

Influence of gonadal sex hormones on behavioral components of the reproductive hierarchy in naked mole-rats

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Abstract

Naked mole-rats (*Heterocephalus glaber*) are fossorial, eusocial rodents that live in colonies which typically include 60–80 individuals. Generally, only one of the females and 1–3 of the males in a colony are reproductives. The reproductives engage in mutual genital nuzzling behavior that is rarely exhibited by subordinates (non-reproductives). Thus, genital nuzzling may represent a mechanism of bonding and/or specific recognition between reproductive individuals. We investigated whether gonadal hormones are involved in the maintenance of genital nuzzling behavior and mating behaviors in isolated pairs of mole-rats and also in established breeding pairs of mole-rats within colonies. We also explored whether sex hormone deprivation would alter the strict partner preference for performance of nuzzling within colonies. Our results indicate (a) considerable variation between pairs in the frequency of nuzzling, (b) a reduction in the frequency of nuzzling following castration of the male and restoration of the ‘baseline’ frequency after replacement of testosterone in castrated males, (c) the failure of either castration or combined castration and ovariectomy to eliminate genital nuzzling in established pairs, and (d) the exhibition of nuzzling behavior by some of the subordinates in all three experimental colonies beginning several weeks after gonadectomy of both of the reproductives. No cases of lordosis behavior were seen during the approximately 109 h of behavioral observations. This is not surprising, since female mole-rats have an approximately 30-day ovulatory cycle, and lordosis only occurs during a peri-ovulatory period of a few hours. A total of 44 cases of mounting behavior were recorded; all these involved breeding males in colonies or males from isolated pairs, and all occurred when males were either gonad-intact or castrated with testosterone replacement. Thus, in contrast to nuzzling behavior, male sex behavior appeared to be eliminated during androgen deprivation.

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Introduction

Naked mole-rats (*Heterocephalus glaber*) are fossorial rodents that live in colonies typically including 60–80, and sometimes more than 200, individuals (Bennett and Faulkes, 2000). These animals exhibit a strict reproductive hierarchy and division of labor. Generally, each colony has only one breeding female (the queen) and one to three breeding males. All other individuals are subordinates, which do not breed but help with maintaining the burrow system and caring for the young. Larger subordinates tend to perform more activities related to colony defense, whereas the smaller animals tend to be more involved

in foraging and digging (Jarvis, 1981; Lacey and Sherman, 1991). Naked mole-rats are included within the family Bathyergidae, a group that encompasses five genera of African mole-rats. The largest genus, *Cryptomys*, includes several species that have reproductive hierarchies remarkably similar to that seen in naked mole-rats, but with smaller colony sizes. The naked mole-rat is the only living member of its genus; three other genera include only solitary-living species (Bennett and Faulkes, 2000).

Subordinate naked mole-rats virtually never exhibit sexual behaviors and show a remarkable paucity of sex differences in behavior and external anatomy as compared to most other mammals (Lacey et al., 1991). It has been suggested that the reduction in sex differences among subordinate naked mole-rats may have evolved in conjunction with a life history pattern

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whereby most mole-rats achieve reproductive fitness as helpers in the colony rather than via direct reproduction (Peroulakis et al., 2002).

Subordinates are not sterile, however, and can become reproductive in the laboratory if they are isolated from the colony and paired with a subordinate of opposite sex. In the field, new colonies are thought to be formed primarily, if not exclusively, by individual subordinates that leave their colonies and pair with animals of the opposite sex that have left a different colony. Dispersal involves above-ground migration and may represent the only occasion on which naked mole-rats leave the confines of their burrows (Braude, 2000; O’Riain et al., 1996). Although the exact mechanism of reproductive inhibition is not known, several lines of evidence suggest that reproductive activity is inhibited in subordinates by the presence of the breeding animals. The queen may have the largest role in this respect since she appears to be the dominant animal in the colony and frequently shoves other individuals (Reeve and Sherman, 1991). Plasma and urinary estradiol and progesterone are consistently higher in breeding females as compared to subordinate females (Faulkes et al., 1990; Smith et al., 1997). Similarly, testosterone levels are higher in breeding than in subordinate males (Clarke and Faulkes, 1998). Since gonadal hormones have never been experimentally manipulated in naked mole-rats, the role of hormones in establishing or maintaining the reproductive hierarchy is completely unknown.

Several behaviors are closely associated with breeding status in naked mole-rats. Mounting by males and lordosis of the breeding female are similar to what is seen in many other rodents; that is, the male approaches the female from the rear and places his forepaws on the female’s back and the female assumes a rigid posture with the hindquarters and tail somewhat elevated, allowing the male to achieve intromission. These behaviors are not frequently observed, however, as they are limited to the peri-ovulatory phase of a relatively long (approximately 30 day) cycle (Jarvis, 1991). Another behavior that is virtually unique to breeders is genital nuzzling, which occurs at all times of the female’s ovulatory cycle and during pregnancy (Lacey et al., 1991). This behavior, in which one individual appears to nuzzle another’s genital area, or in which mutual nuzzling occurs, therefore serves as a reliable indicator of breeding status that can be assessed at any time. In the present study, we determined whether gonadal hormones produced by the breeding animals are required to (1) maintain genital nuzzling behavior between breeding pairs and/or (2) to inhibit genital nuzzling in subordinates. Mating behaviors, though observed infrequently, were also recorded.

