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ORIGINAL INVESTIGATION

Biparental care in C57BL/6J mice: effects on adolescent behavior and alcohol consumption

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Abstract

Rationale Social attachment plays an important role in offspring development. Different parenting experiences during lactation may shape offspring behavior and later alcohol use.

Objectives We tested the hypothesis that differential rearing conditions (single mother, SM or biparental, BP) in the non-monogamous C57BL/6J mice may affect (1) parental behavior during lactation, (2) adolescent behavior, and (3) adolescent initiation of alcohol drinking.

Methods Mice were reared in SM or BP (cohabitation of father-mother since copulation) condition until weaning (postnatal day, PND, 21). Litters from both conditions were filmed during PNDs 6, 9, and 12 and an ethogram was made taking into account nest-, pup-, or self-directed behaviors. At PNDs, 28–29 adolescent animals were evaluated in a modified version of the concentric square field for measurement of behavioral patterns. Other groups of adolescents were tested in a 4-h daily, two-bottle choice alcohol consumption test (10% alcohol vs. water) during 3 weeks (4 days per week).

Results Single mothers spent less time in the nest, left unattended the nest more times, displayed more self-directed and less pup-directed behaviors than BP parents. SM-reared adolescents displayed more anxiogenic-like and less risk-associated behaviors than BP counterparts. The alcohol consumption test indicated a strong effect of rearing condition. Since the fifth day of test, SM adolescents consumed more quantities of alcohol than BP adolescents.

Conclusions During single-mother parenting, pups are left unattended more often, and during adolescence, these organisms exhibited increased anxiety responses. This behavioral phenotype may act as a risk factor for alcohol initiation during adolescence.

Keywords Parenting · Adolescent · Maternal behavior · Paternal behavior · Alcohol

Introduction

In humans, formation of social bonds is essential for individual well-being and is a critical component of mental health. This behavior is so important that the inability to do it is used as a diagnosis of several psychological disorders including autism, social anxiety, and schizophrenia (Volkmar 2001). Naturalistic variations in social rearing conditions can introduce diversity into adult nurturing and attachment behaviors (Ahern and Young 2009). In this sense, parenting and attachment exert a considerable influence on a wide repertoire of the offspring's behavior, including use and abuse of alcohol and other drugs. In fact, maternal care negligence during childhood has been associated with a subsequent increase in problems related with alcohol use and abuse (Widom et al. 1995). The quality and intensity of these early attachment experiences may also have long-term effects. In rats, it has been observed that the low levels of licking and grooming of the offspring were correlated with high levels of self-administration of cocaine and alcohol (Francis and Kuhar 2008). Some epidemiological studies indicate that children raised by single-parent homes are prone to alcohol use and abuse (Fisher et al. 2007; Ledoux et al. 2002; Scalese et al.

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2014), while others do not or show that this effect is weak (McArdle et al. 2002; Nicholson et al. 1999). The profound consequences of drug use and abuse on social attachment are readily apparent, and it has been shown that drugs can negatively affect pair-bonding in monogamous adults (Liu et al. 2010; Walcott and Ryabinin 2017) and mother–infant interactions (Chasnoff 1992; Minnes et al. 2005; Roland and Volpe 1989) and decrease paternal motivation for pups (Wang et al. 2012).

Evidence from human studies indicates that strong social ties, including close parent–child relationships, pair-bonding, healthy family structures, and nurturing peer groups may reduce drug use or abuse (Adrados 1995; Bell et al. 2000; Ellickson et al. 1999). Although reciprocal interactions between drugs of abuse and social behaviors have been thoroughly documented in human and animal studies and their neural mechanisms are being studied, most of the literature is focused in monogamous species and, specifically, analyze adult pair-bonding behavior. It is known that monogamous rodents engage in a suite of complex social interactions including pair-bonding and biparental care of the offspring (Ahern et al. 2011; Ahern and Young 2009; Campbell et al. 2009). However, although maternal care and its associated neurobiology are highly conserved across mammalian species (Feldman 2015, 2016; Rilling and Young 2014), the nature of paternal care both within a species and among different species exhibits phenotypic plasticity. The neural adaptations that take place in new fathers are less canalized, uniform, and hormone-dependent than those that occur in mothers. These changes are shaped, to a great extent, by active caregiving, exposure to the pregnant or lactating female, and specific infant stimuli and, in humans, sociocultural practices, and belief systems (Feldman et al. 2019).

