A Study of Limited Bedding and Nesting on Maternal Behavior for Postpartum Depression

Emma Brezoczky

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A Study of Limited Bedding and Nesting on Maternal Behavior for Postpartum Depression

A Thesis Presented

by

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To the Keck Science Department

of

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Senior Thesis in Neuroscience

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ABSTRACT

Postpartum depression (PPD) affects up to 20% of mothers in the US and can detrimentally affect both the mother and psychosocial development of the child (Pearlstein et al, 2009). So far, research on PPD is limited and the underlying neuropathology remains unclear. Low socioeconomic status is one risk factor that increases the risk of PPD tenfold (Goyal et al, 2010). The low resource limited bedding and nesting (LBN) paradigm used for rodents has the potential to model this risk factor. LBN has not previously been studied with PPD, but observations of disrupted maternal behaviors and depressive phenotypes makes it a promising novel model to study PPD. In this study, pregnant Sprague-Dawley dams were placed in either LBN or control conditions and maternal behaviors were observed for 9 days postpartum (P). Additionally, estrogen receptor alpha (ER-α) expression in the medial preoptic area (MPOA) was measured due to its hypothesized role in maternal behavior and therefore potentially in PPD mechanisms. No differences were found with maternal behaviors between LBN and control dams; however, an effect of time was found for behaviors supporting progressive changes in coordination with pup growth ($p<0.05$). For MPOA ER-α expression, a main effect of condition was found on P2 ($p<0.01$) but disappeared at P9 suggesting LBN dams can recover ER-α expression. While the risk factor of low socioeconomic status may increase the risk of developing PPD, it alone may not predispose mothers to PPD as shown through the LBN paradigm.
INTRODUCTION

Postpartum depression (PPD) is categorized as a major depressive disorder (MDD) appearing within a month after giving birth (Pearlstein et al, 2009). The majority of women postpartum, up to 85%, can experience postpartum blues within 10 days after giving birth but PPD persists in about 15% of these cases and can lead to suicide and infanticide (Pearlstein et al, 2009). PPD can have other severe detriments not only for the mother, infant, and family but later impacts on the child’s psychosocial development which can also burden society (Grace et al, 2003). Previous depression diagnosis is a significant predictor for PDD, but the postpartum brain differs in plasticity and hormone induced physiological changes. Therefore, it is critically necessary for research to focus specifically on the prevention and treatment of PPD instead of grouping PPD with MDD (Olazábal et al, 2013). An obstacle for investigating PPD appears through gaps in diagnosis as mothers in the United States are only screened for PPD once at their six-week postpartum appointment unless they seek additional treatment later on (Gifford et al, 2021). Not only does this time exceed the defined onset within a month but it also fails to capture cases appearing at 6 months or up to a year after giving birth (Pearlstein et al, 2009). In addition to this logistical gap, another obstacle for diagnosing PPD arises due to symptoms that commonly overlap for women during the postpartum period without PPD such as sleep disturbances, changes in appetite and excessive fatigue (Boyd et al, 2005).

Assessing risk factors to practice prevention may be key to minimizing the onset and severity of symptoms of PPD in vulnerable individuals (Pearlstein et al, 2009). Aside from previous MDD diagnosis, other prominent risk factors include estrogen and progesterone fluctuations, poor social support, low income, and low partner support (Corwin & Pajer, 2008; Pearlstein et al, 2009). Another study found that low socioeconomic status raises the risk of PPD up to ten times higher than those with a higher socioeconomic status (Goyal et al, 2010). Once
PPD is diagnosed, treatment options available include cognitive behavioral therapies and, more effectively, antidepressants; however, the use of antidepressants during breastfeeding may be resisted by mothers due to unwanted side effects both on the mother and unknown long-term effects on the infant (Battle et al, 2008).

Generally, PPD has been difficult to study because while clinical and pre-clinical research exists, the pathophysiological mechanisms remain unknown (Mir et al, 2022). Another obstacle for studying PPD is its categorization under MDD, as depression has mainly been studied in male models so there has been limited focus on pregnant or postpartum females (Carini et al, 2013). Animal models have been used to attempt to understand the neural mechanisms underlying PPD, and rodent models have been most successful (Mir et al, 2022). Even with these rodent models, the etiology and mechanisms still cannot be grasped which reinforces difficulties in improving treatment for PPD (Li & Chou, 2016). Rat species that have been used to attempt to study PPD include Sprague-Dawley, Long Evans, Wistar, Hooded Lister, and Fisher rats and the models used rely on estrogen withdrawal related to pregnancy or stress conditions to generate depressive phenotypes (Li & Chou, 2016; Mir et al, 2022). While the use of these models of experimental stress have helped gain insight into potential PPD mechanisms, development of a new naturalistic model for precipitating PPD is still needed (Li & Chou, 2016).

