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EFFECTS OF STRESS EARLY IN GESTATION ON HIPPOCAMPAL NEUROGENESIS AND GLUCOCORTICOID RECEPTOR DENSITY IN PREGNANT RATS

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Abstract—Pregnancy is a time of marked neural, physiological and behavioral plasticity in the female and is often a time when women are more vulnerable to stress and stress-related diseases, such as depression and anxiety. Unfortunately the impact of stress during gestation on neurobiological processes of the mother has yet to be fully determined, particularly with regard to changes in the hippocampus; a brain area that plays an important role in stress-related diseases. The present study aimed to determine how stress early in pregnancy may affect hippocampal plasticity in the pregnant female and whether these effects differ from those in virgin females. For this purpose, adult age-matched pregnant and virgin female Sprague–Dawley rats were divided into two conditions: (1) Control and (2) Stress. Females in the stress condition were restrained during days 5–11 of gestation and at matched time-points in virgin females. All pregnant females received an injection of bromodeoxyuridine (BrdU) on day 1 of gestation and were sacrificed 21 days later. The same procedure was carried out at matched time points in virgin females. Results show that for number of Ki67-immunoreactive (ir) cells and doublecortin (DCX)-ir cells, there were significant interactions between reproductive state (pregnant/virgin) and stress exposure ($p = .05$, $p = .04$, respectively) with control virgin and stressed pregnant females having more Ki67-ir cells than control pregnant females and more DCX-ir cells than stressed virgin females. Results also show that pregnant females had significantly greater glucocorticoid receptor (GR) density in the CA1, CA3 and granule cell layer compared to virgin females. In addition, there was a main effect of stress on GR density in the CA3 region, with stressed females having significantly lower GR density compared to control females ($p = .01$). This work adds to our understanding of how stress and reproductive state affect plasticity in the female hippocampus.

Key words: cell proliferation, reproduction, depression, motherhood, female, pregnancy.

INTRODUCTION

Stress-related disorders, such as depression, occur in 15–20% of women during gestation and the postpartum period (Bennett et al., 2004a,b; Oberlander et al., 2006). Women are especially vulnerable to mood disorders during the perinatal period as this is a time when there are significant biological and behavioral changes with the responsibility of caring for a newborn (Steiner et al., 2003). Consequences of stress-related mood disorders during pregnancy and in the postpartum period can significantly contribute to poor maternal and fetal health outcomes (de Paz et al., 2011; Ponder et al., 2011). Unfortunately, our understanding of how stress affects the physiology and neurobiology of the mother is limited, particularly with regard to the hippocampus; a brain area important for stress regulation and affected by depression (McEwen, 2008).

The transition to motherhood alone, in the absence of stress, affects hippocampal plasticity (Kinsley et al., 2006; Pawluski and Galea, 2006, 2007; Leuner et al., 2007; Pawluski et al., 2010). Previous work has shown that primiparous rats during the postpartum period exhibit decreased dendritic complexity in CA1 and CA3 pyramidal neurons, as well as lower levels of hippocampal neurogenesis, compared to nulliparous and multiparous rats (Pawluski and Galea, 2006, 2007). However, stress itself markedly affects the hippocampus of the maternal brain

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Abbreviations: ANOVAs, analysis of variance tests; BrdU, 5-bromo-2-deoxyuridine; DAB, 3,3-diaminobenzidine; DCX, doublecortin; DG, dentate gyrus; EZM, elevated zero maze; FST, forced swim test; GCL, granule cell layer; GD, gestational days; GR, glucocorticoid receptor; HPA, hypothalamo-pituitary–adrenal; ir, immunoreactive; MR, mineralocorticoid receptor; NDS, normal donkey serum.

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(Brummelte and Galea, 2010). In turn, these effects appear to differ with the timing and duration of the stressor. For example, repeated stress during gestation has minimal effects on hippocampal neurogenesis in the dam shortly after weaning (Pawluski et al., 2012a), whereas repeated stress during the early postpartum period interferes with the rate of hippocampal cell proliferation and increases hippocampal volume in the maternal brain during lactation (Hillerer et al., 2014). Furthermore, administration of high levels of corticosterone during late pregnancy and throughout the postpartum period (a model of maternal depression) leads to decreased hippocampal cell proliferation (Brummelte and Galea, 2010) and reduces dendritic complexity of basal arbors in CA3 pyramidal cells in the mother after weaning (Workman et al., 2013).

