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Intrauterine Position, Parenting, and Nest-Site Attachment in Male Mongolian Gerbils

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ABSTRACT: We housed male Mongolian gerbils, their mates, and foster litters of standardized size and sex ratio in enclosures that provided cover in two locations. Males had been gestated in known intrauterine positions: either between two females (2F males) or between two males (2M males). From Days 1 to 20 postpartum, we examined the frequency with which both males and females were in contact with the pups they were rearing. We found that 2F males spent more time with pups than did 2M males both during entire observation periods and when females were away from the nest. Further, when pups were moved from the nest site, 2M males spent more time than did 2F males in the vacated nest site. We concluded that 2F male gerbils spent more time with pups than 2M males not because of a greater attachment of 2F than 2M males to places of concealment, their mate, or their nest site. Rather, 2F males were more attracted to pups than were 2M males. © 1998 John Wiley & Sons, Inc. *Dev Psychobiol* 32: 177–181, 1998

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In all litter-bearing species that have been examined to date, the intrauterine position that a fetus occupies relative to fetuses of the same or opposite sex has profound effects on its reproductive behavior when adult (Clark & Galef, 1995, in press; vom Saal, 1989). Adult male Mongolian gerbils, the subjects in the experiments reported here, that resided in intrauterine positions between female fetuses (2F males) have abnormal copulatory patterns (Clark, Malenfant, Winter, & Galef, 1990) and reduced genital musculature (Forger, Galef, & Clark, 1966) relative to males that resided in intrauterine positions between male fetuses (2M

males). Perhaps as a consequence, 2F male gerbils are less likely than are 2M males to impregnate unfamiliar females with whom they are paired (Clark, Tucker, & Galef, 1992). Most relevant to the present study, 2F males living with a mate and pups huddle with or sprawl over those pups more frequently than do 2M males, but do not differ from 2M males in the frequency with which they make casual, lateral contact with pups (Clark, DeSousa, Vonk, & Galef, 1997).

The observation that members of one group of male rodents spend more time in contact with young than do males of another group has sometimes been interpreted as indicating that the former males are more parental than are the latter. However, alternative interpretations of variance among males in frequency of pup contact are possible (Alberts & Gubernick, 1990;

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Gubernick & Alberts, 1987; Ostermeyer & Elwood, 1984; Smuts & Gubernick, 1992; Storey & Snow, 1987). For example, 2F male gerbils that spend more time huddling with pups than do 2M male gerbils might be more attracted than are 2M males not to their young, but to the nest site that they share with their offspring and mate. Alternatively, 2F males could be more attracted to their mates than are 2M males, and a tendency of 2F males to stay near mates that nurse young and huddle with them could cause 2F males to increase contact with pups, even if males were not particularly attracted to them. Last, 2F males might simply be more reluctant to leave cover than 2M males. If so, 2F males might tend to stay in the nest site with young when, as is often the case in laboratory test situations, the nest provides the only place of relative concealment in an enclosure. If males tended to huddle with pups when near them, then, the apparent difference in the attractiveness of pups to 2M and 2F male gerbils (Clark et al., 1997) might be an epiphenomenon reflecting a greater reluctance on the part of 2F than 2M males to leave their nest site, their mate, or a place of concealment, not the result of differences between 2F and 2M males in their response to pups, per se.

In the experiment described below, male gerbils from known intrauterine positions, their mates, and newborn gerbils were housed in cages that permitted adults to spend time either in the open, in an enclosed nest site, or in an alternative enclosed area identical to, but separate from, that containing nest and young. We also examined the behavior of 2M and 2F males and their mates in their respective home cages after pups were moved from whichever enclosed area contained the nest to the alternative enclosed area. Our goal was to determine whether 2F male gerbils spent more time with young than 2M males because the former animals were more attracted by young gerbils than the latter, or for some other reason.

METHODS

Subjects

Forty-four 60-day-old female Mongolian gerbils (*Meriones unguiculatus*) were randomly selected from 30 litters born and reared in the vivarium of the McMaster University Department of Psychology (Hamilton, Ontario) to breeding stock acquired from Tumblebrook Farm (Brookfield, MA).