Materials and methods

Animals and housing

With the exception of one individual, all animals were born in our laboratory colonies at the University of Connecticut. These colonies were descended from a breeding stock that originally included 20 animals obtained by us in 1990; the original source of the breeding line was animals captured in Kenya (Lerata colonies) and bred in the laboratory of Dr. J.U.M. Jarvis (University of Cape Town). Data were collected from four colonies (each consisting of a breeding

pair and their subordinate offspring) and four isolated male/female pairs. Three experimental colonies (EX1, EX2, EX3) underwent hormone manipulations; the fourth colony (CON) served as a control. Each colony contained a single breeding female and a single breeding male as well as a variable number of subordinates (Table 1). Our laboratory colonies vary in size from 4 to 17 individuals. These colonies remain smaller than those found in the field because laboratory colonies frequently fail to raise their young (Jarvis, 1991). Small colonies (4–7 individuals) were chosen for the current study so that the behavior of all animals could be simultaneously recorded. All the colonies had raised at least one litter. One of the isolated pairs (P4) was an experienced breeder and her mate; their offspring had been removed more than 1 year prior to the beginning of this experiment. The female of this pair was the original breeding female that was included in the animals we obtained in 1990. The other three pairs (P1, P2, P3) had not bred despite having been housed together for more than 11 months. The animals ranged in age from 3 to 24 years old.

The colonies and pairs were housed in caging systems made up of small (43 × 21 × 16 cm) and large (72 × 15 × 12 cm) polypropylene tubs connected by “tunnels” of acrylic tubing (5 cm i.d.). Each tub was fitted with a tight-fitting Plexiglas lid to reduce air currents. Dried corncob bedding was provided. The diet consisted of primarily sweet potato supplemented with apples, carrots, squash, and cereal mix prepared in the laboratory. Animals were maintained on a 12L:12D light cycle with lights on at 0500–1700 h. The room temperature was maintained at 29–31°C. A heat coil was placed under at least one cage in each colony to provide an area for “basking. Naked mole-rats have very weak endothermic capacity and maintain body temperatures that are only 1–2°C above the ambient temperature; they prefer to maintain body temperatures of approximately 32–33°C when given access to a source of radiant heat (Buffenstein and Yahav, 1991; Goldman et al., 1999; Riccio and Goldman, 2000b).

Behavioral observations

All animals in our colonies are identified by subcutaneous transponders (AVID Corporation, Norco, CA). In addition, animals’ heads were marked with a marking pen for rapid identification during behavioral observations. Animals were observed in 10 min scans, with no more than one scan being done each day on each colony and pair in the experiment. Scans were performed at random times during the daylight period of the room, usually between 1000 and 1600 h. Earlier studies in naked mole-rats failed to reveal significant circadian rhythms of general locomotor activity (Riccio and Goldman, 2000a) or in sleep vs. arousal (Davis-Walton and Sherman, 1994) in most individuals in established colonies; other behaviors were not assessed in these studies. Since naked mole-rats have bouts of rest interspersed throughout the 24h cycle, the animals were disturbed at the beginning of each scan by replacing their current cage lid with a clean, clear lid. This tended to equalize the activity level at the beginning of the scan and increased the probability that interactive behaviors would be observed during the observation period. For the first and second set of scans (baseline scans and first set of scans following castrations), each colony and each isolated pair were scanned 12–18 times in each set (total of 120–180 min/group). In all other sets of scans, each group was scanned 12 times in each set (Table 2). Total observation time for all pairs and colonies combined was 6550 min.

Naked mole-rats exhibit many social behaviors (Lacey et al., 1991). In this experiment, we recorded only behaviors that are unique to breeding pairs: mutual genital nuzzling, male nuzzling female, female nuzzling male, mounting, and lordosis. All of these behaviors take place almost exclusively between the breeders in the colony. Subordinates in a colony are rarely seen nuzzling with

Table 1
Composition of colonies

Colony	Number of subordinate females in colony	Number of subordinate males in colony	Age range of colony members, including breeders (years)
EX1	1	1	5.5–11
EX2	0	3	3–17
EX3	0	5	5–10.5
CON	1	2	4.5–20

Table 2
Chronology of experimental events for reproductive members of colonies and for isolated pairs

