THE ROLE OF DNA METHYLATION IN PHENOTYPIC OUTCOMES OF EXPERIENCING CAREGIVER MALTREATMENT

by

Samantha M. Keller

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Psychology

Spring 2018

THE ROLE OF DNA METHYLATION IN PHENOTYPIC OUTCOMES OF EXPERIENCING CAREGIVER MALTREATMENT

by

Samantha M. Keller

Approved: _____
Robert F. Simons, Ph.D.
Chair of the Department of Psychological and Brain Sciences

Approved: _____
George H. Watson, Ph.D.
Dean of the College of Arts and Sciences

Approved: _____
Ann Ardis, Ph.D.
Senior Vice Provost for Graduate & Professional Education

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Tania Roth, Ph.D.
Professor in charge of dissertation

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Jeff Rosen, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Mary Dozier, Ph.D.
Member of dissertation committee

I certify that I have read this dissertation and that in my opinion it meets the academic and professional standard required by the University as a dissertation for the degree of Doctor of Philosophy.

Signed: _____

Kevin Bath, Ph.D.
Member of dissertation committee

ACKNOWLEDGMENTS

Most importantly, I would like to thank my mentor, Dr. Tania Roth, for taking me into her lab and providing me with immeasurable support and guidance throughout my graduate study. Her leadership, patience, expertise, and friendship have greatly facilitated my growth as a scientist and as a person. I thank my dissertation committee members, Drs. Jeff Rosen, Mary Dozier, and Kevin Bath, for their time, commitment, and invaluable contributions to my research. I thank Dr. Eric Roth for his mentorship in the teaching component of my graduate schooling and for the comedic relief throughout the past five years.

I would like to thank my fellow graduate students Tiffany Doherty, Will Schreiber, Arun Asok, Jen Blaze, Nick Heroux, and Natalia Phillips for their support and friendship. I thank the undergraduate students that I have had the pleasure of working with, particularly Brianna Stanfield, Jennifer Staib, Bella Archer, and Anna Nowak. I also thank the Office of Laboratory Animal Medicine for kindness and excellence in caring for our animals.

I would like to thank my family, especially my parents and siblings Trish, Ricky, and Ray. I am grateful not only for their continued support and encouragement, but also for their contributions to my academic career. Lastly, I thank my partner Evan for his love, support, and for always being there for me during the highs and lows of graduate school.

PREFACE

I, Samantha Keller, hold principle author status for the chapters comprising this dissertation. Each of these chapters have co-authors, whose contribution greatly facilitated my dissertation research, as detailed below.

Chapter 1 is co-authored by my supervisor, Dr. Tania Roth. This manuscript is published in *Environmental Epigenetics* (Vol 2(2), 1-10) in 2016.

Chapter 2 is co-authored by Dr. Tania Roth and Tiffany Doherty. Additional data will be added to this chapter prior to submission for publication.

Chapter 3 is co-authored by Dr. Tania Roth and Tiffany Doherty. This chapter was submitted in March 2018 to *Frontiers in Behavioral Neuroscience* to be considered for publication.

Chapter 4 is coauthored by Dr. Tania Roth and Anna Nowak. Additional data will be added to this chapter prior to submission for publication.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Caregiving received during development plays a critical role in programming brain development and behavioral trajectories. Receiving maltreatment by the caregiver can have grave, life-long consequences including poor physical health, cognitive dysfunction, and increased propensity to develop psychiatric illnesses. While the mechanisms through which early-life adverse experiences are able to induce long-term effects on the individual continue to be investigated, epigenetic alterations have emerged as a promising candidate. Epigenetic modifications, including DNA methylation, are capable of altering gene expression without altering the underlying genomic sequence. Therefore, alterations to the epigenome provide a way for experiences to produce changes at the molecular level that manifest into changes in brain function with implications for behavior.