Numerous litters were delivered by Caesarian section on Day 24 of their 25-day gestation. We used as subjects the first 23 males from 2F intrauterine posi-

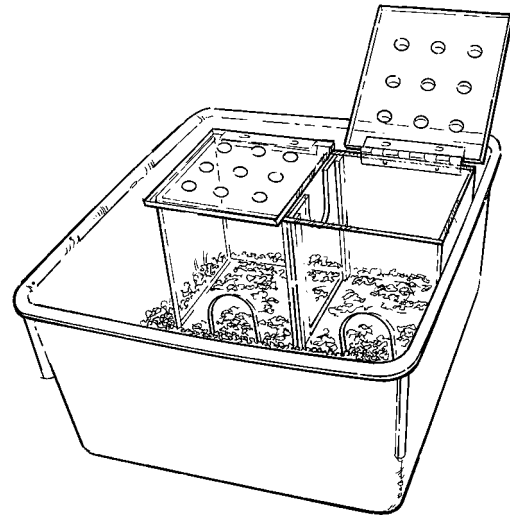


FIGURE 1 The apparatus used in the experiment.

tions and the first 21 males from 2M intrauterine that we discovered. Shortly after birth, each male was permanently marked for individual identification by toe-clipping. Then we placed that male and his littermates with a foster mother that had delivered a litter vaginally within 24 hr of the time of birth of the Caesarian-delivered litter that she was to rear. Details of both delivery by Caesarian section and fostering are provided in Clark and Galef (1988, 1992).

All animals were maintained throughout the experiment in a temperature- and humidity-controlled colony room illuminated on a 12:12 hr light:dark cycle with light onset at 5:00 a.m. Subjects had ad-libitum access to both water and pellets of Purina Rodent Laboratory Chow 5001 (Ralston-Purina, Woodstock, Ontario) throughout life.

Apparatus

During the experiment, we maintained each male–female pair in a 35 × 30 × 15 cm shoe-box cage. The top of each cage was closed with 1.3-cm hardware cloth, and its floor was covered with a thin layer of wood-chip bedding. Cotton batting (5 g) was placed in each cage for nesting material.

A nest box (28 × 12.7 × 12.7 cm) constructed of transparent Plexiglas was placed at one end of each shoe-box cage. This nest box was divided into two identical compartments, separated by a 12.7-cm square, transparent Plexiglas partition (Figure 1).

Two entrance holes, 5 cm in diameter, permitted subjects direct access from the 22.3 × 30 cm open area of each cage into the two compartments of the nest box; a third 5-cm diameter opening cut at the top

of the partition that divided the nest box into separate compartments permitted adult, but not juvenile, gerbils to pass directly from one compartment to the other.

Hinged lids of transparent Plexiglas allowed the experimenter access to each compartment of the nest box, and holes drilled through these lids provided ventilation within the nest box.

Procedure

Rearing and Breeding. All male subjects were weaned at 35 days of age and housed each in a 35 × 30 × 15 cm shoebox cage with 2 or 3 male littermates. When each subject male reached 60 days of age, we marked his back with red ink to easily distinguish him from his mate during periods of observation and placed him, with a subject female of like age, in a cage containing the nest box illustrated in Figure 1.

On the day of parturition of each of the 44 female subjects, we removed each female's litter and replaced it with 4 male and 4 female foster pups taken from females that had given birth on the same day as the subject female. Size and sex composition of litters were equated to control for effects of these variables on the parental behavior of adult gerbils (Clark & Galef, 1986; Elwood & Broom, 1978), and we gave each pair a litter comprised completely of foster pups to control for possible differences in the behavior of adult gerbils toward natural and foster pups.

Observation of Undisturbed Parents and Litters. Each day from Day 1 to Day 20 postpartum we observed adults and the litters they were rearing. Observations began 4 hr after light onset and we recorded, once every 20 s for 15 min, whether each adult was in physical contact with 1 or more of the pups in its cage. The observer scoring behavior was unaware of the intrauterine position in which the male in each pair had been gestated.

Testing of Preference for Nest Site or Litter. On Day 13 postpartum, 2 hr after the end of that day's 15-min observation session, both adults were removed from their home cage and placed in a holding cage, and the pups they were rearing were moved from the nest-box compartment containing the nest to the other side of the partition. Five min later, 1 adult was placed in the open area of the home cage, facing away from the nest box.

During the next 30 min, the amount of time the adult spent both in the nest-box compartment where the pups were now located and in the nest-box compartment that contained the nest was recorded. After testing 1 adult for its preference between nest and lit-

ter, it was returned to the holding cage. Five min later, its mate was introduced into the open area of the home cage. For the next 30 min, we again recorded the amount of time the subject spent in each of the two nest-box compartments. The order in which we tested male and female adults was counterbalanced across litters.