Event	Condition of subjects at time of scans	Day of experiment
Baseline scans	All subjects untreated	n/a
Castration	–	0
CAST 1 scans ^a	Males castrated or sham	17–19
CAST 2 scans	Males castrated or sham	101–119
T capsules inserted	–	156–180
CAST + T scans	Males castrated and bearing T implants	173–195
Ovariectomies	–	243–252
OVX scans	Males castrated and bearing T implants; females ovariectomized	257–272
T implants removed	–	278–298
OVX + CAST 1 scans	Males castrated (no T implants); females ovariectomized	295–315
OVX + CAST 2 scans	Males castrated (no T implants); females ovariectomized	350–370
OVX + CAST 3 scans	Males castrated (no T implants); females ovariectomized	425–432

^a For all sets of experimental scans, days shown indicate days on which days sets of scans were begun. Completion of a set of scans for a given group of animals required 15–30 days.

breeders or with each other, and mounting behavior or lordosis involving subordinates has never been observed in this laboratory or reported by others. We recorded both mounts (male mounts female in normal mating position) and attempted mounts (male mounts from side or head of female).

Nuzzling behaviors involve a “suite” of actions that occur together in a brief sequence. For recording each behavior, criteria were established as follows:

1. *Male nuzzles female.* The suite is initiated when the male and female touch noses while both animals are standing on all four legs. Next, the male proceeds to move along the female’s flank with his nose sliding along her side. When he reaches her hip, he pokes it with his nose and she lifts her leg, exposing her genitals to him. He begins nuzzling her genitals and she rolls onto her side while the nuzzling behavior continues.
2. *Female nuzzles male.* This is the mirror image of male nuzzles female, wherein the suite concludes with the male on his side and the female nuzzling his genitals.
3. *Mutual genital nuzzling.* In this suite of behaviors, either the male or the female initiates a nuzzle, but after one animal rolls over on its side, the other also rolls over, and the male and female nuzzle each other simultaneously (Fig. 1).

In all observations, one must exercise caution not to confuse “feces begging” with nuzzling behavior. Naked mole-rats practice coprophagy, and this behavior, unlike nuzzling, primarily involves subordinates. Although the behavior includes some of the characteristics of nuzzling, it is accompanied by a loud, rhythmic chirping sound, and one can note that the whole body moves when the animal produces the sound (Lacey et al., 1991). In our initial set of behavioral observations (Baseline, see below), we are concerned that we may not have reliably distinguished between feces begging and genital nuzzling. Therefore, the few instances of nuzzling recorded for subordinates in colonies during the Baseline scans may actually have been instances of feces begging. With the experience of our initial observations, this problem was resolved for all further behavioral scans.

Surgery and hormone treatments

Castrations were performed while animals were under avertin anesthesia (30 mg/100 g, i.p.). For implantation and removal of testosterone capsules and for ovariectomies, animals were placed under isoflurane anesthesia. Just prior to

anesthesia, a subcutaneous injection of analgesic (Meloxicam, 50 µg/100 g) was administered.

The testes are retained in the abdomen in naked mole-rats, and abdominal incisions were required for their removal, as was the case for ovariectomies. Abdominal incisions and skin incisions were sutured with surgical thread following removal of the gonads. Animals were returned to their colonies within 4 h following surgery and were accepted by the other members of the colony without incident.

Testosterone (T) capsules were prepared by partially filling silastic tubing (0.078" i.d. × 0.125" o.d.; Dow Corning, Midland, MI) with crystalline testosterone (Sigma) and plugging the ends with short lengths of wooden dowel so that a 10-mm length of hormone-filled tubing was exposed. The ends of the tubing were sealed with medical silastic. Capsules were stored in a solution of zephiran chloride for several days prior to use. Each experimental animal received two of these 10 mm capsules, implanted subcutaneously via a small skin incision in the dorsal neck region. The incision was sutured with surgical thread. As part of an earlier study, we determined that 20 mm testosterone capsules released 38.2 µg testosterone/day (mean value for 5 capsules) when they were incubated in a saline solution at 33°C, which is approximately the mean body temperature of naked mole-rats under our housing conditions (BD Goldman, unpublished data). Since the animals in the present study received two 10 mm T capsules, the rate of testosterone release should have been approximately the same.

Study design

We employed a longitudinal, within-pairs design over the course of approximately 15 months. Naked mole-rats are long-lived rodents, commonly surviving for over 20 years in captivity. A period of several years is generally required to establish a captive colony. Thus, longitudinal studies involving sequential treatments are the most practical design for these animals. The first part of this study consisted of four sets of scans performed on all pairs and colonies: (1) Baseline (all animals gonad intact); (2) CAST 1 (started approximately 2.5 weeks after castration of the breeding male or male member of an isolated pair); (3) CAST 2 (scans started 14–17 weeks after castration of the breeding male or male member of a pair); (4) CAST + T (scans started about 1 week after castrated males received T capsules). The CON colony was also monitored during this phase of the study, and the breeder male was sham-castrated at the time that experimental males were castrated.

