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ORIGINAL INVESTIGATION

Juvenile social isolation affects maternal care in rats: involvement of allopregnanolone

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Abstract
Rationale Social isolation of rats immediately after weaning is thought to represent an animal model of anxiety-like disorders. Socially isolated virgin females showed a significant decrease in allopregnanolone levels, associated with increased anxiety-related behavior compared with group-housed rats.
Objectives The present study investigates whether post-weaning social isolation affects maternal behavior and assesses neuroactive steroid levels in adult female rats during pregnancy and postpartum.
Results Socially isolated dams displayed a reduction in the frequency of arched back nursing (ABN) behavior compared to group-housed dams. In addition, both total and active nursing were lower in socially isolated dams compared to group-housed dams. Compared to virgin females, pregnancy increases allopregnanolone levels in group-housed as well as isolated dams and such increase was greater in the latter group. Compared to pregnancy levels, allopregnanolone levels decreased after delivery and this decrease was more pronounced in isolated than group-housed dams. Moreover, the fluctuations in plasma corticosterone levels that occur in late pregnancy and during lactation follow a different pattern in socially isolated vs. group-housed rats.
Conclusions The present results show that social isolation in female rats decreases maternal behavior; this effect is associated with lower allopregnanolone concentrations at postpartum, which may account, at least in part, for the poor maternal care observed in socially isolated dams. In support of this conclusion is the finding that finasteride-treated dams, which display a decrease in plasma allopregnanolone levels, also showed a marked reduction in maternal care, suggesting that allopregnanolone may contribute to the quality of maternal care.

Keywords Social isolation · Allopregnanolone · Maternal care · Female rat · Finasteride

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Introduction

Increasing evidence suggests that depression and anxiety in the mother elicit negative effects on both parenting and offspring outcomes (Goodman et al. 2014). Some preclinical studies have shown a detrimental effect of chronic social stress on maternal behavior of lactating rats, as well as on the growth, social behavior, and endocrine function of their offspring (Nephew and Bridges 2011; Murgatroyd and Nephew 2013; Babb et al. 2014). Social isolation is a model of prolonged mild stress and is associated with marked behavioral alterations, such as increased locomotor activity, anxiety, and aggression in laboratory animals (Fone and Porkess 2008). We have shown that socially isolated female rats, similarly to males (Serra et al. 2000), manifested an anxiety-like profile in the elevated plus-maze and Vogel conflict tests compared with group-housed controls (Pisu et al. 2013). Accordingly, in female rats, social isolation markedly decreased brain and plasma concentrations of the neuroactive steroid allopregnanolone (Pisu et al. 2013; Pisu et al. 2016),

a positive modulator of γ-aminobutyric acid type A ($GABA_A$) receptors (Majewska 1992) that exerts anxiolytic and antidepressant properties (Bitran et al. 1991, 1995; Khisti et al. 2000). Allopregnanolone concentrations vary across the lifespan, particularly during early life, puberty, or aging (Genazzani et al. 1998; Grobin and Morrow 2001; Shen et al. 2007). Moreover, levels of allopregnanolone are dramatically increased in rat plasma and brain during gestation and in blood of pregnant women (Concas et al. 1998; Bicíková et al. 2002; Paoletti et al. 2006); such fluctuations play a key role in the suppression of maternal hypothalamic-pituitary-adrenal (HPA) axis responses to stress (Brunton and Russel 2011; Brunton et al. 2014). In rats, the maximal levels of allopregnanolone are reached at the end of pregnancy (day 19–20), before dramatically falling immediately before delivery (Concas et al. 1998). It has been hypothesized that the substantial decreases in the concentrations of this neuroactive steroid may confer an arousal state characteristic of the period that immediately precedes delivery, as well as of the early postpartum, which may play a role in predisposition to postpartum depression (Carter et al. 2001). Moreover, the reductions in allopregnanolone levels may be crucial to remove the inhibitory action exerted by this $GABA_A$ receptor-acting steroid on hypothalamic neurons that release oxytocin, which facilitates the initial expression of maternal behavior (Herbison 2001). Allopregnanolone levels parallel those of progesterone that are elevated throughout pregnancy and decline prior to delivery; this fall plays an important role in regulating the expression of maternal behavior. In fact, progesterone treatment after surgical termination of pregnancy in primigravid rats inhibits the onset on maternal behavior (Bridges et al. 1978). It was postulated that progesterone's inhibitory action of the onset of maternal behavior is mediated by the progesterone receptor, since administration of its antagonist RU-486 during pregnancy reversed progesterone's effect (Numan et al. 1999). Mann (2006) had examined the possibility that the inhibitory action of progesterone on maternal behavior may be due to its metabolite allopregnanolone by treating pregnant animals with finasteride, a 5α-reductase inhibitor that blocks the conversion of progesterone to allopregnanolone (Azzolina et al. 1997). The results demonstrated that allopregnanolone is not involved in the inhibitory action of progesterone on maternal behavior, but, intriguingly, administration of finasteride significantly delayed the onset of maternal behavior in females, which also displayed anxiety-like behavior, suggesting a role for allopregnanolone in maternal behavior.