An important mechanism that might influence the quality of maternal care and drive differences related to PPD is estrogen sensitivity in the medial preoptic area (MPOA) of the hypothalamus (Champagne et al, 2003). The MPOA is a brain region involved in the onset and regulation of maternal behaviors in rodents making it a promising target for underlying PPD mechanisms (Champagne et al, 2003; Fang et al, 2018). Estrogen sensitivity is functionally
related to estrogen receptor-α (ER-α) expression in the MPOA (Champagne et al, 2003). ER-α drives structural changes in the MPOA leading to the onset and persistence of maternal behaviors as well as suppression of maladaptive responses to their pups (Champagne et al, 2003; Fang et al, 2018; Numan, 2007). The MPOA actively responds to environmental cues, especially in the postpartum period while adapting to pup growth, making it susceptible to various environmental stressors (Olazábal et al, 2013). ER-α is also significantly activated during the postpartum period and studies have shown that ER-α activation specifically in the MPOA alone is sufficient in inducing a complete onset of maternal behavior (Furuta et al, 2013; Numan, 2007). Further supporting the critical role of MPOA ER-α expression in maternal behavior are studies suppressing ER-α expression in the MPOA leading to reduced pup licking and nursing but an increase in depressive phenotypes, including infanticide, suggesting a role for MPOA ER-α in PPD (Champagne & Curley 2008; Furuta et al, 2013).

The well-studied Limited Bedding and Nesting (LBN) design for rodents can be adopted as a naturalistic approach to model a low resource, stressful environment representing the lower socioeconomic status conditions (Walker et al, 2017). LBN reduces the amount of bedding in a rodent’s environment creating an impoverished cage with a stressful environment due to the lack of bedding to build nests (Walker et al, 2017). Previous research uses LBN to study early life stress adversity on the development of rodent pups and the use of LBN is cited to mimic depressed, severely stressed, abusive, and diminished quality of parental care (Walker et al, 2017, Gallo et al, 2019). Although LBN typically focuses on the pups rather than the dams, and it has not been used before as a model for the low socioeconomic risk factor for PPD, changes in maternal behaviors under LBN conditions have been observed make this model seemingly useful for a novel way to study PPD (Walker et al, 2017, Gallo et al, 2019). LBN has been observed to
increase depressive phenotypes and disrupt maternal behaviors through increased nest exits, licking, and grooming as well as decreased nest digging and time spent with pups (Rincón-Cortés & Grace, 2022). For measuring the quality of maternal care in both LBN and control environments, an important measurement used is the presence of arch-back nursing (ABN) with high presence of ABN being a strong indicator for high quality of maternal care in rodents (Gallo et al, 2019; Walker et al, 2017). Using rodents in the LBN model and observing their behaviors may make it possible to study the neural mechanisms underlying PPD which could enable the improvement of current treatment options for patients.

In this study, LBN will be used as a model for the low socioeconomic risk factor of PPD. LBN has not previously been used for studying PPD in rodents making this study novel. Pregnant Sprague-Dawley dams will be separated into LBN and control groups, and on the day of pup births, maternal behaviors will start to be observed and recorded up to 9 days postpartum to examine behavioral differences between the environments. MPOA samples will also be taken from the dams to determine changes in ER-α expression from these environments. LBN is expected to decrease quality of maternal care through reduced frequency of ABN and other nesting behaviors such as nest digging, and time spent with pups. Additionally, ER-α expression in the MPOA is expected to be reduced in LBN dams, aligning with the expected decrease in maternal care and potential increase in depressive behaviors. By using the LBN model to study the effects during the postpartum period, this study aims to use the PPD social risk factor of low socioeconomic status to investigate changes in maternal behavior and its underlying mechanism of estrogen sensitivity in the MPOA. Should this study be effective in generating a novel PPD model by driving differences in maternal behaviors and ER-α expression, the LBN paradigm will
fill the gap in rodent PPD models and provide a way to better understand the neurobiological mechanisms underlying this harmful disease.

METHODS

Animals

The animals used were female Sprague-Dawley rats (n=48) (obtained from Charles River Laboratories, Wilmington, MA) and were housed in clear polypropylene cages with ad libitum access to food and water. These animals were kept under a 12 hour light, 12 hour dark cycle with temperature (22°C) and humidity held constant. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Delaware.

