Self-grooming induced by sexual chemical signals in male root voles (Microtus oeconomus Pallas)

Honghao Yu, Pengpeng Yue, Ping Sun, Xinquan Zhao

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Grooming is an innate stereotyped behavior that exists in most animal species (Spruijt et al., 1992). Self-grooming behavior in voles follows a sequential pattern: it begins with rhythmic movements of the paws around the mouth and face, over the ears, and descending to the ventrum, flank, anogenital area, and tail (Ferkin et al., 1996; Leonard et al., 2005; Leonard and Ferkin, 2005). Most rodents spend a large amount of time self-grooming. For example, self-grooming spontaneously occurs after feeding and exploratory behaviors (Spruijt et al., 1992). Furthermore, exposure to novelty and stress also triggers grooming (Inglis and Moghaddam, 1999; Jolles et al., 1979; Kalueff and Tuohimaa, 2004, 2005; Windle et al., 1997) and rodents self-groom when they encounter conspecifics or their scent marks (Carter et al., 1989; Ferkin et al., 2001; Leonard and Ferkin, 2005; Steiner, 1973; Witt et al., 1990).

Many hypotheses have been proposed to explain the functions of self-grooming behavior. One hypothesis for the biological function of self-grooming states that it serves to remove ectoparasites and clean the pelage (Geyer and Kornet, 1982; Hart, 1990; Mooring et al., 2000). Alternatively, individuals may self-groom to lower their body temperature (Hainswor, 1967; Thiessen, 1977). A third hypothesis suggests that animals may release excess motivational energy by self-grooming (Castles et al., 1999; Sachs et al., 1988; Witt et al., 1990). Finally, self-grooming may signal a transition from one type of behavior to another (Baker and Aureli, 1997).

Although self-grooming may possess multiple functions in different contexts, self-grooming in mammals following the encounter of a conspecific odor is considered to be a form of olfactory communication (Ferkin and Leonard, 2005). Many mammals self-groom when they encounter the odor of conspecifics (Ferkin et al., 1996; Leonard et al., 2005; Leonard and Ferkin, 2005). Specifically, we investigated the snif...
The odors of unfamiliar siblings (Paz-Y-Miño et al., 2002), odors from unfamiliar opposite-sex conspecifics as compared to the odors of same-sex conspecifics (Ferkin et al., 1996, 2001; Leonard and Ferkin, 2005). Rodents also self-groom more frequently in response to odors from unfamiliar siblings (Paz-Y-Miño et al., 2002).

The odors of most females vary with reproductive state (Michael, 1975; O’Connell et al., 1981; Rajanarayanan and Archunan, 2004). One interesting study found that self-grooming in meadow voles (Microtus pennsylvanicus) is affected both by the groomer’s reproductive state and the reproductive state of the odor donor (Ferkin, 2006); however, this study did not explore the effects of odors from lactating females on male self-grooming behaviors. Endocrine changes may cause excretion of different signals that communicate specific reproductive states. For example, hormone levels differ based on lactation state. Hence, olfactory cues from lactating females differ from those of non-lactating females (Coureaud et al., 2001, 2006; Coureaud and Schaal, 2000; Jacob et al., 2004; Rameshkumar et al., 2008; Spencer et al., 2004). Voles display different self-grooming behaviors when they are exposed to various odor stimuli, potentially due to the reception of different types of chemical signals or the degree of chemical signals. Chemical signals that lead to the performance of different self-grooming behaviors may act as sexual signals. Thus, we were interested in the influence of sexual chemical signals from lactating female root voles on the self-grooming behaviors of males.