Given that social isolation during the juvenile period generates adult rats with an anxious phenotype, and with low levels of brain and plasma allopregnanolone (Pisu et al. 2016), the present study was undertaken to evaluate whether exposure to this social stress alters maternal care in adult lactating dams. Moreover, given that maternal nurturing is influenced by the emotional state of the dam, we evaluated plasma levels of allopregnanolone and corticosterone (a marker of HPA axis function) through pregnancy and postpartum in socially isolated and group-housed dams. We hypothesized that the pattern of secretion of these stress-sensitive neuroactive steroids during pregnancy and postpartum is altered by social isolation and may influence the quality of maternal care. Indeed, in support to this hypothesis, it has been demonstrated that corticosterone affects maternal care (Brummelte and Galea 2010). To further evaluate the role of allopregnanolone in maternal behavior, we assessed maternal care in group-housed dams in which progesterone's conversion to its neuroactive metabolite was blocked by finasteride administration after delivery, in order to decrease allopregnanolone levels and thus mimic the low levels of this neuroactive steroid observed in socially isolated dams. In summary, we hypothesized that chronic stress exposure due to social isolation from weaning to young adulthood may induce physiological and behavioral changes during pregnancy and lactation that result in a reduction in maternal care; such reduction may be related to the altered pattern of allopregnanolone concentrations induced by juvenile social isolation.

Experimental procedures

Animals

Experiments were performed in Sprague-Dawley female rats from our colony, generated from breeders obtained from Charles River (Calco, Italy). All animals were maintained under an artificial 12-h-light, 12-h-dark cycle at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available at all time. Adequate measures were taken to minimize pain or discomfort of animals whose care and handling throughout the experimental procedures were in accordance with the European Parliament and the Council Directive of 22 September 2010 (2010/63/EU) and were approved by the Italian Ministry of Health (no. 1062/2016-PR) according to the Italian Legislative Decree no. 26 of 4 March 2014.

Juvenile social isolation

Male and female Sprague-Dawley rats at 21 days of age, immediately after weaning, were housed for 30 days either in groups of five per cage (59 by 38 by 20 cm; group-housed) or individually in smaller cages (42 by 26 by 15 cm; socially isolated). For the breeding procedure, 51-day-old males and females were paired for 5 days; socially isolated females were bred with socially isolated males, and group-housed females were bred with group-housed males, in order to match the same protocol previously described by Pisu et al. (2013).

The day in which sperm was detected in the vaginal smear was designated as gestational day 0 (G0). Upon gestational status identification, female rats were either singly housed or group-housed (depending on which experimental group they belonged to, socially isolated or group-housed) until gestational day 20, when every rat was singly housed for parturition and subsequent nursing. Separate groups of socially isolated and group-housed female rats were used for the observation of maternal care (n = 11 per experimental group) and to assess hormone levels in virgin controls, during pregnancy (G17), and postpartum (PP3) (n = 15 rats per experimental group) (see Fig. 1a for the experimental timeline).

Finasteride treatment

To assess the role of allopregnanolone in maternal care, a separate cohort of group-housed dams (n = 11 per group) was subcutaneously treated with finasteride (50 mg/kg/2 ml, Steraloids Inc., Newport, RI, USA) once daily (at 17:00 h) for 3 consecutive days, starting from postpartum day 0 (PP0). Control rats received an equal volume of vehicle (sesame oil; Sigma-Aldrich, Milan, Italy). Maternal care was evaluated for each dam from PP1 to PP3; upon the last behavioral observation on PP3 (09:00 h), dams were euthanized for allopregnanolone measurement. A timeline of the experimental procedures is shown in Fig. 1b.

Social isolation during pregnancy

Fifty-one-day-old group-housed males and females were paired for 5 days. Upon gestational status identification (see above), female rats were either singly housed or group-housed during pregnancy, until gestational day 20, when all rats were singly housed for parturition and subsequent nursing. The same groups of socially isolated and group-housed female rats (n = 11 per experimental group) were used for the observation of maternal care and to assess allopregnanolone levels on PP3. A timeline of the experimental procedures is shown in Fig. 1c.