Breeding

For breeding, individual female rats were placed with different individual male rats for a maximum of 4 days. Females were checked daily for the presence of a sperm plug. Once the presence of a sperm plug was identified, it determined the day of conception and thus was assigned as Embryonic (E) Day 1. The day of birth was then assigned as Postnatal (P) Day 0 which was typically on day E23.

Limited Bedding and Nesting

At E19, the female rats were randomly assigned to one of two groups, LBN or control conditions. For LBN, 250 mL of bedding was used, while a standard 4000 mL of bedding was used for the control. The LBN bedding was changed daily for maintaining hygienic conditions. The control bedding was changed once per week to support stable pup-rearing conditions. This
paradigm was approved by the University of Delaware Institutional Animal Care and Use Committee (IACUC).

**Maternal Behavior**

Maternal behavior was recorded through UniFi video software. Automated recordings were taken for three consecutive 10 minute periods (30 minutes total) at 7 am and 3:30 pm during the light cycle and uploaded to a UniFi database. Recordings were taken daily from P0 to P2, and then half of the cohort was used for tissue collection at P2. The other half continued to be recorded for P4, and P7 to P9. Maternal behaviors were then hand scored at each minute for the 30 consecutive minutes for 13 maternal behaviors: pup licking, ABN, blanket nursing, passive nursing, grooming, sleeping, wall climb, approach pups, transport pups, retrieve pups, chase tail, digging, and step on pups (Table 1). Maternal behaviors were scored for each dam so that there were 30 consecutive observations on days P0-P2, P4, and P7-9. The observers scoring were trained and assessed for interrater reliability.

**Table 1. Ethogram of maternal behaviors scored.**

<table>
<thead>
<tr>
<th>Specific behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pup Licking</td>
<td>Dam repeatedly licks pups</td>
</tr>
<tr>
<td>Arch Back Nursing</td>
<td>Nursing with back arched with legs extended, pups visible</td>
</tr>
<tr>
<td>Blanket Nursing</td>
<td>Nursing position on top of the pups like a blanket</td>
</tr>
<tr>
<td>Passive Nursing</td>
<td>Dam laying on side, stomach is exposed to pups</td>
</tr>
<tr>
<td>Grooming</td>
<td>Dam licks self and uses paws to clean head and body</td>
</tr>
<tr>
<td>Sleeping</td>
<td>Dam does not move after 5 minutes, eyes are closed</td>
</tr>
</tbody>
</table>
### Wall Climb
Dam stands or leans against wall, hind legs remain on floor

### Approach Pups
Dam approaches pups

### Transport Pups
Dam moves pups from one spot to another

### Retrieve Pups
Dam brings pups to her from her nesting position

### Chase Tail
Dam chases own tail in a repetitive circular motion

### Digging
Dam digs through bedding material

### Step On Pups
Dam steps on pups

---

**Euthanasia, Perfusion, and Tissue Collection**

Animals were euthanized through an intraperitoneal injection administering an overdose of barbiturate Euthasol® (ANADA 200-071). Anesthesia was used and sufficiency was assessed by no response to toe or tail pinch. For perfusion, a 0.9% saline solution was used to remove peripheral blood from brain tissue. The brain was split into the two hemispheres and one hemisphere was placed in 4% paraformaldehyde for post fixation and the other was micro dissected. Tissue was immediately frozen on dry ice and stored at -80°C until ready for processing. Half of the animals underwent these procedures at P2 as mentioned above, and the remaining half underwent these procedures after completing maternal behavior recordings on P9.

**ER-α staining**

Half brains for immunohistochemistry were placed in 4% paraformaldehyde for 48 hours with fresh paraformaldehyde replacement at 24 hours. Half brains were then transferred to fresh 30% sucrose with 0.1% sodium azide solution daily for 2 consecutive days to dehydrate brain tissue until slicing. The brains were then flash frozen in 2-methylbutane and sliced at 30 microns.
into 5 series on a Leica CM1950 cryostat. Series of medial preoptic area (MPOA) sections were sorted and stained using anti-estrogen receptor α from Sigma (Cat No. 06-935) and Vector Laboratories goat anti-rabbit biotinylated secondary antibody to visualized ER-α. Sections were mounted onto microscope slides and MPOA sections were imaged with Stereo Investigator software. Densitometry staining in the MPOA was then analyzed with ImageJ software. Integrated area densities and % area from 6-8 sections per brain were averaged and used to determine average density of ER-α in the MPOA.

