Neuroscience 303 (2015) 200–210

PRECONCEPTION PATERNAL STRESS IN RATS ALTERS DENDRITIC MORPHOLOGY AND CONNECTIVITY IN THE BRAIN OF DEVELOPING MALE AND FEMALE OFFSPRING

A. HARKER, [a]* **S. RAZA,** [a] **K. WILLIAMSON,** [a] **B. KOLB** [a,b] **AND R. GIBB** [a]

[a] *Canadian Centre for Behavioural Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada*

[b] *Canadian Institute for Advanced Research Program in Child Brain Development, 180 Dundas Street West, Toronto, ON M5G 1Z8, Canada*

Abstract—The goal of this research was to examine the effect of preconception paternal stress (PPS) on the subsequent neurodevelopment and behavior of male and female offspring. Prenatal (gestational) stress has been shown to alter brain morphology in the developing brain, and is presumed to be a factor in the development of some adult psychopathologies. Our hypothesis was that paternal stress in the preconception period could impact brain development in the offspring, leading to behavioral abnormalities later in life. The purpose of this study was to examine the effect of preconception paternal stress on developing male and female offspring brain morphology in five brain areas; medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), parietal cortex (Par1), hippocampus (CA1) and nucleus accumbens (NAc). Alterations in dendritic measures and spine density were observed in each brain area examined in paternal stress offspring. Our two main findings reveal; (1) PPS alters brain morphology and organization and these effects are different than the effects of stress observed at other ages; and, (2) the observed dendritic changes were sexually dimorphic. This study provides direct evidence that PPS modifies brain architecture in developing offspring, including dendritic length, cell complexity, and spine density. Alterations observed may contribute to the later development of psychopathologies and maladaptive behaviors in the offspring.

Key words: paternal, development, prefrontal, nucleus accumbens, hippocampus, Golgi.

*Corresponding author. Address: Department of Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada. Tel: +1-403-393-1394.
E-mail addresses: allonna.harker@uleth.ca (A. Harker), sarah.raza@uleth.ca (S. Raza), kayla.williamson2@uleth.ca (K. Williamson), kolb@uleth.ca (B. Kolb), gibb@uleth.ca (R. Gibb).
Abbreviations: AID, orbitofrontal cortex; ANOVA, analysis of variance; BLA, basolateral amygdala; CA1, hippocampus; Cg3, cingulate cortex; HPA, hypothalamic–pituitary–adrenal; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OFC, orbitofrontal cortex; P21, postnatal day 21; Par1, parietal cortex; PFC, prefrontal cortex; PPS, preconception paternal stress.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.058
0306-4522/

INTRODUCTION

A rich literature has been amassed demonstrating the impact of early life events on the structure and function of the developing brain. Research has shown that the effect of maternal stress on offspring development has been linked to an increased risk of psychopathologies, including attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), major depressive disorder (MDD), schizophrenia, and drug addiction (Beversdorf et al., 2005; Pelham et al., 2007; Khashan et al., 2008; Bale et al., 2010; Franklin et al., 2010; Arnsten, 2011; Dietz et al., 2011; Kolb et al., 2012; Class et al., 2014). Although there is a well-established relationship between maternal stress and mental health, very little is known regarding the effect of preconception paternal stress (PPS) on the developing brain of offspring. In a recent study, we found that PPS had a significant effect on early behavior and stress reactivity in offspring (Mychasiuk et al., 2013a). PPS also altered global DNA methylation levels in postnatal day 21 (P21) offspring. Dietz et al. (2011) found that offspring of fathers that had been exposed to chronic social defeat stress exhibited anxiety-like and depressive behaviors, as well as decreased levels of endothelial growth factor and increased levels of corticosterone. However, these effects were absent when researchers used *in vitro* fertilization (IVF), suggesting limited epigenetic transmission. Epigenetic reprogramming of the germ cells mediated by hypothalamic–pituitary–adrenal (HPA) axis dysregulation was found in offspring of chronically stressed fathers (Rodgers et al., 2013). Braun and Champagne (2014) review substantial literature that examines possible influences of fathers on offspring development. These researchers suggest three possible routes by which transmission may occur; direct paternal care, epigenetic transmission, and interactions between mother and infant, influenced by fathers. The present study investigated brain morphology in P21 offspring of fathers stressed prior to mating. Analyses of spine density, dendritic length, and cell complexity were examined in the following five brain areas, medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), parietal cortex (Par1), hippocampus (CA1), and nucleus accumbens (NAc). These brain regions were chosen because we (and others) have previously found them to be especially sensitive to stress in adult rats (cingulate cortex (Cg3), AID), gestational stress (Cg3, AID, CA1), maternal separation (Cg3, AID, NAcc), and bystander stress (Cg3, AID, CA1) (e.g., Liston et al., 2006;

Muhammad and Kolb, 2011a,b; Mychasiuk et al., 2011a,b). We included an additional region, Par1, because it is extremely sensitive to early experience such as prenatal and infant tactile stimulation (e.g., Kolb and Gibb, 2010), but were unaware of any evidence showing it to be stress sensitive. Indeed, Shors et al. (2001) found no effect of stress on Par1 in adult male and female rats.