Adult social bonding (long-term pair-bonding after mating) can be affected by or may have an impact on drug central effects caused by amphetamine (Liu et al. 2010, 2011; Young et al. 2011, 2014) and alcohol (Anacker et al. 2014a, b; Anacker and Ryabinin 2010; Hostetler et al. 2012; Hostetler and Ryabinin 2014). Alternative studies have focused on how these social experiences affect drug use in the progeny (Anacker et al. 2012). During earlier ontogenetic stages, e.g., childhood and adolescence, there is a greater sensitivity to the reinforcing effects of drugs of abuse such as alcohol (for example see: Miranda-Morales et al. 2014; Pautassi et al. 2009 for infants and Spear 2018 for adolescents). Research conducted in Latin America indicates that 47% of 8-year-old children have already tried alcohol and that at the age of 12, the majority (82%) has already started drinking (Pilatti et al. 2013). A similar result was found in US children: 37% of 8-year-old children have already tried alcohol and the percentage rises to 67% at age 12 (Donovan and Molina 2013). Taking into account the inverse relationship observed between the age of onset and the probability of drug abuse and dependence (Baer et al. 2003), it appears necessary to devote experimental efforts centered on early stages of development such us late childhood or early adolescence.

In the present study, using a non-monogamous strain of mice C57BL/6J, we tested the hypothesis that differential rearing conditions may affect offspring behavior that, in turn, could affect adolescent initiation of alcohol drinking. We explicitly compared the consequences of monoparental and biparental rearing conditions on parental behavior itself as well as the phenotypic profile of the progeny including anxiety-like and risk assessment behaviors and its possible relation with alcohol affinity.

General methods

Experimental animals and rearing conditions

C57BL/6J mice were used for the study. In Experiment 1, 16 litters were reared in a single-mother (SM, eight litters) or biparental (BP, eight litters) condition. Experiment 2 evaluated 32 adolescents derived from SM (eight males and nine females) and BP condition (seven males and eight females). Experiment 3 tested 34 adolescents derived from SM (nine males and 10 females) and BP condition (seven males and eight females). These animals were born and reared in the vivarium of the Instituto M. M. Ferreyra (INIMEC-CONICET-Universidad Nacional de Córdoba, Córdoba, Argentina). The colony was maintained at 22 ± 1 °C under a 12 h/12 h light/dark cycle (lights on at 08:00 A.M.). Animals had ad libitum access to water and food (*Cooperativa* irradiated balanced food for rodents; Pergamino, 1437, Capital Federal, Argentina) and were housed in standard transparent cages for mice (396 × 215 × 172 mm) with corncob used as bedding. The experiments were conducted at 10:00 A.M. The protocols here employed were approved by the Ministry of Animal Care of INIMEC-CONICET-Universidad Nacional de Córdoba and complied with the National Research Council Committee for the Update of the Guide for the and Use of Laboratory (2011).

Rearing conditions For BP condition, a female-male adult couple was maintained in the same cage since pairing. In SM condition, a female–male adult couple was maintained in the same cage until day 18 post-pairing. At that day, the male was removed and located in a new cage. Pups were born after 21–22 days of gestation (postnatal day, PND, 0). Litter sizes of this strain of mice vary (ranging from two to eight, normally), but litters were culled if pups were more than ten. Pups were weaned at PND 21 and housed in same-sex cages. Animals remained behaviorally undisturbed until adolescence (PND 28) when they were tested in the concentric square field or later, since PND 32, for alcohol consumption. Only pups from the first birth of each female from SM or BP condition were used.

Parenting behavioral observation

Litters from both parenting conditions were filmed during PNDs 6, 9, and 12, at 14:00 h. Litter size and weight of pups were registered. For each daily observation, the maternity cage was moved to a different room; pups were removed from the cage; the nest was removed and nest materials and pups were relocated in the cage in a random position. A transparent Plexiglas roof was placed on the cage and the litter was filmed during 60 min. Video data were collected by spot-checks every 10 s. An ethogram was performed and the behavior of the mothers and the fathers were logged according to two categories: (1) nest occupancy and (2) activity. Nest occupancy recorded whether parents occupied the nest or not. The activity category included pup-directed (PD) behaviors [licking and grooming, nest building, nursing or huddling over the pups] and self-directed (SD) behaviors [self-grooming, eating, drinking, exploring or digging]. The frequency of each behavior over PND 6–12 for each rearing condition served as dependent variables.

Concentric square field test

Adolescent mice from SM and BP conditions were evaluated in a modified version of the concentric square field (CSF, first described by Meyerson et al. 2006). This test has been used to test the behavioral profile of rats and mice (Ekmark-Lewén et al. 2010, 2017; Stringer et al. 2017). In addition, CSF has been successfully used to test adolescent mice (Stringer et al. 2017) and also adult mice ranging from 2 to 8- and 11-month old (Ekmark-Lewén et al. 2010, 2017). The apparatus (48 cm × 48 cm) had a central open-field square (serving as the starting area) that was connected to other areas by several corridors. One of these areas was a dark, enclosed area that normally evokes shelter-seeking behavior, whereas the other areas evoke exploration, risk assessment, and risk taking. The front section of the maze had a brightly lit runway, separated from the outside by transparent plastic, and a ramp (12 cm × 10 cm, 20° incline) that was connected to an elevated bridge (30 cm × 10 cm) that was made from wire mesh. The lighting conditions were the following: shelter 0 lx, corridors 20–30 lx, and ramp and bridge 600–650 lx. At PND 28, adolescents were located in the central square and recorded during 20 min. A trained experimenter using JWatcher 0.9 recorded frequency of entries and time spent in each area. Unlike other tests that measure anxiety-like and risk-taking behavior, the concentric square field test is more naturalistic and allows a concurrent and graded set of exploratory activities, instead of imposing a single or binary behavioral option (Karlsson and Roman 2016). As mentioned by Roman and Colombo (2009), frequency, duration, and mean time per visit in the ramp; frequency, duration, and mean time per visit in the bridge; and the coefficient between time in bridge/time in ramp can be assessed as measures of risk assessment behaviors. Measures of risk-taking behaviors are more related to frequency, duration, and mean time per visit in the bridge only (Roman and Colombo 2009; Stringer et al. 2017).