Data Analysis

Maternal behavior scores were analyzed with a two way ANOVA in SPSS to test for main effects of condition and day, as well as interactions between the condition and day. Tukey’s HSD Post Hoc tests were also used for analysis. For determining ER-α expression, a two way ANOVA was run in SPSS where the integrated area densities (IntDen) and percent area from ImageJ were treated as dependent variables and the day (P2, P9) and condition (LBN and control) were independent factors. Differences were considered significant at p<0.05 and a trend was defined as p<0.10.

RESULTS

ABN and Non-Nursing Behaviors

A two way ANOVA was run to determine the effect of bedding condition (LBN and control) and day (P0-P9) on maternal behaviors. There were no significant differences found between groups for ABN, pup licking, grooming, wall climb, approach pups, transport pups, retrieve pups, chase tail, digging, and step on pups (Table 2).
Table 2. ANOVA Results for ABN and Non-Nursing Behaviors

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Condition</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABN</td>
<td>$F(1,216)=0.882, p=0.349$</td>
<td>$F(6,216)=0.671, p=0.673$</td>
</tr>
<tr>
<td>Pup Licking</td>
<td>$F(1,216)=0.055, p=0.815$</td>
<td>$F(6,216)=0.814, p=0.560$</td>
</tr>
<tr>
<td>Grooming</td>
<td>$F(1,216)=1.490, p=0.224$</td>
<td>$F(6,216)=0.212, p=0.973$</td>
</tr>
<tr>
<td>Wall Climb</td>
<td>$F(1,216)=0.001, p=0.981$</td>
<td>$F(6,216)=1.300, p=0.258$</td>
</tr>
<tr>
<td>Approach Pups</td>
<td>$F(1,216)=0.249, p=0.618$</td>
<td>$F(6,216)=0.859, p=0.167$</td>
</tr>
<tr>
<td>Transport Pups</td>
<td>$F(1,216)=1.599, p=0.207$</td>
<td>$F(6,216)=0.456, p=0.840$</td>
</tr>
<tr>
<td>Retrieve Pups</td>
<td>$F(1,216)=0.473, p=0.492$</td>
<td>$F(6,216)=0.787, p=0.581$</td>
</tr>
<tr>
<td>Chase Tail</td>
<td>$F(1,216)=1.660, p=0.199$</td>
<td>$F(6,216)=0.594, p=0.735$</td>
</tr>
<tr>
<td>Digging</td>
<td>$F(1,216)=2.173, p=0.142$</td>
<td>$F(6,216)=0.477, p=0.825$</td>
</tr>
<tr>
<td>Step on Pups</td>
<td>$F(1,216)=0.010, p=0.919$</td>
<td>$F(6,216)=1.128, p=0.347$</td>
</tr>
</tbody>
</table>

Blanket Nursing

A two way ANOVA was run to determine the effect of bedding condition (LBN and control) and day (P0-P9) on blanket nursing. There was no significant effect of the condition on blanket nursing. There was a significant effect of day (P0-P9) on blanket nursing ($F(6,216)=8.187, p<0.001$) (Figure 1). A Tukey HSD Post Hoc test was run and showed that there was a significant different between P0 and P4, P7, P8, P9 ($p$’s<0.05); a difference between P1 and P4, P9 ($p$’s<0.05); and a difference between P2 and P4, P7, P9 ($p$’s<0.05) (Table 3). There was also a trend towards significance for P2 and P8 (Table 3).
Figure 1. Maternal Behaviors from P0 to P9. Mean ± SEM. A two way ANOVA found a main effect of day on (A) Blanket Nursing ($F(6,216)=8.187, p<0.001$), with decreases between P0 and P4, P7, P8, P9 ($p$’s<0.05), P1 and P4, P9 ($p$’s<0.05), P2 and P4, P7, P9 ($p$’s<0.05). (B) Sleep ($F(6,216)=3.110, p=0.006$), with a decrease between P0 and P9 ($p=0.050$). (C) Passive Nursing with a trend towards a significant increase across time ($p<0.10$).

