Alloparenting experience affects future parental behavior and reproductive success in prairie voles (*Microtus ochrogaster*)

Anita Iyengar Stone[a,b], **Denise Mathieu**[a], **Luana Griffin**[a], and **Karen Lisa Bales**[a]

[a]Department of Psychology, University of California, Davis

[b]Department of Biology, Grand Valley State University, Allendale MI

Abstract

Various hypotheses have been proposed to explain the function of alloparental behavior in cooperatively breeding species. We examined whether alloparental experience as juveniles enhanced later parental care and reproductive success in the prairie vole (*Microtus ochrogaster*), a cooperatively breeding rodent. Juveniles cared for one litter of siblings (1EX), two litters of siblings (2EX) or no siblings (0EX). As adults, these individuals were mated to other 0EX, 1EX or 2EX voles, yielding seven different pair combinations, and we recorded measures of parental behaviors, reproductive success, and pup development. As juveniles, individuals caring for siblings for the first time were more alloparental; and as adults, 0EX females paired with 0EX males spent more time in the nest with their pups. Taken together, these results suggest that inexperienced animals spend more time in infant care. As parents, 1EX males spent more time licking their pups than 2EX and 0EX males. Pups with either a 1EX or 2EX parent gained weight faster than pups with 0EX parents during certain developmental periods. While inexperienced animals may spend more time in pup care, long-term benefits of alloparenting may become apparent in the display of certain, particularly important parental behaviors such as licking pups, and in faster weight gain of offspring.

Keywords

alloparental care; cooperative breeding; prairie vole; social experience

INTRODUCTION

Cooperative breeding, or the presence of non-breeding helpers within social groups, occurs in both birds (Cockburn, 2006) and mammals (Jennions and Macdonald, 1994; Solomon and Getz, 1997), occurring in 35 rodent species and 30% of rodent families (Solomon and Getz, 1997). Juvenile mammals (typically defined as animals between weaning and first reproduction, Pereira and Fairbanks, 1993) in cooperative breeding systems assist in rearing offspring that are not their own through activities such as feeding, carrying, allo-suckling, babysitting, and pup thermoregulation (Russell, 2004; Solomon and French, 1997).

Benefits of such alloparental behavior to the helper are unclear, since helpers sacrifice individual reproductive opportunities to remain in their natal group. Three main, non-mutually exclusive hypotheses have been proposed to explain the evolution of alloparental care in singular cooperative breeders (groups in which only one female is breeding). First, helpers may be contributing to their indirect fitness when they care for siblings, who are more likely to survive with the presence of helpers (Hamilton, 1964a, b; Emlen et al., 1991; Emlen and Wrege, 1989). Positive relationships between the number of helpers and surviving infants have been recorded in several mammals (Snowdon et al., 1985; Clutton-Brock et al., 2001; Bales et al., 2000) and may be more likely when young are more related (Griffin and West, 2003). Second, because they decrease the workload of breeders, helpers may benefit indirectly if breeders that receive assistance have higher survivorship (Rood, 1990; Bales et al., 2000), subsequently produce more litters or have shorter interlitter intervals (Solomon, 1991; Powell and Friend, 1992; Bales et al., 2001; Russell et al., 2003).

A third hypothesis is that alloparental care yields direct benefits to helpers by providing experience that will allow them to become more successful parents (Lancaster, 1971; Snowdon and Cronin 2007). This hypothesis predicts that alloparenting experience results in an increased quality or quantity of future offspring. Among primates, tamarins (*Saguinus* sp.), marmosets (*Callithrix* sp.) and vervet monkeys (*Cercopithecus aethiops*) that have experience with younger siblings are more likely to be successful breeders themselves (Tardif et al., 1984; Goldizen, 1987; Fairbanks, 1990). In addition, studies on cooperatively breeding rodents show that alloparental experience can increase reproductive success. Margulis et al. (2005) demonstrated that juvenile female oldfield mice (*Peromyscus polionotus*) which remained in their mother's nest later had better nest-building skills and more surviving pups than females without previous exposure to pups. Male Mongolian gerbils (*Meriones unguiculatus*) with prior experience with younger siblings produced their first litter sooner than inexperienced males, and their pups gained weight more rapidly and had earlier eye opening (Salo and French, 1989). However, alloparental behavior was not measured in either study, and it is unclear whether the quantity of alloparental experience influenced later parental competence or pup development. Altogether, previous support for this hypothesis is perhaps weaker than support for indirect fitness arguments, suggesting the need for further data. Recent theory has also suggested that this field would benefit from the integration of direct benefits arguments of cooperative breeding with more general theory on cooperation (Bergmüller et al., 2007).

Here we test the hypothesis that alloparental experience as juveniles enhances later parental care and reproductive success in the prairie vole (*Microtus ochrogaster*). Prairie voles are small (30-40 g) rodents native to north-central North America. Field studies have shown that prairie voles live in extended family groups (Getz and Hoffman, 1986; Getz and McGuire, 1997). Philopatric juveniles spend as much time in the nest with pups as do parents (Wang and Novak, 1994; Solomon and Getz, 1997), participate in pup licking and retrieval to the nest, and huddle over pups (Solomon, 1991; Wang and Novak, 1992). The presence of juvenile helpers is beneficial to breeders (Solomon, 1991; Hayes and Solomon, 2004) and to pups (Solomon, 1991, 1994), but benefits to the helpers themselves are less clear. In a laboratory study, Wang (1991) did not find differences in parenting between voles that had been exposed to younger siblings and those that had not. However, pups reared with two experienced parents developed slightly faster than those raised by inexperienced parents. Breeder pairs in that study, however, were composed either of two experienced or two inexperienced individuals, making it difficult to examine the effect of experience on different sexes. Furthermore, no studies to date have examined how much exposure to younger siblings (e.g. caring for one sibling litter versus two litters) is necessary for the acquisition of parenting skills. Experience with pups prior to weaning (as little as 2 days) has been associated with greater parental behavior towards unrelated pups in alloparental care tests (Roberts et al., 1998).

In this study, we examined whether voles that had experience as juveniles caring for younger siblings would be more successful as parents than inexperienced voles when they first became reproductive, and if this effect displayed a dose-response. We allowed juveniles to care for one litter of siblings, two litters of siblings or none. Specifically, we predicted that (1) voles that spent more time exposed to and caring for younger siblings would show greater levels of parental behavior than less experienced juveniles; (2) voles that had more exposure to younger siblings would experience greater reproductive success (e.g. larger surviving litters, heavier offspring). Alternatively, exposure to one litter of pups may be all that is necessary, and a dose-response may not be observed. Since not all family members engage in equivalent amounts of alloparental care (Solomon and Getz, 1997), we also were interested in potential sex differences in alloparenting in the natal group, and whether alloparenting experience would have different effects on males and females once they became breeders, as has been found in Mongolian gerbils (Salo and French, 1989). Because pup-naïve female prairie voles are often infanticidal (Roberts et al., 1998; Lonstein and De Vries, 2001; Bales et al., 2004), we expected that if sex differences in the effects of alloparental experience existed, they would be more dramatic in females than in males.