The Roth Lab employs a rodent model, the scarcity-adversity model of low nesting resources outside the home cage, to explore the neurobiological and behavioral consequences of experiencing caregiver maltreatment. This model involves exposing pups to brief but repeated bouts of maltreatment (30 minutes) daily from postnatal days 1-7. Using this model, we have discovered a number of behavioral and epigenetic aberrations induced by caregiver maltreatment. We hypothesize that the maltreatment-induced epigenetic alterations are causally related to behavioral outcomes. However, empirical data demonstrating a causal relationship between epigenetic alterations and behavior in the scarcity-adversity model are lacking.

Female subjects are largely underrepresented in the scientific literature. However, it is well established that the implications of exposure to stress across the lifespan are sex-specific. Indeed, the Roth Lab has identified a number of sex differences in the consequences of maltreatment, with deleterious outcomes of maltreatment being more numerous in female subjects. For example, female rats subjected to maltreatment exhibit increased levels of adverse care toward their own offspring and altered behavior in the novel object and forced swim tests. Reasons underlying this sex disparity are unclear. While there are known sex differences in nurturing care provided toward offspring (dams lick their male pups more than the females) it is not known if sex differences exist in adverse caregiving.

The goals of this dissertation were to: 1) examine the relationship between DNA methylation and phenotype; 2) explore the possibility for the epigenome-altering drug, zebularine, to rescue behavioral outcomes of maltreatment in adult animals; and, 3) explore reasons underlying sex differences in maltreatment outcomes. Chapter 1 reviews data regarding the influence of environmental factors on the epigenome of the female brain and associated behavioral outcomes, particularly in the context of environmental perturbations occurring during development. Experiments comprising this dissertation employed the scarcity-adversity model of low nesting resources outside the home cage. In experiments detailed in Chapter 2, females were subjected to the scarcity-adversity model as infants and then as adults administered zebularine at a dose and schedule previously shown to rescue DNA methylation. The number of adverse behaviors dams directed toward their offspring were recorded. We replicated our previous finding that dams with a history of maltreatment mistreat their own offspring and extended this to show that zebularine administration normalized

maternal behavior in maltreated dams. Further, seven days of zebularine administration disturbed maternal behavior in animals with no history of maltreatment, such that these animals showed enhanced levels of adverse behavior toward their offspring relative to their vehicle-treated counterparts.

To see if zebularine could normalize other behavioral aberrations induced by maltreatment, we next sought to examine the capacity for zebularine to normalize behavior in the novel object and forced swim tests in adult female animals. The same infant manipulations and drug regimen as used in Chapter 2 were utilized for experiments in Chapter 3. Following seven days of drug or vehicle administration, adult female animals were run through a battery of behavioral tests including the open field test, novel object recognition, and the forced swim test. In the forced swim test, maltreated females showed an increased latency to their first bout of immobility, and seven days of ICV zebularine treatment normalized this behavior. We did not find any group differences in the open field test or novel object recognition. Together, data from Chapters 2 and 3 demonstrate the capacity for behavioral outcomes of infant maltreatment to be rescued by a pharmacological intervention in adulthood. Further, these data provide support for a causal link between the epigenetic and phenotypic outcomes of experiencing maltreatment.

Chapter 4 of this dissertation sought to examine potential sex differences in adverse care received in the scarcity-adversity model. To do this, adverse behaviors directed toward pups of each sex in the scarcity-adversity model were recorded. Results indicated that, in the maltreatment condition, female pups received more adverse behaviors from the dam relative to their male littermates. These data lend support to our hypothesis that outcomes of maltreatment in the scarcity-adversity

model of low nesting resources are more numerous for female subjects because they experience higher levels of adverse behavior.

These studies provide seminal evidence supporting a causal link between maltreatment-induced DNA methylation and behavioral outcomes in the scarcity-adversity model of low nesting resources. These experiments also show the experience-dependent nature of the behavioral impact of epigenome-modifying drugs, such as zebularine. Further, results highlight the importance of exploring sex differences in caregiving when interpreting data from models of early-life maltreatment.

