

Exploration in a dispersal task: Effects of early experience and correlation with other behaviors in prairie voles (*Microtus ochrogaster*)



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ABSTRACT

Socially monogamous prairie voles (*Microtus ochrogaster*) display remarkable individual variation in social behaviors, which has been associated with differences in early life experience and neuropeptide receptor densities. These differences are also seen in the wild, where approximately 70% of young voles remain in their natal group as non-breeding alloparents, while the other 30% disperse. We investigated whether natural variation in early parental care could contribute to offspring's willingness to "disperse" (willingness to explore) in a laboratory context. Behavioral differences between dispersers and residents could also provide a way to interpret individual variation in other behaviors commonly observed under laboratory conditions. Breeder pairs ranked as high, medium or low-contact, according to the amount of early parental care they provided to offspring, were used to produce and rear experimental subjects. Effects of early parental care on the offspring's willingness to disperse were seen at post-natal day 21, with high-contact offspring spending more time in the start cage and low-contact offspring spending more time exploring. Variations in parental care were also associated with differences in juvenile and adult behaviors that could potentially encourage philopatry or dispersal behavior in the wild. High-contact offspring displayed less anxiety-like behavior compared to low-contact animals. Low-contact offspring displayed the lowest amount of alloparental care. High-contact offspring spent more time in side-by-side contact with a potential partner compared to medium and low-contact offspring. These results suggest that variations in early parental care can impact weanlings' exploratory behavior, but that philopatry is not driven by high anxiety.

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1. Introduction

Prairie voles (*Microtus ochrogaster*) are small rodents native to the North Central region of the United States (Carter et al., 1995). Unlike many mammals, prairie voles are socially monogamous, biparental and alloparental. Adults establish selective long lasting male-female pair bonds and share the care for offspring; juveniles tend to exhibit spontaneous care-giving toward younger siblings – i.e. alloparental behavior (Carter and Getz, 1993). Although prairie voles are highly affiliative, there is significant individual variation in their behavior, which has been associated with variations in early biparental care and family structure (Ahern and Young, 2009; Perkeybile et al., 2013). Experimental and pharmacological manipulations of early experience and its long term effects on behavior

and physiology have also been demonstrated in prairie voles (Bales and Perkeybile, 2012).

Prairie vole pairs display natural variability in the amount and type of early biparental care provided to offspring. These natural variations in parental care are relatively subtle, do not affect survival of offspring, and are stable from one litter to the next (Perkeybile et al., 2013). However, these variations are likely to provide different social, tactile, thermal and olfactory experiences to offspring during a critical developmental period. This variation has been associated with long term changes in alloparental, intrasexual aggression and anxiety-like behaviors in juveniles and adults, as well as differences in cortical connections, densities and patterns in the primary somatosensory cortex (Perkeybile et al., 2013, 2015; Seelke et al., 2015).

Field studies on the social organization of natural prairie vole populations have revealed that approximately 70% of male and 75% of young female prairie voles remain in the natal nest until death as non-breeding alloparents, sacrificing their reproductive opportunities (Getz et al., 1987). These animals are defined as philopatric. The remaining 30% of male and 25% of female voles disperse from their

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place of birth, leaving at about the same age (4555 days) and moving similar distances (28–33 m). Some animals have been reported to disperse as early as 16 days old and as late as 161 days old. Animals also appeared to make “trial runs” of approximately 20 m before permanently leaving their natal nest (McGuire et al., 1993).

After dispersal, wild prairie voles can follow different paths. Some individuals form a breeding pair, others join established social groups (direct transfer), while others continue to wander (McGuire et al., 2013). Female wanderers are more likely to settle either as single individuals or as part of a male–female pair or communal group, given the need for a stable nest site and lactation (Getz et al., 1994; Getz and Carter, 1996). Wandering males tend to remain wanderers, and have large home ranges that overlap with many individuals, probably to increase the chances of opportunistic mating. Wanderers or residents that engage in extra pair fertilizations show low levels of vasopressin receptors (AVPR1a) in the posterior cingulate/retrosplenial cortex and laterodorsal thalamus, areas that have been implicated in spatial memory (Ophir et al., 2008).

Critical factors that determine whether or not an individual disperses have not been identified. Studies in natural and semi-natural conditions have focused mainly on characteristics at the time of dispersal and have found multiple factors influencing dispersal behavior, providing partial explanations (McGuire et al., 1993; Lin et al., 2006; Lucia et al., 2008). Dispersal was more common at low population densities, following the disappearance of both parents or the mother (in the case of single reproductive females), during the breeding season, from smaller groups, when young individuals became reproductively active at the natal nest, and when potential mates within the natal group were relatives (McGuire et al., 1993). An experimental study (Lin et al., 2006) controlled food availability and risk predation in habitat patches and found that prairie voles were less likely to disperse from high quality patches than from low-quality patches, and that dispersers preferred to settle in similar or higher quality patches. The proportion of offspring that remained philopatric increased as population density increased, however the presence of philopatric individuals at low population densities suggested that other factors might be involved besides habitat saturation (Lucia et al., 2008). Importantly, regardless of the level of food availability (Getz, 1997; Getz et al., 1992), most prairie vole offspring remain as philopatric individuals their entire lives. This suggests that philopatric behavior is not a direct response or an adaptation to low, medium or high food habitats. Data did not support other variables such as competition for mates, sex differences, body weight, or age differences at the time of dispersal as important factors influencing dispersal behavior in prairie voles (McGuire et al., 1993).

Prairie voles reared in the laboratory also show significant variation in behavior, for example in the commonly used alloparental care test. When exposed for the first time to an unfamiliar pup, the majority of sexually naive young prairie voles (approximately 75% of females and 70% of males) show spontaneously alloparental behavior, while the remainder either ignore or attacked the pup (Lonstein and De Vries, 2001; Olazabal and Young, 2005; Ahern and Young, 2009; Roberts et al., 1998). It has also been found that in the laboratory, alloparental behavior decreases with age in females and variability in alloparental response can be associated with other behaviors and oxytocin receptor density (Lonstein and De Vries, 2001; Olazabal and Young, 2005, 2006a,b). Female juveniles that interacted with pups more positively displayed less anxiety-like behavior and were more affiliative to other female conspecifics (Olazabal and Young, 2005). Female adults were more likely to attack pups than juveniles and this infanticidal behavior was associated with high anxiety-like behavior and a preference for spending less time in social contact with other female conspecifics and more time isolated (Olazabal and Young, 2005; Roberts et al.,

1998). It is thus possible that the propensity to disperse is correlated with other behavioral characteristics such as anxiety levels and affiliation.

