Title: OFFSPRING EXPOSURE REDUCES DEPRESSIVE-LIKE BEHAVIOUR IN THE PARTURIENT FEMALE RAT

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Abstract

In women, breastfeeding generally results in reductions in anxiety and increased positive mood. However, approximately 10–15% of women experience depressed mood and increased anxiety during the first year postpartum. Recent research has demonstrated that offspring-exposure is important for the reduction in behaviours related to depression and anxiety in the mother. It remains to be determined whether these effects are due to factors related to pregnancy and/or pup-exposure, are associated with the degree of maternal behaviour by the mother towards offspring, or persist after weaning. To address these questions the present study used four groups of female rats; primiparous, nulliparous, primip-no-pups (primiparous females with pups permanently removed), and sensitized females. Depressive- and anxiety-like behaviours were assessed one week after weaning/pup-exposure (4 weeks after birth for primip-no-pups animals) using the Forced Swim Test for measures of depressive-like behaviour, and the Open Field Test and Elevated Plus Maze for measure of anxiety-like behaviour. Results demonstrate that primiparous females without pup-exposure have increased depressive-like, but not anxiety-like, behaviour compared to primiparous and sensitized females. In addition, kyphotic nursing by primiparous mothers was negatively related to behavioural measures of depression and anxiety. From this work is it clear that pup-exposure acts is important for reductions in depressive-like behaviour in parturient females. Further research is needed to determine the extent of these changes and the neural and hormonal correlates of these events.
Keywords: Forced swim test, parity, sensitization, lactation, licking/grooming, nursing, elevated plus maze, open field test, anxiety, depression
Introduction

The onset of motherhood often results in altered mood and anxiety. In women, the general consensus is that breastfeeding results in reductions in anxiety and increased positive mood [22, 48]. Furthermore, changes in emotionality with motherhood are thought to be adaptive and important for maternal behaviours to occur [21]. Apart from the beneficial effect of motherhood on maternal mood, approximately 10–15% of women experience depressed mood and increased anxiety during the first year postpartum, with a much larger percentage (28-85%) of women exhibiting postpartum blues in the first few weeks post-parturition [46]. Recent research has aimed to determine factors that contribute to altered emotionality with motherhood. Lonstein (2005) has shown that contact with offspring, but not necessarily suckling, is important for lactating mothers to exhibit decreased anxiety early, but not later, in the postpartum period [29]. Others have shown that long after pregnancy and lactation, parturient rats exhibit a reduction in anxiety-like behaviour but these effects appear to be dependent on age, repeated testing, and stage of estrous cycle at the time of testing [12, 31, 51, 54].

Maternal mood during the postpartum period can be dependent on offspring exposure [10, 18]. Mother-infant separation for short durations does not appear to alter maternal mood [18], but not surprisingly the loss of a child can lead to severe grief and depression in mothers and fathers [6]. In animal models, short mother-offspring separations increase maternal behaviours toward offspring, and longer-term mother-offspring separations decrease maternal-offspring interactions [27, 28, 45] and increase maternal depressive-like behaviour [10]. In addition, parous females with offspring
removed shortly after birth (within 24 hours of birth) exhibit decreased motivation 2 months after parturition when tested in a radial arm maze task [38]. From animal models, offspring-exposure appears to be important for the reduction in maternal depression and anxiety, but little is known about the effect of offspring-exposure on depression and anxiety in the nulliparous female. Pup-exposure to ovariectomized sensitized females is inconclusive with reports demonstrating decreased anxiety-like behaviour [1, 42] and others demonstrating no change anxiety-like behaviour [20] compared to nulliparous females without pup-exposure. Thus, the contribution of offspring to anxiety- and depressive-like behaviour in the primiparous and nulliparous females has yet to be thoroughly examined.

Maternal behaviours are also associated with altered emotionality in the mothers and offspring. In women, increased feelings of attachment to their infant are associated with increased positive mood during the postpartum period [22]. In rodent models, the degree of maternal licking of pups during the first week of life is negatively related to the expression of anxiety in adult offspring [13, 54] and increased licking of pups by the dam is associated with decreased depressive-like behaviour in the mother [10]. In addition work has demonstrated that dams bred for either high- or low-anxiety related behaviour (HAB or LAB rats) differ in pup-directed maternal behaviours, such that HAB dams spend more time on the nest and arched-back nursing offspring than LAB dams [37].

