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Infant titi monkey behavior in the open field test and the effect of early adversity

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Abstract

The open field test is commonly used to measure anxiety-related behavior and exploration in rodents. Here, we used it as a standardized novel environment in which to evaluate the behavioral response of infant titi monkeys (*Callicebus cupreus*), to determine the effect of presence of individual family members, and to assess how adverse early experience alters infant behavior. Infants were tested in the open field for 5 days at ages 4 and 6 months in four successive 5 min trials on each day. A transport cage, which was situated on one side of the open field, was either empty (non-social control) or contained the father, mother, or sibling. Infant locomotor, vocalization, and exploratory behavior were quantified. Results indicated that age, sex, social condition, and early experience all had significant effects on infant behavior. Specifically, infants were generally more exploratory at 6 months and male infants were more exploratory than females. Infants distinguished between social and non-social conditions but made few behavioral distinctions between the attachment figure and other individuals. Infants which had adverse early life experience demonstrated greater emotional and physical independence, suggesting that early adversity led to resiliency in the novel environment.

Keywords

anxiety; attachment; early experience; exploration; primate

1 | INTRODUCTION

The open field test is the most commonly employed behavioral assay in animal psychology (Prut & Belzung, 2003) and is primarily used with rodents, for which it was initially developed. The open field is a brightly lit square or circular enclosure with surrounding walls that prevent the animal from escaping. The floor is marked with a grid used to measure movement in the form of number of gridlines crossed, and to determine the location in

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CONFLICTS OF INTEREST
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which the animal spends the longest duration of time. The open field originated as a measure of anxiety-related behavior, which is quantified by comparing the amount of time spent along the periphery of the chamber to the more open center of the field. Based on the predisposition of common laboratory rodents to prefer dark and enclosed spaces, this method is intended to pit the rodent's inclination to explore a novel environment against the desire to avoid the anxiety-inducing condition of an open and brightly lit space (Choleris, Thomas, Kavaliers, & Prato, 2001).

The open field paradigm has been frequently extended to non-rodent species, including calves, sheep, lobsters, rabbits, pigs, chickens, honeybees (Prut & Belzung, 2003), and in non-human primates, the bushbaby (Watson, Ward, Davis, & Stavisky, 1999), the mouse lemur (Dal-Pan, Pifferi, Marchal, Picq, & Aujard, 2011), and the rhesus monkey (e.g., Lavenex, Amaral, & Lavenex, 2006; Rapp, Kansky, & Roberts, 1997; Sun et al., 2006). In these disparate species, the open field test is used in ways distinct from its original purpose. Although the same measures may be taken during the test as are used in rodents, when employed for a species which does not fear bright open space, no motivational conflict exists. In this context, the open field test is often is used to measure reaction to stress-inducing novelty.

In the present study, the open field paradigm was extended to the infant titi monkey (*Callicebus cupreus*). The titi monkey is an arboreal New World primate that lives in small family groups composed of an adult male and female and their shared offspring. Adults formlong-term heterosexual pair bonds (Mason, 1966) characterized by a mutual emotional attachment distinguished by proximity seeking, separation distress, and stress buffering. In the titi family, fathers do the majority of infant care, carrying the infant more than 70% of the time. Infants possess a filial attachment to the father, and show a rise in plasma cortisol when separated from the father, even when still in the presence of the mother. This bond is not reciprocated, as neither the mother nor the father forma parental attachment to the infant (Hoffman,Mendoza, Hennessy, & Mason, 1995; Mendoza & Mason, 1986). While siblings may display alloparental care on rare occasions, in most cases they actively avoid infant carrying (Fragaszy, Schwarz, & Shimosaka, 1982).

Titi monkeys are a neophobic species, reacting strongly and with caution when presented with a novel situation or object. In the laboratory, they show substantial increases in plasma cortisol to changes in the home cage or in the environment outside the home cage, which is mediated by the presence or the absence of the pairmate (Hoffman et al., 1995). Similarly, presence of the attachment figure alters investigation of a novel object, and when removed, object exploration is attenuated (Fragaszy & Mason, 1978). While there is only a minor age effect in attraction to food, the effect of age on approach, contact, and manipulation of a novel object is pronounced, with attraction steadily decreasing with age (Mayeaux & Mason, 1998). The open field test presents the titi infant with environmental novelty sufficient to elicit a distress response, and can thus be used to examine how factors such as age or availability of the attachment figure impact the magnitude of response.

Reactivity to a stressor such as the open field reflects the coordinated activity of integrated central and peripheral neuroendocrine systems which prepare an individual for oncoming threat. The quality of the early social environment has lasting effects on later stress reactivity and health outcomes such that early adversity biases the individual toward greater reactivity (Boyce & Ellis, 2005). In particular, disrupted parent-offspring relationships lead to changes in stress reactivity, as parental behavior shapes development of the neuroendocrine systems that underlie physiological and behavioral responses to stress (Fairbanks, 1996; Francis & Meaney, 1999; Meaney, 2001; Suomi, 2011). Individuals with heightened stress reactivity are subject to negative health outcomes and psychological pathologies (Heim & Nemeroff, 2001), which has led many to directly relate early adverse experiences to negative effects on mental health and wellbeing. However, experiencing negative events in early life may not have unilaterally negative outcomes, as there may be benefits to experiencing some early adversity, particularly in that it may promote later resiliency in stressful conditions (Levine & Mody, 2003; Lyons & Parker, 2007; Parker, Buckmaster, Sundlass, Schatzberg, & Lyons, 2006).

Here, we evaluate the contribution of individual family member presence, prior exposure to early adversity, and the infant's sex and age to behavioral response to the stress-inducing novelty of the open field test. Given that titi infants hold an attachment to their father specifically, we hypothesized that infants would show decreased behavioral indicators of distress and greater exploration when in the presence of their father compared to other individuals. We also examine the effect of early adversity on changes in behavior from 4 to 6 months to evaluate the effect of adversity on development, and characterize the nature of differences between typically and adversely experienced infants.