Studies in non-human primates and rodents have provided substantial evidence that experiences in early life can profoundly impact behavioral and physiological outcomes. Natural variations of maternal care have been extensively studied in rats. During the postpartum period, rat dams display different amounts and types of maternal behavior – licking and grooming frequency (LG) and arched back nursing (ABN) (Francis et al., 2000; Meaney, 2001; Champagne et al., 2003). These variations in maternal behavior have been found to be reliably transmitted from mother to daughter in an epigenetic manner (Francis et al., 1999a) and have been linked to changes in expression of oxytocin receptors (Francis et al., 2000), glucocorticoid receptors (Liu et al., 1997) and long term changes in behavioral response to stress (Francis et al., 1999b), social behavior (Starr-Phillips and Beery, 2014) and spatial learning (Liu et al., 2000b) in offspring. Adult offspring reared by high LG/ABN mothers displayed less fear behavior in a novel environment, enhanced spatial learning and memory in the Morris water maze test and spent significantly more time in social contact with unfamiliar individuals (Francis et al., 1999b; Liu et al., 2000a; Starr-Phillips and Beery, 2014).

As previously described (Perkeybile et al., 2013), characterization of parental care variations in established prairie vole breeding pairs was based on the amount and type of maternal and paternal care delivered to offspring during postnatal day (PND) 1–3. Behaviors used to assess parental style included huddling, non-huddling contact, licking and grooming, nest building and exclusively for the mother different nursing postures (lateral, active and neutral). The amount and type of behaviors were quantified and breeding pairs were ranked in relation to each other using a total parental behavior score (total amount of biparental care) for each pair. Scores were ranked into quartiles, the top ranked quartile became the high-contact breeding pairs, the pairs within the middle quarters became the medium contact pairs and the bottom quarter became the low-contact breeding pairs. These categories of early care were linked to differences in juvenile social behavior (Perkeybile et al., 2013), and adult stress regulation (Perkeybile and Bales, 2015a,b).

In this paper we tested the hypotheses that whether an individual “dispersed” in an experimental task is related to factors in the early social environment that influence behavioral development (Getz et al., 1994), as well as whether this tendency to disperse is related to other behaviors commonly measured in the laboratory. We examined how differences in early parental care may lead to individual variations in adult social behaviors such as the willingness to disperse from the natal nest, alloparental behavior, anxiety like behaviors and social contact behaviors. We predicted that offspring reared by high-contact parents would display decreased anxiety-like behavior and higher exploratory behavior, and therefore show more disposition to disperse. This prediction was based on the decreased anxiety displayed in studies of high LG/ABN rats (Liu et al., 2000a; Liu et al., 1997; Starr-Phillips and Beery, 2014). Offspring reared by low-contact parents were expected to display increases in anxiety-like behavior, and decreased exploratory behavior (again based on the rat data), and to be less willing to disperse. Finally, we predicted that high contact offspring would show both a higher preference for social interaction in general, including alloparental care, and possibly a facilitation of pair-bonding, in a social preference test. We based this prediction on our expectation that offspring of more social parents might be more social themselves; and if more ready to disperse, might also be behaviorally and neurobiologically more prepared to form an adult pair-bond.

2. Materials and methods

2.1. Experimental subjects

Experimental subjects were the offspring of fifteen established breeder pairs of laboratory-bred prairie voles (*Microtus ochrogaster*), descendants of a wild stock originally caught near Champaign, Illinois. These breeders were ranked as high, medium and low breeding pairs by methods previously described (Perkeybile et al., 2013). Prairie vole litters usually range from 2 to 6 individuals; when necessary litters were culled to 4 offspring, ideally 2 females and 2 males. Our sample was composed of 15 high-contact offspring, 21 medium-contact offspring and 18 low-contact offspring. Scorers for parental care ranking were different from scorers for dispersal and other behavioral testing, who were blind to parental care ranking.

On postnatal day one (PND 1) offspring were briefly removed from the home cage, sexed, weighed and dyed for identification with Nyanzol-dye. Individual dye marks on the experimental subjects allowed us to differentiate offspring within a litter and individually record behaviors on behavioral tests and home cage observations.

2.2. Housing conditions

Animals were maintained on a 14:10 light-dark cycle with lights on at 0600. Food (high-fiber Purina rabbit chow) and water were available ad libitum; cotton was provided as nesting material. Breeding pairs and experimental subjects (L1) were kept together until PND 70. For the first three weeks families were housed in large polycarbonate cages (44 × 22 × 16 cm). Because females can mate post-partum and have a short gestation period of approximately 23 days, experimental subjects were exposed to three subsequent litters (L2, L3 and L4). To provide more space, on day of birth of the second litter, families were moved into two large polycarbonate cages (44 × 22 × 16 cm) connected by a plexiglas tube (10.5 cm long × 7 cm diameter). Upon weaning age (PND 21) siblings from L2, L3 and L4 were separated from the family group and housed in same sex pairs in smaller polycarbonate cages (27 × 16 × 16 cm). All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

2.3. Behavioral testing

2.3.1. Dispersal testing

The dispersal tube was a 6 m long polycarbonate tube divided in four segments, 152.5 cm each, and attached to two cages on each end. At one end was the “start cage”, consisting of two large polycarbonate cages connected by a Plexiglas tube, a replicate of their original home cage, and on the other end of the tube was the “end cage”, a single large polycarbonate cage (44 × 22 × 16 cm), with clean bedding, food and cotton for nesting material. The segments of the tube starting from the start cage and proceeding towards the end cage were designated as segment 1, 2, 3 and 4. Bedding, cotton nest, food and water from the original cage were relocated to the “start cage”. Breeding pair, experimental subjects and when present, offspring from subsequent litters (L2, L3 or L4), were transferred to the “start cage” and were given a 15 min acclimation period before starting the test. After the acclimation period, animals were allowed to move freely into the tube. Latency to leave the start cage and time spent at the start cage, each segment and the end cage were recorded for 60 min for each experimental subject and parents, using Behavior Tracker software (www.behaviortracker.com). The location criterion was met when an animal had its two front paws in the location. This test was repeated 5 times starting at PND 21 until PND 70 (See Table 1). Dispersals 3 and 4 were clos-

Table 1
Dispersal testing, age and conditions at the time of the test.

Dispersal test	PND	Presence and age of pups from subsequent litters
1st dispersal test	PND 21	No new pups present
2nd dispersal test	PND 28–29	2nd Litter present, newborn pups
3rd dispersal test	PND 41–42	2nd Litter present, juveniles
4th dispersal test	PND 55–56	3rd Litter present, juveniles
5th dispersal test	PND 69–70	4th Litter present in most groups, newborn pups

est to the mean age of dispersal reported for animals in the wild (45–55 days) (McGuire et al., 1993).