Chapter 1

ENVIRONMENTAL INFLUENCES ON THE FEMALE EPIGENOME AND BEHAVIOR

Environmental factors have long-lasting effects on brain development and behavior. One way experiences are propagated is via epigenetic modifications to the genome. Environmentally-driven epigenetic modifications show incredible brain region- and sex-specificity, and many brain regions affected are ones involved in maternal behavior. In rodent models, females are typically the primary caregiver and thus, any environmental factors that modulate the epigenotype of the mother could have consequences for her current and future offspring. Here we review evidence of the susceptibility of the female epigenome to environmental factors, with a focus on brain regions involved in maternal behavior. Accordingly, implications for interventions that target the mother's epigenome and parenting behavior are discussed.

1.1 Introduction

Epigenetics, a term coined by Waddington in the 1940's, is used to describe gene-environment interactions that influence phenotype (Waddington, 1940). Epigenetic mechanisms include DNA methylation, histone modifications, and microRNAs (miRNAs), and collectively, afford routes for environmental factors to

alter gene activity. DNA methylation refers to the addition of methyl groups onto cytosine residues of a DNA strand. This commonly occurs at dinucleotide cytosine–guanine (CpG) sites, though methylation can also occur at non-CG dinucleotides (Lister et al., 2009; McGowan et al., 2011; Stroud et al., 2014). DNA methylation is catalyzed by a group of enzymes called DNA methyltransferases (DNMTs), of which several types exist. DNMT 1 contributes to the maintenance of DNA methylation by adding methyl groups to hemi-methylated DNA, while DNMT 3a and 3b are able to modulate methylation patterns via *de novo* methylation (L. D. Moore, Le, & Fan, 2013). Typically, DNA methylation results in the suppression of gene expression; however, under some circumstances it can also enhance gene transcription (Chahrour et al., 2008; Guy, Cheval, Selfridge, & Bird, 2011; St-Cyr & McGowan, 2015).

Posttranslational histone modifications comprise acetylation, methylation, ubiquitylation, sumoylation, and phosphorylation of the N-terminal tail of histone proteins. Because DNA is wrapped around histone molecules within nucleosomes, such modifications can either make DNA more or less accessible for transcription (Grant, 2001; B. Li, Carey, & Workman, 2007; Millar & Grunstein, 2006). For example, histone acetylation involves the addition of acetyl groups (via histone acetyltransferases) at lysine residues on the N-terminal tail of histone proteins, decreasing the affinity between the histone and DNA and thereby allowing a more permissive transcriptional state (Grant, 2001; B. Li, et al., 2007; Millar & Grunstein, 2006). Histone deacetylases (HDACs) reverse this process (Grant, 2001; B. Li, et al.,

2007; Millar & Grunstein, 2006). Another mode of epigenetic regulation gaining increasing attention is miRNAs, which are non-coding single stranded RNAs (usually about 22 base pairs in length) capable of exerting gene silencing effects via degradation or destabilization of mRNA (Adlakha & Saini, 2014; Bartel, 2004; Chen & Rajewsky, 2007; He & Hannon, 2004). Some studies also indicate that certain miRNAs upregulate gene expression (Valinezhad Orang, Safaralizadeh, & Kazemzadeh-Bavili, 2014; Vasudevan, 2012).

While epigenetic modifications were once thought to be limited to embryonic development, it has since been discovered that epigenetic modifications in the central nervous system continue to transpire throughout development and into adulthood. Since initial discoveries in the early 2000's, (e.g. (Champagne et al., 2006; Levenson et al., 2006; Skinner & Anway, 2005; Waterland & Jirtle, 2003; Weaver et al., 2004)), investigators have continued to uncover the epigenetic consequences of exposure to various environmental perturbations. Aberrant epigenetic profiles have also been linked to a host of neuropsychiatric disorders (Lutz & Turecki, 2014; Provencal & Binder, 2015; Roth & Sweatt, 2011; Tamura, Kunugi, Ohashi, & Hohjoh, 2007), and epigenetic modifications are increasingly being recognized as important for understanding sex differences in brain development and responses to environmental and psychosocial perturbations. Epigenetic mechanisms are known to mediate sexual differentiation of the brain, and sex differences in DNA methylation resulting from hormonal exposures during the perinatal period are long-lasting and continue to