Alcohol consumption test

At PND 32, adolescent mice reared from both parenting conditions were evaluated for alcohol consumption. The protocol utilized was an adaptation of the one~~s~~ described by Giardino and Ryabinin (2013) and Marianno et al. (2017). The evaluation consisted of a 4-h daily, two-bottle choice ethanol consumption test (10% ethanol v/v vs. tap water) during 3 weeks and 4 days per week (Tuesday to Friday). Throughout the evaluation process, animals had ad libitum access to food and water. Daily, adolescents were weighed (± 0.01 g) and individually housed in an evaluation cage (section size: 23 cm length × 30 cm width × 20 cm height), although they could see and smell (but not touch) a conspecific of the same sex through a plastic divider, located in the other half of the evaluation cage. They had access to two 100-ml bottles, one filled with water and the other with alcohol. No fading procedure was employed before the consumption test. Leakage of any fluid was controlled by conducting pre- and post-session readings of two control bottles that were placed in an empty cage. The position of the alcohol and water bottles was randomly varied across tests to prevent place-preference effects. The sessions began at 11:00 and lasted at 15:00. After each daily session, animals were returned to their home cage. The volume consumed from each bottle was assessed by subtracting the weight of the bottle after the intake test from the volume recorded before the test. The dependent variables employed were alcohol intake (reported as grams per kilogram, g/kg and as ml/kg for total fluid consumption calculation), water intake (ml/kg), total fluid consumption (alcohol + water intake scores, ml/kg), and percentage of alcohol intake preference [(alcohol intake / overall liquid intake) × 100]. The g/kg of alcohol consumed were calculated as: [(bottle weight post-test − bottle weight pre-test) × ethanol density] / (animal weight / 1000). Water and alcohol intake scores in ml/kg were calculated as: [(bottle weight post-test − bottle weight pre-test) / (animal weight / 1000)].

Experimental design and data analysis

Experiment 1 (parental behavior) assessed nest occupancy, pup and self-directed behavior, nursing and nest building behavior (frequency of the observed behaviors), latency to retrieve pups, and latency to build the nest using a two-factor with repeated-measure design [rearing condition (SM or

BP) × days of observation (PNDs 6, 9, and 12)]. Experiment 2 (adolescent behavior profile) evaluated adolescent animal behavior in the CSF (time and frequency in each compartment) and used a factorial design with repeated measures (sex × rearing condition × areas of the CSF), where areas of the CSF were considered as a within factor to allow direct comparisons between the different compartments. Measures of risk assessment and risk-taking behavior were analyzed by a factorial two-way ANOVA (sex × rearing condition). Finally, Experiment 3 tested adolescent consumption scores (total fluids, water, and alcohol scores) using also a factorial design with repeated measures (sex × rearing condition × days of evaluation).

Data from each experiment were analyzed using main, factorial, or repeated-measures analysis of variance (ANOVA). Least significant difference (LSD) pairwise post hoc tests were conducted to analyze significant main effects and significant interactions. Correlation analyses were performed (Pearson correlation coefficient) between variables of parenting (Exp. 1), adolescent behavior (Exp. 2), and alcohol consumption (Exp. 3). The alpha level was set to 0.05. Statistica 8.0v software was used to compute descriptive and inferential statistics.

Results

Parental behavior

To control the possibility for litter effects dependent upon rearing conditions, two main ANOVAs were conducted taking into account the number of pups per litter and pups' body weight. Number of pups per litter was not significantly affected by rearing conditions (mean litter size ± SEM for MP and BP conditions were 6.12 ± .51 and 6.25 ± .73, respectively). In addition, the offspring's body weight was not significantly affected by the rearing conditions (mean pups' weight ± SEM for MP and BP were 6.16 ± .06 and 6.11 ± .11, respectively).