Table 3. Tukey’s HSD Post Hoc Results for Blanket Nursing

<table>
<thead>
<tr>
<th>Day-Day</th>
<th>$p$</th>
<th>Day-Day</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-P1</td>
<td>$p=0.895$</td>
<td>P2-P4</td>
<td>$p=0.015**$</td>
</tr>
<tr>
<td>P0-P2</td>
<td>$p=0.995$</td>
<td>P2-P7</td>
<td>$p=0.050*$</td>
</tr>
<tr>
<td>P0-P4</td>
<td>$p=0.003**$</td>
<td>P2-P8</td>
<td>$p=0.053+$</td>
</tr>
<tr>
<td>P0-P7</td>
<td>$p=0.011**$</td>
<td>P2-P9</td>
<td>$p&lt;0.001**$</td>
</tr>
<tr>
<td>P0-P8</td>
<td>$p=0.011**$</td>
<td>P4-P7</td>
<td>$p=1.000$</td>
</tr>
<tr>
<td>P0-P9</td>
<td>$p&lt;0.001**$</td>
<td>P4-P8</td>
<td>$p=1.000$</td>
</tr>
<tr>
<td>P1-P2</td>
<td>$p=0.998$</td>
<td>P4-P9</td>
<td>$p=0.816$</td>
</tr>
<tr>
<td>P1-P4</td>
<td>$p=0.060+$</td>
<td>P7-P8</td>
<td>$p=1.000$</td>
</tr>
</tbody>
</table>
Sleep

A two way ANOVA was run to determine the effect of bedding condition (LBN and control) and day (P0-P9) on sleep. There was no significant effect of condition on sleep. There was a significant effect of day (P0-P9) on sleep ($F(6,216)=3.110, p=0.006$). A Tukey HSD Post Hoc test showed that there was a significant difference between P0 and P9 ($p=0.050$) (Table 4) (Figure 1). There was also a trend towards significance between P1 and P9 ($p=0.068$) (Table 4).

Table 4. Tukey’s HSD Post Hoc Results for Sleep

<table>
<thead>
<tr>
<th>Sleep</th>
<th>Day-Day</th>
<th>$p$</th>
<th>Day-Day</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-P1</td>
<td>$p=1.00$</td>
<td></td>
<td>P2-P4</td>
<td>$p=0.525$</td>
</tr>
<tr>
<td>P0-P2</td>
<td>$p=0.995$</td>
<td></td>
<td>P2-P7</td>
<td>$p=0.953$</td>
</tr>
<tr>
<td>P0-P4</td>
<td>$p=0.220$</td>
<td></td>
<td>P2-P8</td>
<td>$p=0.401$</td>
</tr>
<tr>
<td>P0-P7</td>
<td>$p=0.738$</td>
<td></td>
<td>P2-P9</td>
<td>$p=0.181$</td>
</tr>
<tr>
<td>P0-P8</td>
<td>$p=0.149$</td>
<td></td>
<td>P4-P7</td>
<td>$p=0.994$</td>
</tr>
<tr>
<td>P0-P9</td>
<td>$p=0.050^*$</td>
<td></td>
<td>P4-P8</td>
<td>$p=1.000$</td>
</tr>
<tr>
<td>P1-P2</td>
<td>$p=0.999$</td>
<td></td>
<td>P4-P9</td>
<td>$p=0.998$</td>
</tr>
<tr>
<td>P1-P4</td>
<td>$p=0.274$</td>
<td></td>
<td>P7-P8</td>
<td>$p=0.981$</td>
</tr>
<tr>
<td>P1-P7</td>
<td>$p=0.803$</td>
<td></td>
<td>P7-P9</td>
<td>$p=0.900$</td>
</tr>
<tr>
<td>P1-P8</td>
<td>$p=0.191$</td>
<td></td>
<td>P8-P9</td>
<td>$p=1.000$</td>
</tr>
<tr>
<td>P1-P9</td>
<td>$p=0.068^+$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: ** $p<0.01$,   * $p<0.05$, + indicates a trend towards significance, $p<0.10$*

Passive Nursing
A two way ANOVA was run to determine the effect of bedding condition (LBN and control) and day (P0-P9) on passive nursing. There was no significant effect of the condition on passive nursing. There was also no significant effect of day (P0-P9) on passive nursing, however there was a trend towards significance ($F(6,216)=1.902, p=0.082$) (Figure 1).

**ER-α Expression**

A two way ANOVA was run to determine the effect of pregnancy with a non-pregnant control (NPC) and bedding condition (LBN and control) on ER-α expression through % area and IntDen. The % area and IntDen were measured on postpartum day 2 (P2) and postpartum day 9 (P9). There was a main effect of condition found for % area on P2 ($F(2,20)=5.897, p=0.010$). A Tukey HSD Post Hoc test revealed a significant difference between the NPC and control ($p<0.01$) (Table 5). There was also a trend towards significance between the NPC and LBN ($p=0.07$) (Table 5). For IntDen, there was a trend towards significance between groups on P2 ($F(2,20)=2.876, p=0.08$) (Figure 2). There were no significant differences found for % area and IntDen on P9 (Figure 2).