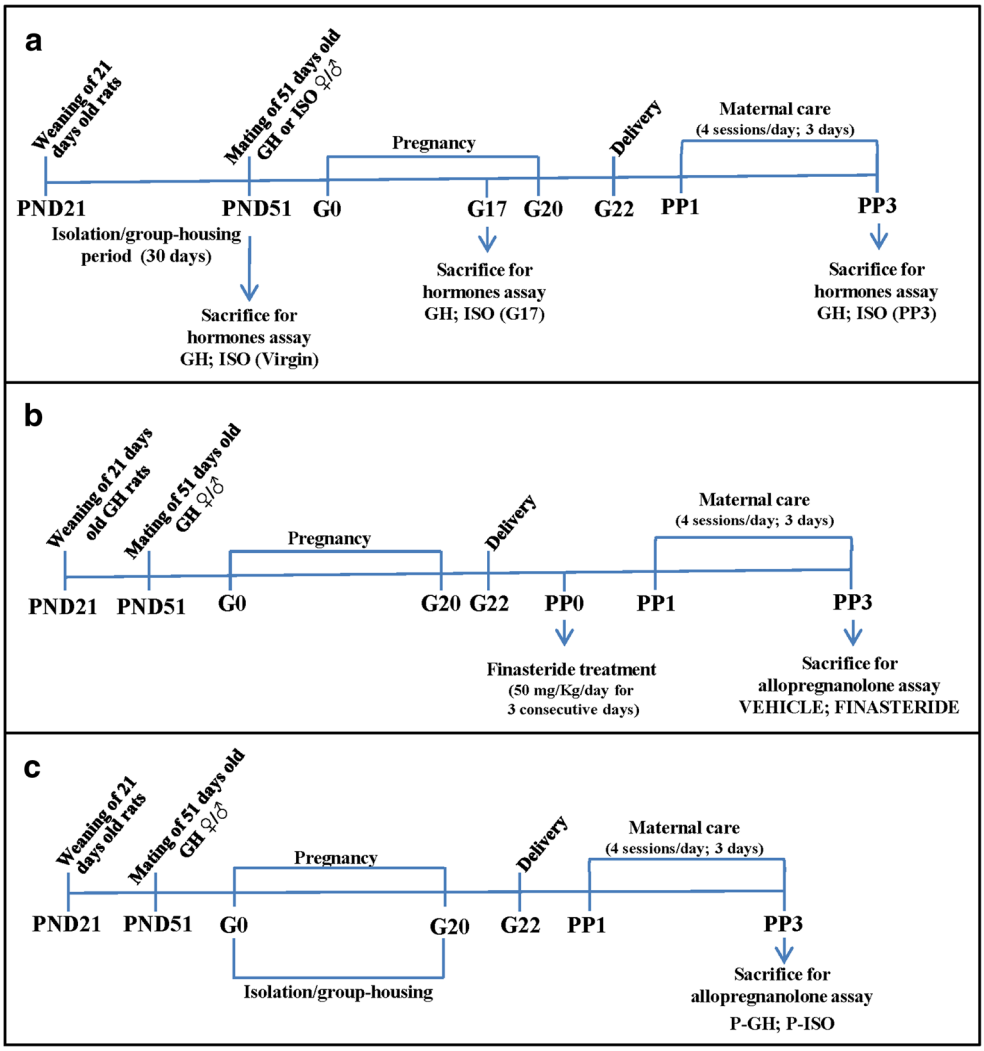

Fig. 1 Timeline of the experimental procedures. **a** Juvenile social isolation. *GH* group-housed, *ISO* socially isolated rats. **b** Finasteride treatment in group-housed rats. **c** Adult social isolation during pregnancy. *P-GH* group-housed rats during pregnancy, *P-ISO* socially isolated rats during pregnancy

Observation of maternal care

Litters were monitored to ensure the presence of an equal number of male and female pups for each mother in order to avoid any sex bias in maternal care. The behavior of each dam was observed in four daily 75-min observation sessions from postpartum days 1 (PP1) to 3 (PP3). Observations occurred at regular times each day with one session during the dark phase (08:00 h) and three sessions during the light phase (09:00, 13:00, and 16:00 h) of the light/dark cycle (lights on at 09:00 h). This distribution was based on the finding that nursing in rats occurs more frequently during the light phase. Data were expressed as percentage of observations in which every pup-related behavior occurred/total behaviors. Within each observation session, the ongoing behavior of each mother was observed 25 times, once every 3 min, and recorded on a checklist. In this way, every mother was observed 100 times per day (25 observations per session, four sessions per day = 100 observations/mother/day).

The following behaviors were scored: (1) total nursing (active + passive + arched-back posture), (2) nursing pups in a blanket posture (active nursing) or (3) in an arched-back posture (ABN), (4) passive nursing, (5) licking and grooming of any pup (LG), (6) nest building, (7) self grooming, (8) staying in the nest in contact with the pups (in nest), (9) resting with no contact with the pups (resting), and (10) eating or drinking. A detailed description of these behaviors is provided by Myers et al. (1989). Note that behavioral categories are not mutually exclusive. For example, LG often occurred while the mother was nursing the pups. The characterization of individual mothers depends upon the reliability of the cohort dataset. To provide more reliable estimates of individual differences in maternal behavior, for each experiment, we observed cohorts of 22 mothers/litters (11 for each experimental group); data were analyzed by Student's *t* test. Maternal care was recorder by an experienced observer blind to the experimental groups until the analysis of results.

Measurement of hormone levels

For measurement of allopregnanolone and corticosterone levels, all animals were sacrificed by decapitation between 10:30 and 12:00 h. Blood was collected from the trunk into K3-EDTA tubes, centrifuged at 900×*g* for 10 min at 4 °C, and frozen at −80 °C until use. Steroid levels were assayed in plasma from the same rats. Estrous cycle in virgin controls was not assessed to reduce manipulation of the animals.

Allopregnanolone was extracted from plasma as previously described (Serra et al., 2000). The combined organic phases were dried under vacuum. The recovery (70 to 80%) through the extraction procedure was monitored by addition of a trace amount (6000 to 8000 cpm, 20 to 80 Ci/mmol) of [^{3}H] allopregnanolone (Perkin Elmer Italia, Monza, Italy) to the plasma samples. Allopregnanolone levels were quantified by radioimmunoassay with a specific antibody raised in rabbit, as previously described (Serra et al. 2000; Purdy et al. 1990).