For nest occupancy, the repeated-measure ANOVA indicated that the BP rearing condition resulted in a significantly higher level of nest occupancy relative to the SM condition ($F_{1,14} = 35.29$, $p < .001$). Independently from rearing condition, nest occupancy significantly increased from day 6 to day 12 ($F_{2,28} = 3.74$, $p < .05$). The nest without any parent was significantly more observed in SM condition ($F_{1,14} = 22.17$, $p < .001$). When nest occupancy was analyzed but only focusing on the mother, this difference was still significant ($F_{1,14} = 6.80$, $p < .025$). Within BP condition, nest occupancy was significantly affected by parents ($F_{2,14} = 7.18$, $p < .01$). The frequency that both of them were observed in the nest was significantly lower than the frequency observed for the mother alone ($p < .0025$). The mother was more frequently observed in the nest than the father was, although this effect did not reach significance ($p = .068$).

Analysis of nest building behavior indicated that BP parents were observed significantly more often building the nest than SM mice ($F_{1,14} = 6.60$, $p < .025$). The time that mothers were observed doing this behavior was similar across both rearing conditions ($p = .82$). Within BP rearing condition, there was no significant difference when contrasting males and females relative to this behavior.

Pup-directed behavior was also significantly affected by rearing conditions, showing higher frequency of behaviors devoted to pups in the BP condition relative to SM condition ($F_{1,14} = 28.99$, $p < .001$). This behavior significantly increased, in both conditions, across days ($F_{2,28} = 4.43$, $p < .025$). PD behavior of mothers from both rearing conditions showed a similar profile. Within BP condition, this behavior was observed significantly more often in mothers than fathers ($F_{1,7} = 12.42$, $p < .01$). Self-directed behavior was observed more times in single mothers, although this effect did not reach significance ($p = .063$). Regarding rearing condition, this behavior significantly decreased at the last day of observation ($F_{2,28} = 4.85$, $p < .025$). Within the BP condition, self-directed behavior did not significantly differ as a function of sex ($p = .19$). Results from parental behavioral profile are depicted in Fig. 1.

Nursing behavior was similar between mothers of both conditions and significantly increased between days 6 to 12 ($F_{2,28} = 12.36$, $p < .001$). When huddling behavior of the father was taken into account (huddling or crouching over the pups) and added to the analysis, the ANOVA indicated a significant effect of the rearing factor: BP parents spent more time over the pups than single mothers ($F_{1,14} = 11.29$, $p < .005$). In addition, nursing and huddling behaviors significantly increased across days of observation in both rearing conditions ($F_{2,28} = 9.46$, $p < .001$). Analyses of latency to retrieve pups and latency to build the nest showed no significant differences between rearing conditions.

Concentric square field test

The ANOVA employed to analyze time spent in the areas of the CSF indicated a significant main effect of the areas of the apparatus ($F_{4,112} = 14.24$, $p < .001$). Adolescents spent significantly more time in the open field and shelter relative to the remaining compartments. Of major importance, the interaction rearing condition × CSF areas also attained significance ($F_{4,112} = 8.45$, $p < .001$). Post hoc analysis indicated that SM adolescents spent less time in the bridge and more time in the shelter relative to BP counterparts. These results are depicted in Fig. 2.

The statistical analysis of frequency of visit to each area of the CSF showed a main effects of sex ($F_{1,28} = 24.76$, $p < .001$) and areas of the CSF ($F_{4,112} = 407.43$,