**Table 5.** Tukey’s HSD Post Hoc Results for % area on P2

<table>
<thead>
<tr>
<th>Condition - Condition</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC – Control</td>
<td>$p=0.007**$</td>
</tr>
<tr>
<td>NPC – LBN</td>
<td>$p=0.070+$</td>
</tr>
<tr>
<td>Control – LBN</td>
<td>$p=0.365$</td>
</tr>
</tbody>
</table>

*Note: ** $p<0.01$, * $p<0.05$, + indicates a trend towards significance, $p<0.10$*
Figure 2. A one way ANOVA was used to measure ER-\(\alpha\) expression. Mean ± SEM, * \(p<0.05\) and + indicates a trend, \(p<0.10\). (A) % area on postpartum day 2. Significant decrease found between NPC and the control \((p=0.007)\) and a trend towards a decrease between NPC and LBN \((p=0.07)\). (B) % area on postpartum day 9. No significant differences found between groups.

DISCUSSION

In humans, low socioeconomic status can increase the risk of PPD up to ten times greater than higher socioeconomic status mothers (Goyal et al, 2010). This study used the LBN paradigm to model the low resource risk factor as a novel rodent investigation of PPD. Maternal behaviors determined the effect of LBN on quality of maternal care, and our results showed that the condition of LBN did not significantly affect maternal behaviors. ER-\(\alpha\) expression in the MPOA was also measured to investigate the hypothesis that the underlying mechanism of PPD was MPOA estrogen sensitivity. Our results showed that the LBN condition did not significantly influence MPOA ER-\(\alpha\) expression. While LBN did not significantly affect maternal behaviors or ER-\(\alpha\) expression in the early postpartum period, an effect of day (P0-P9) was found for blanket nursing and sleeping behaviors. Generally, our findings suggest that the LBN paradigm alone as
a model for low socioeconomic status may not be successful as a rodent model for studying PPD. Additionally, while low socioeconomic status is a risk factor for PPD, the underlying neurobiological mechanisms remain unclear so a rodent model with a predisposition to depression, a major PPD risk factor, may need to be paired with LBN to see any behavioral and mechanistic effects for more effectively modeling PPD.

**LBN Does Not Affect Maternal Behaviors**

No significant differences were found between control and LBN conditions for all 13 maternal behaviors measured in this study. Our findings do not support previous research reporting that LBN disrupts maternal behaviors and increases depressive phenotypes (Gallo et al, 2019; Rincón-Cortés & Grace, 2022; Walker et al, 2017). One major discrepancy in our study that might explain these differences compared to previous research is in frequency of maternal behavior observations. In this study, maternal behaviors were only recorded for 30 minutes during the light cycle with measurements taken at one minute increments. Other studies recorded maternal behavior for both the light and dark cycle and found that the light and dark cycles affected maternal behaviors differently (Rincón-Cortés & Grace, 2022). Some studies also used tracking technology to measure changes in maternal behaviors compared to our hand-scored maternal behaviors which increased the frequency of observations allowing for more data collection and more thorough assessments of changes in maternal behaviors across the early postpartum period (Gallo et al, 2019).

Studies using more frequent observation periods could observe the frequency and duration of maternal behaviors (Walker et al, 2017). One study found that LBN led to fragmented behaviors, meaning there were more initiation attempts of nursing and pup interactions than controls, but that the overall expression of the behaviors were the same between
groups (Walker et al, 2017). In this study, behavior initiations could not be assessed due to our observation and scoring methodology; however, our results do support that overall expression of maternal behaviors were the same for control and LBN dams.

Another factor not considered in this study was defining negative maternal behaviors. A recent study measured an effect of LBN increasing adverse maternal behaviors including time dragging pups, shoving pups away, and having a scattered litter during light and dark cycles (Rincón-Cortés & Grace, 2022). Dragging pups, shoving pups, and scattered litters were not behaviors measured in this study. We found no differences in maternal care between the control and LBN groups through any of our observed maternal behaviors with the absence of the defined negative maternal behaviors. Instead of defining and observing negative maternal behaviors, quality of maternal care was expected to be observed through nursing since arched back nursing (ABN) has previously been shown to be a strong indicator for high quality of maternal care in rodents (Gallo et al, 2019; Walker et al, 2017). Regarding the ABN measurement, our findings align with other studies that reported inconsistent or no differences between LBN and control dams even though the ABN behavior was expected to decrease with LBN (Gallo et al, 2019; Walker et al, 2017).