During the second phase of the study (scan sets 5–8), a subset of the pairs and the three experimental colonies were monitored after the breeding females or female members of isolated pairs were ovariectomized. Thus, for the experimental colonies, both breeding animals were now gonadectomized. Two isolated pairs and the three experimental colonies were monitored after ovariectomies. The CON colony was also scanned: (5) OVX (scans started approximately 2 weeks after ovariectomy of breeder female or female member of isolated pair; breeder male is CAST + T, as in previous scan set); (6)



Fig. 1. Genital nuzzling by breeding pair of naked mole-rats. These animals were photographed at the culmination of the suite of behaviors that typify mutual genital nuzzling.

OVX + CAST 1 (scans started approximately 2 weeks after the T capsules were removed from castrated males). Finally, to establish the long-term response of both subordinates and breeders to the gonadectomy of both breeders, two additional scan sets were performed in the three experimental colonies, and the CON colony: (7) OVX + CAST 2 (scans started approximately 10 weeks after T capsules removed from breeding males); (8) OVX + CAST 3 (scans started about 21 weeks after T capsules removed from breeding males). The time-line of the entire study is given in Table 2.

The female from P3 died shortly following ovariectomy and the female of P4 was not subjected to ovariectomy because of her advanced age; thus, the corresponding post-ovariectomy scans (5–8) were not performed for these pairs. Also, the final two scans (7 and 8) were performed only for the colony animals and not for the isolated pairs, since these scans were primarily conducted to determine whether subordinate colony members might exhibit nuzzling following long-term deprivation of gonadal hormones in both members of the breeding pair.

All experimental procedures were approved by the IACUC of the University of Connecticut.

Analysis

There was considerable variation among the pairs with respect to the frequency of nuzzling and data were not normally distributed. Animals that showed high frequencies of nuzzling under baseline conditions generally continued to exhibit relatively high frequencies under the various treatment conditions. Nuzzling data during the first phase of the study (castration and hormone replacement) therefore were analyzed using the Wilcoxin Signed Ranks Test, a non-parametric statistic for repeated measures. N (number of pairs) = 7 for each of these tests, and two-tailed probabilities are reported. Percent change from baseline was also calculated for each pair. Data from the second phase of the study, which involved long-term observations on a small number of gonadectomized animals, were not analyzed quantitatively, but are discussed below. Also, mating behaviors were not observed frequently enough to justify statistical analysis, but data for mounting behavior are presented, and a suggested pattern in relation to the reproductive hierarchy and to hormone status is discussed.

Results

All pairs exhibited nuzzling behavior during Baseline scans, and the frequency of nuzzling was similar in isolated pairs and breeding pairs in colonies. On average, Male–Nuzzles–Female accounted for 54.7% of the nuzzling incidents observed in pairs and colonies, whereas Female–Nuzzles–Male and Mutual Genital Nuzzling accounted for 23.6% and 21.7% of nuzzling incidents, respectively. The mean nuzzling frequency (all three categories combined) was 15.3 incidents/100 min of observation.

Gonadectomy and testosterone replacement of breeder males in colonies and males in isolated pairs

We first asked whether castration and testosterone replacement of male breeders affect the frequency of genital nuzzling. Seven experimental pairs (four isolated pairs and three in colonies) participated in this portion of the study. All nuzzling incidents were recorded during four sets of scans: one set prior to surgery (Baseline), two post-castration scans (CAST 1 and CAST 2), and one set of scans following testosterone replacement of castrated males (CAST + T). Throughout this interval, we also recorded nuzzling in a control colony, in which the breeding male was sham-castrated at the time of surgery.

Isolated pairs and all of the breeding pairs in colonies exhibited genital nuzzling behavior during this phase of the study (Figs. 2, 3). Castration of the breeder male reduced the rate of genital nuzzling in all pairs ($Z = -2.37$, $P < 0.02$). On average, nuzzling during CAST 1 (scans begun 2.5 weeks post-castration) was reduced by $60.8 \pm 11.0\%$ relative to Baseline (Figs. 2, 3). This decrease was not likely to be due to effects of the surgery, per se, as a nearly identical decline relative to Baseline ($55.2 \pm 10.4\%$) was seen in the second set of post-castration scans, conducted approximately 14–16 weeks after castration. In addition, sham-castration did not reduce genital nuzzling; nuzzling rate in the control colony was increased relative to baseline in both post-surgery scan sets (5% and 64%, respectively).

The response to testosterone replacement was quite variable (Figs. 2, 3). However, compared to rates during CAST 2, overall nuzzling increased significantly when castrated breeder males received testosterone capsules ($Z = 2.03$, $P < 0.05$). Moreover, the rate of nuzzling during CAST + T did not differ from Baseline ($Z = -0.34$, $P > 0.7$). Thus, the rate of genital nuzzling among breeding pairs of naked mole-rats decreases following castration and nuzzling frequency is restored by testosterone replacement in castrated males.