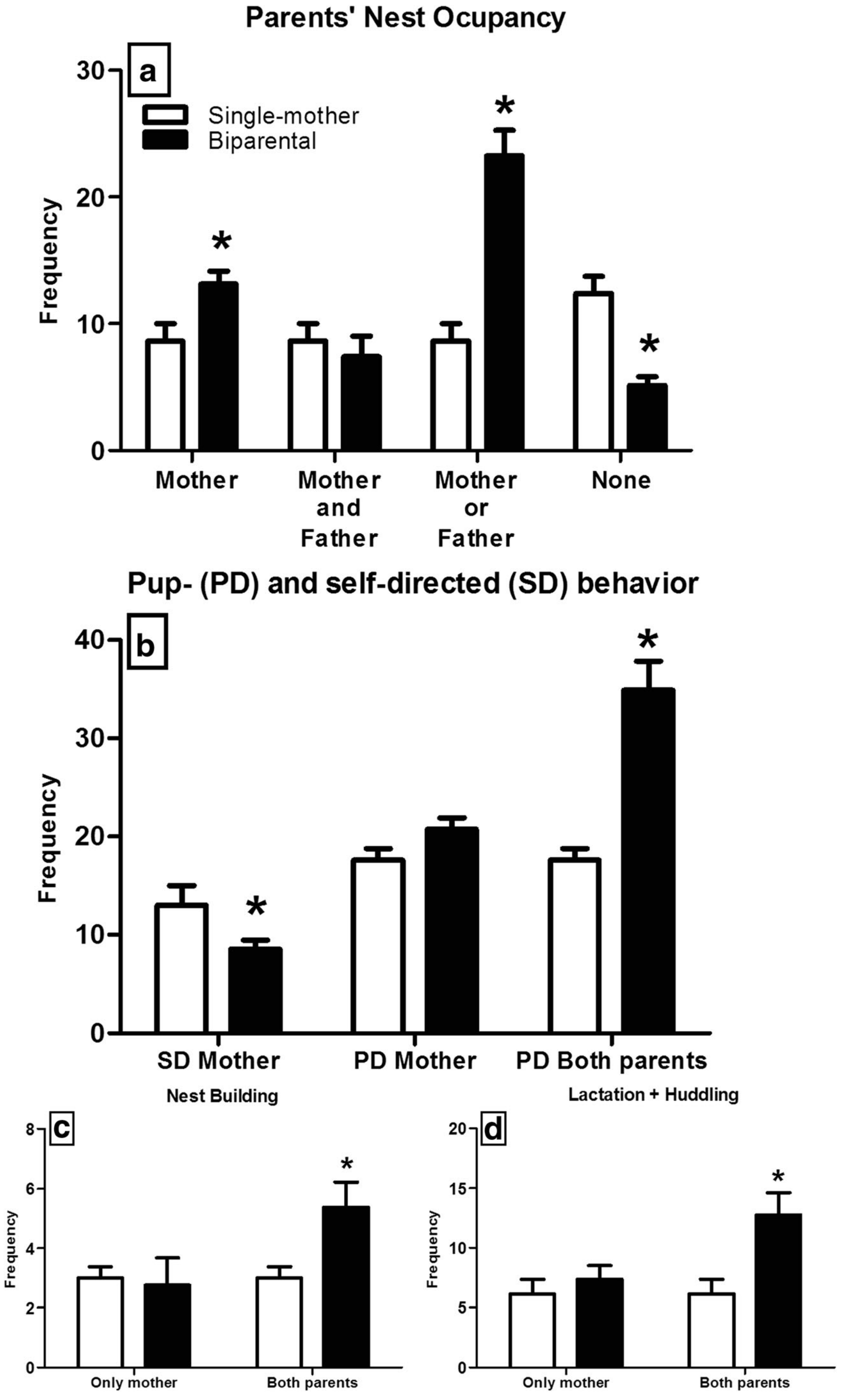

Fig. 1 Total observations of behaviors at PDs 6, 9, and 12 as a function of rearing conditions (single mother or biparental). **a** Parents' nest occupancy category. **b** Pup- (PD) and self-directed (SD) behavior of parents. **c** Nest building behavior. **D** Lactation and huddling behavior. The asterisk (*) sign indicates significant differences between rearing conditions for the specific situations under consideration. Bars represent mean ± SEM

$p < .001$). The interaction of both factors also reached significance ($F_{4,112} = 5.84$, $p < .001$) and indicated that regardless of rearing condition, adolescent females were more active visiting all the areas of the CSF, with the exception of the bridge, than males. In addition, the most visited areas were the open field central arena and the corridors, then the shelter and the ramp and the least frequently visited area was the bridge (all p's < .001).

Regarding risk assessment behavior, the ANOVA for mean duration per visit in the ramp showed significant effects of sex ($F_{1,28} = 22.68$, $p < .001$) and sex × rearing condition ($F_{1,28} = 4.77$, $p < .05$). Male BP adolescents spent more time per visit in the ramp than all the other groups (comparison against SM males reflected a borderline effect, $p = .066$). This risk assessment and risk-taking behavior is also confirmed by the analysis of mean duration per visit in the bridge which indicated a

