

Good Fathers Make Better Mothers

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Abstract

Early life experience, in the form of care received from parents, has been shown to contribute to lifelong changes in offspring's future behavior. Using a biparental rodent model, we investigate the stability of the dyad's parenting across time, the relationships between mother and father's parenting behavior, and the impact early life care has on the future care of an organism's own offspring. We provide evidence that naïve mothers parent more than experienced mothers, but fathers remain stable in the amount of care they provide to their offspring. We also show that naïve mothers' parent like their fathers, but experienced mothers adapt their parenting to their partner's parenting.

Introduction

Early life experience impacts an organism's adult life and shapes individual variation. A large body of literature in rats details the effect of differences in natural maternal care (Liu et al., 1997), and early manipulations such as maternal separation (Plotsky & Meaney, 1993) or handling (Meaney et al., 1989) can produce lifelong changes in physiology and species typical behaviors in the offspring (Liu et al., 1997). A seminal cross fostering study in rats indicates that transmission of these changes is through non-genomic means (Weaver et al., 2004). Rat dams naturally vary in the amount of pup licking and grooming (LG) and arched-back nursing (ABN) they provide to offspring and can be classified into High LG/ABN and Low LG/ABN mothers (Caldji et al., 1998). LG/ABN is passed on from mother to daughter, where daughters of high LG/ABN mothers become high LG/ABN mothers themselves, the same is true for low LG/ABN animals (Francis et al., 1999; Weaver et al., 2004). The amount of care received by these offspring also impacts their future response to stressors and expression of social behaviors (Francis et al., 1999).

Much of the work examining parental care has focused on the mother/child relationship. Humans, however, are often biparental, meaning children are often parented by both parents, a life history that is only exhibited by 3-5% of mammalian species (Kleiman, 1977). Work in biparental rodents, such as the California mouse (*Peromyscus californicus*) and prairie vole (*Microtus ochrogaster*), indicates that variation in paternal care can result in changes to their offspring. In California mice, paternal care is passed from father to son in a manner similar to maternal care in rats, and high paternal care increases intruder aggressive behaviors in all offspring (Becker et al., 2021; Bester-Meredith et al., 1999; Frazier et al., 2006; Gleason & Marler, 2013). In the prairie vole, pups raised without a father exhibit decreases in species typical prosocial behaviors, including lowered amounts of spontaneous alloparental behavior, and delays in partner preference formation, indicating the importance of the father (Ahern et al., 2011; Ahern & Young, 2009). Further work in the prairie vole has also determined that natural variation in biparental care can impact the development of these social behaviors. Male offspring raised by a high care parents exhibit greater amounts of alloparental care, and female offspring

spend more time with their opposite sex partner in partner preference tests (Perkeybile et al., 2013; Razo & Bales, 2016).

We explored several facets of biparental care in the prairie vole model. There is minimal and conflicting literature exploring how prairie vole parenting changes across time, with some evidence stating it remains stable between litters (Ahern et al., 2011; Perkeybile et al., 2013), and some evidence stating it may vary between litters (Rogers et al., 2018). We explored stability in parenting behavior by examining the pup directed behavior in two cohorts of prairie voles. First, we examined the first two litters of newly established breeding pairs. We found that total parental care significantly decreased from litter one to litter two. This decrease was driven by dams reducing the amount of care they provide to their second litter by 15%. As much of the work claiming stability was completed on established breeder pairs, we theorized this change was in part caused by dams transforming from naive to experienced mothers. To determine if experienced breeder pairs remained stable, we then examined the pup directed behavior on litters six-eight in our breeding colony. As expected, we found that parenting behavior was stable between litters in these established breeding pairs. While it is possible that dams are decreasing their behavior due solely to experience, we examined how dams and sires might influence their partner's parenting behavior. We found that pup directed behavior of sires was strongly related to how his partner parented in both litters. However, the relationship between dam and partner behavior was significant only in litter two, suggesting that dams are adapting to their partners behavior with experience. Finally, we investigated how natural variation in early life care impacts the future parenting behavior of the offspring. In line with other biparental rodent models, we expected parental care to be transmitted to the next generation in a sex specific manner, with females displaying parenting behavior similar to their mothers, and males displaying parenting behavior similar to their fathers. However, our data did not support this hypothesis. Dams' parental behavior towards their first litter was significantly related to the amount of care they received from their father. This relationship was no longer observed in the second litter. Sires' parental behavior was not impacted by the early life care they received from either parent. These findings suggest that dams parenting behavior is experience driven, first by how she was parented by her father, then by her experience with her partner.

Methods

Animal Model

Laboratory-bred prairie voles (*Microtus ochrogaster*) were maintained on a 14:10 light-dark cycle with lights on at 0700 during daylight savings and 0600 the rest of the year. Animals were given food (high-fiber Purina rabbit chow) and water *ad libitum*. After weaning on postnatal day 20 (PND20), same sex pairs were housed in small polycarbonate cages (27cm X 16cm X 16cm). Breeding pairs were housed in larger polycarbonate cages (44cm X 22cm X 16cm). All procedures were approved by the University of Virginia's Institutional Animal Care and Use Committee.