An enzyme-linked immunosorbent assay (ELISA) was used to quantify plasma levels of corticosterone (IBL International, Hamburg, Germany). ELISA was performed according to the manufacturer's instructions, using a 96-well plate that was pre-coated with a primary antibody. Each sample was run in duplicate.

Statistical analysis

Quantitative data are presented as means ± SEM. Unpaired Student's *t* test was used to analyze maternal behavior data and allopregnanolone assay in finasteride and isolation during pregnancy protocols; two-way ANOVA considering the housing (group-housed vs. isolated) and status (virgin, pregnancy and postpartum) effects, followed by Newman-Keuls post hoc test, was used to analyze hormone assays data in juvenile social isolation protocol. A value of $p < 0.05$ was considered statistically significant.

Results

Juvenile social isolation decreases maternal care

Maternal care was observed during postpartum days 1–3 in both isolated and group-housed dams. As shown in Fig. 2, socially isolated dams displayed a reduction in the frequency of total nursing (−20%, [t(218) = 3.72, $p = 0.009$], Fig. 2a), active nursing (−18%, [t(218) = 3.01, $p = 0.01$], Fig. 2b), and ABN (−59%, [t(218) = 4.61, $p = 0.001$], Fig. 2c), compared to group-housed dams across all days of the observation period. By contrast, no difference in LG behavior was observed between the two experimental groups ([t(218) = 1.11, $p = 0.27$], Fig. 2d). Moreover, no significant changes in the mean percentage frequency of pup-directed behaviors (nursing in a passive posture; nest building) or non pup-directed behaviors (self-grooming; in nest; resting; eating and drinking) were found between socially isolated and group-housed dams (data not shown).

Juvenile social isolation alters allopregnanolone levels during pregnancy and postpartum

Plasma allopregnanolone and corticosterone were measured in control (virgin) rats, during pregnancy (G17), and on postpartum day 3 (PP3) in both socially isolated and group-housed dams (Fig. 3).

Two-way ANOVA for allopregnanolone revealed a significant effect of status [F(2, 84) = 157.32, $p = 0.0003$], no

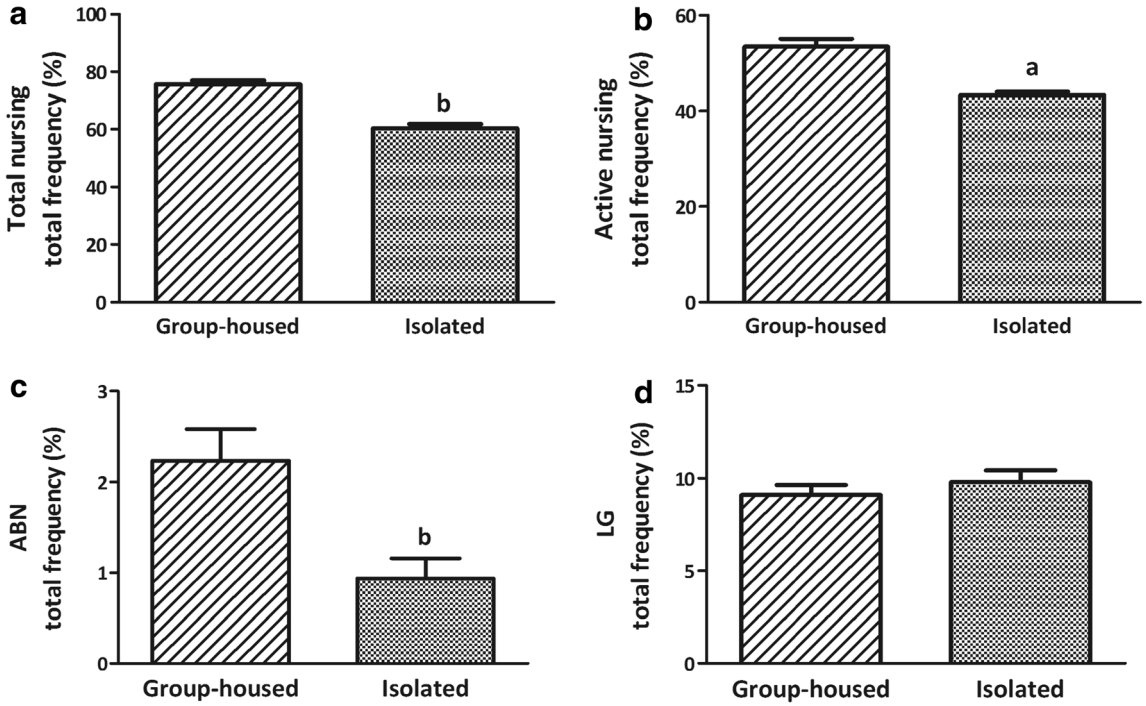

Fig. 2 Juvenile social isolation decreases maternal care behavior in dams. Maternal care was observed during the first 3 days postpartum in isolated and group-housed dams. Behaviors are expressed as a percentage of the number of observations for each behavior/total observations over time (frequency %) across all days of the observation period and are means ± SEM of 11 rats per group. Data were analyzed by unpaired Student's *t* test ($^{a}p < 0.05$; $^{b}p < 0.01$ vs. group-housed dams)