Although it is evident that reductions in anxiety- and depressive-like behaviour occur in parturient rats, it is unclear whether these effects are due to factors related to pregnancy and/or pup-exposure, are associated with the degree of maternal behaviour by
the mother towards offspring, or persist after weaning. To address these questions the present study used four groups of female rats: primiparous, nulliparous, primip-no-pups (primiparous with no pups), and sensitized rats. Animals began testing on behavioural tasks 1 week after weaning/pup-exposure for primiparous and sensitized females which is 4 weeks after parturition for primip-no-pups females and at a matched age in nulliparous females (for specific details see Methods). The forced swim test (FST) was used to assess depressive-like behaviour [32] and the open field test (OFT) and elevated plus maze (EPM) were used to assess locomotor ability and anxiety-like behaviour [43, 50]. It was hypothesized that if reductions in depressive- and anxiety-like behaviour were due to the effects of pup-exposure, primiparous and sensitized rats would exhibit decreased anxiety- and depressive-like behaviour compared to nulliparous and primip-no-pups females. In turn, it was expected that high amounts of maternal behaviours such as licking and/or nursing might be associated with decreased levels of depressive- and anxiety-like behaviour in the mother.

**Methods**

**Animals**

Thirty-six female Sprague-Dawley rats (approximately 70-80 days of age, between 275-300g) were obtained from the UBC Rodent Breeding Unit (Vancouver, Canada) and were used in the study. Rats were initially housed in pairs in opaque polyurethane bins (48 x 27 x 20 cm) with aspen chip bedding and were given Purina rat chow and tap water *ad libitum*. Rats were maintained in a 12h/12h light/dark cycle (lights on at 7:00 a.m.). All protocols were in accordance with ethical guidelines set by the
Female rats were randomly assigned to one of four conditions: nulliparous (n = 9), primiparous (n = 10), primip-no-pups (n = 9), and sensitized (n = 8) rats. All rats were age-matched such that parous rats gave birth to their litter at the same age that sensitized rats were exposed to pups. Nulliparous rats were not sexually experienced. Day of parturition in parous dams and the first day of pup-exposure to virgins for sensitization (sensitized group) was considered Day 0. Primiparous rats gave birth on Day 0 and had their pups weaned on Day 21 after birth. Sensitized rats were exposed to pups for the same duration of time as primiparous females, from Day 0 to Day 21, and became sensitized during this period (for details see below). Primip-no-pups rats gave birth once and had pups removed permanently within 24 hours of birth. Nulliparous rats that were not exposed to pups or pup-related cues and were singly housed in clear polyurethane cages in a separate room from pregnant and mothering/sensitized rats. Once pups were removed from any group, females were housed in the same room away from pups and pup-related cues.

**Breeding**

For breeding, one female and one male were paired in a wire mesh bottom cage until a vaginal plug was released from the female. Females were then individually housed in clear polyurethane bins until day 8 postpartum. Day 0 was designated the day of birth. Within 24 hours of birth, litters were culled to 5 male and 5 female pups for primiparous dams and all pups were removed from primip-no-pups rats. Primiparous dams and pups
were housed in large opaque polyurethane bins (51 x 41 x 22cm) 8 days after parturition until weaning (postpartum day 21).

**Sensitization**

Sensitization (pup-exposure to nulliparous rats) was carried out based on previous work [38, 39, 47]. Briefly, nulliparous females were continuously housed with 3 pups for the same duration of time as primiparous females (22 consecutive days) starting on Day 0. Age of pups at the start of sensitization was 3-5 days and they were supplied from a biological mother not involved in behavioural testing. Every 24 hours, between 8a.m. and 10a.m., milk-replete pups were given to sensitized nulliparous rats, and donor pups were replaced with their biological mother. To confirm that sensitized rats acted maternally, licking of pups and hovering over pups were scored from daily 10 minute video-recordings taking place each morning (between 8 and 10 am) immediately after new donor pups were introduced to the sensitized rats. Spot checks were performed approximately 1, 3 and 24 hours after the milk-replete pups were placed with sensitized rats. Video recordings and spot checks took place until a nulliparous rat acted maternally towards pups (licked and hovered over pups within a 24 hour period). If a female injured a pup at any time the sensitization was terminated for that day and all pups were removed. If this occurred more than 3 times the females was removed from the study. This occurred once.

