiment recovered fast from surgery and were healthy. A similar procedure has been used earlier (Snoeren & Ågmo, 2013).

Apparatus

The seminatural environment used in this study has been described in detail elsewhere (Chu & Ågmo, 2014, 2015). Briefly, it measured 2.8 x 2.4 m and consisted of a complex burrow system and an open area. The burrow consisted of several tunnels (7.6 cm wide and 8 cm high) and 4 nest boxes measuring 20 x 20 cm, 20 cm high, and it was covered by Plexiglas, whereas the open area (2.1 x 1.2 m) was uncovered. Seventy-five cm high walls made escape from the area impossible. There were 4 small openings (8 x 8 cm) between the burrow and the open area. A light-blocking wall of extruded polyethylene foam was used to divide the room in which the environment was installed into two parts, thereby providing the possibility to vary the light intensity in the open area while maintaining the burrow in complete darkness. Infrared lamps provided light for the video camera centered above the burrow. Another camera was centered above the open area.

One high frequency sensible microphone was placed above each corner of the open area making it possible to register vocalizations during experiments. Spectrograms of all vocalizations were analyzed with the Sonotrack® sound analysis system (Metris, Hoofddorp, the Netherlands).

Procedure and design

The rats were given sexual experience before the experiment. One male and one sexually receptive female were placed in a regular copulation cage. They were allowed to copulate until the first postejaculatory intromission. If no ejaculation was reached, the test was terminated 20 minutes after the first intromission. If there was no intromission, the test was terminated after 15 minutes.

The sham and devocalized males and females were tested for the presence or absence of vocalizations, respectively, shortly before (< 72 h) being introduced into the seminatural environment. This was performed in a test set-up in which male and receptive female rats were exposed to each other in different enclosures separated by a wire mesh. The inside of each enclosure was covered with sound-absorbing isolation material of extruded polyethylene foam. A high frequency sensible microphone (Metris, Hoofddorp, The Netherlands) was placed above the cage and adjusted so that all sounds from within the cage were registered while sounds from the adjacent cage were not captured by the microphone. The microphone was connected to a computer with the Sonotrack® sound analysis system.

Shortly before the subjects were introduced into the seminatural environment, they were shaved in different areas of the back and their tail was marked with different numbers of black stripes. The floor in the environment was covered with a 2 cm thick layer of aspen wood shavings. Twelve wood sticks and 3 plastic shelter huts were provided in the open area, and nest building material was put in the nest boxes. About 3 kg of food pellets were provided in a corner of the open area, and 4 water bottles were freely accessible in that corner. The 12:12 h light/dark

cycle was preserved in the open area. During the dark phase, light intensity was about 1 lx at floor level. It was about 180 lx during the light phase. The burrow was maintained in total darkness for the rats but illuminated with 2 infrared lamps for the video camera. The video recorders were activated when introducing the animals at 13:00 on Day 0. Recording was then continuous for a period of 8 days. The subjects were allowed to explore this environment undisturbed for 5 days. The females received an injection of EB on Day 5 and of P on Day 7. USVs were recorded for 12 h following the P injection.

Four groups were used. Each group consisted of 4 females and 3 males. This sex ratio (57 % females) is similar to that reported for adult rats in the wild (Wang et al., 2011). The group size was chosen so that the number of individuals per square meter corresponded to a wild rat population of middle to low density (Calhoun, 1962). The number of devocalized and sham subjects in each group is shown in Table 1. Subjects in the same group came from different cages to ensure that they were unknown to each other at the beginning of observation.

Behavioral observations

In order to assure that the subjects had the choice to interact either with both sham operated and devocalized sexual partners, behaviors were registered for one hour starting when the four females in the group had become sexually receptive. From the video record, we observed the duration and/or the frequency of the behaviors defined in Table 2. The animal that initiated the behavior and the recipient of the behavior were also recorded in order to make it possible to determine the amount of interaction between specific individuals. The Observer XT 10 (Noldus, Wageningen, Netherlands) was used for all observations.