The role of self-grooming has been comprehensively studied in the meadow vole (Microtus pennsylvanicus) and prairie vole (Microtus ochrogaster), and cotton bedding is typically used as an odor stimulus (Ferkin et al., 2001, 2007; Ferkin, 2006; Leonard et al., 2005; Paz-Y-Miño et al., 2002). Mammals emit chemical signals into their surroundings via urine, feces, saliva, or specialized scent glands (Dominic, 1991; Hurst and Beynon, 2004; Novotny, 2003). Thus, chemical signals present in cotton bedding are integrative and can reflect individual olfactory information. Individual chemical signals are produced by specialized scent glands, urine, and feces. Specialized scent glands are distributed across different areas of an individual, including the salivary gland, lacrimal gland, and Harderian gland in the head area, the flank gland in the trunk area, and the anal gland in the tail area. Vaginal fluid and residual feces and urine may also be present in the tail area. Several studies examining the role of chemical signals from specialized scent glands indicated that information contained in head area odor leads to interference in copulation and relays information about species identity, sexual attractiveness, aggression, and territorial boundaries (Austin et al., 2004; Kimoto et al., 2007; Seyama and Uchijima, 2007; Sokolov et al., 1994; Srikanant et al., 2005; Thompson et al., 2007). In contrast, trunk area odor is used for individual discrimination (Johnston and Peng, 2000; Johnston and Rasmussen, 1984) and tail area odor conveys information about sexual attractiveness and mate recognition (Cloe et al., 2004; Johnston, 1974, 1975; Woodley and Baum, 2003; Zhang et al., 2003). Thus, odors from these three areas include different chemical signals, and the influence level of these odors may vary and subsequently induce different types/levels of self-grooming behavior.

Sniffing is one way to collect chemical signals. Research on the relationship between self-grooming and sniffing may reveal connections between self-grooming and chemical signals. The role of self-grooming in chemical communication may be further elucidated using this strategy. In this study, we examined the behaviors of male root voles that received chemical signals from three different body areas of lactating and non-lactating females. By integrating these observations with data on self-grooming behavior, we can derive information on the relationships among odors, odor production areas, and self-grooming. Collectively, chemical signals that induce self-grooming behavior and the role of self-grooming can be investigated using this new methodology.

We tested the hypothesis that sexual chemical signals from females can induce self-grooming behavior in male root voles. The root vole is the only extant Holarctic member of the species-rich genus Microtus and these small interzonal mammals occur in the wet grasslands of both Arctic and temperate zones (Brunhoff et al., 2003). Olfactory communication plays a key role in root vole behavior (Sun et al., 2007, 2008), and root voles display self-grooming behaviors in response to olfactory communication (Sun et al., 2007). In the current study, the sniffing patterns of male root voles in response to odors from the head, trunk, and tail area of lactating and non-lactating females were investigated. Differences in male sniffing patterns and self-grooming in response to odors from lactating and non-lactating females were examined and the relationships among sniffing, odor production area, and self-grooming were analyzed. Support for this hypothesis would indicate that self-grooming is correlated with sniffing of the tail area odor from females.

2. Materials and methods

2.1. Subjects

The female and male root voles used in this experiment were second, third, and fourth generation offspring of field-caught animals captured from a meadow in Menyuan County, China (37°29′ to 37°45′N, 101°12′ to 101°23′E). Laboratory colonies were established at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences (CAS). The voles used in this experiment were born and raised under a long photoperiod (14L:10D. lights on at 08:00 Beijing Standard Time). At 20 days of age, voles were weaned and housed with littermates until they were 30 days old. All animals were housed in clear polycarbonate cages (40 cm × 28 cm × 15 cm) with wood chip bedding and cotton-nesting material. At 30 days of age, animals were separated from littermates and housed singly until they were 60 days old. At 60 days of age, individual root voles were paired with unrelated opposite-sex conspecifics. All voles used in this study were about 90–120 days of age and had successfully produced offspring. Laboratory temperature was maintained at 22 ± 2 °C and food (BLARC, China) and water was provided ad libitum. Cages were cleaned and cotton-nesting material was replaced once a week. For the behavior test, 20 male voles that were 90–120 days old were used as observational animals. Ten lactating females 10–15 days post-birth and 10 non-lactating females were used as sources for individual odor stimuli. The non-lactating females were neither pregnant, nor lactating. Some studies demonstrated that such non-pregnant, non-lactating, parous females which were housed under a long photoperiod will readily mate within a few hours when paired with a male (Taylor et al., 1992; Meek and Lee, 1993). The odor of non-lactating females is moderately attractive to males (Ferkin and Seamon, 1987; Ferkin and Johnston, 1993a,b). All experimental animals were used only once.