2.3.2. Alloparental behavior

Real-time behavioral observations on alloparental behaviors were conducted using Behavior Tracker software. Ten-minute focal samples at the start cage were recorded per experimental subject during their L2, L3 and L4 siblings' first week of life (PND 2–4). We recorded the duration of huddling, pseudo-huddling and non-huddling contact, retrievals and licking/grooming, as well as time the juvenile spent inside or outside the nest. L2 observations were used for the analyses presented here.

We conducted alloparental observations for all subsequent litters (L2–L4), with the aim of seeing how this behavior changed over time, as animals get close to the average age for dispersal. Alloparental behavior decreases with age in females but not in males, young female voles (21–30 days) acted more parentally toward pups than older females, particularly those older than 45 days (age close to dispersal) (Lonstein and De Vries, 2001; Stone et al., 2010; Ophir et al., 2008).

2.3.3. Elevated plus maze

The Elevated Plus Maze (EPM) is a rodent model of anxiety and exploratory behavior, behaviors in this task reflects the conflict between the natural tendency of many rodents to explore a novel environment versus the tendency to avoid exposed areas (open arms) and preference for protected areas (closed arms) (Walf and Frye, 2007). An increase in the time spent in the open arms reflects lower anxiety behavior. Experimental subjects were weighed and tested on the EPM on PND 30. PND30 was chosen as an age which was close to but not overlapping with the first two dispersal tests. The EPM consisted of two opposing closed arms, with a black floor and walls and an open top, and two open arms made of clear Plexiglas perpendicular to the close arm. Each arm was 67 cm in length and 5.5 cm wide, the intersection of the four arms was a center square 5.5 × 5.5 cm, the entire structure was elevated 1 m above the floor.

Animals were placed in the center of the maze and allowed to explore for 5 min. The amount of time the animal spent in the center square, open and closed arms, and time spent auto-grooming were recorded using Behavior Tracker software.

2.3.4. Cohabitation for preference test

Preference tests, as the tests which cause the most neurobiological changes and require the subject to be an adult, were carried out last, on PND80. Experimental subjects were placed in a clean cage polycarbonate cage (27 × 16 × 16 cm) with a stimulus animal of the opposite sex and similar age and size, designated as “partner”. Males were allowed to cohabit with their partner for 30 min and females for 60 min. The cohabitation period is necessary for the development of partner preference, although these periods are too short for pair formation. These short cohabitation periods were designed to observe individual differences in the ability to form a

preference for the partner between high, medium and low-contact offspring; in particular, to see if high-contact offspring would form a preference even when not given sufficient time (DeVries and Carter, 1999). Previous studies have determined that females needed less time than males to form a partner preference (6 h for females, 24 h for males) (DeVries and Carter, 1999). However, many factors including husbandry can affect the length of time that voles take to form a partner preference (Bales et al., 2007, 2011), and in our laboratory, both males and females form preferences faster than in the literature. Thus, we chose short cohabitation periods.

2.3.5. Preference test

Immediately after cohabitation, experimental subjects began a 3 h preference test (PT). Preferences were assessed using the choice apparatus developed by Williams and Carter (Williams et al., 1992). The PT apparatus consisted of three identical polycarbonate cages connected by 2 Plexiglas tubes. The empty cage designated as “neutral” was connected to the other 2 cages, housing the stimulus animals, the “partner” and an unfamiliar animal of the opposite sex designated as “stranger”. The experimental subject was placed in the neutral cage of the testing apparatus, and could freely move throughout the 3 cages for 3 h, the stranger and partner were restricted to its own cage with no direct contact with each other. The tests were video recorded. Scored interactions included side-by-side contact with the partner and the stranger, time in each chamber, and aggression using Behavior Tracker software.

2.4. Data analysis

Data analysis was carried out using generalized linear mixed models in SAS 9.4 (SAS Institute, Cary, NC) with early parental care and sex as the independent variables, parents' behavior in the apparatus as a covariate, and subject identity as the random variable. Post-hocs were carried out by least-squared means. Data were tested for assumptions of ANOVA, and transformed if non-normal using the square root transformation. All tests were two-tailed and significance was set at $p < 0.05$. Sample size used in this study was chosen based on preliminary data, to detect a medium effect size with a power of 0.8. For the dispersal test, variable reduction methods were used to reduce the number of outcome variables to three; therefore, we examined time in the start cage, time in segments one and two, and time in segment four and the end cage.

Pearson's correlations were used to test predictions that behavior in the dispersal task was stable across development, and correlated with other laboratory behavioral tasks. For correlations between dispersal tests, the Benjamini-Hochberg false discovery rate correction was used to account for multiple comparisons (Benjamini and Hochberg, 1995). For correlations between dispersal tests and other behavioral tests, a correction was not used. However, these were strictly limited to correlations with *a priori* hypotheses and only the major variables for each test. Specifically, we correlated variables from dispersal test 1 (time spent in start cage, time spent in segments 1 & 2, time spent in segment 4 and far cage) with total time spent in alloparenting, open arm ratio in the elevated plus-maze, and time spent in side-to-side contact with the partner in the partner preference test.

3. Results

3.1. Dispersal behavior

3.1.1. Early parental care

On the first dispersal test (PND 21), significant differences were found in the time offspring spent at the start cage according to the level of early parental care received ($F_{2,51} = 3.38, p = 0.04$). A post hoc pair-wise comparison indicated that high-contact offspring spent