A reduction in genital nuzzling after castration of the breeder male might be due to a lower rate of nuzzling initiation on the part of the males, a reduction in the initiation by breeder females, or both. To examine this further, we performed separate analyses of Male–Nuzzles–Female, Female–Nuzzles–Male, and Mutual–Genital–Nuzzling. Prior to castration, males initiated more nuzzling than did females. Both Male–Nuzzles–Female ($Z = -2.20$, $P < 0.03$) and Female–Nuzzles–Male

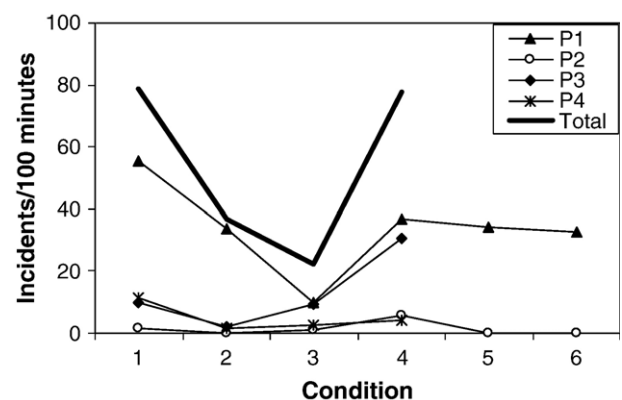


Fig. 2. Frequency of genital nuzzling in 4 isolated male/female pairs of naked mole-rats (incidents/100 min observation time; all three types of nuzzling incidents are combined here—Male–Nuzzles–Female, Female–Nuzzles–Male, Mutual Nuzzling). The numbers on the abscissa refer to experimental conditions which are detailed below. In addition to individual plots for each of the 4 pairs, there is a combined plot showing the summed data for all pairs under each condition. Conditions were: (1) Baseline = scans begun prior to any treatments; (2) CAST 1 = scans begun 2.5 weeks after castration of males; (3) CAST 2 = scans started 14–17 weeks after castration; (4) CAST + T = scans started 1 week after insertion of T capsules in castrated males; (5) OVX = scans begun 2 weeks after ovariectomy of females (castrated male still has T capsule); (6) OVX + CAST 1 = scans begun 2 weeks after removal of T capsules from castrated males. Two of the pairs (P3 and P4) were not examined under conditions 5 and 6.

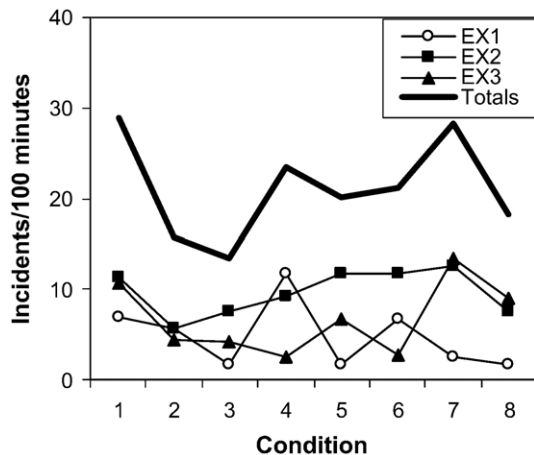


Fig. 3. Frequency of genital nuzzling for reproductive pairs in experimental colonies (EX1, EX2, EX3). In addition to individual plots for each of the 3 colonies, there is a combined plot showing the summed data for all experimental colonies. Conditions were: (1) Baseline = scans begun prior to any treatments; (2) CAST 1 = scans begun 2.5 weeks after castration of breeder males; (3) CAST 2 = scans started 14–17 weeks after castration; (4) CAST + T = scans started 1 week after insertion of T capsules in castrated males; (5) OVX = scans begun 2 weeks after ovariectomy of breeder females (castrated male still has T capsule); (6) OVX + CAST 1 = scans begun 2 weeks after removal of T capsules from castrated males (6 weeks after ovariectomy of breeder females); (7) OVX + CAST 2 = scans begun 11 weeks after removal of T capsules from castrated males (15 weeks after ovariectomy of breeder females); (8) OVX + CAST 3 = scans begun 22 weeks after removal of T capsules from castrated males (26 weeks after ovariectomy of breeder females).

($Z = -2.37$, $P < 0.02$) were reduced following castration of the breeder male. Both categories of nuzzling behavior were also reduced relative to Baseline during CAST 2, but this was significant only for Female–Nuzzles–Male ($Z = -2.37$, $P < 0.02$). Testosterone replacement significantly increased Male–Nuzzles–Female ($Z = 2.21$, $P < 0.03$ for CAST + T vs. CAST 2), but not Female–Nuzzles–Male ($Z = 1.76$, $P = 0.078$). Thus, changes in behavior on the part of both male and female partners contribute to the reduction of nuzzling after castration and to the increase following testosterone replacement. There were no significant effects of treatment on Mutual–Genital–Nuzzling.