Creation of experimental pairs

Seventy-seven breeder pairs were established concurrently using a timed mating paradigm (Kenkel et al., 2019). Prairie vole females experience induced estrous after exposure to

a male. Timed mating is performed by placing a male and female in a breeding cage together for 24 hours with a scoop of the male's dirty bedding. The following day a clear plexiglass divider with holes is placed in the cage, separating the male and female, but allowing them to see and smell each other. Seventy-two hours later, the divider is removed and mating is confirmed. Females were weighed at the time of pairing. All animals were sexually naïve, aged between PND61 and PND103. Typical vole gestation is 21.5 days. Twenty days after mating, pairs were moved into a clean cage, and a small line of fur was trimmed across the males' hips as an identifier. Females were weighed again to confirm weight gain due to pregnancy. Females were assumed to be term pregnant if they gained over 15 grams in weight. Of our 77 breeder pairs, 13 pairs were eliminated from the study as they gained little to no weight, indicating they were not pregnant. Four additional pairs were eventually dropped, as they did not produce a second litter, or one of the parents died for unknown reasons. The final number of experimental pairs was 60.

Additionally, our experimental dyads are offspring of 24 established colony breeders. Of our 120 experimental animals, 94 (48 dams, and 46 sires) had parenting behavior directly scored for their litter. For the remaining 26 animals, though behavior was not directly scored for the litter they were born in, their parents were experienced breeders and had been scored an average of eight of times before or after our experimental animals' birth to generate average parenting care scores which were used in place of direct litter parenting behavior.

Classification of prairie vole parenting

Classification of early life parenting behavior in the prairie vole was performed as in (Perkeybile et al., 2013). Parenting behavior was live scored in the home cage by a trained observer using behavioral software (www.behaviortracker.com). Observers were blind to the history of animals they were scoring. Animals were not disturbed during the observation period. The behaviors of both the dam and sire were simultaneously scored. Behaviors scored are listed in Table 1. Early life observations occurred a total of four times per litter each for 20 minutes. All observations were made between PND1 and PND3, with two observations occurring in the morning and two observations occurring in the afternoon. Observations were completed for litters one and two. Mean duration of pup directed behaviors were summed for each litter to produce a (1) maternal care score, (2) paternal care score, and (3) total care score for each litter, see Table 1.

Assessing parenting behavior in established breeder pairs

As part of standard breeding practice, we routinely score parenting behavior (Table 1) in our breeding colony. Parental behavior was scored on litters six, seven and eight for 19 of our colony dyads to examine the stability of parenting behavior in established breeder pairs.

Statistical analysis

Analysis of parenting behavior was performed in R Studio version 4.2.2. For comparison of total pup directed behavior between litters, a linear mixed model was used with the fixed effect of litter, and a random effect of breeder pair ID to account for within-subject repeated measures. For comparisons of individual parental behavior and effects of early life parenting given to experimental voles, three additional linear mixed models were performed. Each model

consisted of parent and litter as fixed effects and animal ID as a random effect to account for within-subject repeated measures. The third fixed effect in each model was partner's pup directed behavior, grandfather's parental behavior, or grandmother's parental behavior. P-values for linear mixed models were calculated using Satterthwaite method in *afex* R package. Post-hoc analysis was performed using *emmeans* (pairwise) and *interactions* (simple slopes) packages. Plots were created using *afex* and *ggplot* packages (RCoreTeam, 2022).

Results

Experience changes parenting behavior. Total parental care was assessed for 60 breeder pairs at litter one (L1) and litter two (L2). We found that total pup directed behavior of a dyad is significantly different from L1 to L2 ($t(1,116) = 50.76, p < .001$). Post-hoc analysis indicated that total care significantly decreases from L1 to L2 ($t(1, 59) = 3.007, p = .0038$), with parents providing on average 5% less pup directed behavior towards their second litter (Figure 1). We further explored this data set to determine if there was a parent specific decrease in behavior. Using a linear mixed model with litter number and parent as fixed effects and parent ID as a random effect we found a main effect of Litter ($t(1,234) = -4.45, p < .001$), Parent ($t(1,234) = -8.65, p < .001$), and a significant Litter X Parent interaction ($t(1,234) = 4.13, p < .001$). Post-hoc analysis revealed a significant difference between L1 and L2 for dams only ($t(118) = 4.542, p < 0.001$). Dams provide on average 15% less care to offspring in their second litter. Sires do not significantly change their total amount of pup directed behaviors between L1 and L2, $t(118) = -1.295, p = 0.1979$. We also note that dam and sire pup directed behavior is significantly different from each other, with dams providing an average of 32.5% more care to pups in L1 ($t(221) = 8.65, p < 0.0001$). This relationship holds true in L2, ($t(221) = 3.63, p = 0.0003$), but in L2 dams provide only 13.6% more care to pups than sires. A visual display of these relationships is provided (see Figure 2).