Vocalization analysis

The spectrograms of the USVs recorded during the one h of behavioral observation were evaluated manually from the computer record. The number of sound episodes recorded was

counted for each of 4 microphones. We distinguished between 22 kHz calls and calls in the range of 40 - 70 kHz.

Data preparation and statistics

To determine the function of vocalization during sociosexual interactions, we established four kinds of interactions: Between vocalizing males and vocalizing females; between vocalizing males and devocalized females; between devocalized males and vocalizing females; between devocalized males and devocalized females. In addition to recording the behaviors in Table 2, we calculated the lordosis quotient (LQ) was by dividing the number of lordoses displayed by the number of mounts received multiplied by 100.

Male behavioral data were analyzed with two-factor ANOVAs for repeated measures on one factor. The between groups factor was the type of male, sham and devocalized. The within-groups factor was the recipient of male behavior, sham or devocalized female. Female behavior was analyzed in a similar way, with the between-groups factor, type of female having two levels (sham, devocalized). The within-groups factor was the recipient of female behavior, sham or devocalized male. In case of significant interactions, tests for simple main effects were performed as recommended by Winer, Brown, & Michels (1991). All probabilities reported are two–tailed.

Results

General

One “devocalized” female and one “devocalized” male emitted vocalizations during the test before the seminatural experiment. Thus, the devocalization surgery had failed in these animals. Since both rats produced all subtypes of USVs in the same way as the sham rats, they were included among the sham operated subjects.

Because of a beam angle of 12 degrees, the microphones we used in this study were only capable to collect signals from a fraction of the seminatrual environment. In fact only about 10 %

of the surface of the open area was covered. The occurrence of USVs in the entire open area was therefore estimated in accordance to this proportion. As the emitters of USVs were unknown, it was impossible to determine whether the calls were produced by sham males and/or sham females. However, the purpose of this analysis was to determine whether the sham subjects emitted calls during the observation period. The estimated amount of USV episodes in the 40 - 70 kHz range was 470 per h, and the corresponding number of 22 kHz was 9. This means that the proportion of 40 - 70 kHz USVs emitted during copulatory interaction was approximately 98%. Thus, any potential effect of vocalizations is most likely related to 40-70 kHz calls. The very few 22 kHz calls can, thus, be ignored.

Male behavior

The ANOVAs of the frequency and/or the duration of male behavior patterns towards the females failed to detect any difference between sham and devocalized males (*p*s > .10). This applies to the sexual behavior patterns of mounting, intromission and ejaculation as well as to the prosocial behaviors of sniffing and anogenital sniffing and the antisocial behavior nose off. Likewise, the sham and devocalized males showed a similar amount of pursuit of the females. Thus, devocalization did not affect the males' own behavior.

There was no difference in male behavior towards sham and devocalized females (*p*s > .21). This means that the males displayed as much sexual behavior with the devocalized females as they did with the sham females. This was also the case for the pro- and antisocial behaviors recorded. Moreover, the males pursued devocalized females as much as vocalizing females. Thus, sham and devocalized females were equally attractive to the males.

Finally, there was no interaction between Type of male and Type of female (*p*s > .27). Consequently, it must be concluded that sham males and devocalized males interacted equally with sham and devocalized females. The male behavioral data are shown in Figure 1.

Female behavior

All female subjects responded with lordosis to male copulatory acts. There was no difference in frequency of lordosis between sham and devocalized females (p = .81), and the lordosis quotient did not differ (107 ± 9 vs. 128 ± 25, p = .19). The mean latency between P injection and the display of the first lordosis was 265 ± 61 m for the sham females and 205 ± 30 m for the devocalized females (p = .50). ANOVAs of the additional items of female behavior showed that devocalization of the female had no effect on the amount of paracopulatory behavior displayed, or on sniffing the males, pursuit of the males, rejections and nose off (ps > .09). The only significant effect in the factor Type of female was on the duration of anogenital sniffing of the males (3.81 ± 0.75 s vs. 0.96 ± 0.85 s, $F_{1,14}$ = 5.63, p = .02). The sham females sniffed more than the devocalized females. The increase in the duration of anogenital sniffing from about 1 s in devocalized females to about 4 s in sham females has probably no major consequence. It seems that devocalization had very slight effects on the females' own behavior.