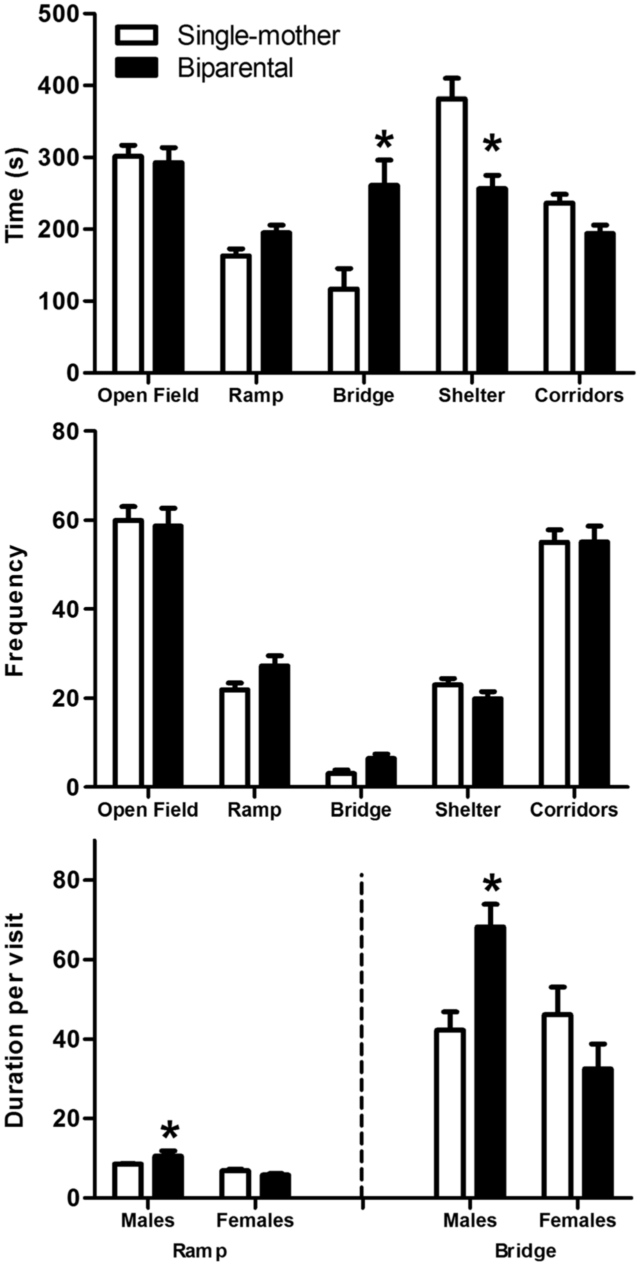

Fig. 2 Performance in the concentric square field (CSF) of adolescents reared by a single mother or both parents. Upper panel depicts the time spent in each area of the CSF. Middle panel depicts the frequency that animals visit each compartment of the CSF and the low panel represents the mean duration per visit in the ramp and bridge areas. The asterisk (*) sign indicates significant differences between rearing conditions for the specific area of the CSF under consideration. Bars represent mean ± SEM

significant main effect of sex ($F_{1,25} = 6.14$, $p < .025$) and the interaction of sex × rearing condition ($F_{1,25} = 9.49$, $p < .01$). BP adolescent males spent more time per visit in the bridge than all the other animals. The coefficient bridge/ramp also attached significance ($F_{1,28} = 8.16$, $p < .01$). The value was significantly higher in BP (1.48 ± .26; mean ± SEM) than SM animals (0.66 ± .11; mean ± SEM). Values higher than 1 indicate that animals spent more time in the bridge relative to the ramp.

Alcohol consumption test

The analysis of total fluid consumption scores (water + alcohol intake) showed significant effects of days of evaluation ($F_{11,330} = 3.12$, $p < .001$). The interaction days of evaluation × rearing conditions also reached significance ($F_{11,330} = 4.53$, $p < .001$). LSD post hoc analysis showed that BP adolescents consumed more fluids on days 1 and 3 and, on the other hand, SM adolescents consumed more fluid on days 9 and 11 of evaluation.

When analyzing water consumption scores, a similar pattern was observed. Days of evaluation ($F_{11,330} = 2.81$, $p < .005$) and the interaction comprising days of evaluation × rearing condition ($F_{11,330} = 3.19$, $p < .001$) significantly affected water intake. BP adolescents consumed more water on day 1 and 3 while SM adolescents showed higher water consumption on days 9 and 11.

Alcohol consumption scores were characterized by a different profile. The ANOVA revealed significant main effects of sex ($F_{1,\ 30} = 14.24$, $p < .001$) and days of evaluation ($F_{11,330} = 2.61$, $p < .005$). Of major importance, rearing condition and days of evaluation significantly interacted ($F_{11,330} = 3.41$, $p < .001$). SM adolescents showed significantly higher alcohol intake scores since day 5 (specifically on days 5, 6, 8, 9, and 11). These results of consumption scores are summarized in Fig. 3.

Correlational analyses

To analyze the interrelation between parenting, offspring adolescent behavior, and alcohol consumption, correlations analyses were performed (Pearson's correlation coefficients with an alpha level set at 0.05). For this purpose, the variables taken into account were the ones significantly affected by rearing conditions. In Exp. 1: nest occupancy by any parent, nest alone, and pup-directed behavior by any parent. In Exp. 2: time in the bridge, time in the shelter, mean duration per visit in the ramp, and bridge. In Exp. 3, alcohol consumption scores during the 12 sessions or during the last eight sessions.

From a correlational perspective, nest occupancy frequency negatively correlated with time spent in the CSF shelter ($r = -.55$; $p < .05$). Nest occupancy also negatively and significantly correlated with alcohol consumption scores of adolescent males during the last eight sessions ($r = -.57$; $p < .05$). When considering nest alone frequency, this variable positively correlated with males' alcohol consumption scores ($r = .60$; $p < .01$).