emerge throughout development (McCarthy et al., 2009; McCarthy & Nugent, 2013; Nugent et al., 2015). Indeed, sexually dimorphic DNA methylation is observed at a multitude of genes throughout the genome (Ghahramani et al., 2014). However, the role epigenetic mechanisms play in sexually divergent behaviors, such as maternal behavior, is less clear. Males and females are known to differ in prevalence rates across a multitude of psychiatric disorders (Kessler et al., 2005) and it has been proposed sex differences in the epigenome contribute to this disparity (Jessen & Auger, 2011). As female subjects are certainly underrepresented in behavioral neuroscience literature (Beery & Zucker, 2011; Klein et al., 2015), and because experiences altering the brain and behavior of females have implications for future generations due to the critical roles of infant-mother interactions and the quality of maternal care in offspring development, we chose to focus this review on data acquired from female subjects.

1.2 Overview of Rodent Maternal Behavior and Circuitry

Before delving into the epigenetics literature, here we mention several maternal behaviors and neuroanatomical substrates that are discussed in various sections of the review. For a more thorough evaluation of these topics, we refer the reader to several excellent reviews (e.g. (Barrett & Fleming, 2011; Kristal, 2009; Numan & Woodside, 2010; Jodi L. Pawluski, Lambert, & Kinsley, 2016)). One of the predominant maternal behaviors observed in laboratory rodents is licking of the pup's

body, with an emphasis on the anogenital area (anogenital licking aids in waste elimination) (Celia L. Moore, 1984; Stern, 1986). Mothers spend a significant amount of time in the nest hovering over pups, engaging in bouts of licking and nursing (Stern, 1986; Stern & Lonstein, 2001). Retrieval of pups becomes necessary as they wander from the nest, and this maternal behavior is elicited by ultrasonic vocalizations emitted by pups (Brunelli, Shair, & Hofer, 1994). Of note, nulliparous females display retrieval behavior after continuous exposure (sensitization) to pups (Lonstein, Wagner, & De Vries, 1999; Stolzenberg, Stevens, & Rissman, 2012). Further, dams will engage in a behavior referred to as tail chasing, in which a dam chases their tail, eventually picking it up and carrying it in her mouth (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Ward et al., 2013). The specific function of tail chasing is not known, but may be related to pup retrieval as dams often engage in this behavior antepartum and outside of the nest area, carrying the tail back to the nest (Ward, et al., 2013). Finally, brain regions involved in maternal behavior include the bed nucleus of the stria terminalis (BNST) (Bosch, Pförtsch, Beiderbeck, Landgraf, & Neumann, 2010; Klampfl, Brunton, Bayerl, & Bosch, 2014; Numan & Numan, 1997), paraventricular nucleus (PVN) (Consiglio & Lucion, 1996; Giovenardi, Padoin, Cadore, & Lucion, 1998; Insel & Harbaugh, 1989), nucleus accumbens (Champagne et al., 2004; M. Li & Fleming, 2003a, 2003b), prefrontal cortex (PFC) (Afonso, Sison, Lovic, & Fleming, 2007; Febo, Felix-Ortiz, & Johnson, 2010; Pereira & Morrell, 2011), medial preoptic area (MPOA) (Champagne, et al., 2006; Alison S. Fleming, Miceli, & Moretto, 1983; A. S. Fleming & Walsh, 1994; Numan & Stolzenberg, 2009; Pedersen, Caldwell,

Walker, Ayers, & Mason, 1994; Pereira & Morrell, 2011), amygdala (A. S. Fleming, Vaccarino, & Luebke, 1980; A. S. Fleming & Walsh, 1994; Numan, Numan, & English, 1993), and hippocampus (Kimble, Rogers, & Hendrickson, 1967; J. L. Pawluski & Galea, 2007). Several of these regions and their role in regards to maternal behavior are depicted in Figure 1.1.