The females sniffed, anogenitally sniffed, rejected and displayed nose off as often with the devocalized male as with the sham male (ps > .21). The duration of female pursuit of the males appeared to be longer for sham than for devocalized males, but this appearance was not confirmed in the statistical analysis ($F_{1,14}$ = 4.49, p = .052). There was one important, significant effect in the factor Type of male: The number of paracopulatory behaviors displayed towards the sham males was superior to that displayed towards devocalized males both with regard to duration ($F_{1,14}$ = 11.03, p = .005) and frequency of episode ($F_{1,14}$ = 9.30, p = .009). The mean duration of each episode of this behavior appeared to be longer when displayed to a sham male than to a devocalized male, but this impression was not confirmed by the ANOVA ($F_{1,14}$ = 4.53, p = .052). Female behavioral data are illustrated in Figure 2.

The only significant interaction between Type of female and Type of male concerned the duration of sniffing of the male ($F_{1,14} = 6.41$, $p = .03$). It appears that the devocalized females sniffed the devocalized male more than they sniffed the sham male, and that sham females sniffed sham males more than the devocalized males. However, analysis of the simple main effect of Type of male within sham female did not reach significance (ps > .09). This was also the case for analysis of Type of male within devocalized female (ps > .10). There was no interaction with regard to any other behavior (ps > .18).

Discussion

A crucial issue in this experiment is whether the sham subjects vocalized during the observation period. If not, there would be no possibility to detect any potential effect of devocalization. The recording of sounds during the observation period showed that the subjects emitted a substantial number of vocalizations in the 40 – 70 kHz range. In the seminatural environment there is no possibility to determine the individual sound emitter, but in the preexperimental sound emission test it was confirmed that all sham subjects vocalized in response to a conspecific of the opposite sex, whereas none of the devocalized animals did so. Prior studies in this laboratory have shown that all sexually active males and all sexually receptive females vocalize when exposed to a member of the opposite sex (Snoeren, Helander, Iversen, & Ågmo, 2014; Snoeren & Ågmo, 2014b; Ågmo & Snoeren, 2015). We assume, then, that all the sham subjects vocalized during the behavioral test.

The current experiment showed that sham males and devocalized males copulated in a similar manner. This result is in agreement with earlier studies (Thomas, Talalas, & Barfield, 1981; Ågmo & Snoeren, 2015), in which no effect of male vocalization was found in any parameter of male sexual behavior (including the number and latency of mounts, intromissions and ejaculations). All these data show that vocalizations do not have any self-regulatory function

in the copulatory behavior of sexually experienced male rats regardless of whether the behavior is studied in standard procedures (one male, one female) or in a seminatural environment housing several males and females.

Present data also show that male rats do not distinguish between vocalizing and silent females. They showed as much sexual behavior with devocalized as with sham females. Likewise, there was no difference in the amount of pro- or antisocial behaviors displayed towards vocalizing and silent females. This observation clearly suggests that female vocalizations are inconsequential, at least with regard to sociosexual interactions with males in a seminatural environment. Similar observations have been made in standard pair tests and in a mate choice procedure in which the male can choose between three females (Snoeren et al., 2014; Ågmo & Snoeren, 2015). Moreover, playback of female vocalizations do not activate approach in male rats (Snoeren & Ågmo, 2013).