MATERIALS AND METHODS

Breeding Colony

Ten pairs of laboratory-bred male and female prairie voles who had had at least one prior litter were used as breeder pairs for the production of juvenile subjects. Voles were descendants of a wild stock originally caught near Champaign, Illinois, and it had been approximately four years since the introduction of wild-born voles to the colony. Stock was systematically outbred. Animals were maintained on a 14 h light: 10 h dark cycle and provided with food (high-fiber Purina rabbit chow) and water ad libitum. Breeding pairs were maintained in two large polycarbonate cages ($44 \times 22 \times 16$ cm) connected via a polycarbonate tube (10.2 cm long $\times$ 7 cm diameter) and provided with cotton for nesting material.

Experimental Design

Subjects—Only litters containing three or more pups were used to generate the juvenile treatment groups. Groups of focal juveniles undergoing different amounts of experience with younger siblings were assigned as follows. The "zero exposure" subjects (hereby 0EX) were housed together with their parents until removed from their parents' cage at 20 days of age (weaning age per standard laboratory procedure), and housed in same-sex/same-age sibling pairs in smaller ($27 \times 16 \times 13$ cm) cages until mating. Because voles exhibit post-partum estrus, and gestation length is 21 days, these juveniles were not exposed to younger siblings. Juveniles in the "one exposure" (1EX) group were not removed from their parents' cage at 20 days of age, but allowed to remain with their parents and provide alloparental care to one subsequent litter of younger siblings until time of weaning. At 20 days, 1EX juveniles received colored-coded aluminum ear tags (National Band and Tag Company, Newport, KY, model 1005-1) for individual identification. Tags were color-coded with non-toxic spray paint that is designed for metal surfaces. Four randomly selected focal juveniles (two males and two females) from each litter were kept with parents and any remaining juveniles were removed. Once their younger siblings reached 20 days, juveniles were removed from the parents' cage and housed in the same manner as 0EX juveniles (same-sex/same-age sibling pairs until mating). 1EX juveniles were approximately 42 days old when removed from their parents' cage. Juveniles in the "two exposure" (2EX) groups were ear-tagged and culled to four, but allowed to remain with parents and care for two consecutive litters of younger siblings. In order to minimize crowding, the first litter of siblings was removed from the parents' cage before the birth of the second litter. 2EX juveniles were approximately 63 days when removed from the parents' cage, and were also housed in same-sex/same-age sibling pairs until mating. Sexual maturity in

prairie voles is reached at 55 days (Carter et al., 1989). However, young females do not become reproductively active as long as they remain with their parents or siblings (Carter et al., 1980; McGuire and Getz, 1981); therefore, females in the 2EX group remained in a non-reproductive state. Mothers also did not mate with their 2EX sons. 0EX, 1EX and 2EX voles were never from the same litter.

Juvenile females housed with parents (even in the absence of younger pups) after weaning may be more responsive to strange pups than those housed with a same-sex littermate (Lonstein and De Vries, 2001). Therefore, differing lengths of exposure to parents is a potentially confounding variable. To control for this difference, we added soiled bedding from the parents' cage during weekly cage changes to 0EX and 1EX juvenile cages once they were removed from the parents' cage (approximately 1/3 soiled bedding to 2/3 fresh bedding per cage), thus maintaining exposure to parental scent and pheromones. Because, as noted above, young females are induced into estrus by the presence of an unfamiliar unrelated male, differing lengths of exposure to parents would *not* have affected reproductive hormones. The decision to remove subjects and house them with parents' bedding, rather than gonadectomizing parents, was made in order to maintain the parents as breeders for the main colony, and to reduce the number of animals necessary for this large project. The decision to remove the focal juveniles from the parents, rather than removing the younger siblings, was made in order to completely eliminate potential exposure of juveniles to younger siblings in the 0EX group (since infants are often born overnight and the focal animals would then exposed for an unknown number of hours).

Matings—When subjects reached 63 days of age, each individual was matched and paired with an opposite-sexed individual that was 63-90 days old from a different or same treatment group. Seven different mating treatments were generated (Table 1). Pairs were housed in single polycarbonate cages ($44 \times 22 \times 16$ cm) and reared under the same conditions as described above. In order to control for possible effects of maternal body weight on reproductive success (e.g. experienced females may be larger and larger mothers may have greater reproductive success), all pairs were weighed at the time of mating. No significant effects of alloparental experience on weight were found (females: $F_{2, 44} = 0.28$, p=0.75; males: $F_{2, 44} = 0.93$, p=0.40). Pairs were considered non-breeders if they failed to produce a litter within 60 days of pairing.

Data Collection

Behavioral Observations—Real-time behavioral observations on alloparental behaviors were conducted on 1EX and 2EX juveniles using behavioral software (Behavior Tracker 1.5, www.behaviortracker.com). We observed 49 juvenile males (27 in the 1EX group and 22 in the 2EX group) and 44 juvenile females (29 1EX and 25 2EX). Observations were conducted on one male and one female from each litter (the other male and female were used for a concurrent study on neurobiological variables; Mathieu et al., unpublished data). Two twenty-minute focal samples were recorded per individual per day, one in the morning and one in the afternoon (thus equally representing time of day across replicates and treatments). Observations were conducted on two days during the pups' first week of life (ranging from days 1-3) and on two days during the second week (days 8-10). A total of four days of observations was thus recorded for each individual, with a total of 160 minutes of data per individual. We recorded the duration of any huddling behavior, pseudohuddling, non-huddling contact, retrievals and licking/grooming (full definitions in Table 2). We also recorded how much time the juvenile spent inside or outside the nest.

Identical parental observations were conducted on the subsequently mated pairs, in order to measure parenting behavior. These observations included additional nursing postures for females such as lateral nursing, neutral nursing and nursing while locomoting (Table 2). These

postures were scored separately based on the possibility that they are under differing neural control (Lonstein and Stern, 1997;1998;Lonstein et al., 1998) and may have differing functional consequences for pups (Lincoln et al., 1980;Stern and Johnson, 1990).

Measures of Reproductive Performance—In order to assess the reproductive success of mated pairs, several variables were recorded for first and second litters. For those pairs producing litters, we recorded: time from pairing until the production of their first litter, litter size at birth, surviving litter size and sex ratios at weaning, and interbirth intervals (Wang, 1991; Solomon, 1994).

Pup Development—We recorded several developmental markers such as: litter weight at birth to the nearest 0.1g (post-natal day 1) and litter weight on days 8, 15 and 20. Daily checks were conducted after the birth of pups in order to determine: the day at least one pup opened one eye, the day all pups opened one eye, day pups first moved out of the nest and day pups first consumed solid food (Solomon, 1991; Wang, 1991; Wang and Novak, 1992, 1994). On days when we conducted both behavioral observations and pup weighing, weighing was always done after observations in order to minimize disturbance to the voles. Growth rate was calculated by subtracting litter biomass between days and dividing it by the original weight.

Analyses

For juvenile treatment groups, we used a 2 (sex) × 3 (treatment) factorial design ANOVA to examine whether sex and degree of exposure to pups (i.e. 1EX group and the second exposure of the 2EX group) affected level of alloparental behavior. We also compared alloparental behaviors for the first exposure and the second exposure for the 2EX group with paired t-tests.

