Behavioral Effects of Early Postpartum Offspring Removal in Rats

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Abstract

The maternal experience has been associated with alterations in behavior and in many different areas of the brain. Soon after giving birth and throughout the postpartum period, maternal behavior and care of offspring in particular have been shown to stimulate the dopaminergic system in postpartum women and rats alike. Around 15% of women who give birth develop postpartum depression (PPD), which has been associated with downregulation of dopamine activity. This experiment tested whether the removal of offspring immediately after parturition would alter the anxiety and depressive-like behavior of dams, as well as the expression of dopaminergic neurons. Adult female Sprague-Dawley rats remained with their pups (pup-remain) or had their pups removed (pup-remove) on postpartum day (PD) 1. As an additional control, age-matched nulliparous rats remained undisturbed except for testing. Depressive-like behavior, anxiety-like behavior, and anhedonia were tested using the forced swim test (FST), open field test (OFT), and sucrose preference test (SPT), respectively. To identify the presence of dopaminergic neurons, brains were processed for tyrosine-hydroxylase (TH), a rate-limiting enzyme that synthesizes dopamine. TH-expression was visualized in the ventral tegmental area (VTA) because of the key role the VTA plays in the reward system and the projection to dopaminergic pathways. Rats that had their pups removed displayed greater depression-like behavior, as measured by immobility in the FST. However, here was no significant effect of experimental group on the optical density of TH expression in the VTA. These results point to the possibility that while pup-remove rats showed increased depression-like behavior, the dopaminergic activity in the VTA associated with pup presence is not driving an antidepressant effect for the pup-remain dams. This work could further our understanding of how the maternal experience affects the brain, and the neurological changes behind PPD.
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Introduction

Around 15% of women who give birth develop postpartum depression, or PPD (Rockhill et al., 2017). PPD is associated with adverse outcomes for both mothers and the development of their offspring (Bonari et al., 2004; Goodman & Gotlib, 1999). While causes of PPD are not well understood, the peripartum period is associated with many neural and hormonal changes. This includes changes in different areas of the brain in terms of reduction or increase in volume, neurogenesis, and cell proliferation (Medina & Workman, 2018). In women, there is a rapid drop in blood concentration levels of estrogen and progesterone immediately postpartum, with these hormones reaching their highest levels at the full term of pregnancy, before returning back to baseline 2-5 days after the baby is delivered (Abou-Saleh et al., 1998). In female rats, blood concentration of progesterone is elevated during pregnancy, reaching its peak at gestation day (GD) 18 before declining. Estradiol, a form of the hormone estrogen, increases in blood concentration from GD 10 to parturition before decreasing rapidly (Anadol et al., 2014; Garland et al., 1987). However, many of these changes are adaptive and are meant to facilitate behavioral changes that occur in order to adapt to taking care of offspring.

After giving birth, female rats will begin to respond differently to pups. Nulliparous rats, or rats without reproductive experience, respond more fearfully to rat pups when compared to primiparous rats, or rats that have given birth for the first time. Nulliparous rats will initially withdraw from or avoid rat pups, whereas after parturition, female rats develop the motivation to respond maternally, and will approach pups quickly if in their presence (Fleming & Luebke, 1981). This shows the large shift in behavior that accompanies the maternal experience. Postpartum rats can also be trained to bar press for pups of their own or foster pups, indicating that offspring are a reinforcing stimulus (Wilsoncroft, 1968). However, this response is
decreased if the mother is unable to touch and retrieve the pups (Lee et al., 2000). This suggests that pup-reinforcement is mediated by the physical exposure to pups.

Offspring exposure can have the effect of reducing behaviors related to depression and anxiety in mothers. A study done by Pawluski et al. (2009) measured the effects of pup exposure by comparing the behaviors of primiparous rats with exposure to pups, primiparous rats with no pup exposure, and nulliparous rats that were sensitized to pups through daily exposure. Primiparous rats with no pup exposure did not experience a decrease in depressive-like effects, whereas primiparous rats and sensitized nulliparous rats did. This demonstrates that the reduction of depressive-like behaviors can be partially attributed to the exposure to pups, rather than just factors related to pregnancy.