**Maternal Behaviour Testing**

The duration of the following maternal behaviours were observed as previously described [36, 38]: licking (body licking and genital licking with the dam off the pups);
licking and nursing (body licking and genital licking with the dam on the pups); kyphosis (arched-back nursing); blanket nursing (dam lies over the pups); passive nursing (dam lies either on her back or side while pups nurse). Observations were made once every 5 seconds for 10 minutes three times per day: between 8-10a.m., 12-2p.m., and 3:30-5:30p.m. on postpartum Days 2 to 8. During scoring of maternal behaviour, dam and pups were left undisturbed in their home cage. Data for each behaviour was aggregated across all test periods.

**Forced Swim Test (FST)**

To assess depressive-like behaviour in the females, the forced swim test was used as previously described [23, 26]. Briefly, the apparatus consisted of a vertical cylindrical glass container (height 45 cm x diameter 28 cm) filled to a depth of 33 cm with tap water at 24 ± 1°C. This depth was sufficient to ensure that animals could not touch the bottom of the container with their hind paws [32]. The forced swim test was conducted over two days and took place at the same time point for all groups; 1 week after weaning/pup-exposure for primiparous and sensitized females (4 weeks after parturition in primiparous rats or 4 weeks after initial pup exposure in sensitized rats), 4 weeks after parturition for primip-no-pups females and a matched time point in nulliparous females. On the first day of the FST, females were introduced to cylindrical glass tank filled with water for 10 minutes, towel dried and returned to their home cage. Twenty-four hours later, animals were exposed to the same experimental conditions for 10 minutes, dried and returned to their home cage. Sessions were videotaped and scored using Best Collection System (Educational Consulting Inc) by an observer blind to conditions. The behaviours scored
in the forced swim test were: (1) immobility — floating with the absence of any movement; (2) struggling — quick movements of the forelimbs such that the front paws break the surface of the water; (3) swimming — movement of forelimbs or hind hind limbs. The number and time spent in escape attempts was also recorded. Escape was defined as swimming to the bottom of the tank and pushing off. To determine whether stage of estrous cycle (proestrus) affected performance, animals were vaginally swabbed after testing (see swabbing details below).

**Open Field Test (OFT)**

To assess locomotor activity and anxiety-like behaviour, females were tested on the OFT 2 or 3 days after the end of the FST as previously described [23]. The apparatus, a 120 x 120 cm$^2$ arena divided into 16 squares of equal dimension with 40 cm high walls, was placed in a dimly lit room (98 lux). The central region of the OFT was 60 x 60 cm$^2$. An animal was placed in the centre of the field and activity was video recorded for a 5-minute period. All animals were tested once between 9am and 1pm. For scoring, a video-tracking system (Anymaze; Stoelting Co., Wood Dale, IL) was used to score the distance travelled, and an observer blind to the groups scored the number of squares, peripheral or central, entered, the number of rears, and the number of fecal boli from each animal. The apparatus was wiped with 70% ethanol and dried between rats. An increased number of central crossings is considered indicative of decreased anxiety-like behaviour, while increased total crossing is considered an index of locomotor behaviour [43]. All females were vaginally swabbed after testing to determine estrous cycle phase.
**Elevated Plus Maze (EPM)**