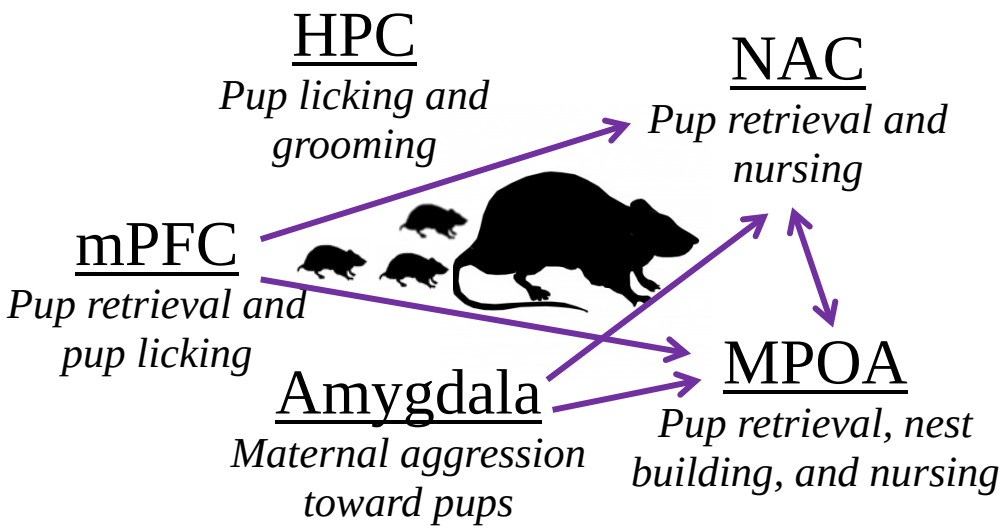

Figure 1.1 Neuroanatomy underlying maternal behavior. This figure illustrates several of the key neuroanatomical regions underlying maternal behavior which are subject to epigenetic modulation by environmental factors as described in this review. Several maternal behaviors each region has been implicated in are also listed. The arrows indicate a simplified neuroanatomical circuit of projections amongst brain regions (Dulac, O'Connell, & Wu, 2014; Numan & Woodside, 2010; Pereira & Morrell, 2011). Abbreviations: HPC= hippocampus; NAC= nucleus accumbens; mPFC= medial prefrontal cortex; MPOA= medial preoptic area. (Figure from Keller and Roth, 2016).

.

1.3 Adulthood and Preconception Psychosocial Stress

Stressors experienced in adulthood are capable of modulating the female epigenome and behavior. In one study that implemented a chronic variable mild stress paradigm, adult female rats were found to have increased levels of the histone acetyltransferase cyclic AMP response element-binding protein (CBP) in the BNST (Sterrenburg et al., 2011). This effect was not seen in male rats (Sterrenburg, et al., 2011). These data suggest an important role of histone acetylation in response to stress exposure that could lead to sex-specific alterations in behavioral outcomes. As further evidence for this notion, in an acute restraint stress paradigm that elicited elevated corticosterone and corticotropin releasing factor (*Crf*) mRNA in the PVN in male but not female rats, males demonstrated elevated CBP levels and females did not (Sterrenburg et al., 2012). In another study that employed a subchronic variable stress paradigm to produce a depression-like phenotype, female mice had increased levels of *Dnmt3a* within the nucleus accumbens (Hodes et al., 2015). Mice with a knock-out of *Dnmt3a* in the nucleus accumbens showed resilience to the subchronic variable stress, providing further support for the concept that *Dnmt3a* overexpression might mediate stress-induced depression (Hodes, et al., 2015). Taken together, these studies show that the female brain can be epigenetically modulated in key components of maternal behavior circuitry by stress exposure. Further research is needed to understand the

functional significance of sex differences in these epigenomic marks induced by these stressors.