We hypothesized that this decrease in pup directed behavior in dams is in part due to their change from naïve to experienced mothers. To test this, we again observed early parental care, this time in experienced colony breeders at litters six (L6), seven (L7), and eight (L8). As previously reported (Perkeybile et al., 2013; Rogers et al., 2018) we found overall model significance ($t(1,52) = 30.19, p < .001$), however, the effect of litter on total dyad care was not significant ($t(1,52) = -0.05, p = .96$), indicating that total dyad behavior is stable for experienced parents (Supplemental Figure 1A). We further assessed if the individual dam or sire pup directed behavior in our colony breeders was different in L6, L7 and L8. In our 19 established pairs, only the main effect of parent ($t(1,36) = -5.634, p < .001$) was significant. Follow up pairwise comparisons were all non-significant, further indicating both parents in an established breeder pair have stable pup directed behaviors (Supplemental Figure 1B). Additional pairwise comparisons of dam and sire revealed that in our established breeders, dams provided an average of 30% more care to pups than sires, matching the pattern we observed in the experimental cohort of breeders.

As previously reported (Finton & Ophir, 2020), we similarly find that sires are driving the variability in total pup directed care. Sires have a wider range of pup directed behavior with an average maximum of 1227 seconds, and an average minimum of 160 seconds across both cohorts, giving them an average range of 1,101 seconds. Dams have an average maximum of

1436 seconds, and an average minimum of 594 seconds, giving them an average range of 842 seconds. Additionally, across all scores in all cohorts, dams provided less than 500 seconds of care only five times.

Mothers adjust to their partners parenting style. To explore the parenting relationship within the dyad, we examined whether the behavior of individual dyad members had moderating effects on each other. We found a main effect of partner's behavior ($t(1,218) = -2.036, p = 0.043$), and a trend in the interaction between litter and partner behavior ($t(1,170) = -1.87, p = .062$). In post-hoc analysis corrected for multiple testing ($p < 0.025$), we found that dam's behavior was not significantly predicted by her partner's behavior in L1 ($t = -2.03, p = 0.042$), but was in L2 ($b = -0.50, SE = 0.12, t = -4.24, p < 0.001$) (Figure 3). Sires had a significant negative relationship with their partner's behavior in both L1 ($t = -2.81, p = 0.01$) and L2 ($t = -3.66, p < 0.001$), which is in line with the idea that the time a mother spends in close contact with her offspring limits the time that dad can spend. Together, these data suggest that in a prairie vole dyad, the dam adapts from L1 to L2 to the parenting style of the sire.

Early life experience influences parenting behavior. Based on prior literature in other rodent models (Francis et al., 1999; Gleason & Marler, 2013), we hypothesized that naïve parents may care for their young (L1) based on their own early rearing experience. In addition, if parenting behavior was passed down from parent to offspring, we might expect to see a sex specific effect. To test this hypothesis, we previously determined the parenting that our 60 naïve breeder pairs received when they were pups. We examined the effects of early life care received from their fathers (referred to as grandfathers) and mothers (referred to as grandmothers) separately. We identified a significant main effect of the grandfathers' behavior ($t(1,216) = 2.33, p = 0.0203$), but not the grandmothers' behavior and a trend for a main effect of grandparent ($t(1,216) = -1.845, p = 0.066$), and the interaction of litter by care received from grandfathers ($t(1,116) = -1.972, p = .051$). Follow up simple slopes analysis indicated that grandfathers' parenting behavior impacted naïve dams' behavior (L1) ($b = 0.26, SE = 0.11, t = 2.33, p = 0.02$), but not experienced dams' behavior (L2) ($b = -.005, SE = 0.11, t = -0.048, p = 0.96$). Grandfathers' parenting did not impact sires' behavior on L1 ($b = 0.08, SE = .11, t = 0.73, p = 0.46$), or L2 ($b = 0.01, SE = 0.11, t = 0.13, p = 0.89$) (Figure 4A). Simple slopes analysis of the grandmothers' parenting behavior indicated that grandmothers did not significantly influence their offspring's parenting behavior as neither dams nor sires had slopes significantly different than zero in L1 ($b = -0.07, SE = .11, t = -0.67, p = 0.50$; $b = -0.1, SE = .14, t = -0.67, p = -0.70$) or in L2 ($b = 0.13, SE = .11, t = 1.08, p = 0.27$; $b = -0.01, SE = .14, t = -0.11, p = -0.91$) (Figure 4B).

Discussion

We demonstrate that first-time prairie vole parents decrease total pup directed behavior from the first to the second litter. Change in care is driven by decreases in maternal behavior. Decline in maternal care with experience is a finding that aligns with the human literature (Boukydis & Burgess, 1982). As proposed by Rogers et al., this decline may be experience based, with mothers becoming more efficient with their care, or experience could lead to reduced sensitivity to cues from pups (Rogers et al., 2018). Based on the findings in this work, we propose that the decline in maternal care may in part be driven by her experience with her partner. Here, we have shown that in a dyad, the behavior of dams is not significantly impacted

by her partner's behavior in their first litter, but this relationship changes in the second litter, with her behavior being significantly predicted by her partner's behavior.