2 | METHODS

2.1 | Subjects

Twenty-five captive-born infant coppery titi monkeys (*C. cupreus*), 12 males and 13 females, housed at the California National Primate Research Center in Davis, California were used in this study. Infants were housed with the mother, father, and any previous offspring that had not yet been removed for pairing in 2.13m by 1.27m by 1.27m cages identical to those previously described (Mendoza, 1999). Animals were fed a diet consisting of New World monkey chow, apples, baby carrots, bananas, raisins, and cottage cheese or rice cereal twice daily at 0830 and 1300 hr and were kept on a 12/12 hr light/dark cycle. Subjects were tested at both 4 and 6 months of age for 5 days each on consecutive weekdays. All procedures were approved by the University of California, Davis Institutional Animal Care and Use Committee and adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates.

2.2 | Materials

The open field was constructed of opaque white polyvinyl chloride and measured 1m long by 1m wide, with a wall height of 1m, and a 6 by 6 square floor grid (Figure 1). The north wall of the open field had a wire mesh grate which allowed visual, auditory, and olfactory access to the stimulus animal. Prior to the start of testing, a piece of brown felt was placed left of the center of the field. This served as a novel object, and one which provided a tactile approximation of contact comfort. A familiar food reward (either a peanut or miniature marshmallow, depending on which the infant had been previously exposed to in the home cage) was placed right of the center of the field. Overhead lights kept the open field brightly lit.

2.3 | Procedure

Between 0600 and 0800 hr, the subject and all stimulus animals in the subject's cage were caught in 0.3m by 0.3m by 0.6m transport cages constructed of opaque white plastic with wire mesh sides. Transport cages were covered with towels and transported to a testing room in separate building so that test animals were isolated from auditory and visual contact with the rest of the colony. At 4 months infants were typically transported with a parent, as titi infants are still intermittently carried by the father at this age. At 6 months, infants are infrequently carried, and were thus transported in a separate transport cage unless they clung to another individual during capture.

Infants were removed from the transport cage with the use of leather catch gloves and placed in the center of the open field. In four successive trials on each day of testing, a transport cage containing the father, the mother, and the oldest sibling still living in the infant's home cage, or an empty cage (a non-social control stimulus) were placed at the wire mesh grate. Stimulus condition order was randomized and each trial lasted 5 min. During testing, all animals not serving as the stimulus were kept in transfer cages placed along the left side of the open field, and were thus within auditory but not visual contact of the test subject. These animals were kept in the same room during testing both to facilitate the testing and in case the infant became too distressed and had to be returned to a parent (which did not happen). Between trials, the infant was repositioned into the center of the open field and the food reward was replaced if consumed during the previous trial.

2.4 | Behavioral assessment

All observations were recorded with a digital camcorder for computer software based behavioral coding. Each video was coded three times, once for infant and non-infant vocalizations, once for frequency of gridline crossing, and once for location within the open field and exploratory behavior such as rearing on hind legs and jumping, and interaction with stimulus objects (for ethogram, see Table 1). Behavioral coding was done using Behavior Tracker 1.5 software (http://www.behaviortracker.com) with an inter-rater reliability of 90% or greater for all behaviors. For location coding, the floor of the open field was divided into three zones: the grate zone, directly adjacent to the grate; the periphery, the remaining 18 squares adjacent to the walls of the open field; and the center, the 16 squares inside the periphery (Figure 1).

2.5 | Early experience categorization

All subjects were categorized as having had typical (TYP) or adverse (ADV) early life experience. Causes for adverse categorization included forms of social trauma, specifically the death of the father, both parents, or chronic physical injury caused by maternal wounding prior to the start of testing. Adverse conditions were not imposed by the authors but were treated as a natural experiment. Seven infants met this definition, five male and two female. Of the seven ADV infants, three experienced the death of the father (their attachment figure) and remained housed with the mother, and one experienced the death of both parents, and was subsequently housed alone with a stuffed animal on which to cling, and given frequent socialization with another orphaned juvenile (the other juvenile did not take part in this study). Three siblings experienced parental wounding by the mother starting shortly after birth, in which repetitive grooming and biting of the anogenital region or tail led to chronic tissue damage. Infant age at the onset of the social trauma ranged from one to 55 days after birth.

2.6 | Data analysis

Because the length of trials varied slightly in number of seconds (276–300), all dependent variables were analyzed as proportions of test duration. The majority of the data was not normally distributed nor was it transformable to normal, so a generalized linear mixed model was utilized, which is robust to violations of the normality assumption (Cerrito, 2005). Degrees of freedom were calculated by the default method for mixed models containing a random term; and were thus roughly based on (Subjects × Days of testing per age × Ages of Testing × Number of trials per test)—Parameters estimated.

The model for each dependent variable included an ID term, sex, age, testing day, order, social condition, early experience, and interaction terms of sex, age, social condition, and early experience. Sex and early experience were nested within the ID term because the sex and early experience of each subjects cannot be independent of that individual. The nested ID term was used as a Random Factor to account for the effect of multiple measurements on each subject as well as to allow for conclusions on the impact of sex and early experience beyond their connection to ID. The terms for testing day and order were included to parse out their effect on behavior, however given their limited importance for interpretation, significance of these factors is not reported. Backward selection was utilized and all nonsignificant effects were removed from each model. Post-hoc analyses utilized least squares means to report the results of t-tests between estimated marginal means. All tests were two-tailed.

In order to examine relative time spent in the center versus the periphery of the open field, a ratio was created by dividing time spent in the center by the sum of time spent in the center, periphery, and grate. A latent variable was created to examine duration of exploratory behavior by summing the number of seconds spent eating, rearing, and interacting with the felt. Frequency of vocalizations made by individuals other than the infant subject were included as a covariate in the model for infant vocalizations to account for effects these non-infant vocalizations had on infant vocalization frequency. Our sample included only two ADV females, thus we were unable to reliably examine sex differences within the ADV group.

3 | RESULTS

3.1 | Locomotor behavior

Gridline crossing frequency was used as a measure of locomotor activity. Frequency of gridline crossing was greater in male compared to female subjects ($F_{1,787} = 13.11$, $p <$

0.001). An interaction of age and early experience ($F_{1,787}$ = 11.28, $p < 0.001$) indicated that TYP subjects crossed more gridlines at 6 months than at 4 months of age (t = 7.52, $p <$ 0.0001), however, this difference was absent in the ADV group (t = 0.55, p = 0.58) (Figure 2a). A sex by age interaction ($F_{1,787}$ = 4.55, $p < 0.05$) indicated that females crossed fewer gridlines than males at 4 months (t = 3.02, $p < 0.01$), but there was no sex difference at 6 months (t = 0.07, p = 0.94) (Figure 2b).