Previous work has demonstrated that anxiety-like behaviour as measured by the elevated plus maze is altered in dams [12, 31, 51]. Therefore to assess the degree of anxiety-like behaviour in nulliparous, primiparous, primip-no-pups, and sensitized females, the EPM was used as previously described [29, 40, 50]. The plus shaped apparatus consisted of two open arms (50 x 10 cm) and two closed arms (50 x 10 cm) with 40 cm high walls and was placed 50 cm above the ground. In a dimly lit (60.5 lux) and non-cued room, each rat was placed in the middle of the maze facing an open-arm and allowed to move freely for 5 minutes. Testing began a minimum of 4 days after the OFT and all females were tested in proestrus due to the known effects of estradiol level on EPM performance [12, 52]. To determine stage of the estrous cycle, females were vaginally swabbed between 8am-9am. Females in proestrus were tested between 10am-12pm that day. Sessions were recorded and scored using a video-tracking system (Anymaze; Stoelting Co., Wood Dale, IL). An observer blind to the groups scored the number of open and closed arm entries, and time spent on the centre square, in an open arm, and a closed arm for each animal. An entry occurred when all four paws of the animal entered an arm. An index of anxiety-like behaviour can be measured by the number of entries and time spent in the open arms where a short duration of time spent in the open arms or a low number of entries into the open arms is indicative of anxiety [40]. The number of closed arm entries is indicative of motor activity [19]. The apparatus was cleaned with 70% ethanol and dried between rats.
**Estrous Cycle Determination**

To determine whether animals were in proestrus, vaginal swabs were done after testing on the FST and OFT and prior to testing on the EPM. Only animals in proestrus were tested on the EPM. Swabs were taken in all rats by gently placing a cotton swab dipped in sterile saline into the vagina and smearing the contents of the swab on a plain slide. Slides were examined under 10x objective of a light microscope (Nikon) and proestrous stage was determined when a majority of cells evident in the vaginal mucus (approximately 70%) were nucleated epithelial cells.

**Data Analyses**

Analysis of FST, OFT, and EPM performance was done using a repeated-measures ANOVA. FST behaviour (immobility, swimming, struggling) per day, EPM area (open arm, centre, closed arm), or OFT area (peripheral, center) were used as within-subjects factors and group (primiparous, primip-no-pups, nulliparous and sensitized) as between-subjects factor. One-way ANOVA tests were calculated with the following dependent variables: body weight, number of escape attempts in the FST, total crossings, central divided by total crossings, distance, time spent immobile, number of rears, and number of fecal boli on the OFT, and number of days between OFT and EPM, distance traveled and time spent immobile on the EPM with group (nulliparous, primiparous, primip-no-pups, sensitized) as the between-subjects variable. An analysis of covariance was done on scores from the FST and OFT using proestrous stage as the covariate. Pearson product-moment correlations were conducted between specific maternal behaviours in primiparous dams and onset of maternal behaviour in sensitized females,
and scores from the FST, OFT, and EPM. *T*-tests were performed on litter size, number of male, and number of female offspring, between groups (primiparous, primip-no-pups).

*Post-hoc* comparisons utilized the Fisher LSD test.

**Results**

There was no significant difference between primiparous and primip-no-pups rats in the size of the litter or number of male and female pups (*p* ≥ .5). Sensitized rats took an average of 5.6 ± 0.8 days of pup exposure before being considered maternal (range 2 to 9 days). There was a significant main effect of weight between groups 1 week after weaning (*F*(3, 31) = 5.24, *p* ≤ .005; Figure 1) with primip-no-pups weighing significantly more than primiparous and sensitized rats (*p*’s ≤ .03), and nulliparous rats weighing significantly more than sensitized rats (*p* ≤ .03) and tending to weigh more than primiparous females (*p* ≤ .06). There were no other significant differences in weight between groups.

**Forced Swim Test:**

Percent of time spent immobile in the FST was significantly altered by pup-exposure. Primip-no-pup females spent a significantly greater percentage of time immobile compared to primiparous females (*p* ≤ .03) and sensitized females (*p* ≤ .03) on the second day of testing (day by group interaction effect: *F*(3, 32)=2.959, *p* ≤ .047, Figure 2). All animals spent significantly less time struggling (main effect of day: *F*(1, 32)=8.448, *p* ≤ .007; Table 1) and less time escaping (main effect of day; *F*(1, 32)=40.863, *p* < .0001; Table 1) on the second day of testing than on the first day of testing. All animals made fewer number of escape attempts on the second day of testing.
(F(1, 32)=57.995, p ≤ 0.000001; Table 1). Duration of time in kyphosis was positively associated with time swimming during testing in the FST (Day 1, r = .66, p = .052; Day 2, r = .76, p ≤ .017; Figure 3), indicating greater time spent in kyphosis was related to more active behaviours in the FST and thus reduced depressive-like behaviours. There was no significant effect of proestrus on percent of time spent swimming, immobile, struggling or escaping. There were no other significant differences between groups or significant correlations between FST measures and specific maternal behaviours (.07 < p < .80; data not shown).