Whereas female vocalizations do not seem to modify male behavior, male vocalizations seem to have some effect on female behavior. In the present experiment, the females displayed more paracopulatory behavior to sham males than to devocalized males. This observation is consistent with other studies showing that females dart more and display more ear wiggling in response to vocalizing males than in response to silent males (McIntosh, Barfield, & Geyer, 1978; Thomas et al., 1981). No other aspect of female behavior was modified by devocalization of the male partner. In a mate choice test, females spent equal time with silent and vocalizing males even though they darted more in response to the vocalizing male (Thomas et al., 1982). Moreover, the playback of male vocalizations do not activate approach behavior in sexually receptive females, and a devocalized male is approached as much as a vocalizing male (Snoeren & Ågmo, 2014a). These observations show that vocalizations do not enhance a male's attractivity.

The fact that playback of male vocalizations in the absence of a male does not activate darting (Geyer, McIntosh, et al., 1978; Snoeren & Ågmo, 2014a; White & Barfield, 1990) combined with the observation that females dart in response to silent males, albeit less than to vocalizing males, show that male vocalizations are neither sufficient nor necessary for the male's capacity to activate darting. There is also a report showing that devocalization of sexually inexperienced male rats do not modify paracopulatory behaviors in likewise inexperienced females (Ågmo & Snoeren, 2015). Since the studies in which an effect of devocalization have been found (including the present study) employed sexually experienced animals it is possible to suggest that male vocalizations only are efficient in females who have associated these sounds with sexual activities. They would, then, enhance paracopulatory behavior because of an acquired association between sounds and sex.

We have previously found a close relationship between the amount of paracopulatory behavior displayed and the number of mounts received from males as well as number of lordosis displayed (Chu & Ågmo, 2014). The substantial difference in the amount of paracopulatory behaviors displayed towards a sham and a devocalized male found here was surprising, considering that devocalization neither affected the number of mounts received nor the number of lordosis displayed. However, it is also known that short episodes of paracopulatory behavior are far less efficient than long episodes for activating mounting and the subsequent lordosis (Bergheim, Chu, & Ågmo, 2015). Consequently, we hypothesized that the females displayed more short episodes to sham males than to devocalized males, and that there was no difference in the number of long episodes. This hypothesis was tested by determining the number of short (< 4 s) and of long (> 13 s) episodes of paracopulatory behavior. The cut off points were taken from our previous study. The females displayed significantly more short episodes of paracopulatory behavior to the sham males than to the devocalized males (8.21 ± 2.72 vs.0.87 ± 0.35, $F_{1,14}$ =

8.26, $p = .01$). The number of paracopulatoy behavior episodes lasting more than 13 s, however, was equal in sham and devocalized males (1.02 ± 0.59 vs.0.27 ± 0.18, $F_{1,14} = 1.50$, $p = .24$). This could, perhaps, explain why the enhanced number of episodes of paracopulatory behavior did not lead to any increase in sexual interaction.

Further support for an effect of male devocalization on paracopulatory behavior comes from a detailed study (Le Moëne, Snoeren, Chu, & Ågmo, 2015) showing that this behavior was increased already at the beginning of estrus. Continuous observation of behavior in the seminatural environment, starting one h after P injection in EB primed females, made it possible to determine the moment of the first display of a lordosis in response to a male mount. During a period of 8 minutes following this first lordosis, the females displayed more paracopulatory behaviors to sham males than to devocalized males. Interestingly, there was no difference in paracopulatory behavior displayed to these males during the 8 min preceding the first occurrence of lordosis. There was no difference in the amount of sexual behavior displayed by the sham and devocalized males, confirming that enhanced paracopulatory behavior lacked any functional consequence.