Social condition predicted frequency of gridline crossing ($F_{3,787}$ = 9.92, $p < 0.0001$). Subjects crossed more gridlines in the empty condition compared to all social conditions, father (t = 3.61, $p < 0.001$), mother (t = 4.93, $p < 0.0001$), and sibling (t = 3.25, p = 0.001) (Figure 2c). An age by social condition interaction ($F_{3,787}$ = 3.13, $p < 0.05$) indicated that subjects crossed fewer gridlines in the empty condition than the sibling condition at 6 months (t = 3.85, p = 0.0001), but there was no difference at 4 months (t = 0.58, p = 0.56).

3.2 | Infant vocalizations

Subjects vocalized more frequently at 4 months compared to 6 months ($F_{1,754}$ = 85.27, $p <$ 0.0001). An age by sex interaction ($F_{1,754}$ = 42.57, $p < 0.0001$) indicated that while there was no sex difference at 4 months, males vocalized less frequently than females at 6 months (t = 11.97, $p < 0.0001$) (Figure 3a).

Social condition predicted infant vocalization frequency ($F_{3,754}$ = 42.75, $p < 0.0001$). Infants vocalized less in the empty condition compared to all social conditions, father (t = 11.20, $p <$ 0.0001), mother (t = 8.87, $p < 0.0001$), sibling (t = 7.18, $p < 0.0001$). Interestingly, subjects' vocalizations differed between the mother and father conditions, with more frequent vocalization in the father condition compared to the mother condition (t = 2.72, $p < 0.01$). A sex by condition interaction ($F_{3,754}$ = 5.00, $p < 0.01$) indicated that males vocalized less than females in the empty (t=6.32, $p < 0.0001$) and the mother (t=2.69, $p < 0.01$) conditions (Figure 3b).

Paralleling the results for locomotor behavior, an age by early experience interaction ($F_{1,754}$=, $p < 0.0001$) indicated that TYP subjects vocalized more frequently at 4 months compared to 6 months (t = 14.53, $p < 0.0001$), however this difference was absent in the ADV subjects (t = 1.12, p = 0.27) (Figure 3c).

3.3 | Location in the open field

An interaction of age and early experience ($F_{1,793}$ = 10.97, p = 0.001) indicated that TYP subjects spent a greater proportion of their time in the center at 6 months compared to 4 months (t = 5.01, $p < 0.0001$), but this was not the case for ADV subjects (t = 0.76, p = 0.45) (Figure 4a). ADV subjects spent a greater proportion of their time in the center at 4 months than TYP infants (t = 3.69, $p < 0.001$), but there was no difference between ADV and TYP infants at 6 months (t = 0.53, p = 0.59). Interestingly, 4 month ADV and 6 month TYP subjects' proportion of time spent in the center were not significantly different (t = 0.21, p = 0.84).

Subjects spent a greater proportion of their time in the center during the empty condition compared to all social conditions, father (t = 3.96, $p < 0.0001$), mother (t = 6.48, $p <$

0.0001), and sibling ($t = 5.40$, $p < 0.0001$). An interaction of early experience and social condition ($F_{1,793} = 3.22$, $p < 0.05$) indicated that in the empty condition ADV subjects spent a greater proportion of time in the center compared to TYP subjects ($t = 4.20$, $p < 0.0001$), but there was no difference in any of the social conditions (Figure 4b).

Importantly, age and early experience interacted ($F_{1,791} = 72.34$, $p < 0.0001$) such that ADV subjects spent less time than TYP subjects in the grate zone at 4 months ($t = 8.61$, $p < 0.0001$), but ADV subjects spent more time in the grate zone than TYP subjects at 6 months ($t = 2.44$, $p = 0.01$). Thus, TYP subjects spent more time in the grate zone at 4 months than at 6 months ($t = 9.19$, $p < 0.0001$) and ADV subjects spent more time in the grate zone at 6 months than at 4 months ($t = 4.58$, $p < 0.0001$). Although still significantly different, 4 month ADV and 6 month TYP subjects had similarly lower durations at the grate (Figure 5a).

A sex by age interaction ($F_{1,791} = 6.67$, $p = 0.01$) indicated that males spent less time than females in the grate zone at 6 months ($t = 2.53$, $p = 0.01$) but there was no sex difference at 4 months ($t = 0.51$, $p = 0.60$). Males spent more time in the grate zone at 4 months compared to 6 months ($t = 2.65$, $p = < 0.01$), but there was no difference in females at 4 and 6 months ($t = 1.05$, $p = 0.29$) (Figure 5b).

Notably, social condition altered duration of time spent in the grate zone ($F_{3,791} = 48.68$, $p < 0.0001$). Subjects spent less time in the grate zone in the empty condition compared to all social conditions, father ($t = 7.18$, $p < 0.0001$), mother ($t = 9.99$, $p < 0.0001$), sibling ($t = 7.32$, $p < 0.0001$). A condition by early experience interaction ($F_{2,791} = 2.90$, $p < 0.05$) indicated that ADV subjects spent less time in the grate zone than TYP subjects in the empty ($t = 5.65$, $p < 0.0001$) and mother conditions ($t = 2.01$, $p < 0.05$), and showed a trend in the father condition ($t = 1.85$, $p = 0.06$), while there was no difference in the sibling condition ($t = 0.05$, $p = 0.96$) (Figure 5c).

3.4 | Exploratory behavior

Duration of exploratory behavior showed an interaction of age and sex ($F_{1,801} = 8.31$, $p < 0.01$) in which male subjects engaged in exploratory behaviors longer than females at 6months (t=5.41, $p < 0.0001$) but not at 4months (Figure 6a). Duration of exploratory behaviors was longer at 6months than 4 months in both male ($t = 10.05$, $p < 0.0001$) and female (t=4.65, $p < 0.0001$) subjects. Age and early experience also interacted ($F_{1,801}$=3.76, $p = 0.05$) (Figure 6b). Subjects had greater duration of exploratory behavior at6months compared to 4months inboth the TYP (t=11.07, $p < 0.0001$) and ADV ($t = 4.38$, $p < 0.001$) groups. In addition, while there was no difference in duration of exploratory behaviors between ADV and TYP subjects at 4 months ($t = 0.79$, p =0.43), there was a trend of TYP subjects having a longer duration of exploratory behavior compared to ADV subjects at 6 months (t=1.76, p=0.07).