Much less is known about the effects of stress during gestation on hippocampal plasticity in the pregnant female. Recent work has shown that repeated stress during late pregnancy increases the number of proliferating cells in the hippocampus of the late pregnant female, but has no effect on new cell survival during gestation (Pawluski et al., 2011). Stress during the last 2 weeks of gestation also results in significantly shorter apical dendrites and fewer apical branch points in CA3 pyramidal cells in late pregnant females (Pawluski et al., 2012c). However, further work is needed to understand how stress early in gestation may affect hippocampal plasticity in the pregnant female.

The hypothalamo-pituitary–adrenal (HPA) system itself is altered with pregnancy and the postpartum period. Previous work demonstrates a gradual elevation in basal corticosterone levels during gestation in the rat (as measured from the dorsal aorta; Waddell and Atkinson, 1994; Atkinson and Waddell, 1995), with reduced morning corticosterone levels in late pregnant females compared to virgin females, even after acute stress (as measured from the tail; Pawluski et al., 2009b, 2011), and increased postpartum corticosterone levels (Leuner et al., 2007; Slattery and Neumann, 2008; Pawluski et al., 2009b). The regulation of the HPA system is due, in part, to the function of glucocorticoid receptors (GRs) in the hippocampus. Little research has investigated how these neural correlates in the hippocampus are altered with the changing HPA system during gestation. One study suggests that there is a decrease in GR density in the hippocampus of lactating rats, in the absence of stress (Meaney et al., 1989) and additional work has shown that there is an increase in GR mRNA levels in the dentate gyrus of pregnant rats (Johnstone et al., 2000). Much more work is needed in this area to determine how reproductive state and stress may affect hippocampal GR density in the maternal brain.

The aim of the present study was to understand how repeated stress early in gestation (days 5–11 of gestation) affects aspects of hippocampal neurogenesis in the pregnant female and to determine how these effects differ from those in virgin females. To do this, age-matched pregnant and virgin female Sprague–Dawley rats were subjected to repeated restraint stress and brains were assessed for hippocampal cell proliferation, number of immature neurons, and rate of new cell survival in the dentate gyrus (DG). Furthermore, our understanding of how stress during gestation affects stress regulation in the hippocampus is unknown. Therefore, an additional aim of the study was to show how stress exposure and reproductive state affects GR density in the CA1, CA3, and DG of the hippocampus, thus providing insight into how the HPA system may be altered at this time. Corticosterone levels were also determined in response to swim stress. Affect-related behaviors were also assessed in virgin and pregnant females as per our previous work (Pawluski et al., 2011). Understanding how stress affects the neurobiology of the adult female during gestation will aid in improving the health and well-being of the mother and child.

EXPERIMENTAL PROCEDURES

Animals

Fifty-five adult female Sprague–Dawley rats were used in the present study (275–325 g, 4–5 months of age, Charles River Laboratories, France). Rats had *ad libitum* access to rat chow (Sniff) and tap water and were initially housed in pairs in clear polyurethane bins with corn cob bedding (12-h:12-h light/dark cycle; lights on at 7:00 a.m.). All protocols were approved by the Animal Ethics Board of Maastricht University in accordance with Dutch governmental regulations (DEC 2009-055) and all efforts were made to minimize animal suffering.

All female rats were age-matched and sexually-naïve prior to random assignment to the following groups: virgin (n = 30) and pregnant (primigravid; n = 25). Animals were further divided into the following conditions: cage control, behavioral control and stressed. Therefore, there were a total of six groups of animals: (1) Pregnant cage control (n = 6), (2) Pregnant behavioral control (n = 10), (3) Pregnant stressed (n = 9), (4) Virgin cage control (n = 7), (5) Virgin behavioral control (n = 11), (6) Virgin stressed (n = 12). Cage control females were left undisturbed in their home cage throughout the study until perfusion. Behavioral control females participated in the behavioral tests and corticosterone analysis (see below). Stressed females were individually restrained three times a day for 45 min in transparent plastic cylinders under bright light (between 8–10 a.m., 12–2 p.m., and 4–6 p.m.). The restraint was done from gestational days (GD) 5–11 in pregnant females and at matched time points in virgin females, as previously described (Ward and Weisz, 1984; Van den Hove et al., 2005; Pawluski et al., 2011).

For breeding of pregnant females, one male and one female were housed together in a wire mesh cage. After a vaginal plug was released, pregnant females were individually housed. Gestation day 0 (GD0) was considered the day a vaginal plug was released. Virgin females were individually housed for the same duration of time as pregnant females. All females were weighed