Stress incurred by a female prior to pregnancy is also capable of modulating brain and behavioral trajectories of her offspring. In adult female rats that underwent a seven-day chronic unpredictable stress regimen, corticotrophin releasing factor receptor type 1 (*Crf1*) mRNA was upregulated in the ova and frontal cortex (Zaidan, Leshem, & Gaisler-Salomon, 2013). A separate group of females were bred two weeks after termination of the same stress paradigm, and their offspring were likewise found to have increased *Crf1* expression in their brain in both infancy (on postnatal day 0, prior to any maternal care received) and adulthood (Zaidan, et al., 2013). This altered gene expression corresponded with behavioral alterations when offspring were adults, including potentiated startle responses and increased locomotor activity in the elevated plus maze (Zaidan, et al., 2013). Preconception-stress-exposed rats and their first-generation female offspring also showed increased corticosterone levels. In contrast, second-generation offspring showed reduced expression of *Crf1* mRNA and decreased corticosterone levels (Zaidan & Gaisler-Salomon, 2015). These data provide evidence that a stressor encountered by an adult female can contribute to the programming of HPA-axis reactivity for several generations.

While stressful experiences can modulate the epigenome and introduce maladaptive behavioral outcomes, other types of experiences can exert adaptive influences on behavior through epigenetic mechanisms. Induction and maintenance of

maternal behavior in response to pup interaction has been proposed to result from experience-driven chromatin remodeling (Stolzenberg & Champagne, 2016). In a maternal sensitization paradigm, which involved repeatedly introducing nulliparous female mice to pups to stimulate maternal behavior, histone acetylation was shown to be a critical mediator for this experience-induced behavioral change (Stolzenberg, et al., 2012). Administration of the histone deacetylase inhibitor (HDACi) sodium butyrate reduced the amount of time required for a nulliparous female to display maternal care toward pups (Stolzenberg, et al., 2012). This pharmacological manipulation also increased gene expression of estrogen receptor β, CBP, and the oxytocin receptor in the MPOA (Stolzenberg, et al., 2012). Furthermore, the HDACi-induced facilitation of maternal behavior and gene expression lasted for a month after initial maternal experience (Stolzenberg, Stevens, & Rissman, 2014). Taken together, these studies provide evidence that the induction of maternal behavior has epigenetic underpinnings and that administration of certain epigenome modifying drugs can have long-term facilitatory effects on maternal responsiveness.

1.4 Gestational Stressors

Epigenetic mechanisms also provide routes through which gestational stressors, either psychosocial or chemical in nature, can affect offspring. For example, prenatal predator exposure is one stressor that has both epigenetic and behavioral consequences. Female adult offspring of pregnant mouse dams exposed to predator

odor demonstrated an enhanced corticosterone response and an increase in anti-predator behaviors (St-Cyr & McGowan, 2015). This behavioral profile corresponded with increased *Crf1* mRNA in the amygdala and decreased Brain-derived neurotrophic factor (*Bdnf*) mRNA and DNA methylation of *Bdnf* exon IV in the hippocampus (St-Cyr & McGowan, 2015). Daily exposure to restraint stress during pregnancy similarly modulates the epigenetic profile and levels of epigenetic regulators in rat offspring. The placenta of fetal offspring exposed to this gestational stress had increased levels of *Dnmt3a* mRNA and enhanced methylation of the 11β-hydroxysteroid dehydrogenase type 2 (*Hsd11b2*) gene promoter (Pena, Monk, & Champagne, 2012). These same animals also displayed reduced levels of CpG methylation within the *Hsd11b2* promoter region and increased methylation at sites within exon 1of the hypothalamus as well as enhanced *Dnmt1* mRNA within the cortex (Pena, et al., 2012). Further, adult female offspring of mouse mothers exposed to chronic unpredictable stress during gestation demonstrated impaired spatial memory capabilities, higher plasma corticosterone levels, decreased levels of H3 acetylation, and increased DNMT1 protein in the hippocampus (Benoit, Rakic, & Frick, 2015).

miRNAs have been gaining attention for their ability to influence gene activity, though limited work has examined miRNAs in the female brain (Ma et al., 2010). Rat dams exposed to stress (restraint and forced swim) during pregnancy demonstrated a decrease in the incidence of tail chasing, and a correlational upregulation of 147 miRNAs and downregulation of 195 miRNAs in their frontal cortex (Zucchi et al.,

2013). Target genes of the affected miRNAs had roles in hormonal regulation, brain pathologies, stress responsivity, and neurotransmission (Zucchi, et al., 2013). While a similar profile of altered microRNA expression was found in the brains of their male offspring, future examination is required to determine if female offspring would likewise show disrupted miRNA profiles.