Subjects jumped clear of the floor of the open field at low rates (mean 0.11 ± SD 0.63). Consistent with increased exploration in the absence of a social stimulus behind the grate, condition had an effect on frequency of jumping ($F_{3,797} = 4.58$, $p < 0.01$) such that subjects jumped more in the empty condition compared to all social conditions, father ($t = 3.15$, $p <$

0.01), mother ($t = 2.88$, $p < 0.01$), and sibling ($t = 2.05$, $p < 0.05$) (Figure 6c). There was no effect of age, sex, or early experience on rates of jumping.

4 | DISCUSSION

The open field test has been frequently used as a test of anxiety-like and exploratory behavior in rodents, and has been extended to a diverse array of species as a method of quantifying response to a novel environment. Here, we evaluated the response of infant titi monkeys to the open field, the effect that presence of individual family members had on this response, and how adverse early experience altered infant behavior.

The open field was developed to capitalize on rodent behavior, but in the arboreal titi monkey, the open field may have little ethological relevance. Rather, in this context it functioned as a standardized novel environment. One concern this poses is that of substrate. Several patterns of movement were seen in infants during testing that are not seen in the home cage, which does not contain a solid, flat bottom. For example, pushing with the hands on the floor and moving backwards, away from the stimulus animal was frequently seen, most often with a subsequent quick return to the grate. Intentional hugging of the walls or movement that maintained location in the periphery was rarely seen, as is the norm in the rodent open field. Thus, there is little to suggest that time in the center versus periphery is meaningful in this species as a measure of anxiety-like behavior. In titis, the instinctive fear response is to move in the vertical plane or to remain still. For this reason, jumping may be a more informative measure. Infants jumped more in the empty condition than all social conditions, which could indicate anxiety-driven attempts to escape the open field rather than a form of exploration. However, jumping was seen infrequently, and when infants did jump, they typically only did so once during the first trial. Because jumping almost invariably (with the exception of one infant that was able to jump out of the testing apparatus) ended with landing back on the floor of the open field, it was seldom seen repeatedly in a testing session.

The largest determinant of infant behavior was whether or not a stimulus animal was present. In the empty condition, infants decreased their frequency of vocalization, spent less time eating and in proximity to the grate, and had increased rates of locomotor and exploratory behavior. This pattern suggests that in the absence of attraction to a social partner behind the grate, infants explore the novel environment at a higher rate, and differs from that seen in human infants, in which presence of the attachment figure promotes exploration of a novel environment (Ainsworth & Bell, 1970).

Contrary to expectation, little difference in behavior was found when the attachment figure, the father, was present compared to the mother or sibling. Only infant vocalizations distinguished between the father and other family members, with infants vocalizing most in the presence of the father. This may indicate a greater intensity of retrieval solicitation to the preferred caregiver, or a heightened emotional response to the inability to make contact. As titi infants begin spending time off their father between 3 and 5 months of age (Fragaszy et al., 1982), our 4 and 6 month time points may not have been early enough in development to fully capture specific reliance on the father. Rather, it suggests that in titis, the presence of any family member results in attempts to make physical contact and solicitation of retrieval. The inability of the infant to make contact is distressing, and leads to heightened vocalization frequency, which is a behavioral stress response to separation from the attachment figure in both filial and adult-adult attachments in titi monkeys (Mason & Mendoza, 1998).

We evaluated sex differences and found that the majority of behaviors indeed differed between males and females. Males had higher rates of locomotion at 4 months, spent more time in the center of the open field and engaged in more exploratory behaviors at both time points, and spent less time vocalizing and in the grate zone at 6 months. These differences are consistent with males being more active or independent than females, particularly by the time they reach 6 months of age. Although titis have little physical or behavioral sexual dimorphism, as is typical in monogamous mammals (Kleiman, 1977), differences in social interest and activity levels have indicated developmental sex differences (Mayeaux, Mason, & Mendoza, 2002). Accelerating activity in females between 6 and 24 months has been suggested as underlying earlier dispersal of females in the wild (Mayeaux, 2008). Interestingly, here we found contrasting evidence suggesting greater male activity earlier in development. It may be that sex differences change over time and our earlier points of measurement led to this difference. Alternately, other factors could be involved, such as sex differences in reactivity to novelty or subtle differences in social attraction.

Age was also an important predictor of infant behavior. Infants spent more time in the center at 6 months compared to 4 months. Proximity maintenance declines with age (Fragaszy et al., 1982), presumably because of both an increase in ambulatory independence of the infant and decreasing dependence on the father. The increase in time spent in the center, and away from the grate likely reflects this decrease. It could also be due to a greater interest in exploration at 6 months, however preference for novelty in titis declines with age starting around this time (Mayeaux & Mason, 1998).

Infant monkeys who had adverse early experience had a response distinct fromanimals that had typical early experiences, one that suggests that adversely experienced infants mature faster. They spent less time in proximity to the grate and more time in the center of the open field. In fact, they did so at 4 months at a rate that was comparable to the typically experienced subjects at 6 months, and they vocalized less frequently. These infants also showed a developmental pattern that was different than that of their more typically experienced peers. While typically experienced infants increased their locomotor behavior and decreased their vocalization frequency with age, adversely experienced infants had heightened locomotion at 4 months that did not increase with age, and fewer vocalizations at 4 months that did not decrease with age. This pattern of behavior is suggestive of earlier emotional and ambulatory independence of the adversely experienced infants. Rather than spending the majority of their time at the grate displaying a separation distress response, these infants spent more time in other areas, moving and exploring the environment. Parental wounding or absence, the two causes of adverse categorization in this study, each promote earlier independence of the titi infant. In the absence of the father, maternal rejection forces the young infant to develop its motor skills and gain physical independence due to lack of a willing carrier. In the case of parental wounding, the infant tries to avoid the individual that engages in the damaging behavior, thus also promoting motor development. In either case, the infant must adapt at an early age to stressful life conditions, and may learn that vocalization does not effectively result in soothing by a responsive father. In this way, the behavior of adversely experienced infants in the open field is indicative of resiliency, as these infants adapt with greater independence earlier in development.