2.2. Experimental apparatus

The behavioral choice maze included three components: an odorant box (30 cm × 30 cm × 30 cm), neutral box (30 cm × 30 cm × 30 cm), and connected tube. The two boxes were made of organic glass and were connected by pellucidly organic glass tubes (20 cm × 7 cm). A switch controlled the passage of experimental voles between the odorant and neutral boxes.
Anesthetized individuals were placed in the center of the odorant box and were provided an odor stimulus.

2.3. Odorant preparation

When the male root vole meets the female root vole in free-living population, the communication between male and female is complex. The communication should include acoustic communication and olfactory communication. The voice and the odor of female and the behavioral responses of female may influence the behavior of male. In present study, we would investigate the effect of odor from the female body on self-grooming behavior of male in olfactory communication context. Thus, we anesthetize females and use them as scent donors, in order to control the impact factors.

When individual odor stimuli were prepared, the experimenter wore latex gloves to prevent transfer of their identity to the scent donor. Ten anesthetized lactating females and 10 anesthetized non-lactating females were used for individual odor stimuli. A 5% chloral hydrate solution was used as the anesthetic, which was injected into the abdominal cavity via a midline ventral median line. The injection dose was 0.5 ml/100 g and the unconscious female was placed on a cultural dish, immediately. The anesthetized individual did not wake until the end of the experiment. The odor of narcotic cannot be detected by male because the narcotic was injected into abdominal cavity. The females lost consciousness in a short time after the narcotic was injected. Thus, the narcotic cannot influence the metabolite in short time and the anesthetized voles were not micturition and defeation. We think the anesthetized voles can provide the same olfactory cues as awaked voles.

2.4. Behavioral test procedure

The behavioral tests were conducted in a separate test room. The temperature, lighting, and aeration conditions of the test room were identical to those in the breeding room. Immediately prior to each trial, we transferred a test male vole from its home cage to the test apparatus; the test animals were allowed to acclimatize for 5 min in the apparatus. We then transferred the individual odor source to the odorant box and opened the switch. Behavior was recorded for 10 min using a Sony Vidicon (D805E) and all videos were copied to a computer when the experiment was finished. The duration and frequency of self-grooming and sniffing behavior performed by the male voles was quantified based on the video. All tests were carried out from 09:00 to 18:00 during the light phase. In present study, the behavior responses of 10 male root voles when they were exposed to the lactating individual odor stimuli were recorded. The behaviors of another 10 male root voles in response to non-lactating female odor stimuli were also recorded.

2.5. Behavioral statistic analysis

The scent glands are distributed in the head, trunk, and tail areas of the animal and self-grooming may be affected differently by chemical signals from these three areas. Thus, we compartmentalized the females into the head region, trunk region, and tail region. The head and neck were considered as the head area, the section of the body between the front and rear legs was considered as the trunk area (excluding the actual rear leg area), and the anogenital area, rear legs, and tail were considered as the tail area. Vaginal fluid and residual feces and urine may also exist in the tail area. Sniffing, which is a rhythmic inhalation and exhalation of air through the nose, is a behavior thought to play a critical role in shaping how odor information is represented and processed by the nervous system. We recorded sniffing behavior when the subject’s nose was close to the individual odor stimulation and displayed a rhythmic sniff. The duration and frequency of sniffs to the three regions were recorded. In voles, self-grooming behavior consists of a cephalo-caudal progression that begins with rhythmic movements of the paws around the mouth and face, over the ears, and descending to the ventrum, flank, anogenital area, and tail (Ferkin et al., 1996; Leonard et al., 2005; Leonard and Ferkin, 2005). Root voles generally groom their anogenital area, head, and flanks. Thus, we recorded self-grooming when subjects rubbed, licked, or scratched any of these body areas.

In order to find the different sniffing and self-grooming responses of males to lactating and non-lactating individual odor stimuli, we first examined the distribution of raw data using the Kolmogorov–Smirnov test. As all data were found to be normally distributed, we then used a two-tailed independent-samples t-test to analyze differences in sniffing frequency and duration of males to the head, trunk, and tail regions of lactating and non-lactating females. An independent-samples t-test was also used to analyze differences in self-grooming of males to lactating and non-lactating from individual odor sources.