In addition to rats, vocalization during copulation is also found in mice (Sales, 1972; White et al., 1998; Whitney et al., 1973). USVs of male mice appear to be attractive to females, since they approach playback of male USVs (e.g. Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2009) and since females spend more time with vocalizing males than with devocalized males (e.g. Pomerantz, Nunez, & Bean, 1983). However, the attractiveness of male USV is independent of gonadal hormones. Females were attracted to male vocalizations regardless of the phase of the estrus cycle (Hammerschmidt et al., 2009). This is a very interesting result, because female mice copulate only within the period of estrus. The fact that they responded to male USV outside of estrus suggests that male vocalizations are not specifically involved in copulatory

interaction. Similar results were also reported in rats. The male's USVs had no consistent incentive value for the sexually receptive female (Snoeren & Ågmo, 2014a). Another example is that adolescent mice emit USVs during sniffing, anogential sniffing and allogrooming (Panksepp et al., 2007). The rate of USVs emitted by those adolescent mice in such a non-sexual context (about 2 - 3 times / s) was very similar to what mice emitted during mating (Gourbal, Barthelemy, Petit, & Gabrion, 2004; Panksepp et al., 2007; White et al., 1998). Moreover, there was a positive correlation between the amount of the USVs and the duration of social interactions (Panksepp et al., 2007), which reveals that emitting USV could be part of any social interaction. It appears that USVs play a limited or no direct role during copulatory interaction in mice, exactly as we observed in rats. On the other hand, in some other rodents, USVs have an important part in copulation. For instance, USVs of male hamsters facilitate female lordotic responding without male presence, as gratuitous lordoses were observed in response to playback or natural vocalization (Floody & Pfaff, 1977). The function of male vocalizations in copulation seems to vary across species even among rodents.

Taken together, present data show that even though USVs emitted by male rats enhance the amount of paracopulatory behavior displayed in sexually experienced females, they do not have any consequence for sexual interaction. Female vocalizations are entirely without effect on male behavior. Since these observations were made in a social context not entirely unlike rats' natural context, and in an environment sharing at least some characteristics of rats' natural habitat, these data should have considerable external validity. We propose, accordingly, that male and female rat USVs are of marginal importance for the regulation of sociosexual interactions. They may be an epiphenomenon without any particular function, like the sounds produced by humans when coughing, sneezing or scratching (Blumberg, 1992; Blumberg & Sokoloff, 2001).

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Table 1

The Number of Sham and Devocalized Subjects in the Four Experimental Groups

Group	**Number of sham males**	**Number of devocalized males**	**Number of sham females**	**Number of devocalized females**
I	1	2	2	2
II	2	1	2	2
III	2	1	3	1
IV	2	1	2	2

Table 2

Description of Registered Behaviors (Chu & Ågmo, 2014, 2015).

Male and female behavior	**Data collected as**	**Behavior description**
Sniffing	Duration	The rat places its snout close to any body part, except the anogenital region, of another rat while its whiskers move briskly.
Anogenital sniffing	Duration	The rat sniffs, occasionally grooms and licks, another rats' anogenital region.
Pursuit	Duration	The rat runs closely behind another rat.
Nose-off	Duration	Facing another rat either standing on 4 legs or while rearing; it includes boxing and teeth showing.
Male copulatory behavior		
Mount	Frequency	The rat stands on its hind legs and places its forepaws on another rat's rump from behind and displays pelvic thrusting.
Intromission	Frequency	Mount associated with penile insertion. The mount is ended by a backward thrust and is followed by genital grooming.

Ejaculation	Frequency	Penile insertion lasts longer than at intromission and is associated with rhythmic abdominal contractions. Dismount is slow and associated with an open arm posture.
Female behavior		
Paracopulatory behavior	Duration and Frequency	Approach to a male followed by runaway, often associated with hops, darts, ear wiggling.
Lordosis	Frequency	Female stands immobile with the back arched downward and the rump pushed upward while the tail is deflected to the side.
Rejection	Frequency	The rat kicks, bites or turns around against its suitor.