Contrary to our prediction, we found that parenting behavior did not appear to be passed down in a sex specific manner. Maternal care was unrelated to the care they received from their mothers, but appeared to be impacted by the care they received from their fathers. There is evidence in human literature that daughters may be more impacted than sons by absent or neglectful fathers (Tither & Ellis, 2008). Pairing our findings of maternal care being affected by their own fathers behaviors, with maternal care being significantly predicted by her partner's behavior in the second litter but not the first, we propose an additional experience-based hypothesis. Female prairie voles initially parent in a manner similar to how they were parented by their father; as they parent in a dyad, females learn what to expect from their partner and adapt their behavior in subsequent litters.

Finally, we found that male parenting behavior does not appear to be related to the amount of early life care they received from either parent. This was an unexpected finding, as other work has shown that behaviors such as alloparenting and partner preference formation are critically impacted by the amount of care a prairie vole receives from both their mother and father (Ahern et al., 2011; Bales et al., 2007; Danoff et al., 2023; Perkeybile et al., 2013; Rogers et al., 2021; Wang & Novak, 1994).

It is possible that alloparenting and parenting of one's own offspring are moderated through different mechanisms in male prairie voles. We have shown here that a male's parenting behavior is significantly impacted by their partner's parenting behavior. This may in part be due to higher care mothers limiting access to pups for fathers, i.e., a father cannot provide high levels of care if his partner is huddled over pups and preventing access.

This work highlights the importance of using a biparental species to model the effects of parental care on offspring outcomes. The data emphasize that the care a mother receives early in life and the support she receives from her partner during her own time parenting can have a significant impact on her offspring.

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Tables and Figures

Table 1: Behavioral ethogram of parental behaviors adapted from Perkeybile et al. 2013

Parental Behaviors	Description
Huddling	All four paws touching the ground; head tucked, back arched
Non-Huddling Contact	In non-huddle contact with another animal
Licking/Grooming	Licking and Grooming pups
Retrieval	Lifting pup in mouth and moving it at least one inch
Maternal Postures	Description
Active Nursing	Pups attached while locomoting around home cage
Lateral Nursing	Laying on side with pups laying in front
Neutral Nursing	Standing over pups in a relaxed, non-huddled position w/o locomotion
Additional	Description
In Nest	Animal is intentionally located within the nest
Out of Nest	Animal is out of the nest