Because the adverse experience was not assigned, other factors may have been involved in the differences seen in these subjects. For instance, there may be an inherited predisposition to heightened stress reactivity inherent in parental wounding, which would then predispose the infant to a different reaction to the open field. Likewise, health may be related to early adversity, and could significantly impact activity level, thereby altering behavior in the open field. Several further caveats must be made regarding the measures used here. Although vocalizations are often considered a useful index of emotionality (Walsh & Cummins, 1976), this may not be the case in this application. One would expect to find that infants are more anxious when left alone without a familiar individual, particularly their father. However, because infants vocalize to solicit retrieval, it follows that they vocalize less in the empty condition in the absence of a social stimulus from which to solicit retrieval. This is not necessarily an indication that infants lack an emotional response to the empty condition. Second, while infants showed a preference for their father in the distressing novel environment only in measures of vocalization, previous research has suggested a clear role of father as a buffer against stressful situations (Hoffman et al., 1995). A possible explanation for this is that physical contact is required to fully soothe the infant. As is the case with mother-infant separation in rhesus macaques and squirrel monkeys, behavioral agitation may result from the ability to see the attachment figure but not make physical contact (Levine, Franklin, & Gonzalez, 1984; Seay, Hansen, & Harlow, 1962; Wiener, Bayart, Faull, & Levine, 1990), and may be an added stressor in and of itself that induces an anxiety-like reaction. The adversely experienced infants, then, having repeated familiarity with this circumstance, may be more adept at subverting the behavioral agitation and moving on to explore the environment. Here, we were unable to examine differences in type of adverse experience and sex in ADV infants because of limited sample size. For example, we were not able to differentiate the effects of parental absence and maternal wounding, nor were we able to examine how the behavior of such infants differs among males and females. Future work should more fully characterize how early trauma relates to specific differences in later behavior, and explore how individual differences in temperament factor into the relationship between early experience and behavior in a novel environment such as the open field.

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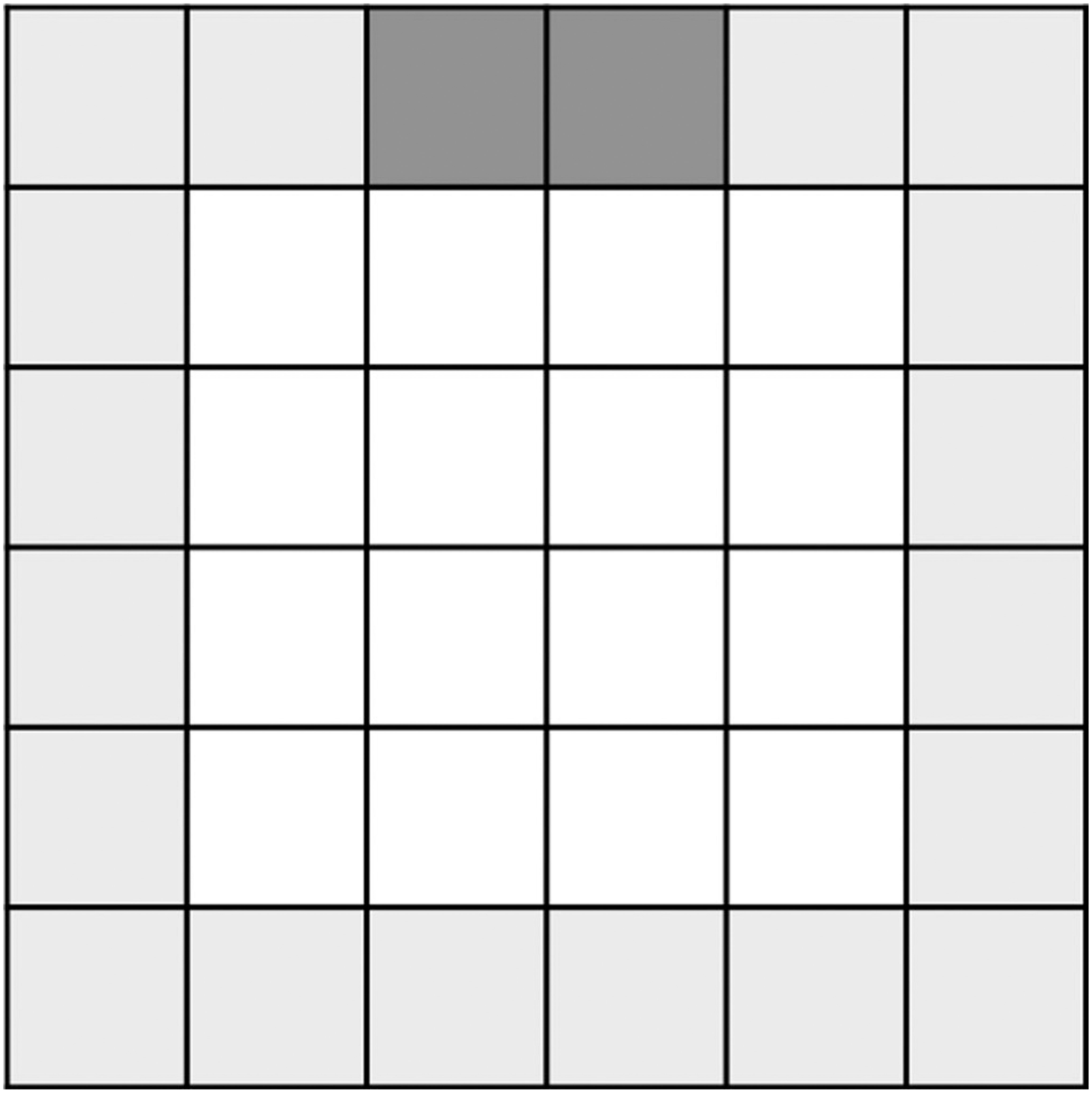

FIGURE 1.
Floor designations of open field for behavioral coding. White, center; Dark gray, grate zone; Light gray, periphery