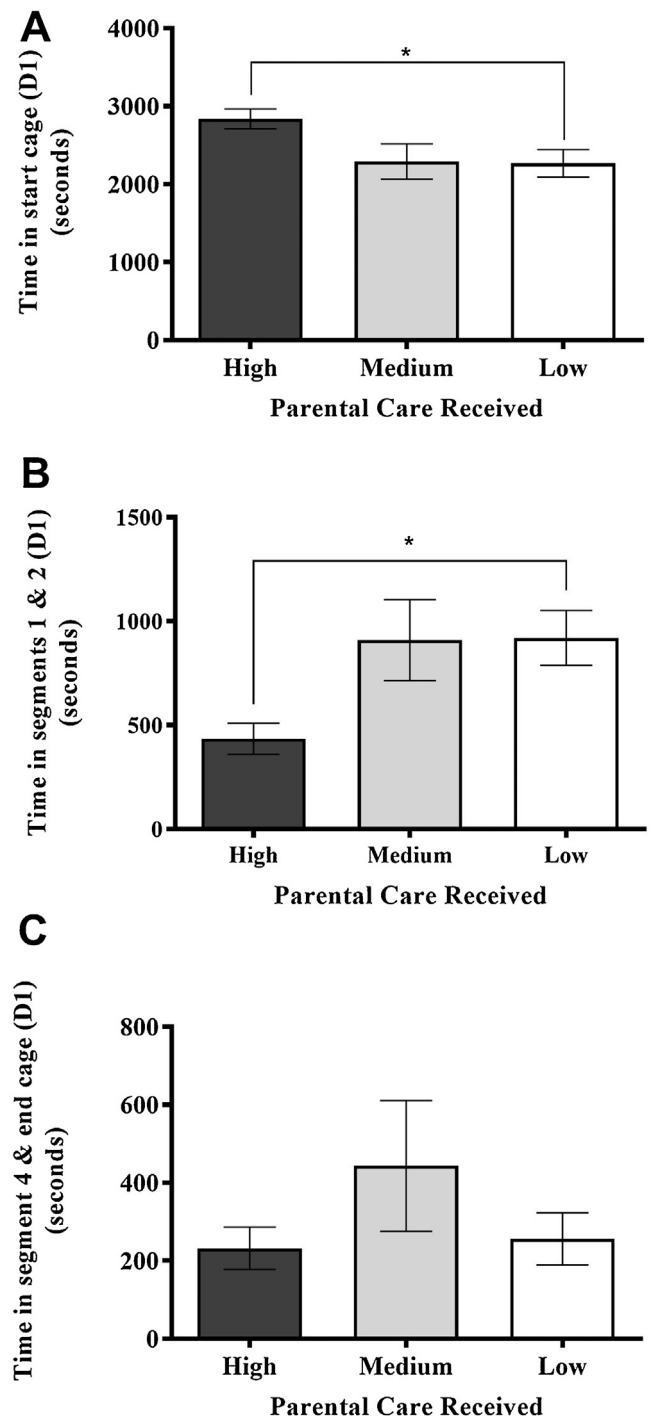


Fig. 1. First dispersal test (D1). In the first dispersal test, offspring time spent at the start cage according to the level of early parental care received (high-contact $n = 15$, medium-contact $n = 22$, low-contact $n = 19$; mean \pm standard errors). (A) High-contact offspring spent significantly more time at the start cage compared to low-contact offspring. (B) Low-contact offspring spent more time exploring segment 1 & 2 compared to high-contact offspring. (C) No significant differences were found in the time offspring spent in segment 4 & end cage.

significantly more time at the start cage compared to low-contact offspring ($t_{32} = 2.52, p = 0.01$), there was a non-significant trend between high and medium-contact offspring ($t_{35} = 1.71, p = 0.09$) and no significant difference between low and medium-contact offspring ($t_{39} = 0.42, p = 0.67$) (Fig. 1A). The amount of time that the mother spent in the start cage was a significant predictor of offspring time there ($F_{1,51} = 7.88, p = 0.0007$); however, the amount of

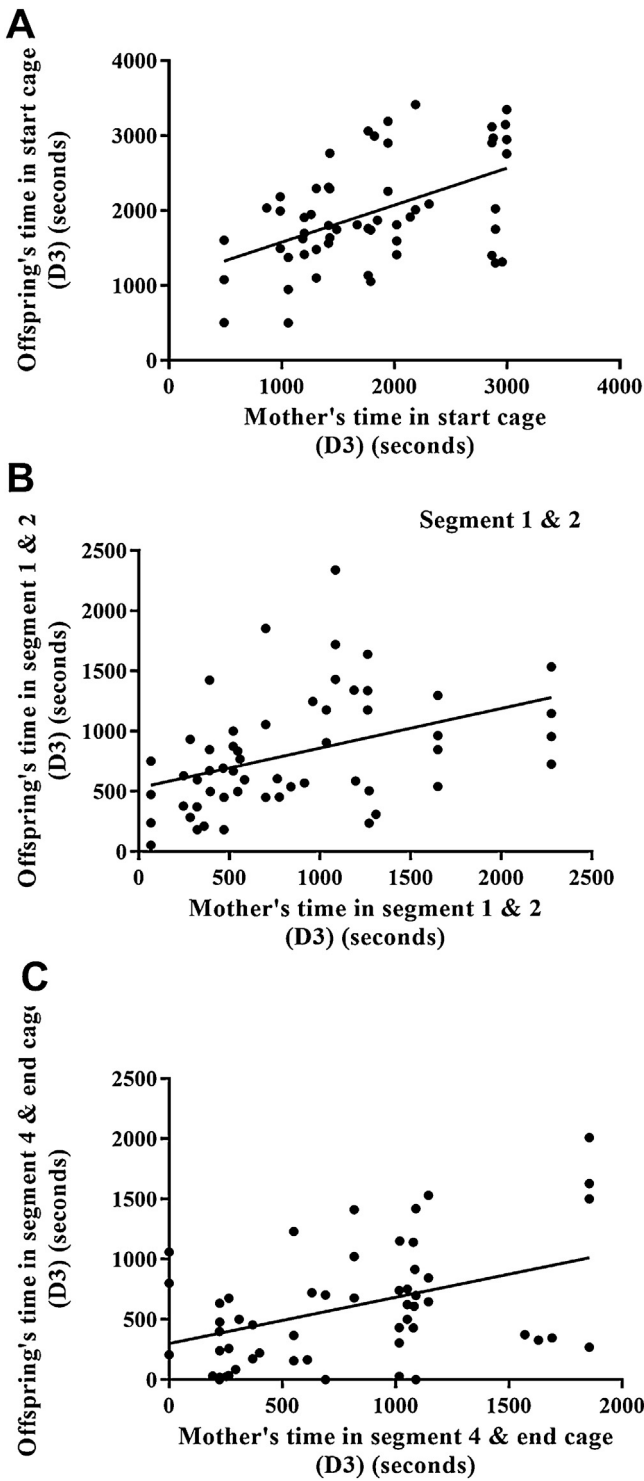


Fig. 2. Third dispersal test (D3); mothers and offspring. In the third dispersal test, there were significant correlations between time spent by offspring in each area and the time the mother spent in the same area (high-contact $n = 15$, medium-contact $n = 21$, low-contact $n = 19$; mean \pm standard errors). (A) Correlation between mothers' and offspring's time offspring spent in the start cage. (B) Correlation between mothers' and offspring's time offspring spent in segments 1 & 2. (C) Correlation between mothers' and offspring's time offspring spent in segment 4 and end cage.