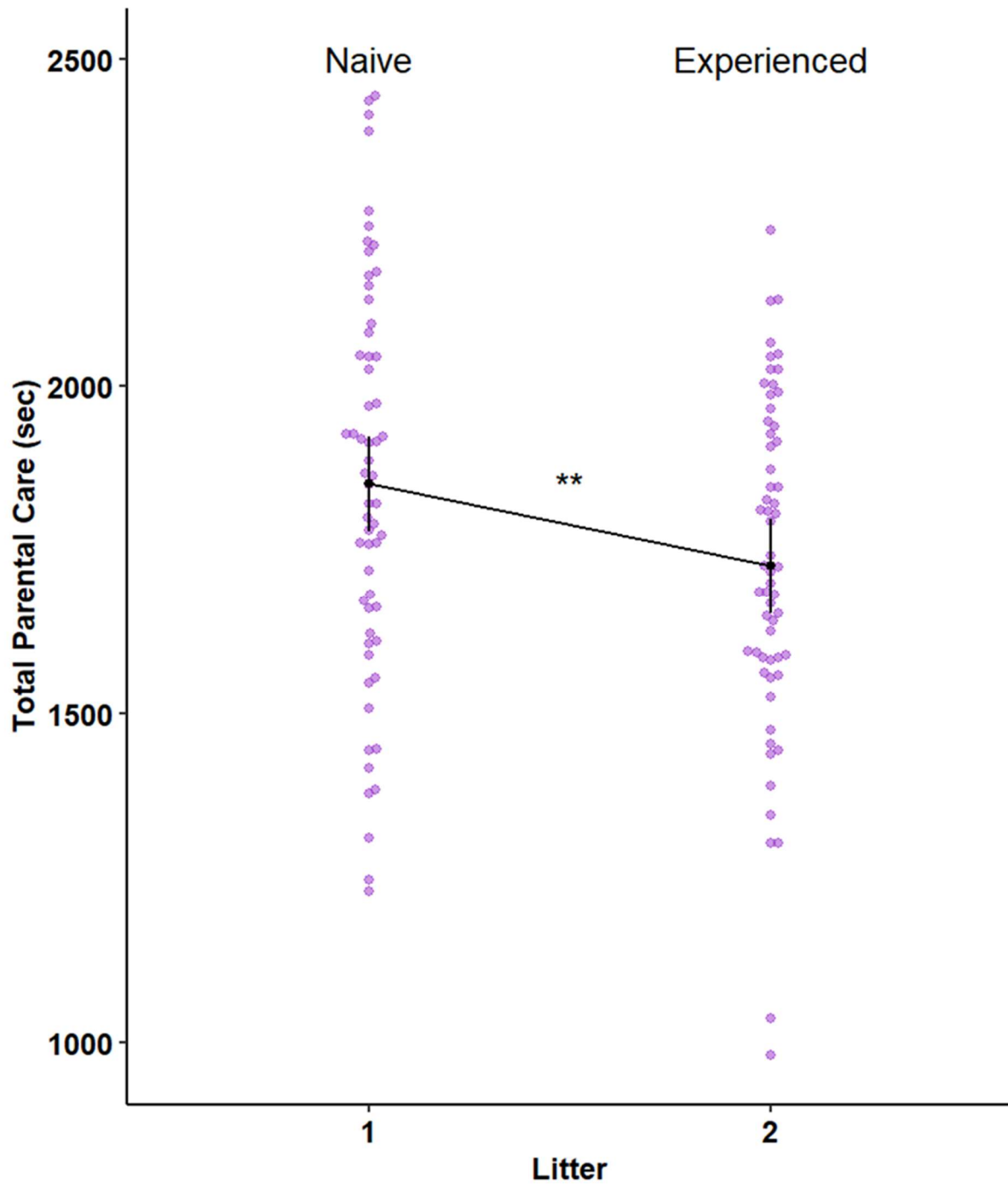


Figure 1: Experience decreases total parenting behavior. Total parental care, in the form of pup-directed behaviors, decreases after the experience gained from the first litter of pups ($t(1, 59) = 3.007, p = 0.00387$). L1 received an average of 126 seconds, or about 5% more pup-directed behaviors than L2