For mated pairs, it was necessary to consider that the behavior of the male and female would be correlated and dependent on each other. We thus analysed "pair treatment", a variable intended to include the treatment of both individuals in the pair, which we indicated by the treatment of the female followed by the treatment of the male (e.g. 1×0 treatment= 1EX female and 0EX male). We used repeated-measures ANOVA, with pair treatment and parity as independent variables considered in each model simultaneously; dependent variables were parental behaviors such as huddling, pseudohuddling and licking of pups.

Separately, we also examined the effect of an individual subject's treatment (0EX, 1EX, 2EX, with no consideration of pair-mate's treatment) on parental behaviors. For these analyses, males and females were analyzed separately. We expected significant effects of individual treatment to be those that were strong enough to be significant regardless of the pair-mate's treatment.

Effects of pair treatment, individual treatment and parity on developmental variables also were examined with ANOVA as described above. All tests were checked for assumptions of ANOVA. When necessary due to non-normality, variables were transformed with a log transformation. When data were non-transformable, Kruskal-Wallis tests were used.

RESULTS

Juvenile Alloparental Behavior: Sex and Treatment Differences

Sex had an effect on time spent in the nest; female juveniles spent more time in the nest than did juvenile males (females: 739.9 ± 34.9 sec.; males: 661.8 ± 42.2 sec.; $F_{1, 64}= 4.32$; $p=0.03$). In addition, 1EX juveniles (being exposed to their first litter of pups) spent more time in the nest than 2EX juveniles in their second exposure ($F_{1, 64}= 5.08$; $p=0.01$). The interaction between sex and treatment on time spent in the nest was not significant ($F_{1, 64}= 0.48$, $p=0.50$).

Within the 2EX group, time spent in nest was greater in the exposure to the first litter of siblings than to the second litter of siblings (paired t-test: t_{31}= 4.12, p=0.0003).

There was no sex difference in total time spent in contact with pups (females: 464.6 ± 35.8 sec; males: 423.2 ± 37.5 sec; $F_{1, 64}$= 1.78, p=0.18). 1EX juveniles spent more time in contact with pups than 2EX juveniles in their second exposure ($F_{1, 64}$= 4.95, p=0.02). The interaction between sex and treatment on time spent in contact with pups was not significant ($F_{1, 44}$= 0.94, p=0.33). Within the 2EX group, total time spent in contact with pups was greater in the exposure to the first litter of siblings than to the second litter of siblings (paired t-test: t_{31}= 2.10, p=0.04).

Parental Behavior as Adults

Females—Total time spent in nest was affected by pair treatment (Kruskal-Wallis: H=12.71, df=6, p= 0.04). Post-hoc Mann-Whitney analyses showed that females in the 0×0 group spent more time in the nest compared to other treatments (Fig 1a). Parity did not affect time spent in the nest (Mann-Whitney: U=1721.0, p= 0.09). Time spent in contact with pups was affected neither by pair treatment (H=7.22, df=6, p= 0.30) nor by parity (U=1759.0, p= 0.21).

Total time devoted to nursing was not affected by pair treatment ($F_{6, 170}$= 0.47, p=0.85) nor by parity ($F_{1, 170}$= 0.01, p=0.91). No interaction between treatment and parity was found for total nursing time ($F_{6, 170}$=0.57, p=0.74). However, pair treatment affected time spent in sit-nursing ($F_{6, 171}$= 4.85, p=0.0001; Fig. 1b). Females in 0×0 pairs engaged in more sit-nursing than females in 2×2 pairs (p=0.019), while females in 1×1 pairs engaged in more sit-nursing than 0×1 females (p=0.003), 1×0 females (p=0.03) and 2×2 females (p< 0.0001). No parity effects were found on sit-nursing ($F_{1, 170}$= 0.15, p=0.69) and there was no interaction of treatment and parity on sit-nursing ($F_{6, 170}$= 1.56, p=0.15).

In addition, a female's individual treatment affected time spent in sit-nurse ($F_{2, 170}$= 3.78, p=0.02), with 1EX females displaying this posture more than 2EX females (p=0.007).

Males—For males, total time spent in nest was affected neither by pair treatment (H=10.06, df=6, p= 0.12), nor by parity (U=1819.0, p= 0.24). Time spent in contact with pups was affected by pair treatment (H=18.04, df=6, p= 0.006). Post-hoc Mann-Whitney analyses showed that males in 0×0 pairs and 2×2 pairs spent less time in contact with pups compared to 0×2 males, 2×0 males and 1×1 males (Fig 2). Parity did not affect time spent in contact with pups (U=1794.0, p= 0.15).

In addition, a male's individual treatment affected time spent licking/grooming his pups (H=6.97, df=2, p= 0.03), with 1EX fathers licking their pups more (1EX: 72.6 ±8.1s; 0EX: 65.7 ±7.3s; 2EX: 53.0 ±8.5 s).

Correlations between Alloparental Behaviors as Juveniles, Parental Behaviors as Adults and Reproductive Success—In both males and females, several components of alloparental care displayed as juveniles and later parental care towards their own pups were significantly correlated. Females that pseudohuddled more over their siblings pseudohuddled more over their own pups (r_{54}= 0.65, p=0.001). Females that retrieved their siblings more retrieved their own pups more (r_{54}= 0.61, p=0.001). In addition, females who spent more time pseudohuddling or retrieving as juveniles had more surviving infants as adults (pseudohuddle: r_{54}= 0.31, p=0.02; retrieve: r_{54}= 0.51, p=0.0002).

Males that huddled and retrieved their siblings more showed more of these behaviors toward their own pups (huddle: r_{50}= 0.72, p <0.001; retrieve: r_{50}= 0.74, p < 0.001). In addition, there was a significant correlation between time spent huddling and retrieving as juveniles and surviving litter sizes of their own (huddle: r_{50}= 0.45, p=0.01; retrieve: r_{50}= 0.43, p=0.001).

Reproductive Success and Pup Development

Reproductive Success—Table 3 summarizes several measures of reproductive success. Several pairs did not produce offspring, even after 60 days of pairing. The highest failure to breed at all was amongst 1×1 pairs. Pairs containing at least one 1EX individual were less likely to breed ($\chi 2$=7.47, df= 2, p=0.02). Pair treatment had no effect on the latency to produce the first litter (H=7.83, df=6, p=0.25) or the second litter (H= 5.93, df= 6, p=0.43).

Litter size at birth was not affected by pair treatment ($F_{6, 99}$= 1.50, p=0.18) nor by parity ($F_{1, 99}$= 1.53, p=0.28); however, there was an interaction between treatment and parity, with 1×0 pairs producing larger first litters (mean: 6.4 ± 0.7 pups; $F_{6, 99}$= 2.24, p=0.04). Pairs with 0EX dads produced larger litters ($F_{2, 107}$= 3.23, p=0.04). Litter biomass at birth was not affected by pair treatment ($F_{6, 93}$= 0.45, p=0.83) nor by parity ($F_{1, 99}$= 2.65, p=0.10). Litter size at weaning was not affected by pair treatment ($F_{6, 93}$= 0.98, p=0.43) nor by parity ($F_{1, 93}$= 1.72, p=0.19). Biomass at weaning was not affected by pair treatment ($F_{6, 91}$= 0.85, p=0.53) but second litters were heavier than first litters at weaning ($F_{1, 91}$= 5.48, p=0.02).