In addition to offspring exposure, many human studies investigated the association between breastfeeding and symptoms of depression in the postpartum period. Symptoms of anxiety and depression at six months postpartum are associated with shorter breastfeeding duration and changing from exclusive breastfeeding to mixed breastfeeding or exclusive bottle feeding (Ystrom, 2012). Mothers with depressive symptoms indicative of PPD also present lower levels of breastfeeding self-efficacy, which influences duration and exclusivity of breastfeeding (Zuban & Foresti, 2013). Additionally, depression scores on the Edinburgh Postnatal Depression Scale decreased significantly from childbirth to three months postpartum in women who maintained exclusive breastfeeding during this time (Figueiredo et al., 2013). Other studies have also suggested that lactation and nursing behavior play a part in reducing stress during the postpartum period. Women that had breastfed their infants thirty minutes prior to a social stress test experienced a suppressed cortisol response compared with women who only held their infants (Heinrichs et al., 2001).
Similarly, rats that were unable to nurse pups through surgical blockade did not show a typical postpartum blunting of corticosterone levels following stress compared to rats that were able to nurse (Stern & Levine, 1972). Furthermore, dams that were exposed to chronic social stress to induce PPD decreased in time spent nursing their pups. Pups of stressed dams also experienced decreased milk intake on days 9 and 16 of lactation (Carini et al., 2013). This suggests that the relationship between stress and nursing is bidirectional, in that experiencing stress gives rise to disruptions in nursing and vice versa. Nursing time was also reduced on PD 2 in rats that were induced to experience depression-like behavior through chronic gestational stress (Leuner et al., 2014).

The behavior of postpartum rats towards pups changes with time and is mediated by the growth of the pups. In the conditioned place preference test, the maternal motivation to be with pups decreases as the mother rats progress further into the postpartum period (Wansaw et al. 2008). This indicates that the motivations that drive the reinforcing aspect of pups may be dependent upon both the developing needs of the pups and the postpartum stage of the mothers. Female rats in the early postpartum period at postpartum day (PD) 8 prefer a pup-associated chamber over a cocaine-associated chamber during a conditioned place preference test (Mattson et al., 2001). However, at PD 10, dams will begin to prefer a cocaine-associated chamber over a pup-associated chamber, though their level of maternal behavior does not differ between PD 8 and 10 (Mattson et al., 2003).

These changes in behavior are reflected by changes in the reward system of the brain. Similar to other studies, Fleming et al. (1994) found that pup-deprived dams prefer a pup-associated box in a conditioned place preference test. Pup-deprived dams that received a dopamine receptor antagonist did not prefer the pup-associated box, whereas pup-deprived dams
that were administered saline still preferred the pup-associated box. This indicates that pup reinforcement is disrupted when dopamine antagonists, which prevents the dopamine from binding to its receptor, are used. The decline in maternal care that occurs as dams progress from early postpartum to late postpartum has also been associated with changes in the dopamine system. Rats in late postpartum at PD 18 showed lowered dopamine receptor expression than early postpartum rats at PD 7. Furthermore, if late postpartum rats at PD 12 to PD 15 were treated with dopamine agonists, a chemical that binds to the dopamine receptor and activates it, they maintained a higher expression of maternal caregiving compared to controls (Grieb et al., 2020). Because pup reinforcement is disrupted when dopamine cannot bind to receptors, and caregiving is maintained with activation of dopamine receptors, this indicates that the dopaminergic system plays a key role in maternal motivation and care. Similarly, increases in depressive-like behavior, which are indicative of PPD, are observed in PD 1 rats through increased FST immobility and PD 1 and PD 3 rats through decreased response in the social approach test (Rincón-Cortés & Grace, 2020). These changes in PD 1 and PD 3 behavior were associated with a reduction in spontaneously active dopaminergic cells in the ventral tegmental area or VTA.