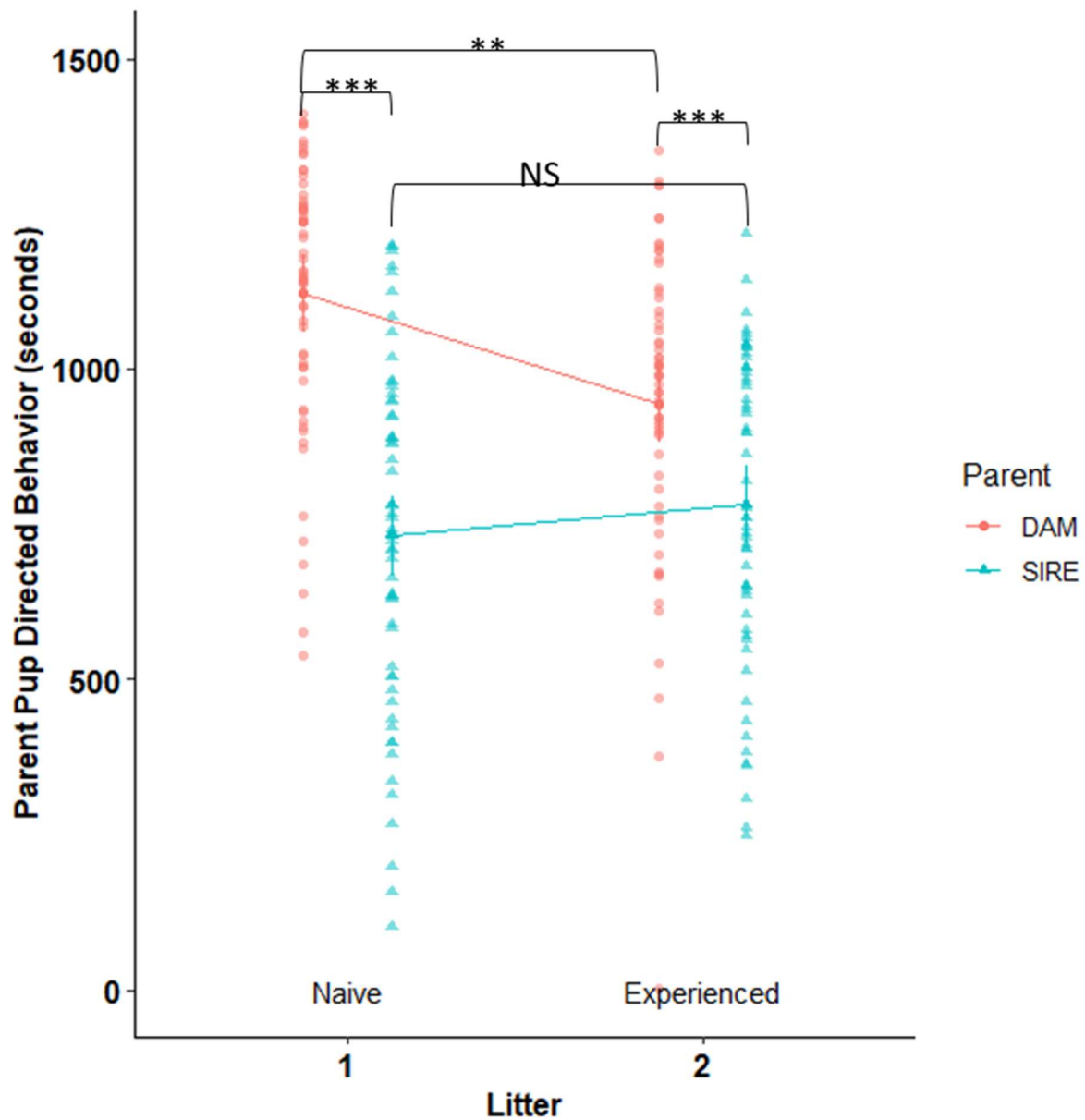


Figure 2: Experience-dependent reduction in parenting is driven by the dam. There was a significant litter by parent interaction ($t(234) = 4.13, p < .001$). Post-hoc analysis revealed a significant difference between L1 and L2 in dams only ($t(118) = 4.542, p < 0.001$) with dams providing 15% less care to offspring in their second litter. Sires do not significantly change their total amount of pup-directed behaviors between L1 and L2 ($t(118) = -1.295, p = 0.1979$). Dams provided 32.5% more pup-directed behavior in L1 ($t(221) = 8.65, p < 0.0001$) and 13.6% more pup-directed behavior in L2 ($t(221) = 3.63, p < .001$).

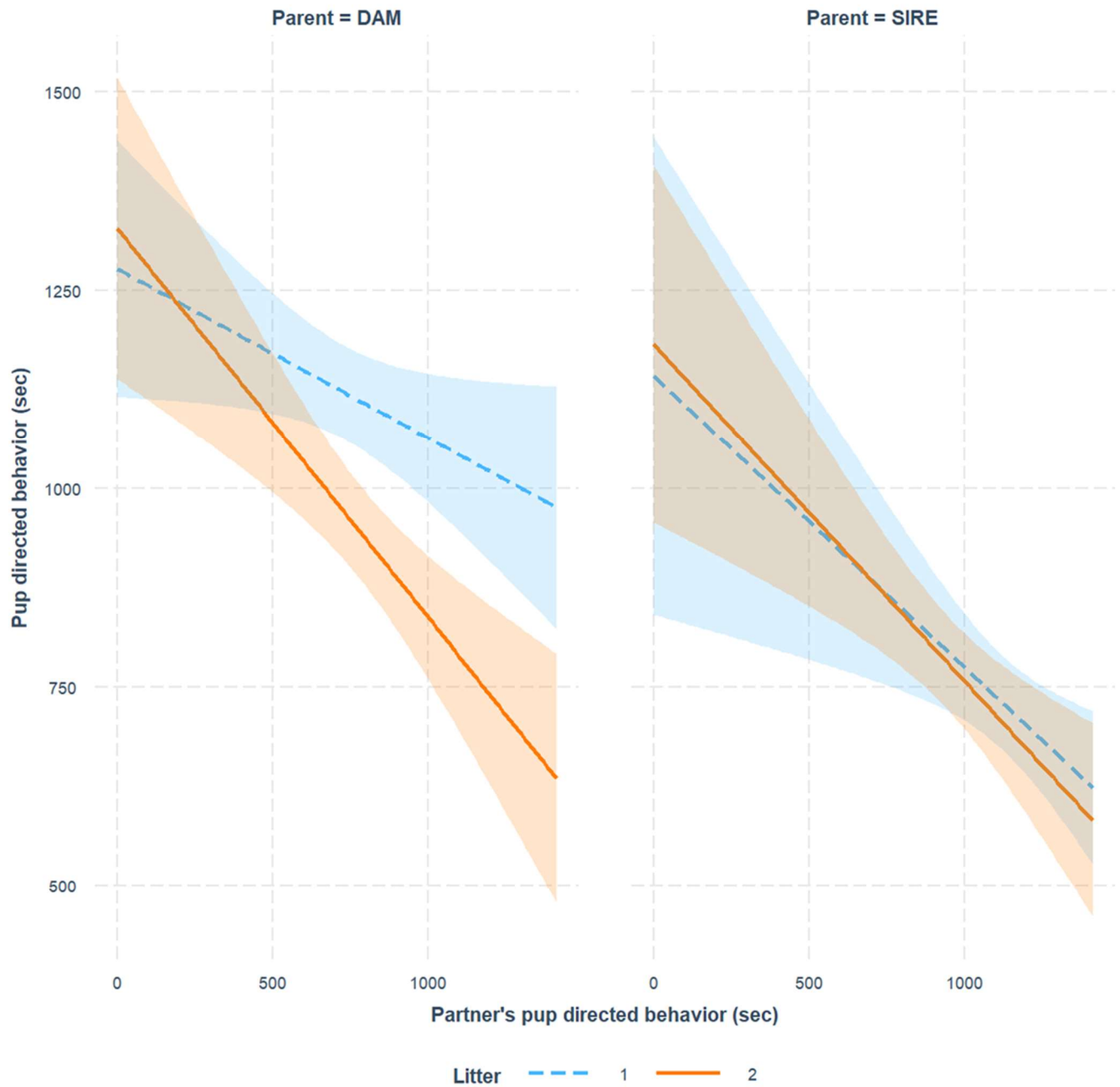


Figure 3: The effect of the partners' behavior. Sire's behavior is significantly predicted by his partner in both litters(L1: $t(59) = -2.75, p = 0.006$, L2: $t(59) = 0.0033, p = .00033$). Dam's behavior is only significantly impacted by her partner in the second litter(L1: $t(59) = -2.035, p = 0.0429$, L2: $t(59) = 0.0004$). Significance set to $p < .025$ to account for multiple comparisons