If self-grooming were induced by olfactory signals obtained via sniffing behaviors, sniffing of the three areas may all contribute to self-grooming. Therefore, to test the hypothesis that self-grooming is induced by sexual chemical signals, a two-tailed partial correlations analysis was used to control two sniffing influence factors, which gave prominence to one sniffing influence factor. We analyzed the correlation between self-grooming and sighs to the tail area by controlling for sniffs to the head and trunk areas. Furthermore, we analyzed the correlation between self-grooming and sniffing directed to the head area by controlling for sniffs to the trunk and tail areas. Finally, we analyzed the correlation between self-grooming and sniffs to trunk area by controlling for sniffs to the head and tail areas.

3. Results

3.1. Male sniffing patterns in response to lactating and non-lactating females

Male sniffing duration and frequency to individual odor stimuli were recorded to analyze the sniffing patterns (reported as a percentage). The results show that the sniffing patterns of males to lactating and non-lactating odor stimuli differ. The sniffing frequencies of males to the head, trunk, and tail areas of lactating females were 39, 18, and 43%, respectively, and the respective sniffing frequencies of males to the head, trunk, and tail areas of non-lactating females were 44, 30, and 26%. The relative sniffing durations of males to the head, trunk, and tail areas of lactating females were 47, 7, and 46%, respectively, and the respective relative sniffing durations of males to the head, trunk, and tail areas of non-lactating females were 55, 22, and 23%.

3.2. Different sniffing and self-grooming responses of males to lactating and non-lactating individual odor stimuli

The results showed that males displayed different sniffing frequencies to the tail and trunk regions when exposed to a lactating female stimulus as compared to a non-lactating individual stimulus (Fig. 1A). Males engaged in more sniffing behaviors to the tail region when they were exposed to lactating female odor stimuli as compared to non-lactating female odor stimuli (n = 10, mean ± SE, 9 ± 0.75 vs. 5.1 ± 0.6, t = 4.064, df = 18, p = 0.001); however, they sniffed the trunk region of lactating female stimuli at high rates compared to stimuli from lactating individuals (3.8 ± 0.39 vs. 5.3 ± 0.49, t = 2.382, df = 18, p = 0.028). Sniff rates to the head region were not significantly different (8.2 ± 1.1 vs. 7 ± 0.75, t = 0.901, df = 18, p = 0.379). Male voles spent different amounts of time sniffing the tail and trunk regions in response to lactating female odor...
stimuli as compared to non-lactating female odor stimuli (Fig. 1b). Males also spent more time sniffing the tail region when they were exposed to lactating female odor stimuli as compared to non-lactating female odor stimuli (30.4 ± 4.04 vs. 10.1 ± 1.9, t = 4.548, df = 18, p < 0.001); however, they spent less time sniffing the trunk region when they were exposed to lactating female odor stimuli as compared to non-lactating female odor stimuli (4.6 ± 0.82 vs. 9.9 ± 2.09, t = 2.356, df = 18, p = 0.030). The sniffing duration of odors from lactating and non-lactating head regions were not different (31.2 ± 7.22 vs. 24.5 ± 4.99, t = 0.787, df = 18, p = 0.441).

Males displayed different frequencies of self-grooming to lactating female odor stimuli as compared to non-lactating female odor stimuli (Fig. 1c). Males engaged in more self-grooming behavior when they were exposed to lactating female odor stimuli as compared to non-lactating female odor stimuli (2.6 ± 0.45 vs. 0.7 ± 0.26, t = 3.642, df = 18, p = 0.002). Male voles spent different amounts of time self-grooming in response to lactating female odor stimuli as compared to non-lactating female odor stimuli (Fig. 1d). Finally, males spent more time self-grooming when they were exposed to lactating female odor stimuli as compared to non-lactating female odor stimuli (6.6 ± 1.28 vs. 1.2 ± 0.53, t = 3.906, df = 18, p = 0.010).

