Behavioural Brain Research 353 (2018) 143–153

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

High nursing demand reduces depression-like behavior despite increasing glucocorticoid concentrations and reducing hippocampal neurogenesis in late postpartum rats

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ARTICLE INFO

Keywords:
Lactation
Suckling stimulation
Postpartum depression
Doublecortin
Ki-67

ABSTRACT

Approximately 15% of women who give birth develop postpartum depression (PPD), and the risk is greater in women who do not breastfeed or who cease breastfeeding early. In some women, early cessation or absence of breastfeeding precedes PPD, but the neuroendocrine mechanisms of this relationship are unknown. We tested whether nursing demand would alter behavioral and endocrine endpoints relevant for depression in postpartum rats. Adult female Sprague-Dawley rats underwent thelectomy (thel; removal of teats), sham surgery (sham), or no surgery (control). Litters were rotated between thel and sham rats every 12 h, yielding a higher nursing burden for sham rats. We investigated behavior in the forced swim test (FST), open field test, and sucrose preference test, and serum corticosterone (CORT) concentrations. Because the hippocampus changes structurally in depression and with maternal experience, we investigated cell proliferation using Ki-67 and hippocampal neurogenesis and immature neuron development using doublecortin (DCX) immunohistochemistry. Sham rats spent less time immobile in the FST compared with control and thel rats. Sham rats also had higher CORT concentrations and fewer Ki-67 cells. Thel rats had more DCX-expressing cells and a greater proportion of mature DCX-expressing cells compared with control and sham rats. These data suggest that greater nursing demand reduces stress-related behavioral responses despite increasing CORT concentrations and suppressing hippocampal neurogenesis. This work is an important step in identifying how lactation buffers behavioral responses to stress and reorganizes stress-related neural circuitry and is crucial for identifying mechanisms of postpartum psychiatric illnesses.

1. Introduction

Lack or early cessation of breastfeeding is associated with a greater risk for numerous diseases in infants and mothers: bottle-fed infants have a higher risk for infections, autoimmune diseases, and psychological illness, and women who bottle-feed have a higher risk for ovarian and breast cancers and metabolic syndrome [reviewed in 1]. Importantly, women who bottle-feed are more likely to develop postpartum depression (PPD) or postpartum depressive symptoms compared with women who breastfeed [2–4]. The CDC and the American Academy of Pediatrics recommend exclusive breastfeeding during the first six months after parturition and continued breastfeeding with supplementation for 1 year or longer [5,6]. Approximately 18.9% of mothers in the U.S. do not breastfeed at all and only 22.3% of mothers breastfeed exclusively for the recommended minimum of 6 months following delivery [6]. The neuroendocrine adaptations that support lactation could promote resilience to psychiatric illness. Thus, research into the relationships among lactation, hormones, and PPD holds critical public health implications.

In most studies that have investigated breastfeeding and depression, the absence or early cessation of breastfeeding was associated with a greater likelihood of depression or depressive symptoms during the postpartum period [e.g., 2–4]. Longitudinal studies suggest a bidirectional relationship between breastfeeding and PPD: in some cases, depressive symptoms preceded cessation of breastfeeding [e.g., 7–9], whereas in other cases, cessation or lack of breastfeeding preceded the development of depressive symptoms [e.g., 2,10]. These latter studies suggest that early cessation of breastfeeding might increase the risk for PPD in some women. Further, cessation of breastfeeding exacerbated an extant depressive episode [11]. In women, breastfeeding (compared

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https://doi.org/10.1016/j.bbr.2018.07.012
Received 27 March 2018; Received in revised form 3 July 2018; Accepted 18 July 2018
Available online 19 July 2018

with formula-feeding) suppressed hypothalamic-pituitary-adrenal (HPA) axis responses to exercise stress [12] and the Trier Social Stress Test [13]. In lactating rats, suckling from offspring during the postpartum period suppressed HPA axis responses to stressors [14]. Because depression is associated with HPA axis dysregulation, it is possible that chronic disinhibition of HPA axis responses to stressors in bottle feeding women increases the vulnerability to PPD.

Repeated or complete removal of offspring increases passive-coping responses (i.e., immobility) in the forced swim test (FST, a task relevant for depression). Long separations (3 h) from offspring increased immobility in postpartum rats compared with brief separations (15 min) and no separation [15]. Further, complete removal of offspring directly after parturition increased immobility in late postpartum rats compared with rats that remained with their offspring [16]. This suggests that absence of offspring increases passive-coping behavior in postpartum rats, but it is unknown whether the increase in passive-coping is due to the absence of nursing itself or the absence of offspring, because offspring provide dams with tactile, social, and chemosensory stimulation. We tested whether the absence of nursing (while maintaining offspring exposure) would alter passive coping in the FST in late postpartum rats. We chose to investigate late postpartum rats because we expected that depression-like behavior would develop due to a chronic rather than acute absence of nursing and to compare with numerous studies investigating depression-like behavior at approximately this time point [15–20].

In women, whole brain volume decreased during pregnancy [21]. Further, cortical and hippocampal volumes decreased during pregnancy, but the hippocampus was the only brain region to partially recover in volume 2 years postpartum [22]. Volume reduction might indicate that these brain regions become more efficient with reproductive experience. Maternal experience also restructures the hippocampus in rats. For instance, postpartum rats have reduced hippocampal neurogenesis compared with nulliparous rats [18,23–26] due to elevated baseline glucocorticoid concentrations during the postpartum period [25]. We therefore investigated whether nursing experience alters cellular proliferation and the density and development of immature neurons in the hippocampus of postpartum rats.