Physical and Behavioral Development of Pups—Pair treatment affected when pups first opened their eyes, with pups born to 1×1, 0×2 and 2×0 parents opening their eyes earlier than pups born to 0×0, 0×1, or 1×0 pairs, though this difference only approached significance ($F_{2, 93}$= 2.06, p=0.06). Pair treatment did not affect the day pups left the nest ($F_{6, 93}$= 0.96, p=0.45) or the first day eating solid food ($F_{6, 93}$= 0.36, p=0.90). Pups from second litters began to eat solid food sooner than pups from first litters ($F_{1, 93}$= 6.29, p=0.01). There was no difference in weaning weight due to pair treatment.

The growth of pups (calculated over the entire pre-weaning period) was greater for litters raised by 1EX fathers (H=7.75, df=2, p=0.02). No significant effect of mother treatment occurred (H=0.91, df=2, p=0.63). The treatment of the pair also did not affect growth rate (H=10.25, df=6, p=0.11). Between postnatal days 1 and 8, pups with 2EX fathers gained weight more rapidly than pups with 0EX or 1EX fathers (H=11.71, df=2, p= 0.002). Between postnatal days 15 and 20, pups with 1EX mothers gained weight faster than pups raised by 0EX or 2EX mothers, though this result only approached significance (H=5.10, df=2, p= 0.07). In addition, between postnatal days 15 and 20, pups with 1EX fathers gained weight faster than pups with 0EX or 2EX fathers (H=9.84, df=2, p= 0.007). There was no difference in weaning weight due to the mother's treatment or the father's treatment.

DISCUSSION

In this study, we tested the hypothesis that alloparental experience as juveniles would lead to greater levels of subsequent parental behavior, greater reproductive success and faster pup development in prairie voles. An association between alloparenting experience and later reproductive success has been found in primates such as vervet monkeys (Fairbanks, 1990), tamarins (Tardif et al., 1984) and oldfield mice (Margulis et al., 2005). Consistent with this hypothesis, pups with fathers that had raised two litters of younger siblings had offspring that gained more weight from days 1 to 8, while pups that had at least one parent that had raised one litter of siblings gained more weight from day 15 to 20. In the wild, faster weight gain and development could result in greater survivorship for those pups, although we could not test that hypothesis in the current study. The fact that there was no difference in weaning weight also suggests that the advantage in weight gain may be transient. Finally, there was a trend for pups to open their eyes faster in litters being raised by parents with a combined two litters of previous alloparenting experience (1×1, 0×2, or 2×0 pairs).

One interesting result concerns the failure to breed of pairs containing at least a 1EX individual (particularly 1×1 pairs). However, when such individuals did reproduce, their pups grew faster

than pups of 0EX or 2EX parents, suggesting that an intermediate level of experience may be optimal in some situations. In addition, 1EX males behaved more parentally towards offspring, licking and grooming them more and, when in 1×1 pairs, spending more time in contact with pups than males in 0×0 pairs or 2×2 pairs. This apparent paradox suggests that while 1EX individuals gain valuable alloparental experience, they may incur a reproductive cost at the level of fertilization or implantation, suggesting a trade-off. While we can only speculate on the mechanism for this reproductive cost, perhaps an initial exposure to pups results in temporary changes in gonadotropin-releasing hormone or progesterone when individuals first experience an alloparenting role.

In addition to the changes found due to the presence or absence of any alloparenting experience at all, behavior as an alloparent was predictive of behavior and reproductive success as a parent. Time spent in alloparental behaviors as a juvenile was positively correlated with larger surviving litters later in life (this was true for both females and males). This could be interpreted to mean merely that good alloparents were also good parents, which resulted in more surviving infants; but it is also possible that the extra time spent in "practice" was what determined the greater eventual reproductive success for these individuals. Although 2EX individuals were exposed to pups for a longer time compared to 1EX juveniles, in actuality these voles spent less time interacting with pups (Figs. 1b and 2) and this may explain their lower reproductive success compared to 1EX voles.

Our behavioral observations on juveniles indicated that 1EX individuals were more alloparental than 2EX individuals, displaying more contact with pups. This suggests that alloparental behaviors do not increase in a linear fashion with experience, that inexperienced animals may actually engage in longer durations of care, and that exposure to one litter of siblings may constitute sufficient alloparenting experience in this species. Furthermore, given that alloparenting levels decreased with a second exposure (both cross-sectionally and longitudinally within individuals), it is possible that a second exposure to pups inhibited alloparenting, possibly due to overhabituation to a no longer novel stimulus. This is unlikely, however, since female prairie voles raised in their natal nest with up to five litters of siblings later behaved more parentally in alloparental care tests compared to those with no prior sibling exposure (Lonstein and De Vries, 2001). In that study, among the females exposed to siblings, no relationship existed between quantity of exposure and performance in alloparental care tests (Lonstein and De Vries, 2001). Another possibility is that differences between the 1EX and 2EX group in alloparental care are related to their age at the time of exposure to siblings (1EX: 21-42 days; 2EX: 42-63 days by their second exposure). Juveniles may simply change with age from being responsive to pups to ignoring them. In laboratory studies, younger female prairie voles (21-30 days) acted more parentally toward pups (in alloparental care tests) than older females, particularly those older than 45 days (Lonstein and De Vries, 2001). In the wild, approximately 70% of male and female prairie voles remain in the nest until death (McGuire et al., 1993). However, those that do disperse leave the nest around 45-55 days (McGuire et al., 1993). These juveniles would correspond most closely to those in our 1EX treatment group. Perhaps exposure to one litter of siblings is the optimal amount of alloparenting needed for future parental competence in prairie voles.

Although previous reports suggest no sex difference in alloparenting within the natal group (Solomon and Getz, 1997) or that juvenile males may spend more time in the nest than juvenile females (Wang and Novak, 1994), an interesting finding of this study was that juvenile females spent more time in the nest than did males (though females did not necessarily huddle or spend more time in contact with the pups). Results from alloparental care tests with unrelated pups show that pup-naïve females generally behave less parentally (and often are infanticidal) than males when presented with an unrelated pup (Roberts et al., 1998; Lonstein and De Vries, 2001). This could result from sex differences in fear reactions to a novel stimulus, since high

anxiety is correlated with low affiliative behavior (Olazabal and Young, 2005). Sex differences in anxiety and fear could lead to differences in alloparenting with strange pups. However, anxiety is unlikely to explain sex differences in alloparenting one's own siblings; an alternate explanation might be sex differences in the effectiveness of infanticide as a strategy for intrasexual competition.