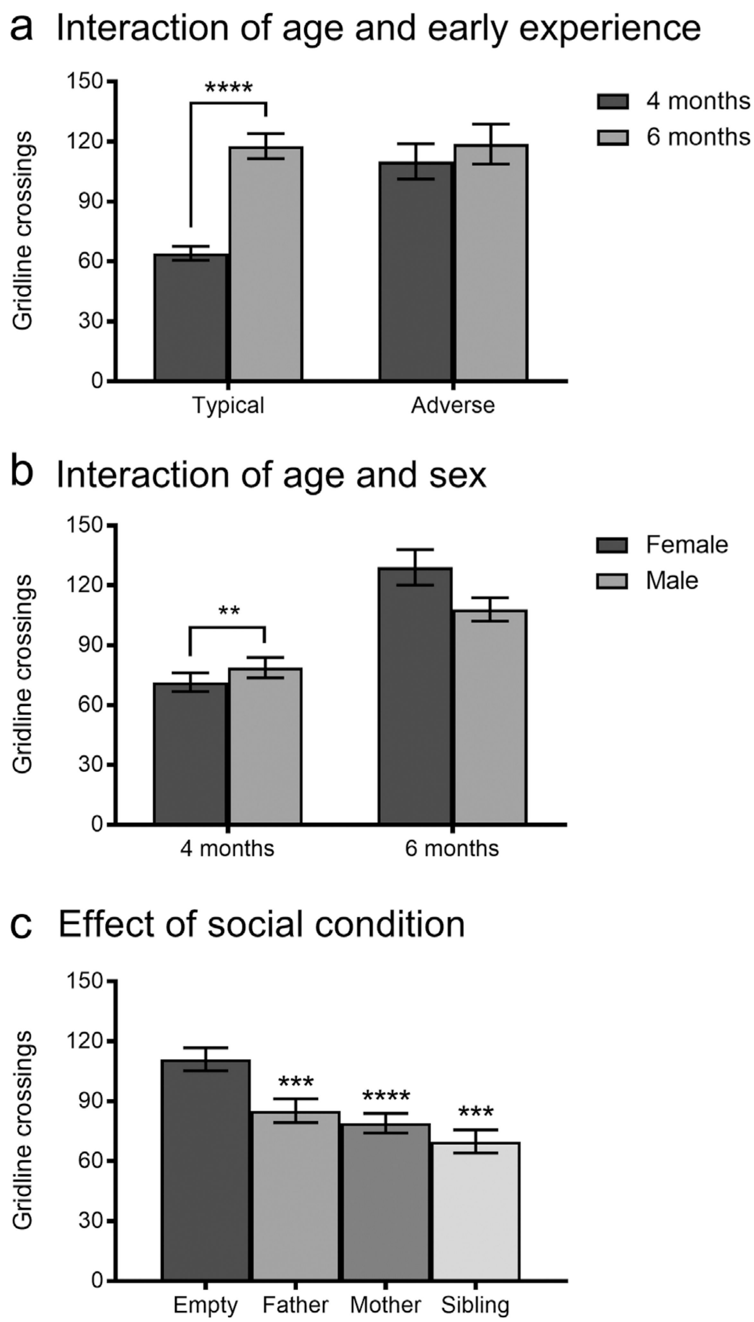

FIGURE 2.

Locomotor behavior. (a) Mean (±SEM) infant gridline crossing was greater at 6 months compared to 4 months in TYP but not ADV subjects. (b) Mean (±SEM) infant gridline crossing was greater in male compared to female subjects at 4 months. (c) Mean (±SEM) infant gridline crossing was greater in the empty condition compared to all social conditions. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

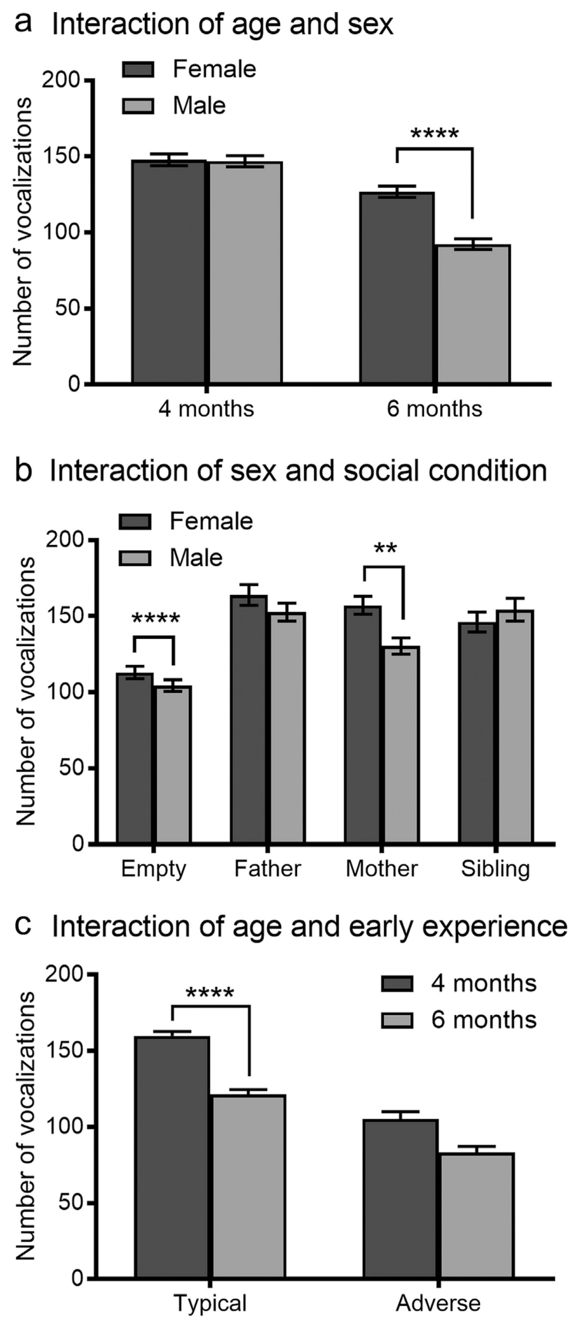

FIGURE 3.