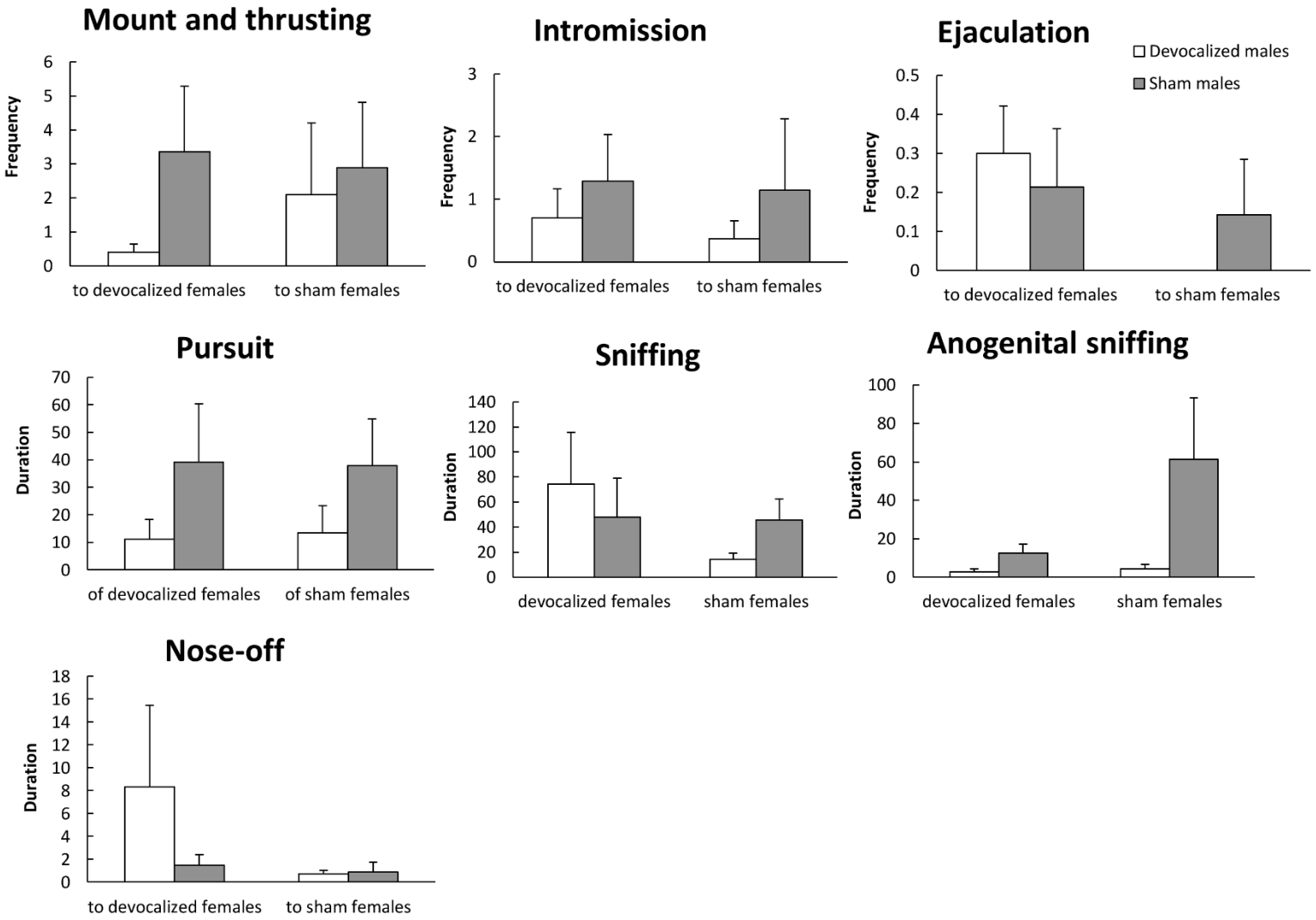

*Figure 1.*Sociosexual behaviors of vocalizing and devcalized males directed towards vocalizng and decocalzied females. Frequency is expressed as occurrences per hour and duration is expressed in seconds. Data are mean + SEM.