The dopaminergic projections in the brain are mediated by the VTA. The VTA plays a key role in the mesolimbic reward system in which it projects dopaminergic pathways to the nucleus accumbens, prefrontal cortex, and other forebrain regions (Nestler et al., 2015). This pathway is critical in motivation, reward-related behavior, attention, and is the same circuitry targeted by food and addictive drugs. The motivating aspects resulting from the maternal experience, such as the increased rewarding aspects of exposure to pups and maternal care, are
linked with the functioning of the VTA and the mesolimbic dopamine system (Numan et al., 2009).

As noted above, exposure to offspring and maternal caregiving are highly rewarding to the maternal rat and can have antidepressant-like effects. In the study previously mentioned by Pawluski et al. (2009), dams that remained exposed to their pups during the entire postpartum period showed decrease in depression-like behavior when compared with dams that had their pups removed. However, this study did not explore the neural components of this effect and behavioral assays were conducted four weeks after parturition, when the reinforcing aspects of pup exposure and contact have been diminished. Additionally, women who are diagnosed with PPD must show symptoms before the fourth week postpartum in order to fit DSM-V criteria (DSM-5; American Psychiatric Association 2013). From a neuroendocrine perspective, this time period may align more closely with the early postpartum period in the rat (that is, within the first week postpartum). The present experiment tested whether early postpartum removal of offspring would alter anxiety and depressive-like behavior of dams as well as the expression of dopaminergic neurons in the VTA. To address this, three groups of female rats were used; primiparous rats that remained with their pups (pup-remain), primiparous rats that had their pups removed (pup-remove) on PD 1, and nulliparous rats that remained undisturbed except for testing. To test whether early postpartum offspring removal would change depressive-like behavior and anxiety-like behavior, the forced swim test (FST) and open field test (OFT) were used. A lack of interest or pleasure in normally pleasurable activities, or anhedonia, is also a common symptom in depression and PPD. Anhedonia was tested using the sucrose preference test (SPT). Finally, to identify the presence of dopaminergic neurons, brains were processed for tyrosine-hydroxylase (TH), a rate-limiting enzyme that synthesizes dopamine, and then assessed
for optical density in the VTA. It was hypothesized that without pup exposure, rats would show an increase in depressive and anxiety-like behavior and a decrease in TH expression.

Methods

Animals

Thirty-six female and nine male Sprague Dawley rats, approximately 60-70 days old, were purchased from Charles River Laboratories (Raleigh, NC, USA). Rats were housed in same-sex pairs in clear polycarbonate cages (27 cm x 48 cm x 29 cm) with microfilter tops and Aspen chip bedding. Females were single housed on gestational day (GD) 0. The first day nulliparous rats were single housed was considered their day 0 and they remained in single housing for the duration of the experiment to align with pregnant rats. Cages included one red or yellow polycarbonate tunnel for enrichment. Colony rooms maintained a 14:10 light/dark cycle with lights on at 6:00AM and off at 8:00PM. Rats were given access to pellets (Lab Diet 5P76 Irradiated ProLab IsoPro RMH 3000) and water ad libitum unless otherwise specified. All procedures adhered to the National Institutes of Health (NIH) ethical guidelines and were approved by the University at Albany Institutional Animal Care and Use Committee (IACUC). Rats were allowed 7 days to habituate to the facility before being handled. All female rats were then randomly assigned to nulliparous (control), pup removal, or pup remain.

Breeding

Two female rats were placed in the cages of each of the nine males overnight. Vaginal lavages were conducted in the morning to either determine stage in the estrus cycle or confirm pregnancy through the presence of sperm. The day sperm were present was considered
gestational day (GD) 0. Pregnant rats were weighed, single housed, and remained undisturbed until parturition apart from weekly weighing and cage changes. Females that did not become pregnant were monitored through vaginal lavages and placed with the same male until she became pregnant.

Day of birth was considered postpartum day (PD) 0. Within 2 hours of giving birth, litters were weighed and sexed. Within 24 hours, dams in the pup remain group had their litters culled to standardize sex ratio (5 females and 5 males) and size (10 total). Pups beyond 10 were either fostered into other litters or euthanized. Rats in the pup removal group had all pups removed as soon as they were found, with checks being performed every 2 hours starting on GD 21. If fewer than 5 males or 5 females were born in a litter, pups were fostered from another dam that had given birth the same day. This practice is used often to control for litter size, weight, and sex ratio. Dams will readily accept new pups, and the act of fostering does not interfere with study effects (Lohmiller & Swing, 2006).