Presence or absence of alloparenting experience as juveniles had some effects on later parental behaviors. Females in the 0EX treatment, paired with 0EX males, spent the most time in the nest, suggesting once again that inexperienced animals, particularly with inexperienced partners, may devote more time to infant care than more experienced, proficient animals. Primiparous mammal mothers are often more protective of their infants, resulting in greater amounts of time spent with them (primates: reviewed in Fairbanks, 1996; rodents: reviewed in Fleming et al., 1996). However, males in 0×0 pairs spent less time in contact with pups, suggesting that differences in the females' behavior could also be compensatory for lack of help by the male. Nursing postures also varied by previous alloparenting experience. In rats, nursing with a fully arched back provides the most milk to the pups (Lincoln et al., 1980; Stern and Johnson, 1990). Sit-nursing, which was lowest in experienced females with experienced mates, might represent a less optimal nursing posture.

Consistent with these observations, 1EX males (but not females) spent more time licking and grooming their pups. This suggests that although as juveniles, females may spend more time in the nest compared to males, stronger or longer-lasting effects of juvenile experience might occur in males. Licking and grooming is also a particularly significant reproductive behavior in regard to pup development (Meaney, 2001). In line with these observations, differences in paternal care have significant effects on prairie vole pup development. When fathers are absent from the natal group, latencies to eat solid food and move out of the nest are greater for pups. In addition, mothers show less parental care when fathers and juveniles are present (Wang and Novak, 1992).

Variables such as litter size, litter biomass at birth and days to first litter were not affected by alloparenting experience. This differed from the findings of studies in two other rodents. Female oldfield mice that stayed with their mother and helped care for one litter of siblings had more surving pups of their own as adults (Margulis et al., 2005). Male Mongolian gerbils with prior experience with one litter of younger siblings produced their first litter sooner than inexperienced males (Salo and French, 1989). In the current study, however, prairie vole pups with 1EX parents gained more weight later in development than did other pups, suggesting that there may be subtler benefits to alloparenting experience. Similarly, pups with 2EX fathers gained more weight earlier in development. Pups with 1EX and 2EX parents also tended to open their eyes sooner (Table 3). Similarly, Wang (1991) found that pups reared with two experienced parents developed slightly faster than those raised by inexperienced parents. This suggests that although overt parenting behaviors (at least as measured by observers) may not be affected in a linear fashion by prior alloparenting, more subtle linear effects may be occurring, and that these can translate into effects on pup development. For example, voles that have alloparenting experience may build better quality nests that retain more heat. In Mongolian gerbils, fathers that had early experience with younger siblings built higher-quality nests than inexperienced fathers (Salo and French, 1989). Nest-building activities were not measured in the present study. Furthermore, the magnitude of the effects seen on pup development might be greater under different conditions. The voles were raised under a controlled laboratory environment, with free access to food, water and nesting material, and free from predators. We might expect that the subtle differences documented in this study would translate into greater differences in pup development under harsher, more variable conditions in the wild. For example, alloparenting effects on pup growth have been found when prairie voles were housed at challenging temperatures (Solomon, 1991).

For cooperatively breeding mammals (particularly rodents), benefits provided by alloparents to parents and offspring have been demonstrated (Solomon, 1991, 1994; Solomon and Getz, 1997; Bales et al., 2000), but benefits to the alloparents themselves have been more difficult to discern. This study shows that juvenile prairie voles, even when housed in an environment free of most stressors, appear to accrue some benefits via alloparenting experience. These effects, however, are not necessarily linear and exposure to one litter of siblings may be sufficient to affect later parental competence and reproductive success. In addition, the effects seen later in life may be subtle. In species with biparental care such as callitrichid primates and prairie voles, alloparenting experience may be especially important for males (Tardif et al., 1984; Goldizen, 1987). An important direction for future research is to examine the differential effects of early experience, including alloparenting experience, on males and females of cooperatively breeding species.

Acknowledgments

REFERENCES

Bales K, Dietz J, Baker A, Miller K, Tardif SD. The effects of allocaregivers on fitness of infants and parents in callitrichid primates. Folia PrimatoL 2000;7:27–38. [PubMed: 10686485]

Bales KL, O'Herron M, Baker AJ, Dietz J. Sources of variability in numbers of live births in wild golden lion tamarins (*Leontopithecus rosalia*). Am. J. Primatol 2001;54:211–21. [PubMed: 11468751]

Bales KL, Pfeifer LA, Carter CS. Sex differences and developmental effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. Dev. Psychobiol 2004;44:122–131.

Bergmüller R, Johnstone RA, Russell AF, Bshary R. Integrating cooperative breeding into theoretical concepts of cooperation. Behav. Proc 2007;76:61–72.

Brown, JL. Helping and Communal Breeding in Birds: Ecology and Evolution. Princeton University Press; Princeton: 1987.

Carter CS, Getz LL, Gavish L, McDermont JL, Arnold P. Male-related pheromones and the activation of female reproduction in the prairie vole (*Microtus ochrogaster*). Biol Reprod 1980;23:1038–1045. [PubMed: 7008851]

Carter CS, Witt DM, Manock SR, Adams KA, Bahr JM, Carlstead K. Hormonal correlates of sexual behavior and ovulation in male-induced and postpartum estrus in female prairie voles. Phys. Behav 1989;46:941–948.

Clutton-Brock TH, Russell AF, Sharpe LL, Brotherton PNM, McIlrath GM, White S, Cameron EZ. Effects of helpers on juvenile development and survival in meerkats. Science 2001;293:2446–2449. [PubMed: 11577235]

Cockburn A. Prevalence of different modes of parental care in birds. Proc. R. Soc. B 2006;273:1375–1383.

Emlen ST, Reeve HK, Sherman PW, Wrege PH, Ratnieks FLW, Shellman-Reeve J. Adaptive versus nonadaptive explanations of behavior: the case of alloparental helping. Am. Nat 1991;138:259–270.

Emlen ST, Wrege PH. A test of alternative hypotheses for helping behavior in white-fronted bee-eaters of Kenya. Beh. Ecol. Sociobiol 1989;25:303–319.

Fairbanks LA. Reciprocal benefits of allomothering. Anim. Behav 1990;40:553–562.

Fairbanks, LA. Individual differences in maternal style: causes and consequences for mothers and offspring. In: Rosenblatt, JS.; Snowdon, CT., editors. Parental Care: Evolution, Mechanisms, and Adaptive Significance. Academic Press; San Diego: 1996. p. 579-611.

Fleming, AS.; Morgan, HD.; Walsh, C. Experiential factors in postpartum regulation of maternal care. In: Rosenblatt, JS.; Snowdon, CT., editors. Parental Care: Evolution, Mechanisms, and Adaptive Significance. Academic Press; San Diego: 1996. p. 295-332.

Getz LL, Hoffman JE. Social organization in free-living prairie voles, *Microtus ochrogaster*. Behav. Ecol. Sociobiol 1986;18:275–282.

Getz LL, McGuire B. Communal nesting in prairie voles (*Microtus ochrogaster*): formation, composition and persistence of communal groups. Can. J. Zool 1997;75:525–534.

Goldizen AW. Facultative polyandry and the role of infant carrying in wild saddle-back tamarins (*Saguinus fusicollis*). Behav. Ecol. Sociobiol 1987;20:99–109.