While the LBN paradigm used in this study did not appear to affect maternal behaviors, increasing observation periods and considering aversive behaviors in measuring maternal behaviors may provide more intricate details about differences in maternal behaviors. Future studies should consider increasing the frequency of observation periods and including negative maternal behavior measurements to determine the effectiveness of this LBN paradigm for studying PPD in rodents.
Nesting Behaviors Alterations During the Postpartum Period

While the condition of LBN did not significantly differ from the control group for maternal behaviors, there were changes across time for blanket nursing, passive nursing, and sleeping. There was a significant decrease between postpartum day 0 and postpartum day 9 for both blanket nursing and sleeping. Blanket nursing had the greatest occurrence of differences as there was significantly more blanket nursing on postpartum day 0 compared to four later days, 4, 7, 8, and 9 whereas there was only one decrease found between postpartum day 0 and postpartum day 9 for sleep (Figure 1A, 1B). While passive nursing did not have any significant differences between days, there was a trend towards a significant increase (Figure 1C). This difference in passive nursing may be due to a subtle shift in maternal behavior of nursing over time which aligns with studies that reported changes in maternal behavior can be subtle and progressive in coordination with pup growth (Cramer et al, 1990). In this case, we observed a subtle increase in passive nursing following a decrease in blanket nursing which may be due to the growth in pups making passive nursing a more feasible nursing position.

Interestingly, our results show significant behavioral changes appear in the early postpartum period, as soon as postpartum day 4 for blanket nursing, which challenges studies reporting that maternal behaviors do not change until after postpartum day 10 with older pups (Cramer et al, 1990; Reisbick et al, 1975). However, this may help explain why significant differences only appeared for two maternal behaviors and not the other 11 maternal behaviors observed in this study since significant changes may not appear until after postpartum day 10 such as increased pup licking and exploration (Reisbick et al, 1975). Additionally, the incomplete observation periods, as discussed before, may have also contributed to the small magnitude of differences between days (P0-P9) as well as the few numbers of behaviors.
affected. Increasing the duration and frequency of the early postpartum observation periods within postpartum days 0-9 and beyond postpartum day 9 could provide a more complete picture of subtle shifts in maternal behaviors during the postpartum period.

One reason why there were only differences across time instead of between conditions may be due to the window of testing. In humans, many women experience postpartum blues, but only up to 15% of these cases may persist as PPD between week 2 and a year postpartum (Pearlstein et al., 2009). In rodents, a similar postpartum blues has been reported where postpartum rats exhibit depressive-like behavior including increased anxiety, reduced social motivation, and increased forced swim test immobility during the first week postpartum (Rincón-Cortés & Grace, 2020). These depressive-like behaviors were no longer observable in the late postpartum period after postpartum day 21 aligning with the fact that many postpartum blues cases resolve beyond the early postpartum period (Rincón-Cortés & Grace, 2020). In this study, maternal behaviors were not observed beyond postpartum day 9, so it is possible that the LBN group and control group do not differ significantly in the early postpartum period observed due to both groups experiencing postpartum blues at the same time in this early postpartum window. In future studies, maternal behavior observations need to be continued beyond postpartum day 9 into postpartum day 21 to look for an effect of LBN on the persistence of depressive phenotypes into PPD for these dams.

**LBN MPOA Recovers ER-α Expression in the Early Postpartum Period**

ER-α expression in this study was measured through values of percent area and integrated density comparing nonpregnant controls (NPC), pregnant controls, and LBN dams.
Measurements of percent area represented the area of ER-$\alpha$ expression in the MPOA sections and integrated density measured an average value of ER-$\alpha$ expression in the MPOA sections.