3.3. The relationship between sniffing and self-grooming

The partial correlation analysis showed that the frequency of self-grooming was significantly correlated with the frequency of sniffs to the tail area (n = 20, r = 0.5745, p = 0.013; Fig. 2); however, the frequency of self-grooming and the frequency of sniffs to the head area (r = −0.0913, p = 0.719), trunk area (r = −0.1756, p = 0.486), and tail area (r = 0.3778, p = 0.122) were not significantly correlated. The results of the partial correlation analysis also show that the duration of self-grooming and the duration of sniffing to the head area (r = −0.0913, p = 0.719), trunk area (r = −0.1756, p = 0.486), and tail area (r = 0.3778, p = 0.122) were not significantly correlated.

4. Discussion

In the present study, lactating and non-lactating females were used as the sources of individual odor stimuli. Therefore, the odor signals were integrative. Chemical signals originate from specialized scent glands that distribute odor in head area, trunk area and tail area of the root vole. Male sniffing patterns in response to individual odorant stimuli differed in frequency and duration based on location (i.e., head, trunk, and tail). Of the three areas, the head area secretes the most complex chemical signals, and chemical signals from the tail area are more complex compared to those from the trunk area (Austin et al., 2004; Kimoto et al., 2007; Seyama...
and Uchijima, 2007; Sokolov et al., 1994; Srikantan et al., 2005; Thompson et al., 2007; Johnston and Peng, 2000; Johnston and Rasmussen, 1984; Cloe et al., 2004; Johnston, 1974, 1975; Woodley and Baum, 2003; Zhang et al., 2003). Our results showed that males were most interested in head and tail area odor, and did not prefer trunk area odor. The observed sniffing patterns suggest that scent glands in the head area include important chemical signals and may be central for reproductive and social behaviors in the vole. The results also showed that male sniffed the trunk region of non-lactating female stimuli at high rates compared to stimuli from lactating individuals. The difference of male in response to the trunk area odor from lactating and non-lactating females indicates that root vole may have special scent gland in their trunk. However, this guess should be investigated using scent collected from the specific areas on lactating and non-lactating females with swabs. The different distributions of sniffing frequencies and duration in response to individual odor stimuli may be a result of the various scent glands, which are distributed across different areas of the female. Specialized scent gland secretions relay different chemical signals to the recipient. The distribution of male sniffing behavior across different parts of the body may be advantageous for receiving chemical signals.

In the present study, different sniffing and self-grooming responses of males to lactating and non-lactating female odor stimuli were observed. Males spent more time sniffing and performed more sniff behaviors in response to the lactating females’ tail area as compared to that of non-lactating females. Males also spent more time self-grooming and engaged in more self-grooming behaviors in the presence of lactating female odor stimuli as compared to non-lactating female odors. These results indicate that the odor of lactating females is more sexually attractive than that of non-lactating females. The increased self-grooming behavior of male root voles in response to lactating female individual odor may reflect sexual motivation. Research on short-tailed field voles (Microtus agrestis) indicates that lactating voles can mate with males (Breed, 1969). Mating during lactation is due to continuous secretion of estrogen (Breed, 1969). In nature, female meadow voles are usually both pregnant and lactating throughout the entire breeding season (Keller, 1985; Tamarin, 1977). Postpartum estrus exists in the root vole and based on observations in our laboratory, lactating female root voles can mate with males. Therefore, we suggest that lactating female root voles may also mate with males in nature.