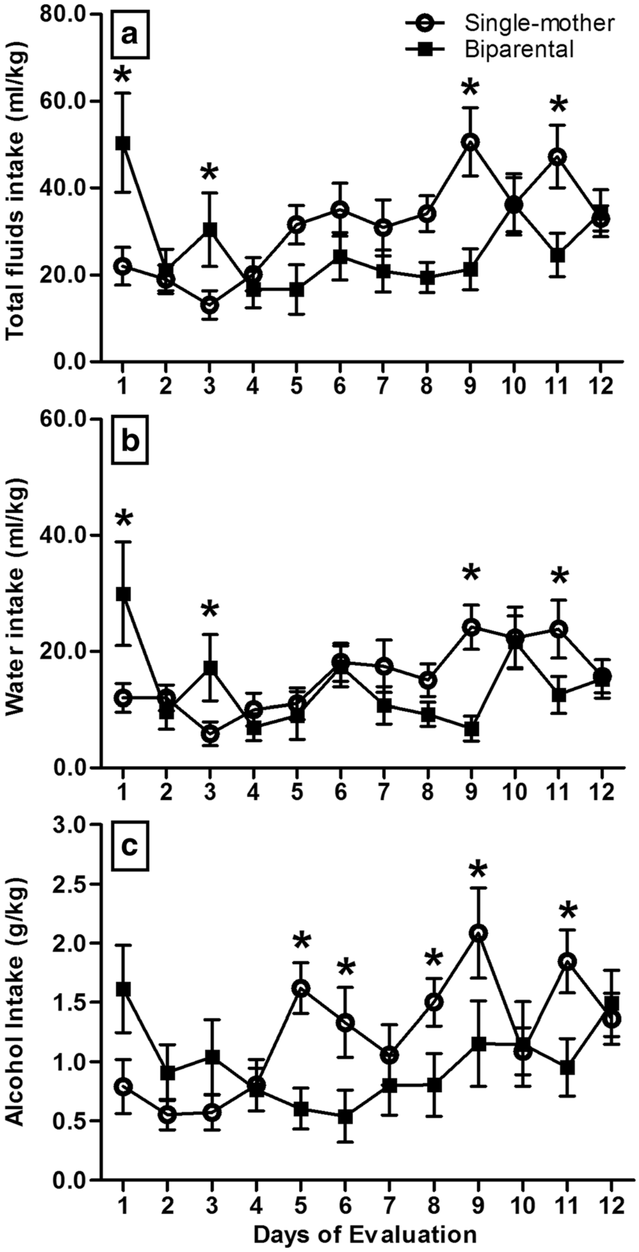

Fig. 3 Fluid intake scores of adolescent mice reared by single mother or both parents during 12 sessions. **a** Total fluids intake scores. **b** Water intake scores. **c** Alcohol intake scores. The asterisk (*) sign indicates significant differences between rearing conditions for the specific evaluation day under consideration. Values represent mean ± SEM

Discussion

The main findings of the present study can be summarized as follows: (i) a distinct parenting behavioral profile was observed in C57BL/6J adult mice as a function of the presence or not of the father during lactation. (ii) Single-mother parenting in this strain of mice evidenced an anxiogenic phenotype on the offspring and less risk-associated behaviors with higher rates of alcohol consumption during adolescence.

Different studies indicate that rats and mice, including the C57BL/6J strain here utilized, do not form selective social bonds between mates, limiting their utility as animal models for the study of more complex family dynamics (Saltzman et al. 2017). Despite the fact that many strains of rats and mice do not provide BP care, the results here reported provide positive evidences relative to paternal care in C57BL/6J mice. In general, maternal behavior was similar across rearing conditions. Instead, when behaviors of fathers were taken into account, significant differences emerged. Our first experiment indicated that fathers actively participated in caregiving evidenced in time devoted to nest building, time in contact with pups, and huddling behavior. A similar result has been observed in the biparental monogamous rodent, the prairie voles: SM-reared pups experience significantly less care and are more likely to be unattended in the nest in comparison to BP-reared counterparts (Ahern and Young 2009). These differences are not due to neglected maternal behavior: pup-directed behavior of dams was similar between both rearing conditions. The observation of the high frequency of pups being alone in the nest within the SM condition is consistent with the findings in BP species. In turn, this suggests that SM mothers would be unable to compensate completely for partner's absence (Ahern and Young 2009). In our experimental model, cohabitation of the male with female since copulation and active exposure to parturition and his own pups (phenomenon called *Pups Sensitization*, Feldman et al. 2019) seemed to result in the expression of paternal behavior. Also in line with studies in BP rodents (Ahern et al. 2011; Lonstein and De Vries 1999; Wang and Novak 1992), fathers and mothers were observed to occupy the nest similar amount of times during the first two postnatal weeks. In this altricial species, nest occupancy during the first two postnatal weeks is essential for survival. Thermoregulation and directed pup behavior (i.e., licking and grooming and adult-pup interaction) usually take place in the nest context (Meaney 2001).

An interesting finding was that differences in adolescent behavior emerged after been raised with the presence or not of the father. SM adolescents spent a greater time in a dark shelter area and less time in the highly illuminated bridge compartment. As predicted and demonstrated by Meyerson et al. (2006), the most evident contrast in this test was the time between the bridge and the shelter areas. It is well known that rodents tend to avoid open, illuminated, and elevated areas, as often seen in tests such as the elevated plus maze test (Doremus et al. 2004) and the light/dark test (Bourin and Hascoet 2003; Miranda-Morales et al. 2014). Hence, the ramp and bridge areas can be considered as anxiogenic zones while the shelter as a secure area. On the other hand, performance in the ramp and bridge is also associated to risk assessment and risk taking. It has been previously reported that this strain of mice has a low risk threshold (Augustsson and Meyerson 2004). We did not find any difference in the frequency that animals visit each area, but a clear avoidance of risk areas and a propensity to seek shelter was found in SM adolescents. In addition, this group of animals showed less risk-associated behaviors such as less time per visit in the ramp and bridge and lower bridge/ramp ratio.