**Open Field Test:**

Primiparous females made significantly fewer central/total crossings than nulliparous rats (p < .007; main effect of group: F(3, 32) = 2.89, p ≤ .05; Figure 4). All animals made significantly more peripheral crossings than central crossings (main effect of area; F(1, 32)=667.51, p < 0.00001; Table 2). There were no other significant differences between groups in number of peripheral and central crossings, distance traveled in peripheral and central areas, time spent immobile, amount of rearing, and number of fecal boli while in the OFT (.1 < p < .9). An analysis of covariance on number of central/total crossings and total number of crossings using proestrous stage as the covariate, revealed no significant effect of proestrous on performance (.24 ≤ p ≤ .39).

There was a significant negative correlation between time spent in kyphosis and number of peripheral crossings (r = -.7, p ≤ .036; Figure 5), indicating that more times spent in kyphosis was related to less time engaged in thigmotaxic or anxiety-like behaviour. There was a strong tendency toward a significant negative correlation between
kyphosis and total number of crossings ($r = -0.66 \ p \leq .054$). There were no other correlations between specific maternal behaviours in primiparous dams and onset of maternal behaviour in sensitized females with measures of the OFT ($p \geq .06$).

**Elevated Plus Maze:**

Elevated plus maze performance was significantly different between groups (area by group interaction: $F(6, 64)=2.74, p \leq .02$). Nulliparous rats spent significantly less time in closed arms than all other groups ($0.0001 \leq p \leq .02$) and significantly more time in the centre than primip-no-pups females ($p \leq .03$; Figure 6). All groups made significantly more closed arm entries than open arm entries (main effect: $F(1, 32)=272.53, p \leq 0.001$). There were no significant interactions or differences between groups on distance traveled, time spent mobile, number of open arm entries, and time spent in open arms ($0.1 \leq p \leq .7$). There were no significant correlations between number of days after OFT EPM testing occurred and any measures on the EPM ($0.1 \leq p \leq 1.0$). Because all rats in all groups were tested during proestrus, there may have been differences between the number of days between the OFT and EPM between groups. Analysis revealed that sensitized females had significantly more days between OFT and EPM testing compared to primiparous females only ($p \leq .02$; primiparous $4.7 \pm 0.5$ days, primip-no-pups $7.4 \pm 1.2$ days, nulliparous $7.4 \pm 0.8$ days, sensitized $9.1 \pm 1.6$ days). There were no significant correlations between specific maternal behaviours in primiparous females or onset of maternal behaviour in sensitized females and measures on the EPM ($0.1 \leq p \leq 1.0$).
Discussion

The current findings demonstrate that there is an antidepressant-like effect of offspring-exposure in parturient females four weeks after parturition. We found removal of pups within 24 hours of parturition increased depressive-like behaviour, long after birth in parturient females compared to primiparous females with pup-exposure and sensitized females. This effect was particular to depressive-like behaviours as we did not find similar effects with offspring exposure on anxiety-like behaviours or on overall locomotor activity. Primiparous females made significantly fewer central/total crossings on the OFT and there were significant body weight differences between groups with both sensitized and primiparous rats weighing less than primip-no-pups females and nulliparous rats. In addition, duration of time in kyphosis was positively associated with time swimming during testing in the FST and negatively associated with number of peripheral crossings on the OFT, indicating that kypothsis is negatively related to both depressive- and anxiety-like behaviours.

The antidepressant-like effect of offspring-exposure is attenuated in dams without pup-exposure.

In the present study the antidepressant-like effect of pup-exposure was evident in primiparous females exposed to pups and sensitized virgin females compared primiparous females with no pup-exposure (primip-no-pups group), when tested on the FST four weeks after parturition. These findings support and expand previous work demonstrating that mother–pup separation leads to increased depressive-like behaviour in
the dam [10] and altered motivation to complete a cognitive task [38]. For example, Boccia et al (2007) demonstrated that mother-pup separation leads to increased depressive-like behaviour in the dam at the time of weaning, but only with longer periods of mother-pup separation (3 hours/day, but not 15 min/day, from postpartum days 3-14). [10]. In the present study we expanded this model by using primiparous females that have pups removed permanently within 24 hours of birth (primip-no-pups). Our findings suggest that pup-exposure acts as a kind of antidepressant in modulating mood in the parturient female.