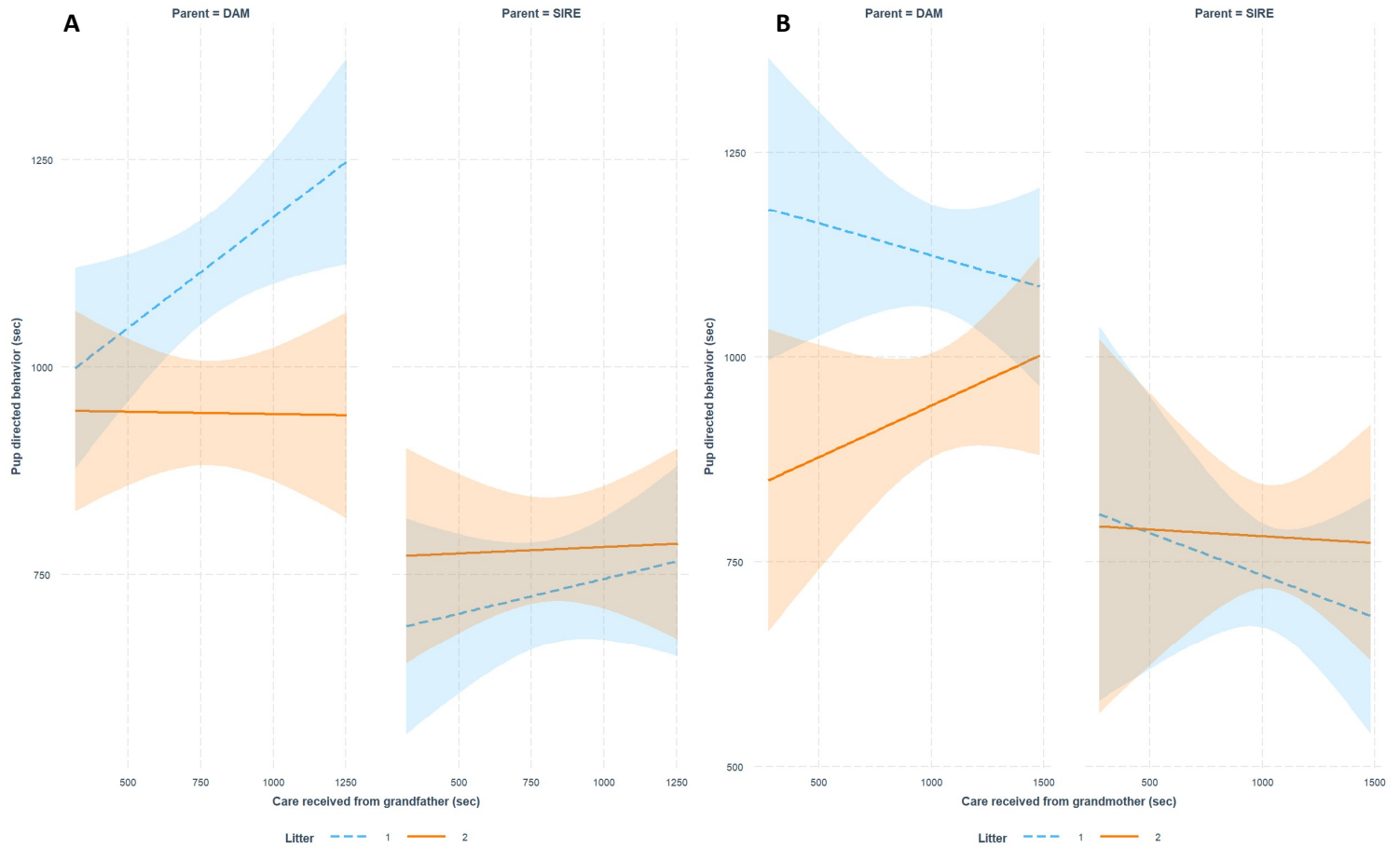
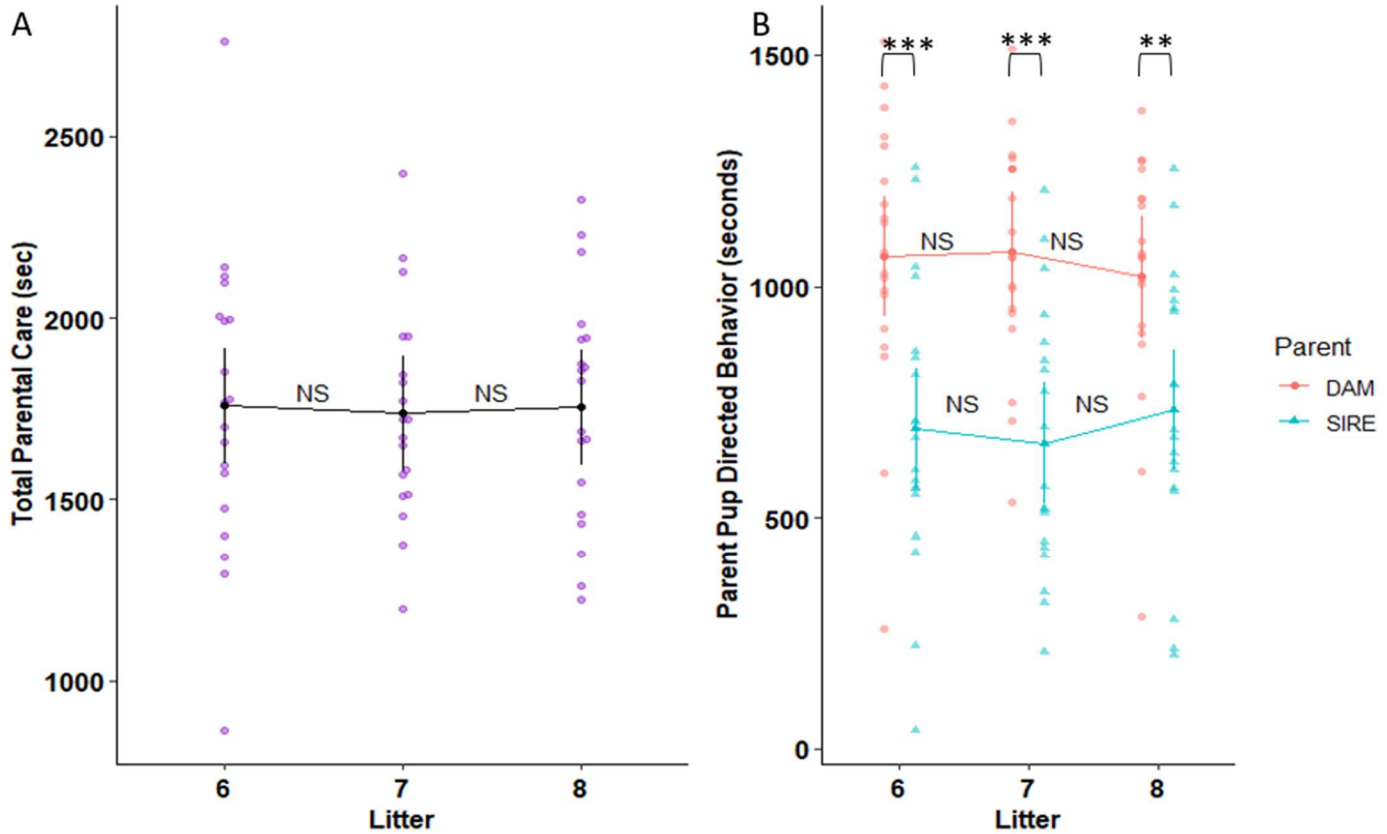