**Sucrose Preference Test (SPT)**

The SPT is used to determine anhedonia in rats through measuring the consumption of a low concentration of sugar water relative to the consumption of regular water. Food and water were removed at 7PM, one hour before the start of the 12h dark phase. At 8PM two bottles, one bottle containing water and one containing a 1% sucrose solution in tap water, were placed in each cage. After 12 hours at 8AM, the bottles were removed, and the food and water were returned to the cages. Bottles were weighed before and after the test. Rats were tested for sucrose preference on GD 17 (or the equivalent for nulliparous rats) and PD 2 (or equivalent; Table 1).
For each test, sucrose and water bottles were alternated in position to avoid the development of a side preference. Sample sizes were pup removal: n=12, pup remain n=12, and control: n=12.

Open Field Test (OFT)

The OFT is used to assess locomotor ability and anxiety-like behaviors in rats. Rats were placed in a 72 cm x 54 cm rectangular chamber, with the center defined as the middle 36 cm x 18 cm. Rats were brought from colony rooms to behavior rooms immediately prior to testing. Once in the room, they were placed in the corner of the chamber and recorded for behavior while experimenters remained out of sight. After 10 minutes, rats were removed and placed back in their home cages. Rats were tested on PD 2 (or equivalent for nulliparous rats) between 9:00AM-11:00AM (Table 1). This test occurred two days after the removal of offspring for the removal group and one day after culling in the remain group. Chambers were wiped with Clidox-S, a sterilant, and dried between tests. Rats were scored on center entry, time spent in center, and total distance traveled using Ethovision XT (version 11.5, Noldus, Leesburg, Virginia, USA), an automatic scoring software.

Forced Swim Test (FST)

The FST is used to assess depression-like behavior in rats. Each rat was tested twice over two consecutive days. Rats were placed in 41 cm high, 19 cm diameter cylindrical tanks filled with water at a temperature of 25°C (±1°C). Water depth was 29 cm, allowing rats to touch the bottom of the tank with their tails without allowing them to rest on their hind legs. On the first day, rats were placed into tanks and allowed to swim for 15 minutes. 24 hours later, rats were placed into tanks again for 5 minutes. After testing, rats would be lifted from the water, towel-
dried, and returned to their home cages. Cages were also lined with clean paper towels that covered bedding. Rats were tested on PD 4 and PD 5 (or the equivalent for nulliparous rats; Table 1). Water was replaced between each rat, and tanks were washed with mild soapy water at the end of each testing day. All testing was recorded on video, to be scored for time spent immobile using Ethovision automatic scoring software.

**Tissue Collection**

On PD 6, rats were injected with an overdose of the anesthetic sodium pentobarbital and placed back into their home cage. After the anesthetic had taken effect, the rat was tested with a toe pinch for pain response. If rats exhibited a pain withdrawal response after one injection of sodium pentobarbital, additional, small amounts of pentobarbital were administered to ensure that all rats undergoing perfusion reached a deep plane of anesthesia. An incision was made to allow access to the heart, followed by a small incision in the right atrium to allow blood, saline, and paraformaldehyde to exit after traveling through the cardiovascular system. At this time, blood was collected from the heart. A winged needle was inserted into the left ventricle of the heart and used to slowly pump 60 mL of saline, and then 120 mL of paraformaldehyde through the circulatory system. After these perfusions, the rat was placed in a guillotine to decapitate. The brain was removed from the skull and placed into 10 mL of paraformaldehyde for 24 hours at 4°C. Brains were then cryoprotected with 30% sucrose in 0.1M phosphate buffer and stored at 4°C. Brains were sectioned at 50 μm using a vibratome (Leica VT 1000 S, Leica, Buffalo Grove, IL, USA) and stored in antifreeze at -20°C until tissue was processed immunohistochemically.
Immunohistochemistry