Previous work has reported that maternal licking of offspring is associated with depressive-like behaviours in dams, but only in dams repeatedly separated from their pups for extended periods of time throughout lactation, including the days maternal behaviour was assessed [10]. It has been well documented that maternal separation can alter maternal licking of offspring, such that brief separations increase licking and long separations decrease licking [27]. Thus in the previous study the association between maternal licking and depressive-like behaviour was dependent on maternal-offspring separation and was not evident in dams that were not separated from their offspring [10]. In the present study we did not find a relationship between maternal licking and depressive-like behaviour in dams given normal maternal-offspring interaction, however we did find that time spent in kyphosis was positively correlated with swimming behaviour in the FST (see below). Therefore the findings of the present study are consistent with Boccia et al (2008) demonstrating that there is no relationship between licking and depressive-like behaviour in dams with normal mother-pup interactions.
We did find a positive association between time spent in kyphosis (arched-back
nursing) and percentage of time spent swimming, suggesting decreased depressive-like
behaviour in mothers that spent more time in kyphotic nursing postures. It is interesting
to note that serotonin plays a role in the mediation of swimming behaviour in the FST
[16, 17] and aspects of kyphosis, such as the suckling-induced prolactin release [8, 44].
Therefore serotonergic functioning during kyphosis may be important for swimming
behaviour in the FST and maternal emotionality. We found a negative association
between time spent in kyphosis and peripheral and total crossings on the OFT, suggesting
decreased mobility and potentially reduced anxiety-like behaviour.

**Effects of pup-exposure to nulliparous females on depressive- and anxiety-like
behaviour.**

Although pup-exposure via pup-directed behaviours or cues related to pups is
important for emotionality in primiparous females, it does not appear that pup-exposure
significantly alters emotionality in nulliparous females alone. We did not find a
significant difference in depressive- or anxiety-like behaviour between nulliparous and
sensitized females 1 week after 22 days of pup exposure in the present study. These
findings are in partial agreement with previous work demonstrating that pup-exposure
does not lead to reductions in anxiety in sensitized females [20]. However, others have
found that there are reductions in anxiety-like behaviour in nulliparous sensitized females
[42]. Therefore, more work is needed to determine how pup-exposure alone or cues
related to pup-exposure affect emotionality in nulliparous females and to determine the
time course of this possible connection. It seems plausible that physiological changes associated with pregnancy, parturition and lactation play a significant role in maternal emotionality. For example, parturition-related hormonal changes are implicated in anxiety and depression [51, 52] as well as altered mood states related to motherhood such as postpartum depression [2-4, 9, 25, 33, 55]. It is also possible that because we examined emotional behaviours in sensitized females one week after exposure to pups, that differences in emotional behaviour are not retained for as long as is evident in parturient females.

**Offspring exposure is related to body weight in parturient and non-parturient females.**

We also found significantly decreased body weight in primiparous and sensitized females compared to primip-no-pups dams, and between sensitized females and nulliparous females 1 week after 3 weeks of pup-exposure and/or 4 weeks after parturition. Previous literature has reported altered body weight in the dam with lactation and weaning [15]. However, to our knowledge this altered body weight in sensitized females has not been previously reported. The *decrease* in body weight between sensitized and nulliparous females may be due to increased demands related to pup-exposure such as maternal behaviours, but are not likely due solely to lactation as sensitized females do not lactate. The *increase* in body weight in primip-no-pups dams compared to primiparous and sensitized females is likely associated with altered physiology due to the lack of the mother-infant interactions via lactation and pup-directed maternal behaviours.
Are there reductions in anxiety-like behaviour with motherhood after weaning?