Figure 4: Effect of early life care on offspring parenting behavior. A: Grandfather's parenting behavior impacted dams' behavior on the first litter ($b = 0.26$, $SE = 0.11$, $t = 2.33$, $p = 0.02$), but not their second litter ($b = -.005$, $SE = 0.11$, $t = -0.048$, $p = 0.96$). Grandfather's parenting did not impact sire's behavior on litter 1L1 ($b = 0.08$, $SE = .11$, $t = 0.73$, $p = 0.46$), or litter 2L2 ($b = 0.01$, $SE = 0.11$, $t = 0.13$, $p = 0.89$). B: Neither Dam nor Sire is impacted by the direct care received by the grandmother in L1 ($b = -0.07$, $SE = .11$, $t = -0.67$, $p = 0.50$; $b = -0.1$, $SE = .14$, $t = -0.67$, $p = -0.70$) or in L2 ($b = 0.13$, $SE = .11$, $t = 1.08$, $p = 0.27$; $b = -0.01$, $SE = .14$, $t = -0.11$, $p = -0.91$).



Supplemental Figure 1: Established prairie vole parents have stable pup-directed behavior. *A*: Total parental care is stable for experienced breeder pairs. In 19 of our experienced colony breeders, parental care behaviors in litters six, seven, and eight were scored, and we found there was no statistically significant difference in care between L6, L7, or L8 ($t(52) = 30.19, p < .001$). *B*: Parents in established breeder pairs have stable pup-directed behavior. Pairwise comparisons of each litter found no differences between L6, L7, or L8 for either dam or sires' pup-directed behaviors, all $p > .66$. Pairwise comparisons indicate that dams provide an average of 30% more pup-directed behavior than sires. $p > .0001 = ***$, $p > .001 = **$, $p > .05 = *$