Gonadectomy of females, or of both members of the breeding pair

Importantly, although castration of the breeding male reduced genital nuzzling, nuzzling was not eliminated in any pair. In addition, nuzzling was exclusive to the breeding pair following castration of the male: within the three experimental colonies, there were no incidents of genital nuzzling involving subordinates during either CAST 1 or CAST 2. Thus, based on this measure, castration of the breeder male did not change the social relations within the colony, as measured by nuzzling behavior, of the pair.

This led us to ask whether genital nuzzling would be eliminated after ovariectomy of the breeding female, or when both members of the pair were gonadectomized. Additional scans were therefore performed on two of the isolated pairs, the

three experimental colonies and the control colony. Female members of the isolated pairs and breeder females of the experimental colonies were ovariectomized, and the previously castrated males retained their T capsules for one set of scans (OVX); the T capsules were removed from the males for the second set of scans (OVX + CAST 1). Ovariectomy of the breeder female eliminated genital nuzzling in one isolated pair (the pair with the lowest rate of nuzzling prior to any manipulations), but had no consistent effect on the other pairs. Overall mean nuzzling rate was 10.8/100 min during the OVX scan, compared to 13.2/100 min during the immediately prior, CAST + T set and 17.2/100 min during Baseline. Removing the T implant from the males also did not eliminate genital nuzzling: approximately 6 weeks after ovariectomy of breeding females and 2 weeks after T capsules were removed from their castrated male partners (OVX + CAST 1), we recorded an average of 11.3 nuzzling incidents/100 min in the breeder animals.

During the OVX scan, there was one incident of genital nuzzling involving a subordinate, with a subordinate male nuzzling the breeding male. However, during the first set of scans in which both the male and female breeders were gonadectomized and without hormone supplement (OVX + CAST 1), no incident of genital nuzzling on the part of subordinates was observed in either the control or experimental colonies. To determine whether the suppression of nuzzling involving subordinates would be maintained over longer intervals after gonadectomy of both breeders, the three experimental and one control colony pairs were observed in two additional scan sets, one at 15 weeks after ovariectomy (OVX + CAST 2) and another at 26 weeks after ovariectomy (OVX + CAST 3). No genital nuzzling involving subordinates was recorded in either of these two scan sets in the control colony. Interestingly, however, nuzzling involving subordinates was observed in all three experimental colonies. Specifically, nuzzling involving subordinates accounted for 27.7% and 24.1% of all nuzzling incidents during the last two scan sets, respectively (Fig. 4). This contrasts with less than 5% for all other scanning periods of the study with the exception of the Baseline scans. However, as described above, genital nuzzling may not have been reliably distinguished from feces begging during the Baseline scans, and this might have inflated the number of recorded instances of nuzzling by subordinates. During the final two scan sets, we recorded 13 incidents in which the breeder female participated in nuzzling with a subordinate, 6 nuzzling incidents involving the breeder male and a subordinate, and one case of nuzzling involving two subordinates.

Sexual behaviors

No lordosis was observed during any of the scans. We rarely observe lordosis in our colonies, as the behavior only occurs during a span of a few hours in each approximately 30-day ovulatory cycle. Mounts and attempted mounts were observed, and there was considerable variation between groups. Nevertheless, some patterns did emerge. Mounting behavior was never observed for any of the subordinate males in colonies. Further, mounts were never observed for any of the isolated