Infant vocalizations. (a) Mean (±SEM) infant vocalization frequency was greater in female compared to male subjects at 6 months. (b) Mean (±SEM) infant vocalization frequency was greater in females in the empty and mother conditions. (c) Mean (±SEM) infant vocalization frequency in was greater at 4 months compared to 6 months in TYP subjects but did not differ in ADV subjects. ** $p < 0.01$, **** $p < 0.0001$

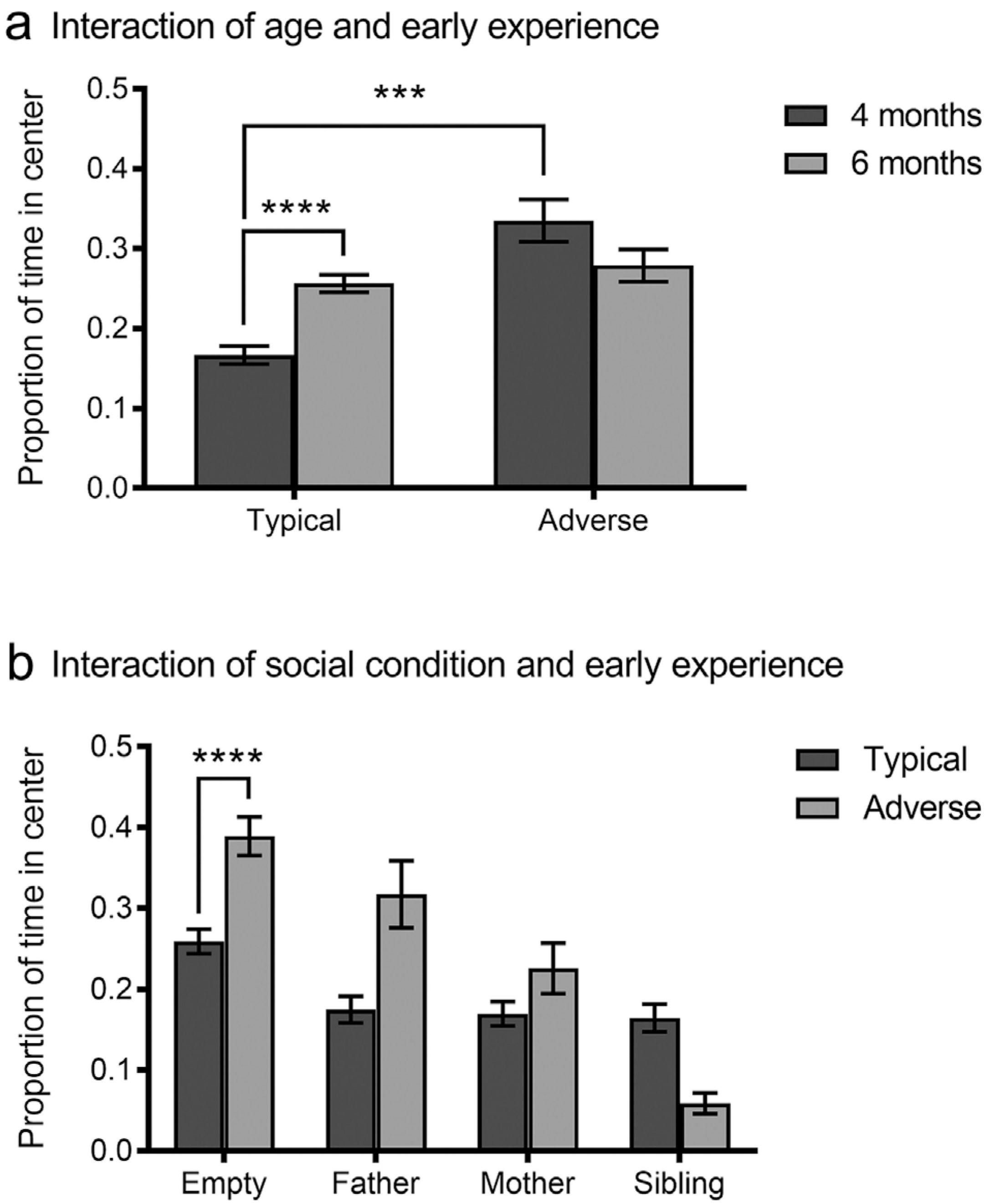

FIGURE 4.

Time in center. (a) Mean (±SEM) proportion of time infants spent in the center of the open field was greater at 6 months compared to 4 months in TYP subjects but not ADV subjects, and was greater in ADV subjects compared to TYP subjects at 4 months. (b) Mean (±SEM) proportion of time in the center was greater in ADV compared to TYP subjects in the empty condition. ***$p \leq 0.001$ ****$p \leq 0.0001$