Tissue containing the ventral tegmental area (VTA) was selected from each animal. Sections containing the anterior VTA were within bregma ranges of -5.28 to -5.64 whereas sections containing the posterior VTA were within the bregma of -5.76 to -6.12 (Harris & Aston-Jones, 2003; Wouterlood et al., 2018). The tissue was processed using an antibody targeted to tyrosine hydroxylase (TH) to label dopaminergic neurons. Between each step, tissue was rinsed with 0.1M PBS or 0.1M PBS-T (0.1M PBS/0.4% Triton-X) for 10 minutes, 3 to 5 times. First, tissue was incubated in a 0.6% H₂O₂ in dH₂O solution, then blocked with 1.5% normal goat serum in 0.1M PBS-T. Tissue was then incubated for 24 hours at 4°C in a TH primary antibody solution (1:2000 rabbit Anti-Tyrosine Hydroxylase, Abcam) with 0.1M PBS-T and 3% normal goat serum. The next day, tissue was incubated in secondary solution (1:500 biotinylated goat anti-rabbit, Vector) with 0.1M PBS for 24 hours at 4°C. To improve visualization by amplifying the signal, tissue was then incubated in avidin-biotin solution (1:1000 A; 1:1000 B, Vector) with 0.1M PBS for 4 hours at room temperature. It was then rinsed in sodium acetate buffer (2.38g sodium acetate per 100mL dH₂O) and developed in DAB solution (3,3'- diaminobenzidine tetrahydrochloride hydrate, Sigma-Aldrich).

Tissue was mounted onto slides and dehydrated using EtOH solutions of progressively stronger concentrations (50%, 70%, 95%, 100%) and xylene (Fisher Scientific). Slides were then coverslipped using permount (Fisher Scientific).

Microscopy

All slides were coded prior to analysis. An experimenter blind to group assignment analyzed sections in the anterior VTA for optical density TH labeling. Pictures of the VTA were
taken at the same exposure at a 10x objective using a Zeiss Axio Imager.A2 microscope (Zeiss, Thornwood, NY, USA). Optical density was assessed by placing three circular ROIs each with an area of 13029.3 um² on each side of the VTA and quantifying the average darkness of the image subtracted from the darkness of an unstained piece of tissue. Each animal had 2-3 sections, each with 6 ROIs total. Sample sizes were pup-remove: n = 7, pup-remain: n = 10, and nulliparous: n = 11.

**Statistical Analyses**

For behavioral data, comparisons between the three groups were analyzed using one-way ANOVA with group (pup-remove, pup-remain, and nulliparous) as the between-subjects factor. Time spent immobile in the FST for tests done on both days was analyzed using repeated measures ANOVA with immobility as the within-subjects factor. Total distance traveled, latency to first center entrance, and duration of time in the center for OFT were analyzed using repeated measures ANOVA with area (center, periphery) as the within-subjects factor. TH optical density was analyzed using repeated measures ANOVA with average density of the three ROIs as the within-subjects factor. Following significant ANOVA, post hoc comparisons utilized the Fisher LSD test. Statistical analyses were done using Statistica (v. 13, TIBCO, Palo Alto, CA, USA). Analyses were considered significant when \( p < 0.05 \).

<table>
<thead>
<tr>
<th></th>
<th>70-80 Days</th>
<th>GD 0-22</th>
<th>PD 0-1</th>
<th>PD 2</th>
<th>PD 4</th>
<th>PD 5</th>
<th>PD 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pup-remove</strong></td>
<td>Breeding</td>
<td>Pregnant</td>
<td>Pups removed</td>
<td>OFT &amp; SPT</td>
<td>FST 1</td>
<td>FST 2</td>
<td>Perfusion</td>
</tr>
<tr>
<td><strong>Pup-remain</strong></td>
<td>Breeding</td>
<td>Pregnant</td>
<td>Pups culled</td>
<td>OFT &amp; SPT</td>
<td>FST 1</td>
<td>FST 2</td>
<td>Perfusion</td>
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<tr>
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<td>Handling</td>
<td>Undisturbed</td>
<td>Undisturbed</td>
<td>OFT &amp; SPT</td>
<td>FST 1</td>
<td>FST 2</td>
<td>Perfusion</td>
</tr>
</tbody>
</table>