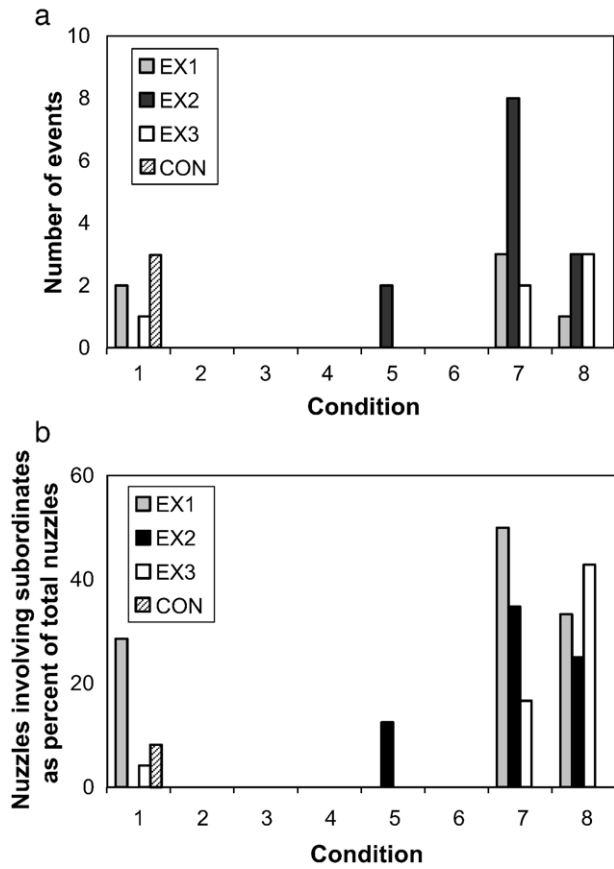


Fig. 4. Genital nuzzling by subordinates in experimental and control colonies of naked mole-rats. (a) The height of each bar indicates the total number of nuzzling incidents involving a subordinate under each of the 8 conditions. The conditions are the same as those listed for Fig. 3. For conditions 1 and 2, there were 14–18 scans/colony for each condition. For scans 3–8, each colony was scanned 12 times under each condition. (b) The height of each bar indicates the fraction of nuzzling incidents involving a subordinate expressed as a percentage of all nuzzling incidents observed. Note that the amount of genital nuzzling recorded for subordinates under condition 1 (Baseline) may have been artificially inflated through mistaken recording of instances of feces begging as genital nuzzling (see Materials and methods).

pairs or experimental colonies under the 5 conditions where the experimental males were castrated and were not receiving hormone replacement. For the same experimental groups, there were 19 mounts and 15 attempted mounts distributed among the 3 conditions where males were either intact or castrated and receiving testosterone replacement (Baseline, CAST + T, OVX + T). The breeding male of the CON colony exhibited 7 mounts during the course of the experiment; all of these occurred during the scan series when the experimental colonies were OVX + CAST 1. There were also 3 attempted mounts by the male of the CON colony; these occurred during the scans when experimental animals were CAST + T.

Discussion

The social bathyergid mole-rats exhibit what may be the strictest reproductive hierarchy known to exist in mammals, with reproductive activity limited to a single pair or to a very

few animals in each colony (Bennett and Faulkes, 2000). The mechanism for reproductive inhibition of the subordinates has not been determined. Gonadal steroids have not been experimentally manipulated in any published studies involving bathyergid species; thus, it is not known whether sex hormones contribute to maintenance of the bond between the breeding animals or to the ability of the breeders to inhibit reproduction in subordinates. In the current study, we examined the effect of sex hormones on genital nuzzling, a behavioral component that is generally performed only by the breeding animals in naked mole-rat colonies and which may therefore contribute to the maintenance of the reproductive hierarchy. Mating behaviors were also recorded.

The low frequency of mating behaviors (no lordosis, 26 mounts, 18 attempted mounts over all experimental and control groups in over 109 h of observation) confirmed our suspicion that it would not be feasible to utilize observations of these behaviors as reliable indicators of reproductive status. However, mounting behavior did appear to be eliminated by castration and then restored by subsequent testosterone treatment. This contrasts with the failure of castration to eliminate genital nuzzling between reproductives or in isolated pairs. We are not aware of any data on male mounting frequency as a function of the stage of the female's ovulatory cycle in naked mole-rats, but it seems likely that cycle-related variation in mounting frequency might be present. If so, the extreme variation in mounting behavior between groups observed under identical hormonal conditions might have occurred because some of the groups were observed at times of the female's ovulatory cycle when mounting is absent or infrequent.

It is common for captive colonies of naked mole-rats to cease producing offspring and breeding may resume after several years without any births. In these cases, it is virtually always the same reproductive animals involved both before and after the years without births (BD Goldman and SL Goldman, personal observations). These observations suggested that (a) the breeding female and male can maintain their reproductive bond over long periods of time without the production of offspring and (b) the reproductives are able to maintain inhibition of reproductive activation of the other colony members during this time, by a mechanism that is not known. The results of the present study indicate that although gonadal hormones may modulate the frequency of genital nuzzling, they are not required for maintaining the reproductive bond between previously established pairs: genital nuzzling continued to be expressed between male and female breeders long after both animals had been gonadectomized.

We have not yet determined whether gonadal hormones have a role during the establishment of a new breeding pair. We also do not know whether the frequency of genital nuzzling varies at different times of the ovulatory cycle or during pregnancy. The CON colony showed a sharp decrease in frequency of nuzzling after birth of a litter, but with only a single incidence of pregnancy during the study, we cannot conclude whether this change was causally related to reproductive state. The gestation period for the species is approximately 66 days (Jarvis, 1991), so the CON female was pregnant during the time when the

second set of experimental scans was conducted but not during the first experimental scans or the baseline scans. Nuzzling frequency was higher during all these scans as compared to those carried out after birth of the litter.