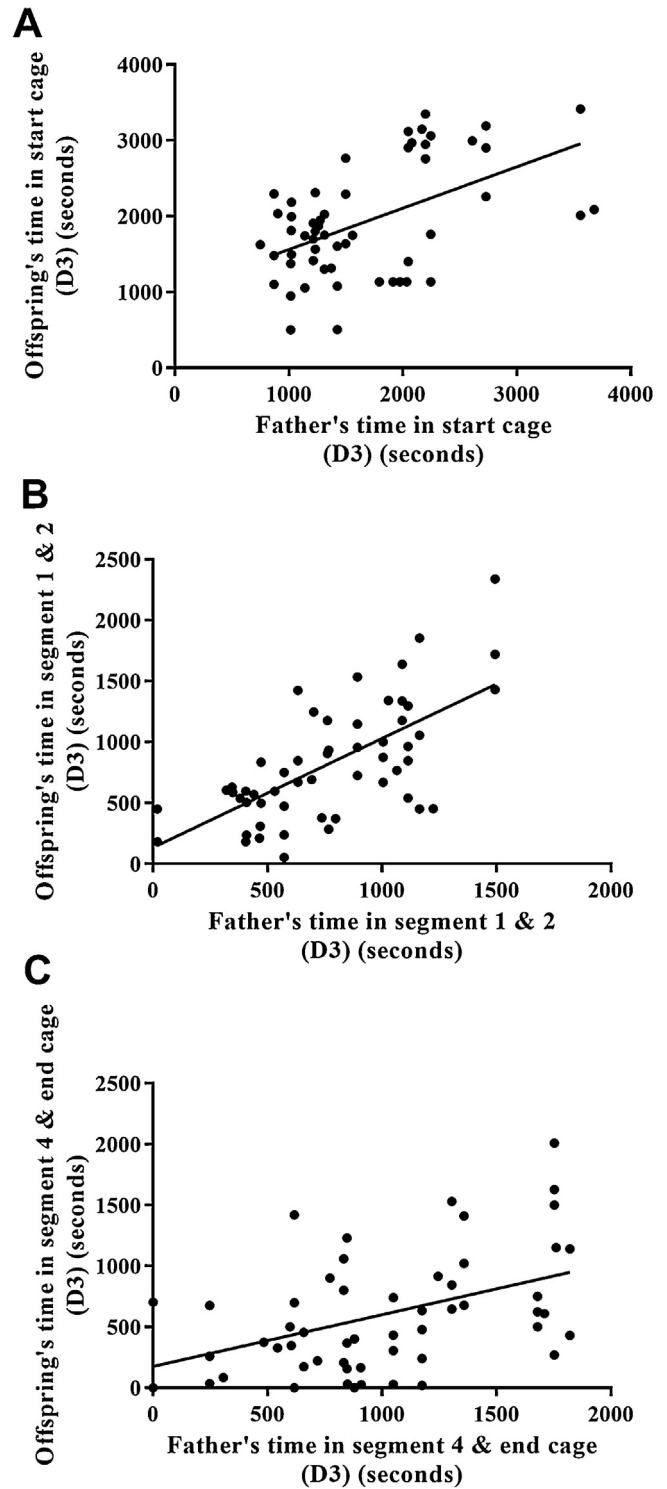


Fig. 3. Third dispersal test (D3); fathers and offspring. In the third dispersal test, there were significant correlations between time spent by offspring in each area and the time the father spent in the same area (high-contact $n = 15$, medium-contact $n = 22$, low-contact $n = 19$; mean \pm standard errors). (A) Correlation between fathers' and offspring's time offspring spent in the start cage. (B) Correlation between fathers' and offspring's time offspring spent in segments 1 & 2. (C) Correlation between fathers' and offspring's time offspring spent in 4 and end cage.

Table 2

Pearson correlations between the dispersal tests, demonstrating temporal consistency in offspring dispersal behavior. P-values reflect a Benjamini-Hochberg false discovery rate correction. * = significant with a two-tailed probability following correction.

Dispersal behavior					
Dispersal	First	Second	Third	Fourth	Fifth
Start cage					
First	1	$r_{55} = 0.58, p = 0.0005^*$	$r_{54} = 0.37, p = 0.007^*$	$r_{54} = 0.39, p = 0.005^*$	$r_{50} = 0.39, p = 0.01^*$
Second		1	$r_{54} = 0.33, p = 0.01^*$	$r_{54} = 0.38, p = 0.007^*$	$r_{50} = 0.22, p = 0.12$
Third			1	$r_{54} = 0.47, p = 0.001^*$	$r_{50} = 0.60, p = 0.0005^*$
Fourth				1	$r_{50} = 0.39, p = 0.007^*$
Fifth					1
Segments one and two of the dispersal tube					
First	1	$r_{55} = 0.35, p = 0.07$	$r_{54} = 0.15, p = 0.39$	$r_{54} = 0.28, p = 0.13$	$r_{50} = 0.23, p = 0.21$
Second		1	$r_{54} = 0.07, p = 0.76$	$r_{54} = 0.26, p = 0.12$	$r_{50} = -0.05, p = 0.81$
Third			1	$r_{54} = -0.03, p = 0.83$	$r_{50} = 0.29, p = 0.12$
Fourth				1	$r_{50} = 0.21, p = 0.24$
Fifth					1
Segment four and end cage					
First	1	$r_{55} = 0.37, p = 0.0006^*$	$r_{54} = 0.17, p = 0.24$	$r_{54} = 0.25, p = 0.09$	$r_{50} = 0.16, p = 0.2$
Second		1	$r_{54} = 0.55, p = 0.005^*$	$r_{54} = 0.26, p = 0.08$	$r_{50} = 0.26, p = 0.09$
Third			1	$r_{54} = 0.39, p = 0.005^*$	$r_{50} = 0.42, p = 0.005^*$
Fourth				1	$r_{50} = 0.59, p = 0.001^*$
Fifth					1

time that the father spent in the start cage was not a significant predictor.

According to the level of early parental care received, significant differences were also found in the time offspring spent exploring segments one and two of the tube ($F_{2,51} = 5.17, p = 0.009$); a post hoc comparison showed that low-contact offspring spent more time exploring segments one and two compared to high-contact offspring ($t = 2.56, p = 0.01$), while medium-contact and low-contact offspring did not differ (Fig. 1B). Sex and a sex by treatment interaction were not significant. The amount of time that the mother spent in segments one and two was a significant predictor ($F_{1,51} = 18.82, p < 0.0001$); the amount of time that the father spent in segments one and two was not significant.

No significant differences due to early parental care received were found in the time offspring spent in segment four and end cage ($F_{2,51} = 2.98, p = 0.09$) (Fig. 1C). Sex and a sex by treatment interaction were not significant, nor were the time spent by the mother and father in those segments.

Mixed model ANOVAs for the effects of early parental care, sex, and their interaction were not significant for dispersal tests 2–5. We also conducted Pearson correlations between the location variables (start cage, segments 1–2, segment 4 and end cage) for each dispersal test. Subjects showed high correlations in these variables between tests, and thus high temporal individual consistency in their dispersal behavior across time, especially for the variables “start cage” and “segment four and end cage”. Correlations between the dispersal tests are shown in Table 2.