*Table 1.* Timeline of experiment and behavioral testing.
Results

Pup removal increased depressive-like behavior

Pup removal did not significantly alter depressive-like behavior in FST 1 ($F = 2.407, p = 0.107, \eta^2 = 0.138$; Fig. 1A). There was a significant effect of group on time spent immobile in FST 2 ($F = 5.858, p = 0.007, \eta^2 = 0.281$; Fig. 1B). Post-hoc comparisons showed that pup-remove rats spent significantly more time immobile than pup-remain rats ($p = 0.008$) and nulliparous rats ($p = 0.003$; Fig. 1B).

Figure 1. Forced Swim Test. A) Mean ± SEM of cumulative duration of time spent immobile in FST 1. There was no significant effect. B) Mean ± SEM of cumulative duration of time spent immobile in FST 2. There was a significant increase in time spent immobile for the pup-remove group compared to the pup-remain and nulliparous groups.
Removal of pups did not significantly alter sucrose preference at GD 17 ($F = 0.282, p = 0.747, \eta^2 = 0.018$) or PD 2 ($F = 0.942, p = 0.4, \eta^2 = 1.885$). However, an effect of time for all groups was seen in the preference for sucrose from GD 17 to PD 2 ($F = 15.394, p = 0.0004, \eta^2 = 0.325$; Fig. 2).

In the OFT, there was no significant effect of group on anxiety-like behavior. Experimental conditions did not alter duration in center ($F = 0.408, p = 0.669, \eta^2 = 0.024$; Fig 3A) nor latency to center ($F = 0.701, p = 0.503, \eta^2 = 0.041$). However, there was a significant effect of group on total distance moved (cm) in the OFT ($F = 4.297, p = 0.022, \eta^2 = 0.207$; Fig 3B). Post-hoc

*Figure 2. Sucrose Preference Test. Mean ± SEM of percentage of the total liquid consumed that was sucrose on GD 17 compared to PD 2. There was a significant decrease in sucrose consumption across time for all groups.*

*Pup removal did not alter anxiety like behavior*

In the OFT, there was no significant effect of group on anxiety-like behavior.
comparisons showed that nulliparous rats traveled a significantly greater total distance than pup-remain rats ($p=0.006$).

*Figure 3. Open Field Test.* A) Mean ± SEM of cumulative duration of time (sec) spent in the center of the OFT. B) Mean ± SEM of total distance traveled (cm) in the OFT. Nulliparous rats travelled a larger total distance compared to pup-remain moms.

**Pup removal did not alter TH expression in the VTA**

Optical density measures of TH expression in the VTA were not significantly altered by experimental condition ($F = 0.858$, $p = 0.435$, $\eta^2 = 0.062$; Fig. 4).
Discussion

The maternal experience is categorized by a shift in behavior that favors the exposure to and care of offspring. Rats in the early postpartum period are especially reinforced by exposure to offspring and can experience depressive-like behavior when offspring are removed. However, the effects of pup exposure on behavior and the accompanying neural changes are not entirely understood. This study looked at the effects of pup exposure on depressive and anxiety-like behavior and TH expression in the VTA. Rats that had their pups removed displayed increased immobility in the second day of FST compared to pup-exposed rats and nulliparous rats. This suggests that the removal of pups increased depressive-like behavior. This effect on behavior was specific to depressive-like behavior, as there was no significant effect of offspring removal.
on anhedonia or anxiety-like behaviors. Pup removal also did not significantly alter TH expression in the VTA.

For FST 1, there was no significant effect of group on immobility. This is to be expected, as FST 1 acts as a pre-test stage used to acclimate the rats to the testing conditions. During FST 2, time spent immobile increased for pup-remove rats compared to pup-remain and nulliparous rats. These results are interpreted as an increase in depressive-like behavior for pup-remove rats. This is consistent with the results found in the study mentioned previously, conducted by Pawluski et al. (2009), in which primiparous females without pup exposure displayed increased immobility four weeks after parturition compared to primiparous rats exposed to pups and sensitized nulliparous rats. This consistency across the progression of the postpartum period solidifies the role of offspring exposure on depressive-like behavior in rats.