The relation that develops between members of a naked mole-rat colony may bear similarity to pair bonding that occurs in other monogamous species. Prairie voles are monogamous and have been perhaps the most widely studied rodent with respect to factors that contribute to pair bonding. In prairie voles, gonadal hormones are not crucial for pair bonding as assessed by monitoring how much time an experimental subject spends in physical contact with a stimulus animal. Although mating does facilitate the development of male aggression toward strangers that is associated with pair bonding (Carter et al., 1995; Williams et al., 1994), bonds can be formed between gonadectomized males and females (DeVries et al., 1997). Neither male nor female voles exhibited a significant preference for gonad-intact as compared to gonadectomized stimulus animals of opposite sex. In similar tests, voles that were allowed to become familiar with an individual of their own sex showed a preference for the familiar animal over an unfamiliar vole of the same sex. However, when allowed a choice between the familiar, same sex vole and an unfamiliar animal of the opposite sex, the test vole spent an approximately equal amount of time with the familiar, same sex individual and the unfamiliar vole of opposite sex (DeVries et al., 1997). These results suggest that the method typically used for assessing pair bonding in voles does not strictly measure a reproductive bond between animals of opposite sex, but also measures a general tendency to prefer familiar individuals; both sex and familiarity can influence the outcome of the laboratory tests for bonding in prairie voles. This is different from the measure that we have used in naked mole-rats, since subordinate animals rarely nuzzle despite extensive familiarity. We did not consider that preference tests such as those employed with voles would prove useful in naked mole-rats, since both reproductive and subordinate naked mole-rats spend considerable time huddling together in their nest chamber, and a preference for the breeding animals to cohabit specifically with each other is not apparent.

Both social factors and early hormone exposure influence the sex orientation of partner preference in the zebra finch, another strictly monogamous species. Zebra finches that were reared in the absence of adult males showed a reduced preference to pair with individuals of the opposite sex (Adkins-Regan and Krakauer, 2000). Manipulation of gonadal hormones before or soon after hatching also resulted in a shift in partner preference in genetically female birds, so that they either showed little sex preference or actually spent more time with other females, whereas untreated females spent more time with males (Adkins-Regan, 1999; Adkins-Regan and Wade, 2001). We do not know whether sexual preference is a factor in the establishment of genital nuzzling between mole-rats. Our colonies are established by pairing one male with one female subordinate, with the two individuals usually being taken from different colonies. It remains to be determined whether genital nuzzling would be expressed between members of a same-sex, isolated pair. In the last two sets of colony scans performed in the present study,

during which there appeared to be an increase in nuzzling by subordinate animals in the three experimental colonies, 7 of the 20 incidents involving subordinates occurred between animals of the same sex. These observations suggest that there may not be a strict sexual preference with respect to nuzzling partners; however, in the field reproductive bonding most likely initially becomes established between animals that have dispersed from their home colonies. Thus, it will be of interest to examine sex preferences under conditions that approximate more closely to what probably occurs in nature, that is, in isolated and unfamiliar pairs.

The function of genital nuzzling in naked mole-rats is unknown. It is possible that the behavior represents a special mechanism for individual recognition. The two best studied species of colonial mole-rats, naked mole-rats and Damaraland mole-rats (*Cryptomys damarensis*), are xenophobic and readily attack unfamiliar animals of their own species (O’Riain and Jarvis, 1997; Jacobs et al., 1998). It has been suggested that naked mole-rats recognize other members of their colony on the basis of a communal colony odor (O’Riain and Jarvis, 1997). This could be reinforced by the habit of these animals to spread urine on their bodies after urinating in a communal toilet area (Lacey et al., 1991). However, the experiments reported to date do not rule out recognition based on familiarity with individual odors as an alternative to familiarity with a colony odor. In Damaraland mole-rats, it appears that xenophobia is based on a relatively short ‘memory’ (2–3 weeks) for the individual odors of other members of the home colony (Jacobs et al., 1998), and the same mechanism for recognition may form the basis for incest avoidance in that species (Bennett, 1994; Burda, 1995; Jacobs and Kuiper, 2000).

In naked mole-rats, nuzzling may reinforce individual familiarity and/or provide additional reinforcement of a “reproductive bond” specifically between the breeding animals. In this context, it is not clear whether the decrease in nuzzling following castration of the male has biological significance. We observed considerable variation between colonies in frequency of nuzzling by the breeding pairs. This variation between colonies was apparent before initiation of treatments and under all the experimental conditions. Indeed, the variation between colonies was greater than the change in nuzzling frequencies evoked by castration and hormone replacement. Yet, all the colonies had raised litters successfully, suggesting that the absolute frequency of nuzzling behavior may not be a critical variable for reproductive success. Nevertheless, it is likely that nuzzling does serve some role for maintenance of the reproductive hierarchy, and none of the treatments eliminated nuzzling between reproductives. The apparent decrease in nuzzling following castration must be interpreted with caution in view of the possibility that the Baseline scan data were contaminated by cases of feces begging that may have been recorded as nuzzling. However, nuzzling frequencies increased following testosterone replacement in castrated males, providing further evidence for a role of testosterone in determining nuzzling frequency.

Perhaps most interesting was our observation that despite the persistence of genital nuzzling even following gonadectomy of

both breeders in all three experimental colonies, nuzzling by subordinates was eventually observed under this condition. This suggests that the presence of one or both of the breeders normally inhibits nuzzling by subordinates and that the secretion of gonadal hormone is required to achieve the full extent of this inhibition.

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