The present study found that primiparous females made significantly fewer central/total crossings on the OFT, which may be considered indicative of an increase in anxiety-like behaviour. This is not consistent with previous findings indicating either that parous dams have decreased anxiety [12, 31] or no significant difference in anxiety-like behaviour at weaning when compared to nulliparous females [29]. However, we did find that kyphosis was significantly related to fewer peripheral crossing suggesting that maternal behaviour may affect anxiety-like behaviour. In addition, consistent effects of motherhood on reducing maternal anxiety have not been reported. For example, Lonstein (2005) reports reductions in anxiety-like behaviour only during early lactation and not during late lactation and at weaning, while others found reductions in anxiety-like behaviour in parous rats months after weaning with repeated testing on the EPM [31]. However, others have found that a reduction in anxiety-like behaviour in the dam compared to the virgin female is evident as early as 2 to 4 weeks after weaning [12, 53].

There are a number of possible reasons for discrepancies between our findings and those of others (for review see [30]). These possibilities include the duration of testing, single versus repeated testing, possible differences in illumination, prior testing in a stressful paradigm, and stage of estrous cycle [34]. For example, Wartella et al (2003) tested rats for 30 minutes on the OFT [53], whereas the present study and Byrnes and Bridges (2006) tested rats for 5 minutes on the OFT [12]. In addition, Lonstein (2005) tested rats for 10 minutes on the EPM [29], whereas Byrnes and Bridges (2006), as well as the present study, tested animals for 5 minutes [12]. The length of time tested on an
apparatus may affect an animal’s behaviour as the animal may become more accustomed to their environment and therefore explore more over longer periods of time. Repeatedly testing animals, as Love et al (2005) have done, may differentially decrease anxiety-like behaviour in the animal as they become familiar with the task and apparatus and explore more and it may be the case that primiparous rats show increased habituation to novel situations relative to nulliparous rats [14].

It has also been suggested that the level of illumination in the testing room [24, 35] and the differences in illumination between open and closed arms of the EPM [41] have a significant impact on anxiety-like behaviour. Both Byrnes and Bridges (2006) and Lonstein (2005) report the use of high levels of illumination during testing, however it is unclear what level of illumination was used in other studies [12, 29]. We have found effects on the EPM and OFT with the levels of illumination that we used in the present study [11], however differences in illumination illicit different results [24, 35]. For example, the frequency of open arm entries and the amount of time spent in an open arm of the EPM is increased with lower levels of illumination (such as 0 to 1 lux) compared to higher levels of illumination (such as 30, 100, or 300 lux) with no differences in overall locomotor activity [24, 35]. Greater differences in lux between open and closed arms also result in greater avoidance of the open arm in the EPM [41]. Therefore, differences in illumination may contribute to discrepancies in work on anxiety-like behaviour.

Differences in hormone levels significantly alter anxiety-like behaviour [49] and may have contributed to differences in behavioural tests seen here. From work done on
ovariectomized females receiving hormone replacement it is clear that ovarian hormones are significantly related to anxiety- and depressive-like behaviour in the females [49]. In addition, reproductive experience results in decreased circulating 17beta-estradiol and prolactin levels during proestrus [12] and a significant decrease in anxiety-like behaviour during proestrus in primiparous compared to nulliparous females [12]. However, the role of hormones on anxiety-like performance with reproductive experience remains unclear as others have shown that regardless of estrous cycle stage differences in anxiety-like behaviour exist between parous and virgin females [31, 53]. These differences may be due to the age of the females [12], the strain of the rat or procedural differences mentioned above. It should also be noted that it is well documented that previous stressors can affect behavioural performance (for example [5, 7]. Therefore, testing in the FST prior to testing in the EPM and OFT, as was done in the present study, may have impacted anxiety-like behaviour in the present study. In addition, virgin and maternal animals may have responded differentially to the initial swim stress in the FST and differentially altered their subsequent behaviour in the OFT and EPM.

Conclusions

In conclusion, the present study adds to a growing body of research investigating how motherhood affects the brain and behaviour. Here we demonstrate that pup-exposure is important for maternal emotionality; the antidepressant–like effect of pup-exposure is attenuated in dams that are not exposed to pups postpartum. This suggests that aspects
related to pup-exposure, lactation and motherhood are important for maternal emotionality.
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Table 1. Mean (± SEM) percentage of time spent in a behaviour on the Forced Swim Test. Animals spent significantly less time struggling (p ≤ .007), less time escaping (p ≤ .000001), and made fewer number of escape attempts (p ≤ .000001) on the second day of testing than on the first day of testing. * denotes Day 2 significantly different from Day 1.