The behavioral profile of SM adolescents could be interpreted as a high anxiogenic basal response. Research with humans and non-human animal models demonstrate that infants raised in adverse environments experience long-life challenges in their ability to generate and regulate normal affective and anxiety responses. Much of what is known about the impact of early life experiences on affective and social development comes from studies on non-human primates raised without their mothers (Gottlieb et al. 2013), rodent models of maternal separation (Bergman 2019; Rincel and Darnaudery 2019), or with different qualities of maternal care during early childhood (Curley and Champagne 2016; Meaney 2001). Few studies have started to address the role that the social group or family structure, in which the organism develops, has on anxiety responses. Recently, Bliss-Moreau et al. (2017) evidenced that 3- to 4-month-old infants raised in restricted social environments (defined as the number of individuals in one's social group) had significantly higher heart rates, lower respiratory sinus arrhythmia, and a more potent stress response, compared to infants raised in unrestricted social environments. These results emphasize the importance of social group and the number of individuals of the group in which subjects are raised for later affective processing. The hypothesis that SM adolescents exhibit heightened levels of anxiety has received support through a recent study that we have conducted using similar rearing conditions but when assessing adolescent behavior via the elevated plus maze (Pasquetta et al., in preparation).

The differences here reported in mono- and biparental behavior throughout the first 2 weeks of lactation seemed to directly impact on offspring adolescent behavior. Indeed, there is converging evidence that differences in family structure do exert long-lasting effects. Alloparental behavior can be also affected, where BP-reared females (Ahern and Young 2009) and males (Wang and Novak 1994) exhibit higher levels of spontaneous alloparenting. Relative to pair-bonding, BP-reared females were observed to form stable partner preferences more quickly than SM-reared females (Ahern et al. 2011). Similarly, the loss of a social bond partner results in increased anxiety-like behaviors in the elevated plus maze and the light/dark box test and increases depressive-like behaviors in the forced swim test (Sun et al. 2014).

Relative to adolescent initiation of alcohol consumption, SM-derived adolescents showed, after the first week of consumption, higher alcohol drinking scores. To our knowledge, this is the second study addressing offspring alcohol consumption as a function of differential parenting. Ryabinin and colleagues have established the socially monogamous prairie vole as an animal model to study the reciprocal relationship between social relationships and alcohol intake (Anacker et al. 2011a, b, 2012, 2014a; Anacker and Ryabinin 2013; Hostetler et al. 2012; Walcott and Ryabinin 2017, 2019). Nevertheless, when they addressed the consequences of early life family structure upon subsequent alcohol use, they found that this social factor did not significantly affect any measure of alcohol drinking (Anacker et al. 2012). Instead, our results indicate, at least in this animal model, that single-mother parenting is a relevant risk factor for alcohol initiation use during adolescence. Animal models of poor or diminished parenting, e.g., maternal deprivation, have been successful in highlighting the importance of this sensitive period for the later prevalence for alcohol use and abuse (Gondre-Lewis et al. 2016; Odeon and Acosta 2019; Portero-Tresserra et al. 2018). This lends support to the literature regarding human adolescence alcohol drinking (Fisher et al. 2007; Ledoux et al. 2002; Scalese et al. 2014; Sharmin et al. 2018; Tomcikova et al. 2015). Our results emphasize that also the structure of the family and amount of time devoted to the offspring care during the neonatal and infancy period plays a significant role relative to subsequent alcohol affinity. The positive significant correlation between frequency of nest alone and alcohol drinking in male adolescents support this hypothesis. When SM adolescents' behavior in the CSF and their alcohol intake profile are taken together, it could be hypothesized that the anxiogenic-like phenotype of these subjects has a greater propensity for alcohol intake during adolescence. In other words, the anxiolytic effects of alcohol could be underlying the exacerbated intake of the drug of MS adolescents. The present finding is consistent with previous studies in mice (Lopez et al. 2011) and rats (Fernández et al. 2016) whereas an anxiogenic state during adolescence enhances subsequent alcohol consumption.

In conclusion, our study shows that single-mother parenting implies that offspring is more time exposed and unattended which, in turn, induces an anxiogenic phenotype and low-risk assessment/risk-taking-associated behaviors during adolescence. This behavioral phenotype is a risk factor plausible to increase alcohol drinking. Further studies have been conducted devoted to analyze the neurobiology underlying theses effects and to continue exploring how family structure during early developmental stages may influence offspring's behavior and response to alcohol.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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