3.2. Parents' exploratory behavior

3.2.1. Mothers and offspring

During the first dispersal test there was a positive correlation between the mothers and offspring's latency to leave the start cage ($r_{55} = 0.40, p = 0.002$); this correlation was stronger between mothers and female offspring ($r_{27} = 0.58, p = 0.001$). For the first and third dispersal tests mothers' exploratory behavior predicted the offspring's exploratory behavior. During the first dispersal test, mothers' presence in each segment was positively correlated with the time offspring stayed there, for the start cage ($r_{55} = 0.39, p = 0.003$), segments one and two ($r_{55} = 0.45, p = 0.0004$) and segment four and end cage ($r_{55} = 0.47, p = 0.0002$). For the third dispersal test, mothers' exploratory behavior positively predicted the time offspring spent in the start cage ($r_{54} = 0.50, p < 0.0001$)

(Fig. 2A), segments one and two ($r_{54} = 0.41, p = 0.001$) (Fig. 2B) and segment 4 and end cage ($r_{54} = 0.50, p < 0.001$) (Fig. 2C). For the second dispersal test, there were no significant differences in relation with either parents' exploratory behavior for any of the locations. For the fourth dispersal test, there was a trend for time mothers and offspring spent in segments one and two ($r_{54} = 0.25, p = 0.072$). For the fifth dispersal test, the time that mothers and offspring spent in segment 4 and the end cage was positively correlated ($r_{50} = 0.47, p = 0.001$). Other comparisons between the mother's location and the offspring location were not significant.

3.2.2. Fathers and offspring

For the third dispersal test, fathers' behavior positively predicted the time offspring spent in every location, start cage ($r_{54} = 0.57, p < 0.0001$) (Fig. 3A), segments one and two ($r_{54} = 0.67, p < 0.0001$) (Fig. 3B) and segment 4 and end cage ($r_{54} = 0.45, p = 0.0006$) (Fig. 3C). For the fourth dispersal test, there was a significant correlation between the time fathers and offspring spent at the start cage ($r_{54} = 0.299, p = 0.026$). For the fifth dispersal test, the time that fathers and offspring spent in segment 1 and 2 was related ($r_{50} = 0.379, p = 0.006$). Other comparisons between the father's location and the offspring location were not significant.

3.3. Body weight

At PND 1 offspring' body weight (Table 3a) did not differ between the three groups of parental care ($F_{2,51} = 0.85, p = 0.432$), or by sex ($F_{1,51} = 0.49, p = 0.488$); however, there was a significant sex by treatment interaction ($F_{2,51} = 3.38, p = 0.042$). Medium contact male offspring weighed more than high contact male offspring ($t_{17} = 2.36, p = 0.022$) and medium contact female offspring ($t_{21} = 2.70, p = 0.009$). All other post-hoc comparisons were non-significant.

Table 3a

Weights (mean \pm standard error) on postnatal day 0. Medium contact male offspring weighed more than high contact male offspring and medium contact female offspring. All other post-hoc comparisons were non-significant.

Parental care received	Female	Male
High contact	3.411 \pm 0.168	3.253 \pm 0.211
Medium contact	3.266 \pm 0.067	3.801 \pm 0.149
Low contact	3.550 \pm 0.142	3.441 \pm 0.176

At PND 30, significant differences were found according to the parental care received ($F_{2,46} = 5.41$, $p = 0.008$); high-contact offspring had lighter body weights compared to medium-contact offspring ($t_{31} = 3.71$, $p < 0.001$), while low-contact and high-contact offspring did not differ ($t_{32} = 1.53$, $p = 0.132$). Medium and low-contact offspring also did not differ. Day 30 body weight also differed by sex ($F_{1,46} = 5.33$, $p = 0.025$), with males weighing more (Table 3b). The sex by treatment interaction was not significant ($F_{2,46} = 2.85$, $p = 0.068$).

3.4. Elevated plus maze

The proportion of time spent in the open arms of the EPM was different according to the amount of early parental care received ($F_{2,52} = 3.39$, $p = 0.04$). There was no effect of sex or sex by treatment interaction. A post-hoc comparison showed that high-contact offspring's open arm ratio was greater compared to low-contact individuals ($t_{32} = 2.5$, $p = 0.01$). There was also a non-significant trend between high and medium-contact individuals ($t_{34} = 1.71$, $p = 0.09$), and no significant difference between medium and low-contact individuals ($t_{38} = 0.99$, $p = 0.32$) (Fig. 4). The effects of early parental care received on total entries in the plus-maze (frequency of open arm, center, and closed arm together) was non-significant, as was sex and a sex by treatment interaction (effect of early parental care received, $F_{2,49} = 0.78$, $p = 0.4650$; effect of sex, $F_{1,49} = 0.28$, $p = 0.5990$; sex by early parental care interaction, $F_{2,49} = 0.46$, $p = 0.6353$; Table 4).

3.5. Alloparental care

Alloparental variables were reduced to a single outcome variable by adding together the times for non-huddling contact, lick/sniffing and pseudo-huddle behaviors. Significant differences in alloparenting were found according to level of parental care received ($F_{2,50} = 11.74$, $p < 0.0001$) and a significant sex by parental care interaction ($F_{2,50} = 4.00$; $p = 0.024$); while the main effect of sex was not significant. After splitting by sex in order to better characterize the data, post-hoc pairwise comparisons showed that medium contact females displayed more alloparental behaviors compared to high ($t_{16} = 2.07$, $p = 0.048$) and low-contact females ($t_{17} = 3.26$, $p = 0.003$), while no significant difference was found between high and low-contact females ($t_{17} = 1.12$, $p = 0.270$; Fig. 5A). Low-contact males displayed the lowest amount of alloparental behaviors compared to high ($t_{13} = 4.22$, $p = 0.0003$) and medium contact males ($t_{20} = 3.08$, $p = 0.005$), while no significant difference was found between medium and high-contact males ($t_{17} = 1.80$, $p = 0.084$; Fig. 5B).

3.6. Preference test

3.6.1. Early parental care

The time subjects spent in side-by-side contact with their partner (the slightly more familiar animal) during the preference test was significantly different by level of parental care ($F_{2,51} = 3.57$, $p = 0.035$). Post-hoc pairwise comparisons revealed that high-contact individuals engaged in more side-by-side contact with their partner compared to medium ($t_{34} = 2.26$, $p = 0.028$) and low-contact individuals ($t_{31} = 2.45$, $p = 0.018$). Medium- and low-contact individuals did not differ significantly ($t_{37} = 0.293$, $p = 0.770$). Sex and the sex by treatment interaction were not significant.