<table>
<thead>
<tr>
<th>behaviour</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming</td>
<td>50.39 ± 2.85</td>
<td>54.70 ± 1.99</td>
</tr>
<tr>
<td>Struggling</td>
<td>12.51 ± .80</td>
<td>9.34 ± .97  *</td>
</tr>
<tr>
<td>Escaping</td>
<td>3.18 ± .45</td>
<td>0.59 ± .19  *</td>
</tr>
<tr>
<td>No. Escape attempts</td>
<td>3.47 ± .41</td>
<td>0.94 ± .35  *</td>
</tr>
</tbody>
</table>
Table 2. Mean (± SEM) peripheral and central crossings on the Open Field Test. All animals made significantly more peripheral crossing than central crossings ($p \leq 0.00001$). There were no other significant differences between groups in number of peripheral and central crossings, distance traveled in peripheral and central areas, time spent immobile, amount of rearing, and number of fecal boli while in the OFT ($0.1 \leq p \leq 0.9$).

<table>
<thead>
<tr>
<th></th>
<th>No. of Peripheral Crossings</th>
<th>No. of Central Crossings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>75.33 ± 5.54</td>
<td>16.67 ± 3.60</td>
</tr>
<tr>
<td>Primiparous</td>
<td>78.00 ± 3.74</td>
<td>6.30 ± 1.85</td>
</tr>
<tr>
<td>Primip-no-pups</td>
<td>75.22 ± 3.60</td>
<td>11.78 ± 2.32</td>
</tr>
<tr>
<td>Sensitized</td>
<td>78.63 ± 6.88</td>
<td>12.88 ± 2.78</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Mean (± SEM) body weight (g) 1 week after weaning. Primip-no-pups females weighed significantly more than primiparous and sensitized females (p’s ≤ .03), and nulliparous females weighed significantly more than sensitized rats (p ≤ .03) and tended to weigh more than primiparous females (p ≤ .06). # denotes significantly different from nulliparous and primip-no-pups.

Figure 2. Mean (± SEM) percentage of time spent immobile on the FST on the second day of testing. Primip-no-pups females spent a significantly greater percentage of time immobile compared to primiparous females (p ≤ .03) and sensitized females (p ≤ .03) on the second day of testing.

Figure 3. Correlation between duration of time in kyphosis and percent of time spent swimming the FST during testing. Duration of time in kyphosis was positively associated with time swimming during testing in the FST on (a) Day 1 and (b) Day 2 (Day 1, r = .66, p ≤ .052; Day 2, r = .76, p ≤ .017).

Figure 4. Mean (± SEM) number of central/total crossings on the OFT. Primiparous females made significantly fewer central/total crossings than nulliparous rats (p < .007).

Figure 5. Correlation between duration of time in kyphosis and number of peripheral crossings on the OFT. There was a significant negative correlation between time spent in
kyphosis and number of peripheral crossings ($r = -0.7$, $p \leq 0.036$), and a tendency toward a significant negative correlation between time spent in kyphosis and total number of crossings ($r = -0.66$, $p \leq 0.054$).

**Figure 6.** Mean ($\pm$ SEM) time spent in closed arms, open arms, and centre of the EPM. Nulliparous rats spent significantly less time in closed arms than all other groups ($0.0001 \leq p \leq 0.02$), and more time in the centre than primip-no-pups females ($p \leq 0.03$). * denotes nulliparous rats significantly different from all other groups. ‘a’ denotes nulliparous rats significantly different from primip-no-pups females.
Figure 1

Mean ± SEM total body weight (g)

- Nulliparous
- Primiparous
- Primip-no-pups
- Sensitized
nulliparous  primiparous  primip-no-pups  sensitized

Percentage of time spent immobile in the FST
(Day 2)

Figure 2
Figure 3

a) 

![Graph showing the relationship between time spent in kyphosis (s) and percent of time swimming in the FST on Day 1.](image)

b) 

![Graph showing the relationship between time spent in kyphosis (s) and percent of time swimming in the FST on Day 2.](image)
Mean (+ SEM) time spent in an area of the EPM (s)

Figure 6

- nulliparous
- primiparous
- primip-no-pups
- sensitized

Closed Arm Centre Open Arm