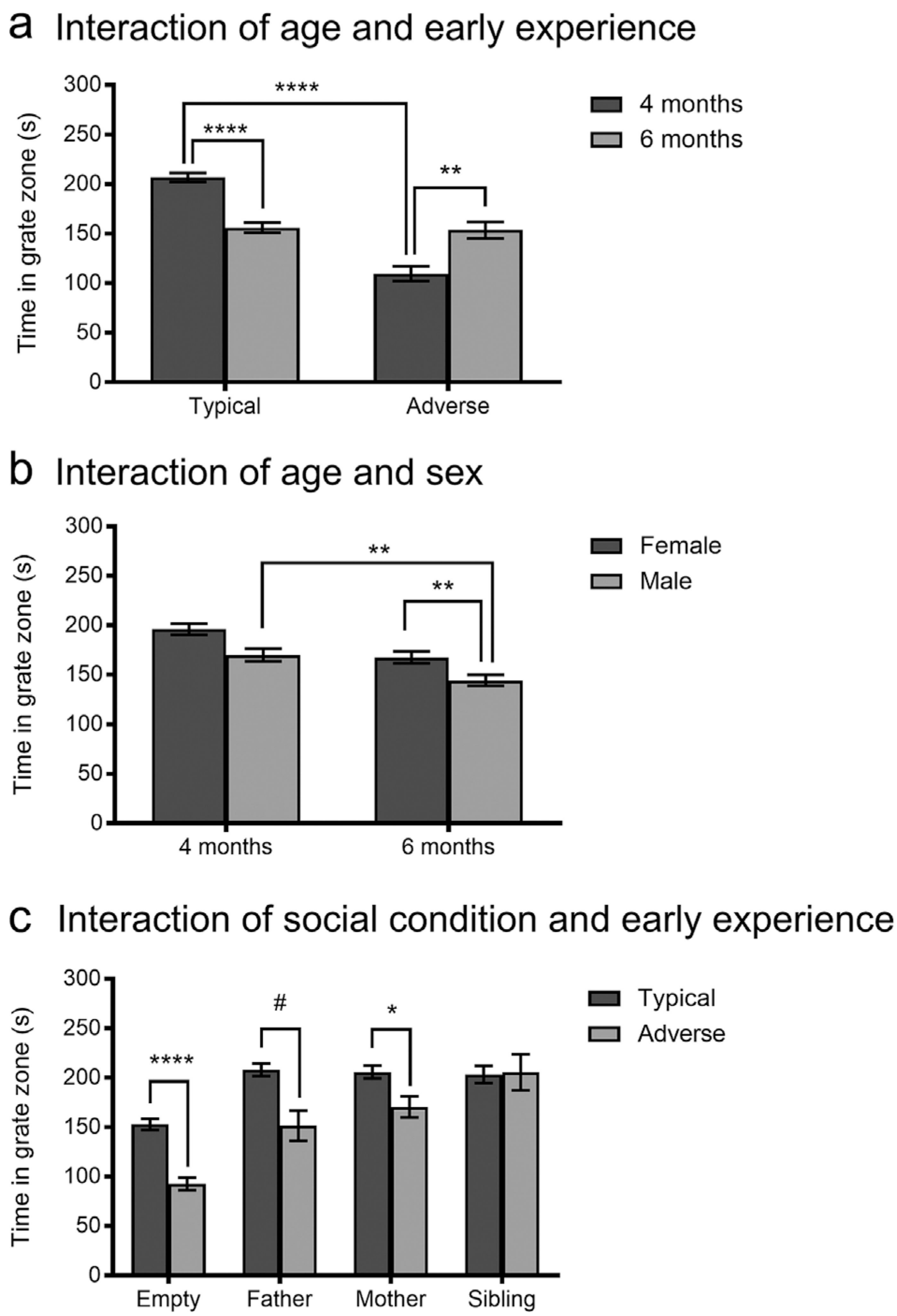

FIGURE 5.

Time in grate zone. (a) Mean (±SEM) duration of time in the grate zone was greater in TYP subjects at 4 months compared to TYP subjects at 6 months and ADV subjects at 4 months, and was greater at 6 months than 4 months in ADV subjects. (b) Mean (±SEM) duration of time in the grate zone was greater in males at 4 months compared to 6 months, and greater in females compared to males at 6 months. (c) Mean (±SEM) duration of time in the grate zone was greater in TYP compared to ADV subjects in the empty condition and mother condition, and showed a trend in the father condition. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, #$p = 0.06$

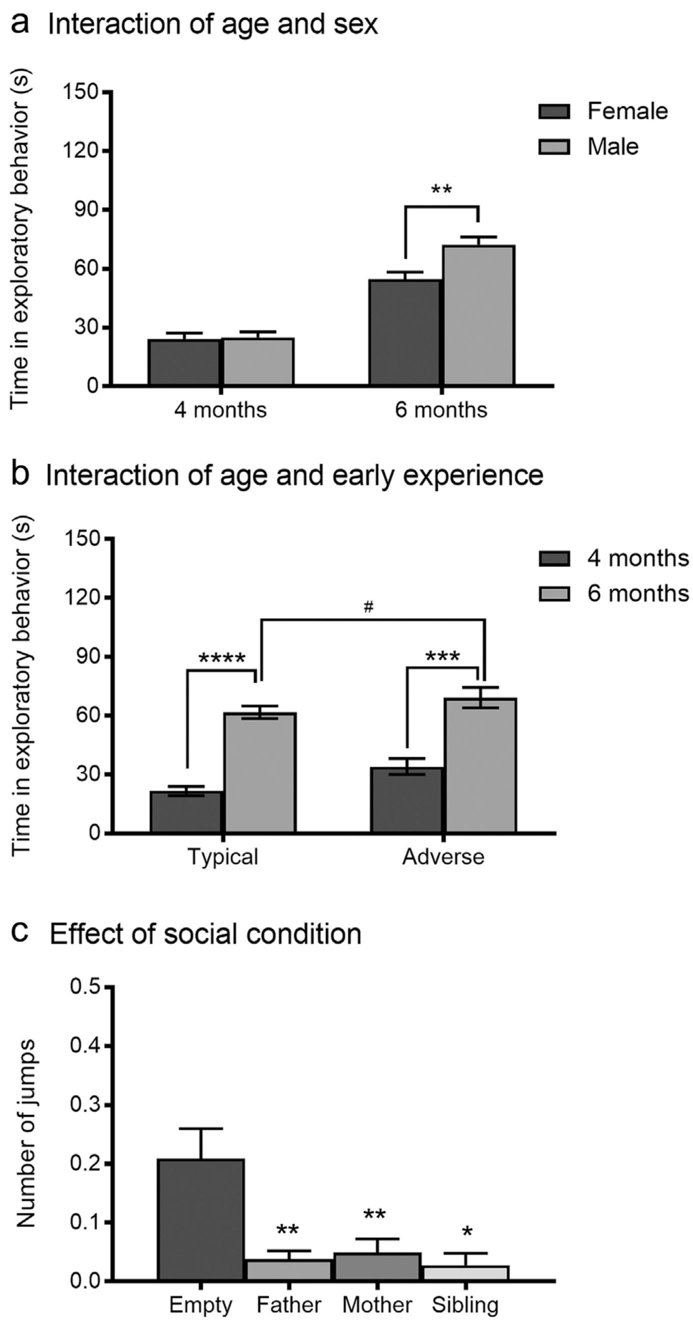

FIGURE 6.
Exploratory behavior. (a) Mean (±SEM) duration of time spent in exploratory behavior was greater in males than females at 6 months. (b) Mean (±SEM) duration of time spent in exploratory behavior was greater at 6 months compared to 4 months in both TYP and ADV subjects. The difference between duration of exploration in TYP compared to ADV subjects at 6 months approached significance (c) Mean (±SEM) number of jumps was lower in all social conditions compared to the empty condition. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, #$p = 0.07$

TABLE 1

Ethogram for behavioral coding of open field behavior

Behavior	Description	Measure type
Center	Both of infant's hands are within the 16 center squares	Duration
Periphery	Both of infant's hands are within the outer 18 squares	Duration
Grate zone	Both of infant's hands are within the two squares directly in front of the grate	Duration
Touch grate	Infant touches grate with hand, foot, or mouth	Duration
Eating	Infant touches food with hands or mouth. Turned off after 3 sec if not being manipulated or consumed	Duration
Jump	Infant springs clear of the floor	Frequency
Rear	Infant rises on rear legs with hands in air or on wall	Frequency
Touch felt	Infant touches felt with hands, feet, or mouth	Duration
Tail lash	Infant swipes tail from side to side, usually a sign of agitation	Duration
Infant vocalization	Infant makes discreet separation vocalization	Frequency
Non-infant vocalization	Mother, father, or sibling makes an isolation peep or alarm call	Frequency
Gridline cross	Infant moves both hands across a gridline	Frequency