Numerous studies within our lab have examined the effects of gestational stress on these specific brain areas (Mychasiuk et al., 2011a,b, 2012; Muhammad et al., 2012). Recently, we found that PPS significantly influenced early behavior, and had a significant effect on DNA global methylation levels in the prefrontal cortex (PFC) (Mychasiuk et al., 2013a,b). We aim to further our understanding of the effect of paternal stress in the preconception period and the impact of this type of stress on these previously examined brain areas.

EXPERIMENTAL PROCEDURES

Animals

All procedures were conducted in accordance with the Canadian Council of Animal Care and were approved by the University of Lethbridge Animal Care and Use Committee. Ten female Long-Evans rats were mated with 10 male Long-Evans rats (six paternal stress and four control). All pairs successfully mated resulting in 131 pups (78 Paternal Stress (PPS): 54 Control (C)). Animals were given access to food and water *ad libitum* and were maintained on a 12-h light/dark schedule (lights on from 07:30 to 19:30 h) in a temperature controlled (21°) breeding room.

PPS

Paternal stress was administered a total of 27 consecutive days prior to the mating session. Stressing consisted of placing the male rat ($n = 6$) on an elevated Plexiglas® platform (1 m tall, 21 × 21 cm) in a brightly lit room for 30 min. (Wong et al., 2007). Following the stressing procedure, rats were transported back to their home cages. Stressing sessions occurred at 9:00 A.M. and 3:00 P.M. During stressing sessions, control males ($n = 4$) were removed from their home cages for the 30 min, but did not participate in the stressing paradigm. Following the 27 days of stressing, paternal stress and control males were immediately mated with females. This was the only exposure that female dams had with the stressed male rats. Subsequent to mating, female dams were housed in shoebox cages with another female in the same experimental condition (ex. control–control vs. paternal stress–paternal stress). Female dams were separated and housed individually prior to birth of pups, following a weight gain $\geqslant$100 g. Female dams remained individually housed following the birth of their litter. Weight gain during pregnancy did not differ between female dams mated with paternal stress males or paternal control males. Average litter size for dams of the paternal control pups was 13.25 ± 1.9 whereas dams mated with paternal stress males had litters of 13.0 ± 1.6. We chose 1–2 male and female pups from each litter to reduce the possibility of litter effects. There were no other differences in litter characteristics to report. The remaining pups were used in parallel experiments.

Stressing paradigm. Wong et al. (2007) developed a stressing paradigm that has been shown to induce significant chronic stress in rats, supported by analysis of corticosterone levels and observation of behavioral effects (consistent urination and/or defecation during the stress procedure). We chose to follow this stressing protocol in order to forego unnecessary stress related to blood collection for corticosterone analysis, extensively examined previously by Wong and associates.

Anatomy

Perfusion and staining. Histological processing was performed on P21. Animals were administered an overdose of sodium pentobarbital solution (i.p.) and perfused with 0.9% saline solution intracardially. The brains were extracted from the skull, brain weight was recorded and brains were preserved in bottles containing Golgi–Cox solution for 14 days in the dark. Following this period, brains were transferred to 30% sucrose solution for a minimum of 3 days. A Vibratome was used to section the brains at a thickness of 200 μm, and sections were mounted on gelatin-coated slides. Brain sections were then processed for Golgi–Cox staining according to the procedures outlined by Gibb and Kolb (1998).

Dendritic analyses. Relevant cells in five brain regions (see Fig. 1) were identified at low power (100×), and individual pyramidal cells from layer III were traced using camera Lucida (at 250×) in areas Cg 3 (mPFC), AID (orbitofrontal cortex), Par1, CA1, as defined by Zilles (1985). The same manner was used to identify and draw medium spiny neurons in the shell of the NAc. A total of 10 cells, five from each hemisphere, were drawn from each brain region of every animal. The mean of the five cells per hemisphere was used as the unit of analysis. Both apical and basilar dendritic branching were drawn for Cg3 and Par1. Basilar (no apical) dendritic branching was drawn for AID and CA1 because the plane of sectioning truncated many apical fields in AID and there was excessive blood vessel artifact obscuring much of the apical fields in CA1.

To meet the criteria for analysis, the dendritic tree of the cell had to be intact, well impregnated with stain and not obscured by blood vessels, astrocytes, or stain precipitations. The cell's dendritic arbor was quantified using two methods. First, dendritic complexity was estimated using branch order (based on the total number of branch bifurcations) (Coleman and Riesen, 1968). Second, a Sholl analysis was used to estimate dendritic length (a transparent grid of concentric rings, equivalent to 25 μm apart was placed over dendritic drawing and the number of ring intersections were counted) (Sholl, 1956).

Spine density. Apical and basilar dendrites were drawn from Cg3 and Par1 and basilar dendrites were drawn from AID and CA1 at 1000× from 10 neurons