Griffin AS, West SA. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 2003;302:634–636. [PubMed: 14576431]

Hamilton WD. The genetical evolution of social behaviour. I. J. Theoret. Biol 1964a;7:1–16. [PubMed: 5875341]

Hamilton WD. The genetical evolution of social behaviour. II. J. Theoret. Biol 1964b;7:17–52. [PubMed: 5875340]

Hayes LD, Solomon NC. Costs and benefits of communal rearing to female prairie voles (*Microtus ochrogaster*). Behav. Ecol. Sociobiol 2004;56:585–593.

Jennions MD, Macdonald DW. Cooperative breeding in mammals. TREE 1994;9:89–93.

Lancaster JB. Play-mothering: relations between juvenile females and young infants among free-ranging vervet monkeys (*Cercopithecus aethiops*). Folia Primatol 1971;15:161–182.

Lincoln DW, Hentzen K, Hin T, van der Schoot P, Clarke G, Summerlee AJS. Sleep: a prerequisite for reflect milk ejection in the rat. Exp. Brain Res 1980;38:151–162. [PubMed: 7358101]

Lonstein JS, De Vries GJ. Social influences on parental and nonparental responses in virgin female prairie voles (*Microtus ochrogaster*). J. Comp. Psych 2001;115:53–61.

Lonstein JS, Simmons DA, Stern JM. Functions of the caudal periaqueductal gray in lactating rats: maternal, aggressive, sexual, and fear behaviors. Behav. Neurosci 1998;112:1–17.

Lonstein JS, Stern JM. Role of the midbrain periaqueductal gray in maternal nurturance and aggression: c-Fos and electrolytic lesion studies in lactating rats. J. Neurosci 1997;17:3364–3378. [PubMed: 9113892]

Lonstein JS, Stern JM. Site and behavioral specificity of periaqueductal gray lesions on postpartum sexual, maternal, and aggressive behaviors in rats. Brain Res 1998;804:21–35. [PubMed: 9729249]

Margulis SW, Nabong M, Alaks G, Walsh A, Lacy RC. Effects of early experience on subsequent parental behavior and reproductive success in oldfield mice, *Peromyscus polionotus*. Anim. Behav 2005;69:627–634.

McGuire B, Getz LL. Incest taboo between sibling *Microtus ochrogaster*. J Mammal 1981;62:213–215.

McGuire B, Getz LL, Hoffman JE, Pizzuto T, Frase B. Natal dispersal and philopatry in prairie voles (*Microtus ochrogaster*) in relation to population density, season and natal social environment. Behav. Ecol. Sociobiol 1993;32:293–302.

Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu. Rev. Neurosci 2001;24:1161–1192. [PubMed: 11520931]

Olazabal DE, Young LJ. Variability in "spontaneous" maternal behavior is associated with anxiety-like behavior and affiliation in naïve juvenile and adult female prairie voles (*Microtus ochrogaster*). Dev. Psychobiol 2005;47:166–178. [PubMed: 16136562]

Pereira, ME.; Fairbanks, LA. Juvenile Primates: Life History, Development, and Behavior. The University of Chicago Press; Chicago: 1993.

Powell RA, Friend JJ. Helping by juvenile pine voles (*Microtus pinetorum*), growth and survival of younger siblings, and the evolution of pine vole sociality. Behav. Ecol 1992;3:325–333.

Roberts RL, Miller AK, Taymans SE, Carter CS. Role of social and endocrine factors in alloparental behavior of prairie voles (*Microtus ochrogaster*). Can. J. Zool 1998;76:1862–1868.

Rood JP. Group size, survival, reproduction, and routes to breeding in dwarf mongooses. Anim. Behav 1990;39:566–572.

Russell AF, Brotherton PNM, McIlrath GM, Sharpe LL, Clutton-Brock TH. Breeding success in cooperative breeding meerkats: effects of helper number and maternal state. Behav. Ecol 2003;14:486–492.

Russell, AF. Mammals: comparisons and contrasts. In: Koenig, WD.; Dickinson, JL., editors. Ecology and Evolution of Cooperative Breeding in Birds. Cambridge University Press; Cambridge: 2004. p. 293

Salo AL, French JA. Early experience, reproductive success, and development of parental behavior in Mongolian gerbils. Anim. Behav 1989;38:693–702.

Snowdon CT, Cronin KA. Cooperative breeders do cooperate. Behav. Proc 2007;76:138–141.

Snowdon CT, Savage A, McConnell PB. A breeding colony of cotton-top tamarins (*Saguinus oedipus*). Lab. Anim. Sci 1985;35:477–480. [PubMed: 3932776]

Solomon, NG.; French, JA. Cooperative Breeding in Mammals. Cambridge University Press; Cambridge: 1997. p. 390

Solomon, NG.; Getz, LL. Examination of alternative hypotheses for cooperative breeding in rodents. In: Solomon, NG.; French, JA., editors. Cooperative Breeding in Mammals. Cambridge University Press; Cambridge: 1997. p. 199-231.

Solomon NG. Current indirect fitness benefits associated with philopatry in juvenile prairie voles. Behav. Ecol. Sociobiol 1991;29:277–282.

Solomon NG. Effect of the pre-weaning environment on subsequent reproduction in prairie voles, *Microtus ochrogaster*. Anim. Behav 1994;48:331–341.

Stern JM, Johnson SK. Ventral somatosensoty determinants of nursing behaviors in Norway rats. I. Effects of variations in the quality and quantity of pup stimuli. Phys. Behav 1990;47:993–1011.

Tardif SD, Richter CB, Carson RL. Effects of sibling-rearing experience on future reproductive success in two species of Callitrichidae. Am. J. Primatol 1984;6:377–380.

Wang, Z. Ph.D. dissertation. University of Massachusetts; Amherst: 1991. Effects of social environment and experience on parental care, behavioral development, and reproductive success of prairie voles *(Microtus ochrogaster)*.

Wang Z, Novak MA. Influence of the social environment on parental behavior and pup development of meadown voles (*Microtus pennsylvanicus*) and prairie voles (*Microtus ochrogaster*). J. Comp. Psychol 1992;106:163–171.