significant effect of housing condition [F(1, 84) = 0.667, $p = 0.402$], and a significant interaction between factors [F(2, 84) = 7.807, $p = 0.004$]. As expected (Pisu et al. 2013, 2016), social isolation decreased allopregnanolone levels in virgin females compared to the respective group-housed controls (−58%, $p < 0.05$, Fig. 3a). Compared to virgin females, pregnancy increased allopregnanolone levels in group-housed as well as isolated dams, and such increase was greater in the latter group (+679 vs. +198%, in socially isolated and group-housed rats, respectively, $p < 0.05$, Fig. 3a). As expected (Concas et al. 1998), allopregnanolone levels decreased after delivery (PP3) compared to pregnancy levels, and this decrease was more pronounced in isolated dams with respect to group-housed dams (−66 vs. −43%, respectively, $p < 0.05$, Fig. 3a).

Two-way ANOVA for corticosterone revealed a significant effect of housing [F(1, 84) = 3.751, $p = 0.01$], no significant effect of status [F(2, 84) = 0.786, $p = 0.752$], and a significant interaction between factors [F(2, 84) = 6.796, $p = 0.03$]. As expected (Pisu et al. 2016), social isolation decreased plasma corticosterone levels in virgin females compared to the respective group-housed controls (−56%, $p < 0.05$, Fig. 3b).

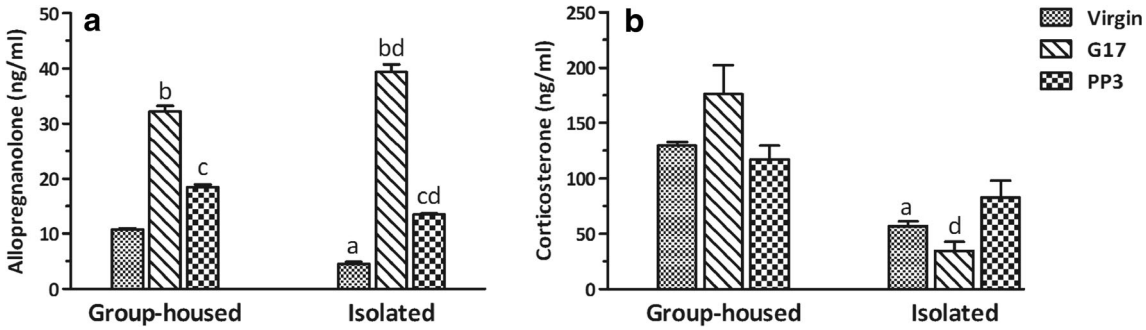

Fig. 3 Allopregnanolone and corticosterone levels during pregnancy and postpartum in group-housed and socially isolated dams. **a** Allopregnanolone and **b** corticosterone were measured in plasma samples from virgin control rats, at day 17 of pregnancy (G17) and at postpartum day 3 (PP3) in group-housed and socially isolated dams. Data are expressed as ng/ml and are means ± SEM of values from 15 rats per group. Data were analyzed by two-way ANOVA followed by Newman-Keuls post hoc test. $^{a}p < 0.05$, $^{b}p < 0.01$ vs. the respective virgin group; $^{c}p < 0.01$ vs. the respective pregnancy group; $^{d}p < 0.05$ vs. the respective group-housed counterpart

Moreover, compared to the respective virgin controls, pregnancy decreased corticosterone levels in socially isolated rats (−40%), while it increased them in group-housed rats (+35%); however, these effects did not reach statistical significance ($p = 0.08$ and $p = 0.42$, respectively; Fig. 3b). Compared to the respective pregnant rats, group-housed dams showed a slight decrease (−33%) in corticosterone levels at PP3, while in isolated rats, corticosterone levels increased by 141%, although this effect was not statistically significant ($p = 0.40$ and $p = 0.16$, respectively; Fig. 3b).

Decreased allopregnanolone following finasteride treatment reduces maternal care

To evaluate a putative role for allopregnanolone in maternal care behavior, group-housed dams were subjected to finasteride treatment (50 mg/kg, s.c.) for three consecutive days starting from PP0. Overall, finasteride administration reduced maternal care behavior (Table 1). A significant decrease in the frequency of total nursing (−15%, [t(218) = 2.18, $p = 0.031$]), active nursing (−18%, [t(218) = 2.49, $p = 0.014$]), and ABN (−66%, [t(218) = 2.62, $p = 0.009$]) has been observed in finasteride-treated dams, compared to vehicle-treated dams. Similar to the results obtained in socially isolated dams, finasteride treatment did not affect LG behavior [t(218) = 1.28, $p = 0.21$] (Table 1); moreover, no significant changes in the mean percentage frequency of pup-directed behaviors (nursing in a passive posture, nest building) or non pup-directed behaviors (self-grooming, in nest, resting, eating and drinking) were found between finasteride and vehicle-treated dams (data not shown). As expected (Concas et al. 1998), finasteride administration decreased plasma allopregnanolone levels in these animals (−35%, [t(20) = 3.899, $p = 0.001$] vs. vehicle-treated dams, Fig. 4).