RESULTS AND DISCUSSION

Observation of Undisturbed Parents and Litters

Nonintrusive observations of the behavior of the 44 gerbil pairs containing males from known intrauterine positions are presented in Figure 2. As is obvious from inspection of Figure 2, (a) the intrauterine position that a male had occupied as a fetus affected his response to pups, $F(1, 42) = 4.84, p < 0.3$; (b) the response of males to pups varied as a function of pup age, $F(19, 798) = 3.17, p < .001$; and (c) there was a significant interaction between the intrauterine position of males and the age of pups in the amount of time that males spent in contact with them, $F(19, 798) = 1.60, p < .05$. These observations show that, even when male gerbils can stay under cover without remaining in contact with pups, males from 2F intrauterine positions spend more time with pups than do males from 2M intrauterine positions. This finding is not consistent with the hypothesis that a greater reluctance on the part of 2F than 2M males to leave cover was responsible for the greater time spent with young by 2F than by 2M males observed in standard laboratory cages (Clark et al., 1997).

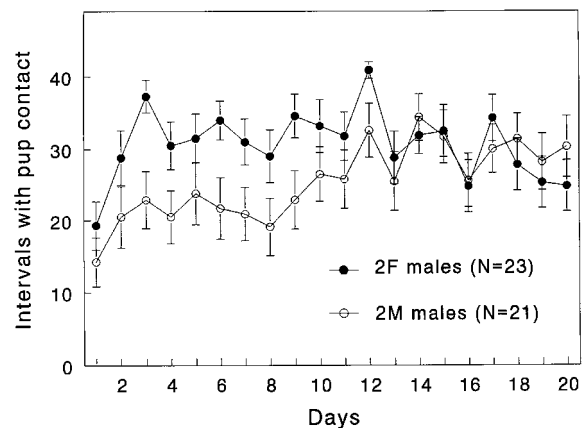


FIGURE 2 Mean number of 20-s intervals (out of 45) that male Mongolian gerbils from 2M and 2F intrauterine positions spent in contact with pups on each of Days 1 to 20 postpartum. Error bars indicate standard errors of the mean.

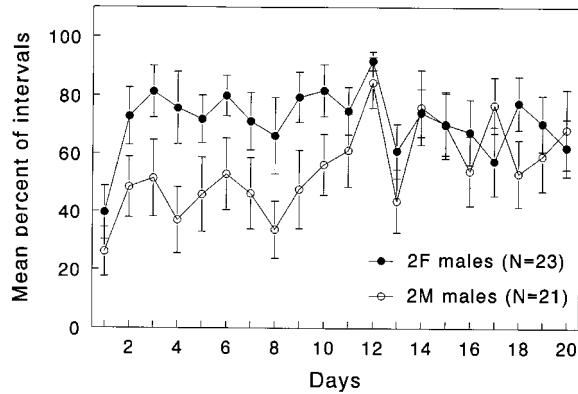


FIGURE 3 Mean percentage of 20-s intervals when female gerbils were not in contact with the pups that their male 2F and 2M mates were in contact with the pups. Error bars indicate standard errors of the mean.

As is evident from inspection of Figure 3, 2F males were more likely than were 2M males to stay with pups when their mates were out of the nest, Student's *t* test, $t(42) = 2.75, p < .01$. This finding is not consistent with the hypothesis that greater attraction of 2F than of 2M males to their mates was responsible for the greater attendance of 2F than of 2M males on young.

As Figure 4 shows, there was no difference in the frequency with which females mated to 2F and 2M males were away from their young, $F(1, 42) = .32, n.s.$, no change over days in the frequency with which these females were away from their litters, $F(19, 798) = .88, n.s.$, and no significant interaction between the variables, $F(19, 798) = .76, n.s.$ Clearly,