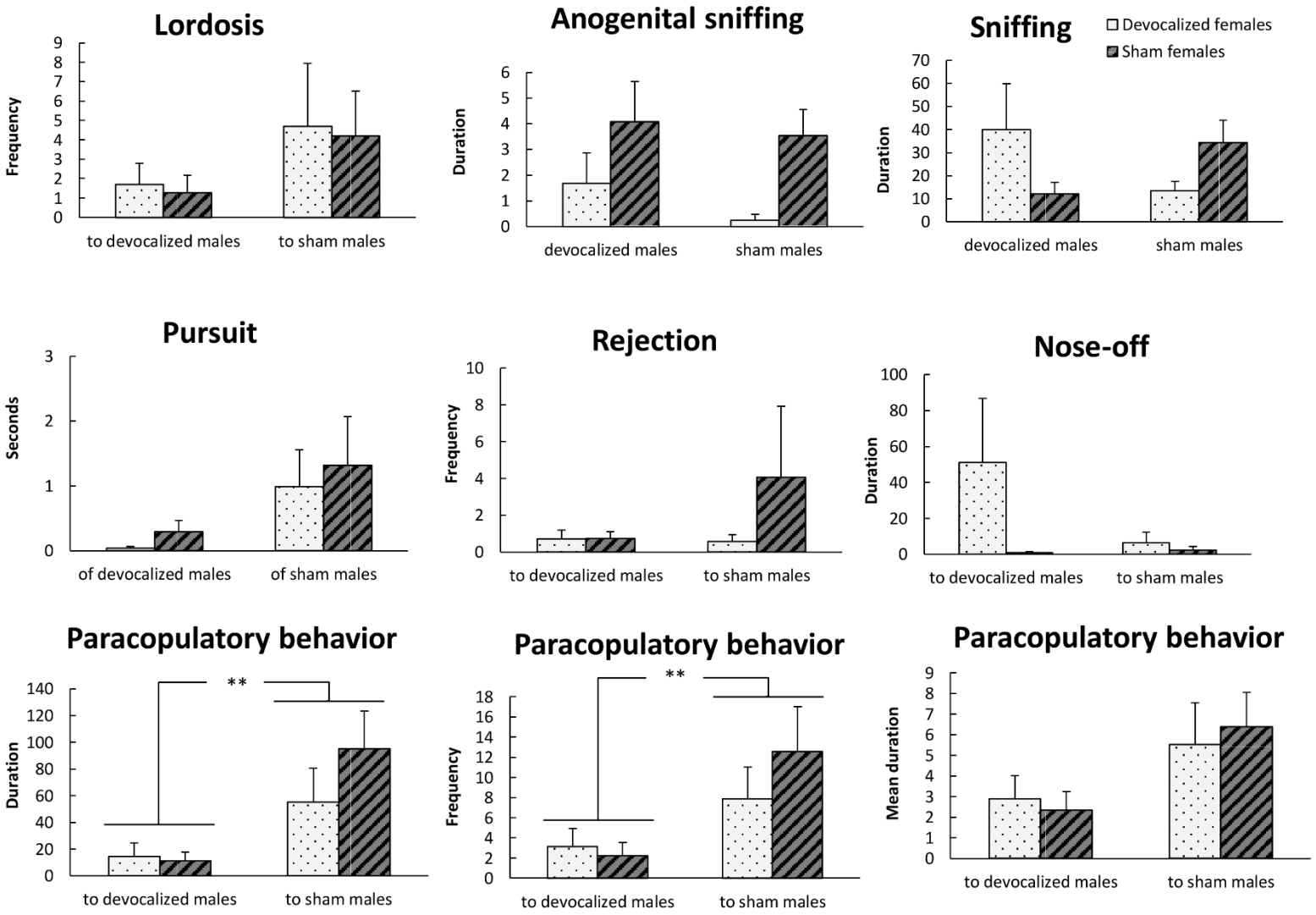

*Figure 2.*Sociosexual behaviors of vocalizing and devcalized females directed towards vocalizng and decocalzied males. Frequency is expressed as occurrences per hour and duration is expressed in seconds. Data are mean + SEM.** repeated measures, $p < 0.01$.