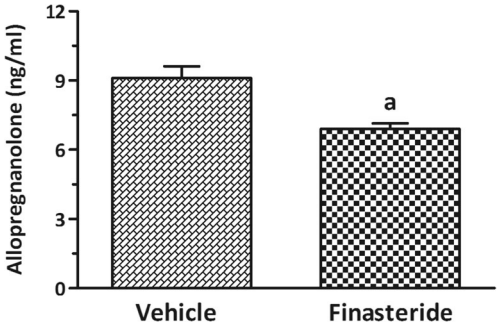

Fig. 4 Finasteride treatment during postpartum decreases allopregnanolone levels in group-housed dams. Group-housed dams were treated once daily for three consecutive days starting on postpartum day 0 (PP0) with finasteride (50 mg/kg/2 ml, s.c.) or an equal volume of vehicle (sesame oil) and were sacrificed on PP3. Data are expressed as ng/ml and are means ± SEM of values from 11 rats per group. Data were analyzed by Student's *t* test. [a] $p < 0.01$ vs. vehicle-treated dams

Adult social isolation during pregnancy failed to decrease maternal care

To ascertain if the reduction in maternal care is due to the stress induced by isolation rearing starting from a juvenile period or to the isolation housing throughout pregnancy, a group of female rats, normally reared in group until mating, was socially isolated from mating and for the entire pregnancy period; maternal care was assessed during postpartum days 1–3. Adult social isolation during pregnancy failed to affect frequency of total nursing (+14%, [t(218) = 0.86, $p = 0.389$]), active nursing (+20%, [t(218) = −1.49, $p = 0.149$]), ABN (+15%, [t(218) = −0.51, $p = 0.608$]), and LG (−6%, [t(218) = 0.92, $p = 0.358$]) (Table 2). Likewise, no changes in the mean percentage frequency of pup-directed behaviors (nursing in a passive posture, nest building) or non-pup-directed behaviors (self-grooming, in nest, resting, eating

Table 1 Finasteride treatment during postpartum decreases maternal care behavior in group-housed dams

Maternal care	Vehicle Frequency %	Finasteride Frequency %
Total nursing	66.03 ± 3.03	56.20 ± 3.31[a]
Active nursing	43.74 ± 2.34	35.68 ± 2.22[a]
Arched-back nursing	2.99 ± 0.67	1.03 ± 0.32[b]
Licking/grooming	7.75 ± 0.63	6.60 ± 0.66

Maternal care was observed on postpartum days 1 to 3 in finasteride and vehicle-treated dams. Finasteride (50 mg/kg in 2 ml of sesame oil) was administered s.c. once daily for three consecutive days, starting at PP0. Behaviors are expressed as a percentage of the number of observations for each behavior/total observations over time (frequency %) across all days of the observation period and are means ± SEM of 11 rats per group. Data were analyzed by unpaired Student's *t* test. [a] $p < 0.05$, [b] $p < 0.01$ vs. vehicle-treated dams

Table 2 Adult social isolation during pregnancy does not alter maternal care behavior

Maternal care	Group-housed Frequency %	Isolated Frequency %
Total nursing	66.64 ± 4.10	76.17 ± 4.66
Active nursing	34.78 ± 1.37	41.78 ± 1.45
Arched-back nursing	0.88 ± 0.17	1.01 ± 0.22
Licking/grooming	10.91 ± 0.58	10.15 ± 0.55

Group-housed rats were mated and, upon mating verification, were either housed in isolation for the entire pregnancy time or were group-housed until gestational day 20, when every rat was separated for parturition and subsequent nursing. Maternal care was observed in dams from postpartum days 1 to 3. Behaviors are expressed as a percentage of the number of observations for each behavior/total observations over time (frequency %) across all days of the observation period and are means ± SEM of 11 rats per group. Data were analyzed by unpaired Student's *t* test

and drinking) were found between the two experimental groups (data not shown). Allopregnanolone levels, measured on PP3 after the last behavioral observation, did not differ between dams group-housed or socially isolated throughout pregnancy [t(20) = 0.927, p = 0.361] (Fig. 5).

Discussion

In the present study, we show that social isolation of female rats, from weaning to young adulthood, decreased maternal care behavior. This effect is accompanied by changes in the pattern of secretion of plasma allopregnanolone during pregnancy and postpartum, suggesting that this neuroactive steroid may play a role in regulation of maternal care behavior. In support of this hypothesis, we show that blocking the conversion of progesterone to allopregnanolone with the 5α-reductase inhibitor finasteride reduced maternal care behavior in group-housed dams. Moreover, we further show that being exposed to social isolation stress early in life, from weaning to adulthood, has long-lasting consequences for future motherhood, as opposed to being exposed to this stress later in life and only during the entire time of pregnancy.