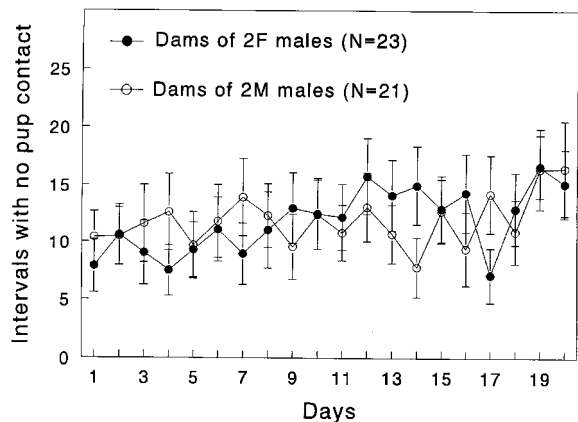


FIGURE 4 Mean number of 20-s intervals (out of 45) that female Mongolian gerbils mated to males from 2M and 2F intrauterine positions spent out of contact with their pups on each of Days 1 to 20 postpartum. Error bars indicate standard errors of the mean.

the difference in the frequency with which 2F and 2M males stayed with pups when their mate was away from the nest (illustrated in Figure 3) did not result from differences in the frequency with which the mates of 2F and 2M males were away from pups they were rearing.

Testing of Preference Between Nest Site and Litter

For various reasons, we could not collect data from all 44 males and females to determine their preferences between nest site and litter when their litters were displaced from their nest on Day 13 postpartum. Problems in developing the preference test prevented testing of 3 pairs; scheduling errors resulted in 5 pairs not being tested on Day-13 postpartum, and 5 females retrieved their litters to the nest after we had moved the young to the alternate compartment of the nest box. We could not collect useful data from these 8 males and 13 females.

As can be seen in Figure 5, there was a significant interaction between male intrauterine position and the

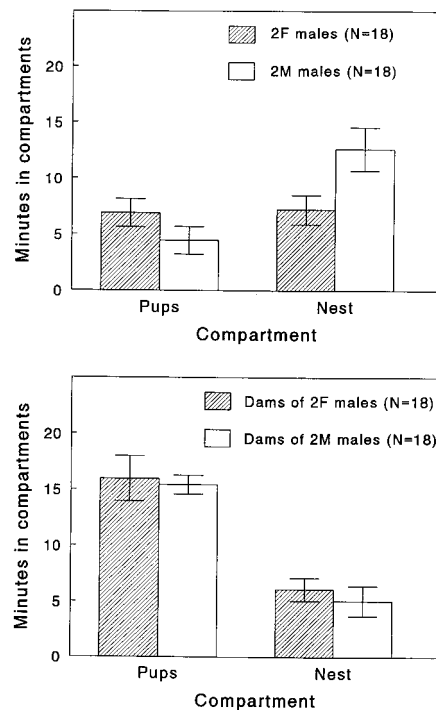


FIGURE 5 Mean number of minutes that (upper panel) male Mongolian gerbils from 2M and 2F intrauterine positions and (lower panel) their mates spent during a 30-min preference test in nest-box compartments that contained either their nest or the litter that they were rearing. Error bars indicate standard errors of the mean.

amount of time that males spent with pups and in the nest site, $F(1, 34) = 5.82$, $p < .02$: 2M males spent more time in the vacated nest than did 2F males, $t(35) = 2.33$, $p < .02$, and males from 2F and 2M intrauterine positions spent equal time with displaced pups, $t(35) = 1.38$, n.s. Further, individual 2F males were significantly more likely than were individual 2M males to spend more time in the nest-box compartment containing pups than in the nest-box compartment containing the nest, Fisher's exact probability test, $p = .02$. These findings clearly contradict the hypothesis that a greater attachment of 2F than of 2M males to the nest site is responsible for the greater amount of time that 2F than 2M males spend in contact with pups when they are undisturbed in the nest (Figure 1).

Females mated to both 2M and 2F males behaved alike in that, when given a choice between nest and pups, both classes of females spent more time in the nest-box compartment containing pups than in the nest-box compartment containing the nest, $F(1, 29) = 24.10$, $p < .0001$. Again, differences in the behavior of males from different intrauterine positions could not be attributed to differences in the behavior of their mates.

GENERAL DISCUSSION

The results of the present experiment indicate that the difference in frequency with which male gerbils from different intrauterine positions are observed in contact with conspecific young (Clark et al., 1997) is not an indirect result of differences in their tendencies to remain in contact with the nest, under cover, or near their respective mates. Our results suggest, to the contrary, that 2F males spend more time in contact with pups than do 2M males because they find pups more attractive than do 2M males. This is, we believe, the first demonstration that variation in the frequency with which adult male rodents contact young reflects differences in their response to young rather than differences in other aspects of their behavior.

NOTES

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