There were differences found between conditions with percent area on postpartum day 2 for ER-$\alpha$ expression (Figure 2A). A trend for differences between groups was also found using the integrated density measure on postpartum day 2 supporting these percent area results. Pregnant dams were found to have significantly lower ER-$\alpha$ expression at postpartum day 2, shortly after birth, compared to NPC, which may suggest a mechanism for postpartum blues. Dams have been observed to have postpartum blues as expected in the postpartum period and this may align with previous research that found lower ER-$\alpha$ expression corresponded to depressive phenotypes (Agrati & Lonstein, 2016; Champagne & Curley, 2008; Furuta et al, 2013). However, for LBN at postpartum day 2, LBN only trends towards a significant difference from NPC, whereas the control significantly differs, suggesting that there may be some effect from LBN on ER-$\alpha$ expression in the MPOA (Figure 2A). Differences in MPOA ER-$\alpha$ sensitivity have been shown to align with differences in maternal behaviors but there were no behavioral differences found between conditions in this study (Olazábal et al, 2013). This in part may result from the observation point at two days postpartum since significant changes in maternal behaviors were not observed until postpartum day 4 in this study, and after postpartum day 21 in other studies (Rincón-Cortés & Grace, 2020). Additionally, the effect of condition on MPOA ER-$\alpha$ expression disappeared at postpartum day 9 for both percent area and integrated density which supports the lack of differences found for maternal behaviors. This suggests that LBN dams can recover ER-$\alpha$ expression within the early postpartum period preventing differences in maternal behaviors (Figure 2B).
The MPOA is a brain region critical in the onset and regulation of maternal behaviors and as LBN recovered ER-α expression within the early postpartum period, no differences in maternal behaviors were found. Since this study was limited to measuring ER-α expression in the MPOA, it is unknown if other underlying mechanisms through other receptors or brain regions are responding to the LBN condition allowing this recovery. Previous research studying the relationship between ER-α and stress conditions have identified ER-α to be a major regulator for stress resilience related to depressive behaviors (Eid et al, 2020; Qu et al, 2020). In these studies, instead of chronic ethological stress that occurs with LBN, chronic unpredictable stress (CUS) was used and included random exposures to wet bedding, social defeat, restraint, cage tilting, light changes, and noise disturbances (Eid et al, 2020; Lorsch et al, 2018; Qu et al, 2020). The CUS model precipitated depressive phenotypes but the use of ER-α agonists ameliorated the depressive phenotypes and restored neurogenesis in the hippocampus (Qu et al, 2020). While in the MPOA, ER-α expression plays a role in the regulation of maternal behaviors, ER-α pro-resilience effects in other brain structures for stress and depressive phenotypes may mediate the effects of LBN stress (Lorsch et al, 2018; Qu et al, 2020). The pro-resilience effect of ER-α in other structures may also help protect the MPOA from LBN stress which could explain why LBN dams are able to recover ER-α expression in the MPOA. Additionally, this study only observes the effects of LBN in the early postpartum period, so it is unknown if the resilience of LBN MPOA ER-α expression persists into the late postpartum period to protect LBN dams from later PPD onset.

Another important consideration in studying PPD is individual differences, such as the major predictor of a previous depression diagnosis (Pearlstein et al, 2009). Specifically, in the MPOA early life experience such as the frequency of a pup being licked can later impact both
the pup’s ER-α sensitivity as well as its own maternal behavior (Champagne et al, 2003). Additionally, LBN has been demonstrated in several studies to generate depressive-like behaviors in the pups born into this environment, so it may be more informative to return the LBN pups to the LBN condition when they become pregnant dams (Walker et al, 2017). Aside from returning LBN pups to the LBN condition as pregnant dams, other preclinical models for depression including learned helplessness, social instability, social defeat stress, or social transmission of stress can be used to generate a rodent model for the previous depressive diagnosis PPD risk (Guruajan et al, 2019). Additionally, learned helplessness, social instability, social defeat stress and social transmission of stress can all help model risks associated with low socioeconomic status including poor social support, low income, and low partner support making them good models for our LBN paradigm (Guruajan et al, 2019; Pearlstein et al, 2009). Pairing the previous depression phenotype risk through preclinical depression models with the low socioeconomic status risk in our LBN paradigm can improve our novel ethological model to investigate the mechanism of ER-α sensitivity in the MPOA for PPD (Guruajan et al, 2019; Pearlstein et al, 2009).

CONCLUSION

This study aimed to determine the effects of the LBN paradigm on maternal behavior as a novel model to study PPD. No differences in maternal behaviors were observed between LBN and control dams suggesting that LBN potentially may not be the best model to study PPD on its own. Differences in MPOA ER-α expression were found at postpartum day 2, however LBN recovered ER-α expression by postpartum day 9. The lack of differences between ER-α expression by postpartum day 9 supported our maternal behavior results. Differences in methodology for recording maternal behavior may explain why no effect of condition was found
on maternal behavior. Additionally, ER-\( \alpha \) stress resilience and individual differences in MPOA ER-\( \alpha \) expression may contribute to the LBN recovery of ER-\( \alpha \) expression. This study only studied the effects of LBN in the early postpartum period but no LBN effects in the late postpartum which is a time when PPD can appear clinically. Future studies should also consider individual differences such as early life events or use preclinical depression models paired with LBN to create a more promising model with the novel use of the LBN paradigm to study PPD.
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