This is the first study to examine the sexual attractiveness of lactating female root voles as compared to females in other reproductive stages; although a similar study examining the sexual attractiveness of lactating female meadow voles (Microtus pennsylvanicus) was performed using a male preference test (Ferkin and Johnston, 1995). Anogenital odors were used as a stimulus in the previous study and the scent likely contained a mixture of odors from urine, feces, and vaginal fluids, and possibly other sebaceous or apocrine glands (Ferkin and Johnston, 1993a,b). Anogenital odors are similar to odors from the tail area, which were used in the present study. The results show that lactating meadow vole females are the most attractive to males during lactation days 1 and 2. These first 2 days of lactation correspond to the time when females are in postpartum estrus. Later in lactation, the female meadow vole's odor becomes less attractive and lactating females are moderately attractive to males at other stages (days) during lactation. In meadow voles, lactation lasts 14–16 days (Keller, 1985), but the sexual attractiveness of lactating meadow voles was determined only until day 9 in the Ferkin and Johnston (1995) study. Our results indicate that odors from the tail area of lactating females are more attractive to males than those of non-lactating females. Since the lactating root vole is at the end of lactation, but does not resemble a non-lactating female with respect to odor, the female may be closer to returning to that stage than a female at an earlier point in lactation. Our results are similar to those from the study of Ferkin and Johnston (1995). Our findings, however, suggest that sexual attractiveness of the lactating root vole at 10–15 days of lactation remains stronger than that of non-lactating females. Thus, lactating female root voles are preferred for a longer duration as compared to female meadow voles. This may be an important difference between the two species of voles.

Some experiments have been designed to address the function of self-grooming in meadow voles. Results of these studies demonstrate that self-grooming may be an indicator of the groomer’s social status and may affect mate choice (Ferkin et al., 1996; Leonard and Ferkin, 2005). Self-grooming behavior may be mediated by exogenous melatonin, prolactin, and testosterone (Ferkin et al., 2007; Leonard et al., 2005). The amount of time a male meadow vole spends self-grooming is also affected by the male’s reproductive state and the reproductive state of the odor donor (Ferkin, 2006). In the present study, we assessed the correlation between self-grooming and sniffing to the head, trunk, and tail areas, respectively. The partial correlation analysis showed that the frequency of self-grooming was significantly correlated with the frequency of sniffs to the tail area. In contrast, the frequency of self-grooming and the frequency of sniffs to the head area and trunk area were not correlated. The results of the partial correlation analysis also show that the duration of self-grooming and the duration of sniffing to the head area, trunk area, and tail area are not correlated. The results of this study indicate that self-grooming is induced by the tail area odor in female root voles. Thus, the results support the hypothesis. Since the self-grooming was induced by sexual chemical signals, we hypothesize that self-grooming may reflect the groomer’s sexual motivation and may facilitate sexual interactions in root voles. Previous studies have indicated that males show interest in and attract females for reproduction by self-grooming (Ferkin et al., 1996, 2001). In present study, males self-groomed more in response to the tail area odor from lactating females than from non-lactating females. The results indicated that lactating females were more attractive to males late in lactation than the non-lactating females and the male root vole showed interest in lactating females. The increasing self-grooming may cause the release of chemical cues from the body and increase the active space. The male root vole can be detected by female root vole in this active space and the released odors allow the groomer to transmit information about features of its identity and sex to nearby conspecifics (Ferkin et al., 1996; Halloran and Bekoff, 1995). This would allow female root vole to respond to the odors of the groomer in an appropriate manner. Previous study indicated that self-grooming may be a tactic used by animals to communicate sexual interest to nearby opposite-sex conspecifics (Ferkin et al., 2001). Self-grooming may also produce odors that reduce intersexual agonism between male groomers and female conspecifics (Harriman and Thiessen, 1985; Payne, 1977; Thiessen and Harriman, 1986). Self-grooming might be a sexual signal that releases odors that serve as a greeting or invitation to nearby opposite-sex conspecifics. Therefore, the increasing self-grooming of male root voles in response to lactating female reflect sexual motivation and the odor from body by self-grooming can facilitate sexual interactions in root voles. A notable result of this study is that the frequency of self-grooming was significantly correlated with the frequency of sniffs to the tail area, although the duration of self-grooming and the duration of sniffing to the tail area was not correlated. The possibility exists that enough chemical signals can be obtained in one sniffing bout during a very short time, and these chemical signals may cause the root vole to respond by self-grooming.

In conclusion, the findings of this study support the hypothesis and suggest that self-grooming is induced by sexual chemical signals and may reflect sexual motivation. The role of self-grooming may also facilitate sexual interactions in root voles.
Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Nos. 30500073 and 30870370) and the China Postdoctoral Foundation (20070420525) to PS.

References


References

the China Postdoctoral Foundation (20070420525) to PS.

Science Foundation of China (Nos. 30500073 and 30870370) and