Wang Z, Novak MA. Alloparental care and the influence of father presence on juvenile prairie voles, *Microtus ochrogaster*. Anim. Behav 1994;47:281–288.
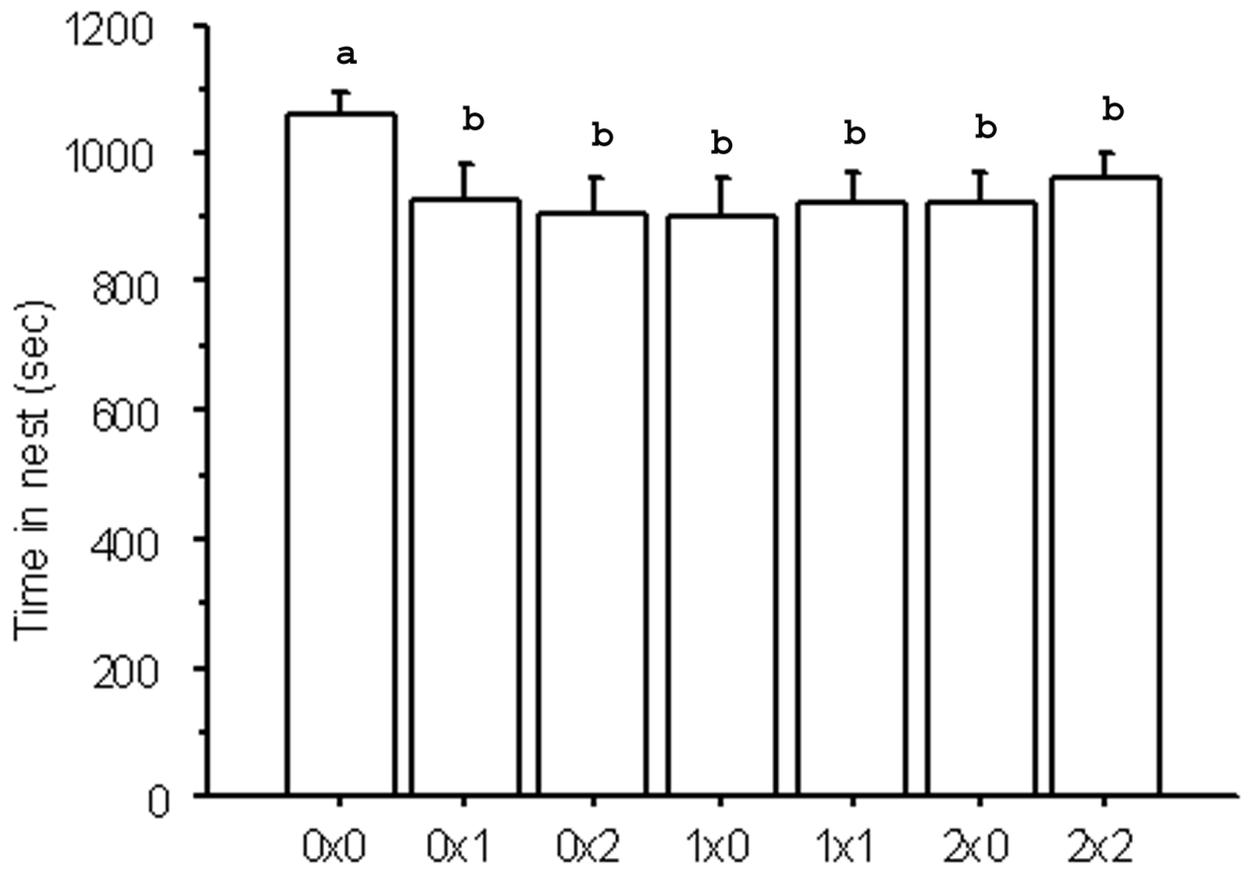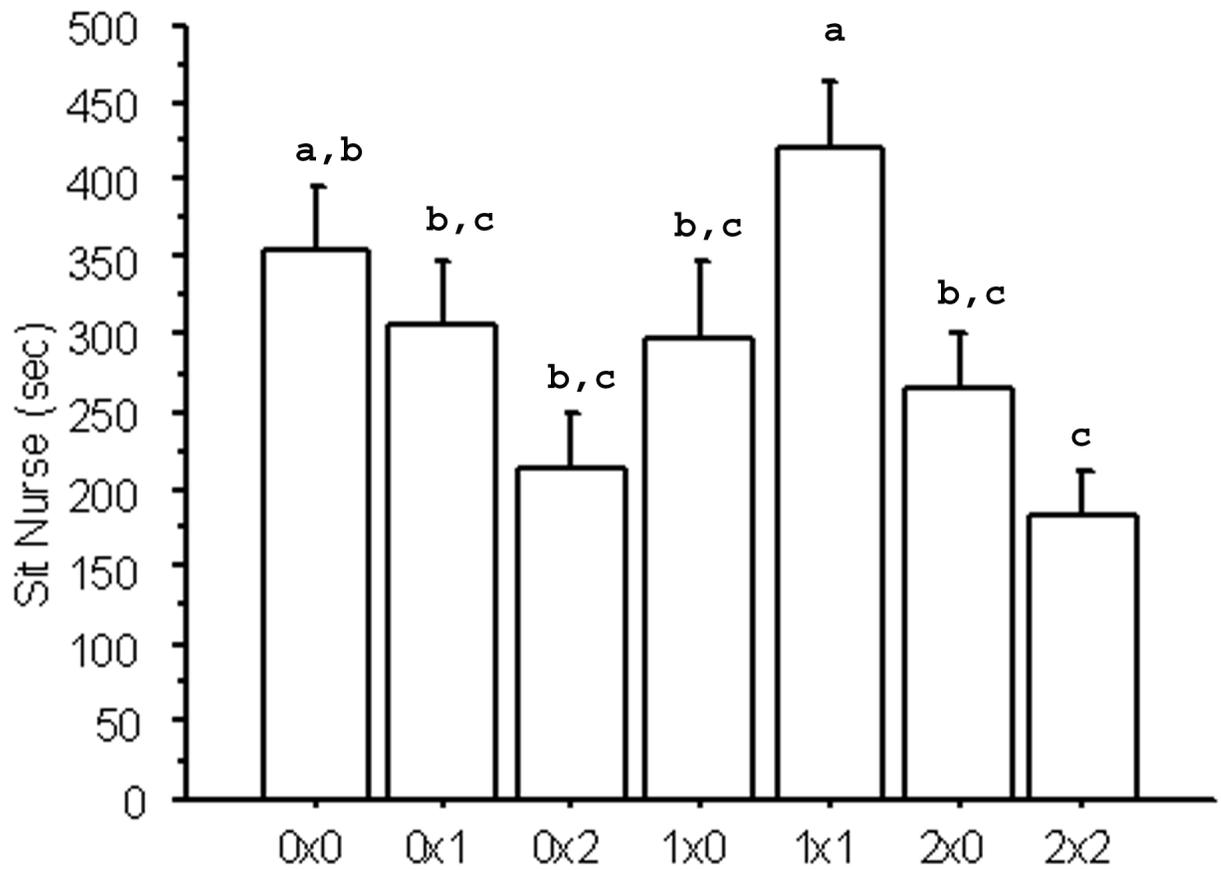
Figure 1.
Duration of a) female time spent in nest and b) female time spent in sit nursing according to pair treatment. On the X axis, the first treatment refers to the mother's treatment and the second treatment to the father's. 1×1 females spent more time sit-nursing compared to several other treatments.
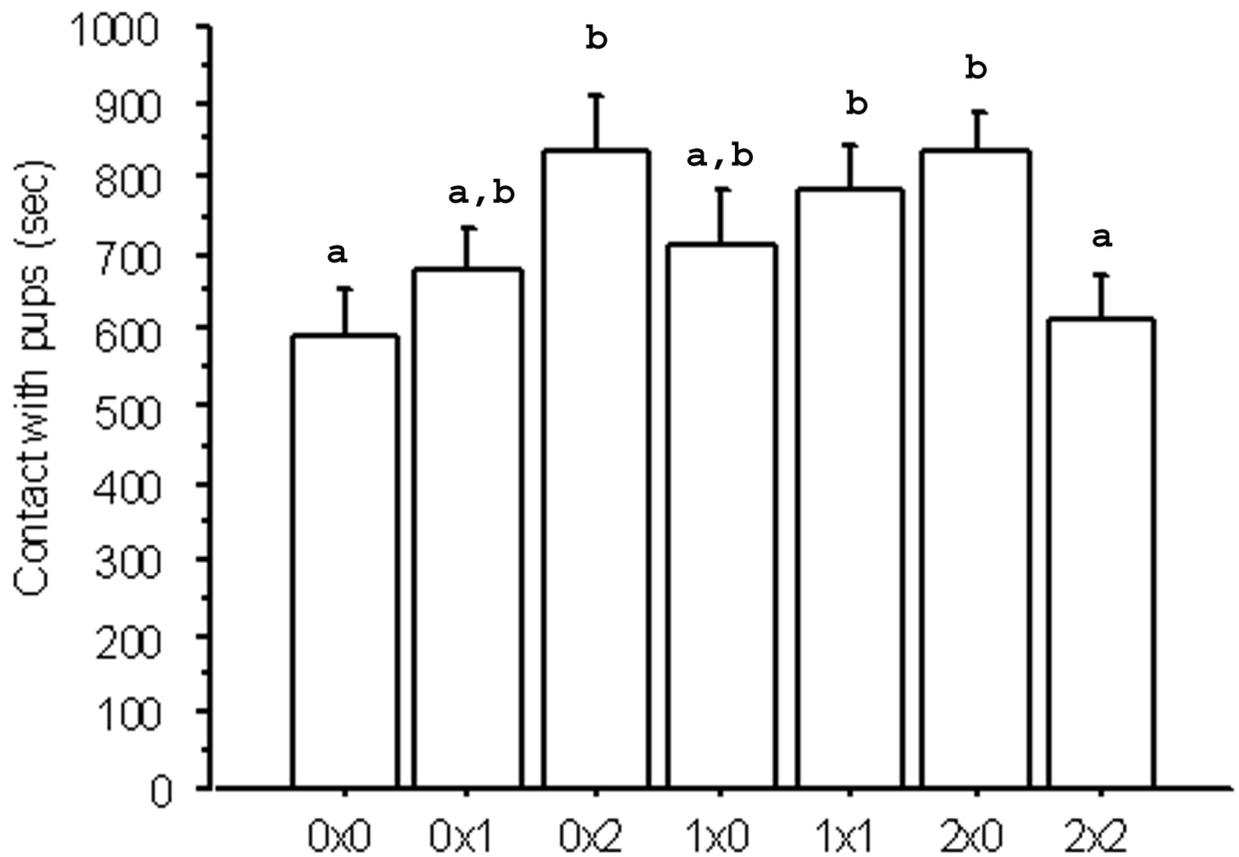
Figure 2.
Duration of male time spent in contact with pup (includes non-huddling contact, huddling, licking/grooming pup). On the X axis, the first treatment refers to the mother's treatment and the second treatment to the father's.