Despite the differences in depressive-like behavior shown in the FST, there was no significant effect of experimental condition on sucrose preference, or grams of sucrose consumed. This suggests that removal of pups did not induce anhedonia, or decrease the ability to experience pleasure. While this is a common symptom of depression, and sucrose anhedonia is a validated test for use in animal models relevant for depression, it is possible that early postpartum offspring removal does not induce anhedonia in rats. Additionally, depression may not always be accompanied by anhedonia (Ho & Sommers, 2013). However, there was an effect of time on sucrose preference, in that all experimental groups decreased in sucrose preference from GD 17 to PD 2. This result is unexpected, as preference for sucrose usually increases through time as rats become habituated to the testing paradigm and the presence of an additional bottle during testing. While a decrease in sucrose preference from later pregnancy to early
postpartum has been observed in other studies (Green et al., 2009), this would not explain the
decline observed in nulliparous rats as well.

In the OFT, there was no significant effect of group on the duration of time spent in the
center of the field, nor on the latency of the rats to enter the center of the field. These results are
interpreted as there being no significant effect of group on anxiety-like behavior. These data are
again consistent with the results found in the study mentioned previously conducted by Pawluski
et al., (2009), which found no effects of offspring exposure on anxiety-like behaviors. These
results are in contrast with prior studies that suggest that the early postpartum period and
exposure to pups is associated with reduced anxiety-like behaviors (Lonstein, 2005; Love et al.,
2005). However, there was a significant effect of group on total distance moved (cm) in the OFT.
Nulliparous rats travelled a significantly greater total distance than pup-remain rats. Typically,
the total distance travelled in the OFT is interpreted as a reflection in overall locomotor activity.
The measure of total distance travelled in the OFT is also used as a control for the FST, which
relies on motor activity to assess depressive-like behavior. While the difference in total distance
travelled was significant between these two groups, this does not invalidate the results of the
FST, as the total distance travelled by pup-remove rats is not significantly different from either
pup-remain or nulliparous rats. This difference in locomotor activity may also be a result of the
increased metabolic demands of nursing mothers.

Optical density measures of TH expression in the VTA were not significantly different
among the experimental groups. This was unexpected, considering the various prior studies that
link the dopaminergic system with maternal caregiving and exposure to offspring (Fleming et al.,
1994; Grieb et al., 2020; Numan et al., 2009). However, prior research concerning maternal
behavior and its rewarding aspects have suggested that the systems controlling the rewarding
aspects of offspring are linked but separate from the mesolimbic dopamine system (Mattson et al., 2003). Additionally, the results of the present study might be explained through the mechanisms suggested by a study conducted by Caba et al. (2019) that found differences in response to pup removal and exposure in the activation of cells in the VTA, measured through cfos, but no differences in the number of dopaminergic cells, which were also identified with TH-labeling. The present study may then demonstrate that despite the effect of pup removal on depressive-like behavior, the dopaminergic activity is not driving the antidepressant effects of offspring exposure. While the present study found no significant results in optical density, it would be valuable to count the individual TH-expressing cells in the VTA to see if the same or different results are found. It would also be interesting to investigate other brain regions involved in the mesolimbic dopamine system, such as the nucleus accumbens where the VTA releases dopamine into, to assess if they are involved in the behavioral changes following pup removal or exposure.

Moving forward, more research needs to be done to assess the relationship between maternal caregiving and the reward system. While these aspects of the maternal rat are certainly linked, it is most likely a complex relationship that varies across the postpartum period. Knowing more about the mechanisms behind the changing maternal brain could lead to a higher level of understanding and potential interventions of PPD.

In conclusion, this study revealed increased depressive-like behavior in the early postpartum rat following pup removal, but no significant differences in TH expression in the VTA. These results might then suggest that despite the effect of pup removal on depressive-like behavior, dopaminergic activity as assessed by optical density of TH in the VTA is not driving the depressive-like effects of offspring removal. This work is important in furthering our
understanding of how the maternal experience affects the brain, and the neurological changes that underly PPD.
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