Social isolation decreased the frequency of maternal ABN across the first 3 days postpartum. ABN, characterized as the only active nursing posture, with the dam being fully engaged in a quiescent kyphotic posture related to the quality and quantity of pup stimuli, is, per se, an important parameter for discriminating the quality of maternal behavior (Caldji et al. 1998; Stern and Johnson 1990). In addition to ABN, the total time spent on nursing and the active nursing are lower in socially isolated dams compared to group-housed ones. An important determinant in the quality of maternal care is the emotional state of the dam; in agreement, it has been reported that the amount of maternal care directed towards the pups correlates with the innate or stress-induced mother's anxiety

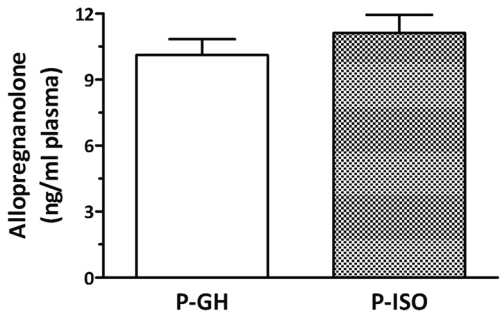

Fig. 5 Social isolation during pregnancy fails to change postpartum allopregnanolone levels in rats. Female rats were either singly housed (P-ISO) or group-housed (P-GH) during pregnancy, until gestational day 20, when every rat was singly housed for parturition and subsequent nursing. Allopregnanolone levels were assessed on PP3. Data are expressed as ng/ml and are means ± SEM of values from 11 rats per group. Data were analyzed by Student's t test

(Carini and Nephew 2013; Clinton et al. 2007; Smith et al. 2004). Accordingly, socially isolated virgin female rats manifest an anxiety-like profile in the elevated plus-maze and Vogel conflict tests. In fact, in these animals, the time spent and the number of entries into the open arms of the maze were reduced by 53 and 42%, respectively (Pisu et al. 2013). Likewise, social isolation also significantly reduced (−44%) the punished consumption of water in the Vogel conflict test (Pisu et al. 2013). Fittingly, nursing percentage is different between naïve GH dams that were left undisturbed during lactation (Fig. 2) and vehicle-treated GH dams that were manipulated during the first 3 days after parturition, in order to be injected with vehicle (Table 1). The stress due to the injection procedure may very likely have decreased maternal care behavior to levels similar to those induced by social isolation. Accordingly, it has been shown that even the stress due to gestational manipulations influences maternal behavior (Popoola et al., 2015).

Social isolation did not affect the frequency of LG. Decreased maternal LG has been associated with increased behavioral responses to stress, a consequence of an alteration of the epigenome at the glucocorticoid receptor gene promoter in the hippocampus of the offspring (Liu et al., 1997; Meaney, 2001; Weaver et al., 2004). Our previous study on offspring of isolated parents indicated that these animals show an increased basal HPA activity and enhanced negative feedback regulation in association with upregulation of glucocorticoid receptor expression in the hippocampus (Pisu et al., 2013).

Socially isolated female rats also have reduced basal concentrations of allopregnanolone in both cerebral cortex and plasma (Pisu et al. 2013, 2016), a condition associated to depression and altered emotional state (Pisu and Serra 2004; Girdler and Klatzin 2007; Girdler et al. 2012). Although the fluctuations in plasma allopregnanolone levels that occur during pregnancy and after delivery (Concas et al. 1998) show a similar trend in socially isolated and group-housed rats, allopregnanolone levels during pregnancy are increased to a greater extent in socially isolated rats, compared to the group-housed counterpart. It is widely accepted that during pregnancy, both basal and stress-stimulated HPA axis activities are blunted to protect the developing fetus from the adverse effects of glucocorticoids, and that allopregnanolone plays a critical role in this regulation (Brunton and Russell 2008, 2011). Given that socially isolated females have lower basal allopregnanolone levels compared to group-housed controls (Pisu et al. 2016), they may be required, during pregnancy, to increase allopregnanolone levels to a greater extent compared to group-housed controls, in order to counteract the altered emotional state and the HPA hyperresponsiveness to glucocorticoids induced by social isolation (Pisu et al. 2013, 2016) and thus ensure a successful pregnancy.

During the postpartum period, the reduction in allopregnanolone content is more pronounced in isolated dams,

resulting in circulating levels that are significantly lower in this latter group, compared to group-housed dams. As stated, allopregnanolone is one of the most potent and efficacious positive allosteric modulators of $GABA_A$ receptor function (Majewska 1992; Lambert et al. 1995), and its administration to animals, either systemically or intracerebroventricularly, induces marked anxiolytic and antidepressant effects (Bitran et al. 1991, 1995; Khisti et al. 2000). Accordingly, a decrease in brain and plasma allopregnanolone concentrations, induced by long-term treatment with ethinyl estradiol plus levonorgestrel, two of the most widely used steroids in the hormonal contraceptive pill, or by ovariectomy or administration of finasteride is associated with anxiety-like behavior in female rats (Follesa et al. 2002; Porcu et al. 2012; Smith et al. 1998; Zimmerberg and Farley 1993). Thus, the greater reduction in allopregnanolone concentrations, observed during lactation in socially isolated dams compared to group-housed dams, may play a role in regulating not only the basal emotional state (Serra et al. 2000; Pisu et al. 2013; Backstrom et al. 2014) but also the quality of maternal behavior. In support of the involvement of allopregnanolone in the quality of maternal care is the observation that administration of finasteride also decreases the mean frequency of maternal care in group-housed dams and, as expected (Concas et al. 1998), reduces plasma levels of allopregnanolone. Moreover, the evidence that social isolation of adult female rats limited to the entire period of gestation, a condition that does not induce anxiety (de Brito Guzzo et al. 2015), failed to decrease both maternal behavior and allopregnanolone levels, further sustains our hypothesis of the involvement of allopregnanolone in maternal care.