Table 1

Study design, detailing sample sizes for parental treatment groups according to degree of exposure to pups as juveniles. In the text, the first treatment refers to the mother's treatment and the second treatment to the father's (e.g. 1×0 treatment= 1EX mother and 0EX father). N= 57 pairs

Females	Males		
	0EX	1EX	2EX
0EX	7	9	10
1EX	7	8	-
2EX	8	-	8

Table 2

Ethogram for scoring juvenile-pup interactions and parent-pup interactions

Postures	
Huddling	All four paws on ground. Legs holding self up over pups. Arched back, head tucked.
Pseudohuddling	A more relaxed version of huddling. Standing over pups in a crouched position. For parental behaviors toward own pups, can be lying on pups.
Lateral Nursing (moms only)	Lying on side with pups.
Active Nursing (moms only)	Female has pup(s) attached behind her. Can be walking or be stationary.
Sit-nurse (moms only)	Female sitting down, nursing pups in front of her. May be licking pups
Non-huddling contact	Vole in contact with pups and quiescent.

Other Behaviors	
In nest	Inside nest area and/or is in contact with pups and other parent.
Out of nest	Does not meet above criteria
Licking and sniffing	Licking and sniffing pups, including anogenital licking.
Retrieving	Lifting pup in mouth and carrying at least one inch.
Total time spent in contact with pups	Includes huddling, pseudohuddling, nursing, non-huddling contact and licking/sniffing
Non-pup directed	Self-grooming, grooming partner, locomoting, drinking, eating.

Table 3

Reproductive performance of parental treatment groups. Means ± SE. Numbers in bold are significantly different at p<0.05. Numbers in parentheses indicate total number of pairs that were set up as breeding pairs.

Treatment	Proportion that reproduced	Days to first litter	Litter size at birth	Litter biomass at birth (g)	Litter size at weaning	Litter biomass at weaning
0×0 (n=8)	87.5%	23.4 ± 0.3	6.1 ± 0.3	20.4 ± 0.8	5.3 ± 0.3	113.3 ± 3.8
0×1 (n=12)	**75%**	25.4 ± 0.9	5.2 ± 0.4	18.5± 1.6	4.9 ± 0.5	110.2± 8.5
0×2 (n=11)	91%	24.8 ± 0.5	4.9± 0.3	18.3± 1.1	4.4± 0.4	97.9± 6.2
1×0 (n=10)	**70%**	24.7 ± 0.5	5.7 ± 0.5	19.6± 2.0	5.2± 0.3	102.9± 5.5
1×1 (n=16)	**50%**	24.5 ± 0.4	4.9 ± 0.4	17.8± 1.5	4.8 ± 0.6	104.6± 8.5
2×0 (n=10)	80%	25.5 ± 0.7	5.4± 0.4	18.8± 1.0	5.0± 0.5	99.7± 5.4
2×2 (n=8)	100%	24.9 ± 1.2	5.2 ± 0.3	19.1± 1.3	4.9 ± 0.4	102.0± 5.3

Table 4

Rates of pup development according to parental treatment. Means ± SE. Numbers in bold are significantly different at p<0.05. Numbers in parentheses indicate total number of pairs that produced litters

Treatment	Latency to open eyes (days)	Latency to leave nest	Latency to eat solid food
0×0 (n=7)	10.1 ± 0.3	12.7 ± 0.9	15.3± 0.5
0×1 (n=9)	10.0 ± 0.4	11.5 ± 1.5	15.4± 0.5
0×2 (n=10)	**8.9 ± 0.4**	10.9± 0.7	14.0± 0.3
1×0 (n=7)	9.2 ± 0.5	10.3 ± 1.8	15.2 ± 0.8
1×1 (n=8)	**8.5 ± 0.3**	11.2 ± 0.9	15.1 ± 0.4
2×0 (n=8)	**8.9 ± 0.3**	11.2 ± 0.9	14.1± 0.6
2×2 (n=8)	9.5 ± 0.3	10.8 ± 0.4	15.4± 0.5