The total time subjects engaged in side-by-side contact with both stimulus animals (partner and stranger) during this test was also significantly different according to the level of early parental care received ($F_{2,51} = 4.20$, $p = 0.020$). Post-hoc pairwise comparisons showed that high-contact individuals spent more time in side-by-side contact with stimulus animals com-

Table 3b

Weights (mean \pm standard error) on postnatal day 30. At PND 30, significant differences were found according to the parental care received; high-contact offspring had lighter body weights compared to medium-contact offspring, while low-contact and high-contact offspring did not differ. Day 30 body weight also differed by sex, with males weighing more. The sex by treatment interaction was not significant.

Parental care received	Female	Male
High contact	28.363 \pm 0.723	30.517 \pm 0.808
Medium contact	30.947 \pm 0.510	36.001 \pm 1.135
Low contact	31.507 \pm 1.351	31.067 \pm 1.550

Table 4

Frequency of total entries (mean \pm standard error) in the elevated plus-maze (non-significant).

Parental care received	Female	Male
High contact	44.222 \pm 6.521	42.0 \pm 7.967
Medium contact	39.556 \pm 6.876	31.168 \pm 4.35
Low contact	37.9 \pm 4.859	40.556 \pm 6.677

pared to medium ($t_{34} = 2.21$, $p = 0.032$) and low-contact ($t_{31} = 2.80$, $p = 0.007$). Medium-contact and low-contact animals did not differ significantly in total contact ($t_{37} = 0.722$, $p = 0.474$). No significant treatment or sex differences, or treatment by sex interaction were found in the time spent on side-by-side contact with the stranger animal (Fig. 6).

3.7. Correlation between behaviors

The total time offspring spent in side-by-side contact during the preference test tended to be positively correlated with the time offspring spent at the start cage during the first dispersal test ($r_{53} = 0.264$, $p = 0.054$). There was also a positive correlation between alloparental behavior and non-anxiety like behavior (open arm ratio) ($r_{54} = 0.285$, $p = 0.035$). Other correlations were not significant.

4. Discussion

Our findings showed that variations in parental care had an effect on offspring's social and exploratory behavior as juveniles and adults. Natural variations in early parental care influenced the willingness of young offspring (PND 21) to explore a novel environment rather than spending time inside their start cage. Despite our prediction that individuals raised by high-contact parents would show lower anxiety and therefore disperse earlier, offspring reared by high-contact parents dispersed less and stayed longer in the start cage than did the medium and low-contact offspring. On average, high-contact offspring spent around 80% of the total time of the first dispersal test in the start cage. Social behavior might be an important determinant of the likelihood of dispersal in prairie voles, in that young individuals that tend to socialize with other members of their group would be less likely to disperse (Bekoff, 1977). Therefore we propose that high-contact offspring spent more time at the start cage as a preference for social interaction with their family members and not as an anxiety-like response to the dispersal tube. This hypothesis is supported by the evidence that high-contact offspring displayed less anxiety-like behavior, and similar levels of exploration, when tested in the elevated plus maze, and higher social behaviors across their life span, including high alloparental behavior during the juvenile period and affiliative behaviors when tested in an adult preference test, compared to low-contact offspring.

The exploration of new environments may be of particular importance in dispersing voles, making them more likely to cover larger distances and to settle away from their natal site (Hoset et al., 2010). Medium and low-contact offspring showed more disposi-

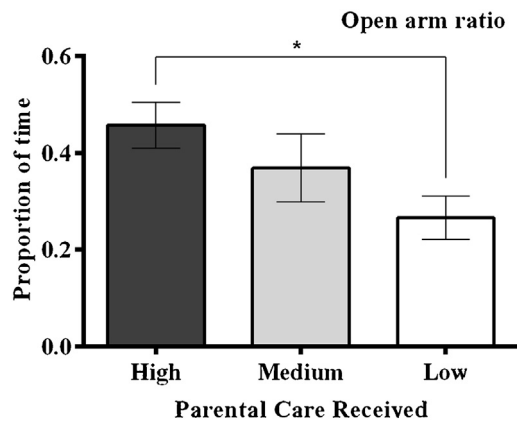


Fig. 4. Elevated Plus Maze. High-contact offspring open arm ratio was greater compared to low-contact offspring (high-contact $n = 15$, medium-contact $n = 22$, low-contact $n = 19$; mean \pm standard errors).

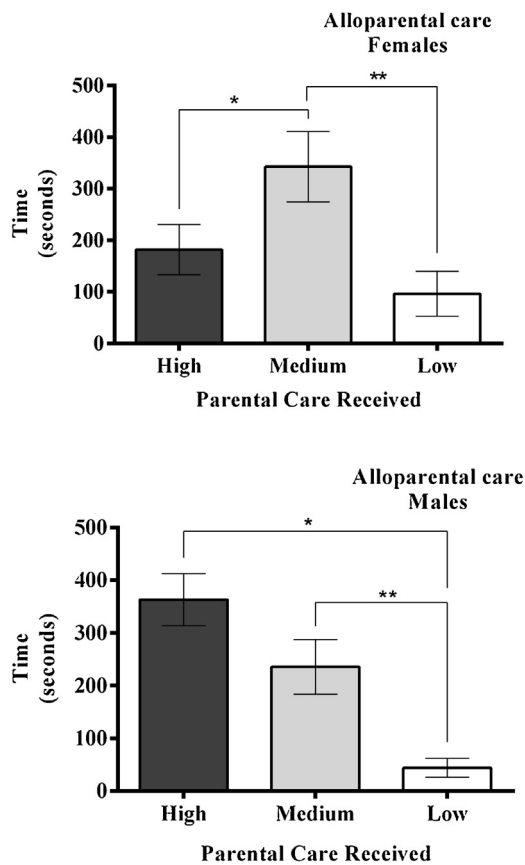


Fig. 5. Allopatal care. Allopataling towards younger siblings was significantly different according to care received from parents (high-contact $n = 15$, medium-contact $n = 22$, low-contact $n = 19$; mean \pm standard errors). There was a significant sex by parental care interaction on allopataling.