To test whether manipulation of nursing would alter stress-related behavioral and endocrine responses relevant for depression in late postpartum rats, we used thelectomy, a minimally invasive surgery to remove the teats, which inhibits milk letdown [14,27]. Wiesner and Sheard [28] were the first (to our knowledge) to use thelectomy in 1933 to determine whether absence of nursing influenced maternal behavior. Since then, thelectomy has been used for several decades to investigate the effects of nursing on maternal care [29], ingestive behavior and metabolism [30], anxiety-like behavior [27], maternal aggression [31,32], and neuroendocrine function [14,33]. Thelectomy, however, has not been used to understand how nursing contributes to depression-like behavior or structural brain plasticity following maternal experience. The use of thelectomy allowed us test the effects of nursing on these endpoints while dams were exposed to offspring throughout the postpartum period. We maintained offspring exposure by rotating litters to intact, nursing dams every 12 h and assessed passive-coping behavior in the FST, sucrose preference, and non-stress corticosterone (CORT) concentrations. Because estrogens can influence depression-like behavior [34], we identified the resumption of estrous cyclicity through daily lavage samples beginning on PD 8 [18,35]. Finally, we used Ki-67 immunohistochemistry (IHC) to quantify proliferating cells and DCX IHC to quantify the number and developmental stage of immature granule cells in the dentate gyrus (DG). We hypothesized that the absence of nursing would increase immobility in the FST and would increase hippocampal neurogenesis.

2. Methods and materials

2.1. Animals

Thirty-two female and 9 male Sprague Dawley rats were purchased from Charles River Laboratories (Stone Ridge, NY, USA). Upon arrival, rats were housed in same-sex pairs in clear polycarbonate cages (27 cm x 48 cm x 29 cm) with microfilter top and Aspen chip bedding. Females were single housed on gestational day (GD) 0. Cages included one red polycarbonate tunnel until breeding (for males) or until surgery (for females). Colony rooms maintained a 12:12 light/dark cycle with lights on at 7:00 AM. The light intensity in the vivarium and test rooms was 130–325 lux at cage level and was maintained by a time-controlled lighting system (Edstrom). The light intensity was consistent throughout the study. Rats had *ad libitum* access to pellets (Lab Diet 5P76 Irradiated ProLab IsoPro RMH 3000) and water unless otherwise specified. Rats were allowed 7 days to habituate to the facility. Thereafter, all females were handled 5 min per day for 5 d and then randomly assigned to receive thelectomy (thel), sham surgery (sham), or no surgery (control). Following breeding, 6 rats (thel: n = 3, sham: n = 2, control: n = 1) spontaneously aborted prior to GD 14 and were excluded from the experiment. Thus, final sample sizes were thel: n = 8, sham: n = 8, and control: n = 10. Offspring were weaned on PD 26 to ensure that all dams had exposure to offspring throughout the experiment and to avoid the possible disruption due to removal of

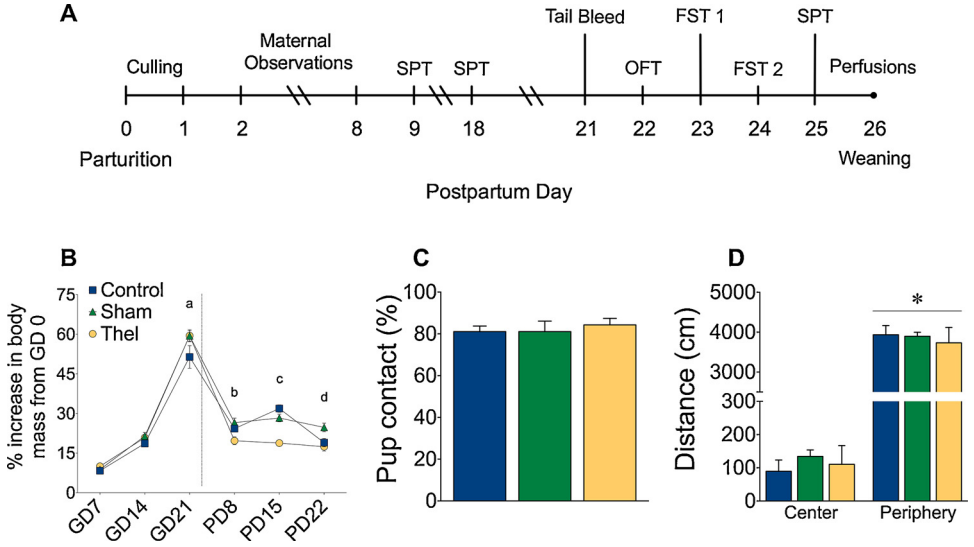

Fig. 1. A) Timeline of experimental procedures during the postpartum period. Litters were culled PD 0 – 1. Maternal observations were conducted PDs 2–8. On PDs 9, 18, and 25, rats were presented with a two-bottle choice test to assess sucrose preference (SPT). Rats were tail bled on PD 21 to assess non-stress CORT concentrations. Rats were tested in the open field test (OFT) on PD 22 and the forced swim test (FST) on PDs 23 – 24. Timeline is not to scale. B) Percent change ± SEM in body mass throughout gestation and postpartum ([a]thel and sham compared with control, *ps* < 0.005; [b]thel compared with sham, $p < 0.008$; [c]thel compared with sham and control, *ps* < 0.001; [d]sham compared with thel and control, *ps* < 0.03). C) Mean + SEM time in contact with offspring. D) Mean + SEM distance in the center and periphery of the OFT (*compared with center, $p < 0.001$).