The observation that postpartum plasma allopregnanolone levels in socially isolated dams (Fig. 3) and those in vehicle-treated dams (Fig. 4) are very similar may mislead the results. However, it must be taken into account that these experiments were conducted in different groups of rats and in different seasons. Seasonal changes for circulating steroid hormones have been reported in mice and rats (Pyter et al., 2007; Otsuka et al., 2012; Cahill et al., 2013). To the best of our knowledge, no evidences for a seasonal alteration in basal allopregnanolone levels have been reported yet; however, we cannot rule out the possibility that similar alterations may occur and may have contributed to the differences in basal allopregnanolone levels in our experimental groups. Another important factor that may account for differences in basal levels of allopregnanolone is that hormone assays were performed with different batches of antibody, which result in a slightly different standard curve; thus, the reported values are related to the respective standard curve and are not "absolute" values per se.

To our knowledge, this is the first evidence for a role of allopregnanolone in regulation of maternal care behavior in the postpartum period. A previous study has shown that finasteride administration during pregnancy increased anxiety-like behavior and delayed the onset of maternal behavior towards foster pups in rats (Mann 2006), further suggesting that the emotional state of the animal influences maternal behavior. However, neither allopregnanolone levels nor a detailed examination of maternal care were assessed in this study. The abrupt decrease in allopregnanolone levels that occurs immediately before parturition and persists during the postpartum period has been linked to postpartum mood disorders in which mother-infant interactions are impaired (Brummelte and Galea 2016). Indeed, lower circulating allopregnanolone levels were detected in women experiencing postpartum "blues," compared to euthymic women (Nappi et al. 2001). Thus, it is tempting to speculate that the more pronounced decrease in allopregnanolone levels, observed in juvenile socially isolated dams vs. group-housed dams at postpartum, may exacerbate the emotional state in these animals, which, as a result, fail to provide a good quality care to their offspring. Accordingly, chronic stress increased anxiety-related behavior in lactating mice and rats (Maestripieri et al. 1991; Nephew and Bridges 2011). A detailed behavioral study on the emotional state during pregnancy and after delivery would be required to assess if the poor maternal care, exhibited by socially isolated dams, is attributable to their anxiety state.

Both preclinical and clinical studies suggest that elevated levels of corticosterone are associated with anxiety and disruption in maternal care (Brummelte et al. 2006; Carini and Nephew 2013; Essex et al. 2011). As expected (Pisu et al. 2016), we found that basal corticosterone levels in virgin isolated female rats were significantly lower than the ones in the group-housed counterpart. In socially isolated rats, corticosterone levels further decline during pregnancy, compared to those in virgin rats; however, during lactation, they were increased at PP3, compared to G17 pregnancy values. By contrast, corticosterone levels in group-housed rats increased during pregnancy and declined in postpartum (Brummelte and Galea 2016). Indeed, ANOVA results found a significant effect of housing condition (juvenile social isolation vs. group-housing) and a significant interaction between housing and status (virgin vs. pregnancy vs. postpartum), although the post hoc test did not reach significance. Thus, we cannot rule out the possibility that the different patterns of corticosterone secretion during the postpartum period, observed in socially isolated vs. group-housed dams, might have contributed, at least in part, to the altered maternal behavior observed in socially isolated dams. Accordingly, previous studies have demonstrated that corticosterone alters maternal care (Brummelte and Galea 2010). In addition, socially isolated virgin female rats showed a greater corticosterone response to foot-shock stress, compared to group-housed shocked pairs (Pisu et al. 2016), in agreement with the idea that priming the HPA axis with chronic social isolation increased sensitivity to acute stress (Serra et al. 2000, 2005; Hawkley et al. 2012). This enhanced HPA response may in part counteract the

physiological stress hyporesponsiveness of lactation that allows the dam to effectively care for her developing offspring (Lightman 1992).

Conclusions

In conclusion, chronic social isolation from weaning to adulthood in female rats decreases maternal care; this effect is associated with lower allopregnanolone levels at postpartum, which may account for the poor maternal care observed in socially isolated dams. This hypothesis is supported by the finding that finasteride-treated group-housed dams, which display a decrease in allopregnanolone levels, also show a marked reduction in maternal care, compared to vehicle-treated dams. To the best of our knowledge, this is the first observation of a link between allopregnanolone concentrations and the quality of maternal care. These results may be relevant to our understanding of the neurobiological mechanisms underlying maternal care, a behavior that has profound and enduring molecular, behavioral and psychological effects on the offspring (Francis et al. 1999; Weaver et al. 2004; Champagne and Curley 2009; Meaney and Szyf 2005).

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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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