(A) Medium contact females displayed more allopatal behaviors compared to high and low-contact females, while no significant difference was found between high and low-contact females. (B) Low-contact males displayed the lowest amount of allopatal behaviors compared to high and medium contact males, while no significant difference was found between medium and high-contact males.

tion to explore and spent more time in the first two segments of the dispersal tube compared to high-contact offspring. Medium and low-contact offspring also displayed increased anxiety-like behavior when tested in the elevated plus maze, which could be useful

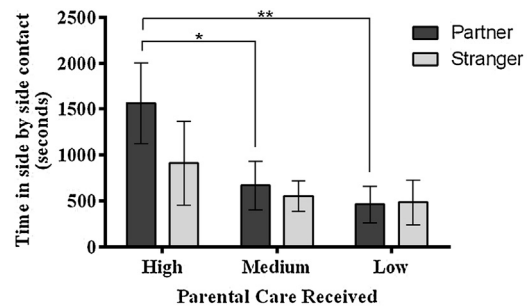


Fig. 6. Preference Test. The time subjects spent in side-by-side contact with their partner (the slightly more familiar animal) during the Preference Test was significantly different by level of parental care, as was the total time subjects engaged in side-by-side contact with both stimulus animals (partner and stranger). High-contact $n = 15$, medium-contact $n = 21$, low-contact $n = 19$; mean \pm standard errors.

to detect predators as increased movement rates during dispersal may increase the risk of predation (Yoder et al., 2004).

However, there are a number of alternative explanations for this finding. Rather than seeking additional social behavior per se, high-contact offspring might be seeking contact, which itself can be highly rewarding (Maruyama et al., 2012; Sailer et al., 2016; Ellingsen et al., 2016). Another hypothesis, that high contact offspring are less exploratory, was not supported by our analysis of total movement in the elevated plus-maze. Yet another possibility is that because offspring often followed parents through the tube (see following paragraph), perhaps the high contact parents spent more time in the start cage and less in the tubes, and influenced offspring behavior directly that way. However, while a comparison of time that mothers spent in the start cage during the day 21 dispersal test did find an effect of treatment ($F_{2,53} = 4.23$, $p = 0.020$), it was low contact mothers that spent the most time in the start cage (HC mothers = 2446.93 ± 114.06 s, MC mothers = 2237.73 ± 206.23 s, LC mothers = 2880.11 ± 114.94 s). Time spent by fathers in the start cage during the first dispersal test was not significantly predicted by their level of parental care ($F_{2,53} = 2.43$, $p = 0.098$; HC fathers = 2511.40 ± 136.78 s, MC fathers = 2455.36 ± 233.14 s, LC fathers = 1961.0 ± 151.98 s). Future research should seek to disentangle these alternative explanations.

The parents' exploratory behavior in the tube was also a consistent influence on offspring dispersal behavior. During the first dispersal test, the mothers' behavior influenced the time offspring spent at the start cage, segments of the dispersal tube and end cage. Offspring at 21 days old still nurse occasionally; therefore it is possible that the offspring were following their mothers intending to nurse or that offspring were attached to the mother nipples while she moved around the dispersal tube (Arias personal observation). It is characteristic for low-contact offspring to receive more nursing while the mother is moving, and for high-contact offspring to nurse more in passive postures (lateral nursing and neutral nursing) (Perkeybile et al., 2013). These behaviors could provide a partial explanation of why high-contact individuals spent more time at the start cage and low-contact individuals inside the dispersal tube at PND 21. Nevertheless, both mothers and fathers played a role influencing dispersal behavior during the third dispersal test third (PND 41–42), only fathers during the fourth test (PND 55–56) and combined influence during the fifth dispersal (PND 69–70). The third and fourth dispersal tests were important during this study as they were close to age of dispersal reported for animals in the wild (45–55 days) (McGuire et al., 2013).

This parental influence is supported by observations in wild prairie voles, as well as in other species. As prairie vole offspring reached the age for dispersal, they were more likely to disperse following disappearance of the parents from the natal nest com-

pared to when the parents stayed at the natal nest (McGuire et al., 1993). Balb c/J mice fathers and female offspring also showed a positive relation in exploratory behavior in a novel environment (Alter et al., 2009). In birds, a study in the great tit (*Parus major*) found that natal dispersal was positively correlated with parent-offspring's exploratory behavior in a novel environment; with fast-exploring parents having offspring that dispersed furthest and dispersers being faster explorers than local individuals. These results suggested that parents influenced offspring natal dispersal either through shared genetic background and/or through parental behavior (Dingemans et al., 2003).

Alloparental behavior has been shown to be sensitive to early experience and rearing environment in prairie voles (Bales et al., 2004; Ahern and Young, 2009; Perkeybile et al., 2013). Prairie voles reared by single mothers experienced a decrease in parental contact, left unattended 10% of the time, compared to offspring reared by both parents, left unattended less than 1% of the time. Offspring reared by single mothers showed significantly lower levels of pup-directed alloparental care (define as time immobile over pups and time licking/grooming) and spent most of their time away from the pups, in comparison to biparental reared adult females (Ahern and Young, 2009). High-contact individuals were involved in more alloparental (sniffing and non-huddling) and play behavior (tumbling) when interacting with a novel infant and juvenile, and displayed less anxiety-like behavior (autogrooming) compared to low-contact offspring (Perkeybile et al., 2013). A cross-fostering study in prairie voles demonstrated a sex-dependent and non-genomic transmission of alloparental behavior, in which active maternal care predicted increases in active alloparental behavior in male offspring (Perkeybile et al., 2015). Consistent with these results, we found that overall increased amounts of early parental contact received by high and medium-contact offspring resulted in juvenile offspring engaging in more alloparental behaviors with the new born siblings, compared to low-contact offspring who spent the least amount of time interacting with the pups. When sexes were considered separately, high-contact males and medium-contact females provided the most alloparental care compared to the individuals from the other groups. In addition, individuals that showed increased alloparental behavior also displayed less anxiety-like behavior; this result is consistent with previous research showing that young females which engaged in more positive interactions with pups also displayed less anxiety-like behavior, while females that displayed high anxiety behavior were more likely to attack or ignore pups (Roberts et al., 1998; Olazabal and Young, 2005).

The amount of time that adult offspring spent in side-by-side contact with a possible partner, regardless if it was the “stranger” or “partner”, was predicted by early parental contact. Individuals reared by high-contact parents showed the highest levels of total time in side-by-side contact with the partner and total time in side-by-side contact with both the stranger and partner. Offspring reared by medium and low-contact parents spent less time in side-by-side contact with the partner and the stranger compared to the high-contact offspring. It is possible that increased early parental contact facilitates pair bonding interactions in adult offspring. Prairie vole offspring reared by single mothers, as adults, exhibit a delayed onset of partner preference formation compared to biparentally-reared animals (Ahern and Young, 2009).

In conclusion, our data support the hypothesis that naturally occurring variation in parental care can predict long-term changes in juvenile and adult social behaviors; however, offspring exploratory and dispersal behavior are especially influenced by parental exploratory and dispersal behavior at the time of dispersal. In addition, early parental care was predictive of later anxiety-like and pair-bonding behavior.

Author contributions

RAdR participated in research design, carried out all data collection and analysis, wrote and edited the paper. KLB participated in research design, data analysis, and editing of the paper.

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Competing interests

The authors report no conflicts of interest.

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