Chemical perturbations during gestation likewise affect female offspring (Dolinoy, Huang, & Jirtle, 2007; Panagiotidou, Zerva, Mitsiou, Alexis, & Kitraki, 2014). Bisphenol A (BPA) is an endocrine disrupting chemical gaining increasing attention for its widespread use and association with the development of diseases (Calafat, Ye, Wong, Reidy, & Needham, 2008; Melzer, Rice, Lewis, Henley, & Galloway, 2010; Midoro-Horiuti, Tiwari, Watson, & Goldblum, 2010; Richter et al., 2007). BPA is of particular concern for females due to its ability to modulate estrogen and alter epigenetic profiles (Kim, Hsiao, & Kraus, 2001; Lee & McEwen, 2001). Female offspring exposed to BPA, either during gestation alone or during both gestation and early postnatal development grew up to spend less time performing nurturing maternal behaviors towards their own offspring, and similarly adult females administered BPA demonstrated fewer maternal behaviors toward their offspring (Della Seta, Minder, Dessì-Fulgheri, & Farabollini, 2005; Kundakovic et al., 2013; Palanza, Howdeshell, Parmigiani, & vom Saal, 2002). BPA exposure also modulates levels of epigenetic regulators within brain regions involved in maternal behavior, which could underlie the observed deficits in maternal behavior in BPA-exposed

females. Specifically, levels of DNMT1 and DNMT3a were altered within the hypothalamus and PFC of juvenile female mice prenatally exposed to BPA (Kundakovic, Gudsnuk, et al., 2013). Gestational and early postnatal exposure of rats to endocrine disrupting chemicals including estradiol benzoate and methoxychlor resulted in elevated estrogen receptor α (*ER*α) mRNA and increased DNA methylation in the POA (Gore, Walker, Zama, Armenti, & Uzumcu, 2011). In adulthood, these perinatally-exposed animals also experienced the advancement of reproductive senescence (Gore, et al., 2011).

While antidepressant drugs mitigate depressive-like behavior in adult animals, developmental antidepressant exposure can have deleterious effects (Boulle et al., 2016; Schroeder, Lin, Crusio, & Akbarian, 2007). Adult rat females that were prenatally exposed to fluoxetine displayed enhanced depression-like behavior, as assessed via the forced swim test (Boulle, et al., 2016). Changes in the hippocampus of these females included decreased *Bdnf* exon IV mRNA and increased histone 3 lysine 27 trimethylation (Boulle, et al., 2016). The presence of *Bdnf* mRNA was negatively correlated with immobility time in the forced swim test, suggesting that the observed epigenetic profile in these animals contributed to the phenotypic outcomes associated with developmental fluoxetine exposure (Boulle, et al., 2016). Taken together, data highlighted in this section illustrate that gestational perturbations certainly have influences on neurobiological and behavioral outcomes in female offspring.

1.5 Rearing Environments

Rearing environments of rodent pups have long been recognized for their profound influence on the development of behavior, including maternal behavior (Brunelli, Shindledecker, & Hofer, 1989; Moretto, Paclik, & Fleming, 1986; Plotsky & Meaney, 1993; Stanton & Levine, 1990; Stern, 1986). Female Long-Evans rats demonstrate a natural variability in their quality of maternal care, with some females exhibiting high levels of licking/grooming (LG), and others displaying low levels of LG (Liu et al., 1997). This variability in maternal care is generationally transmitted, as female rats who were exposed (either born or cross-fostered) to a low-licking and grooming mother in their infancy demonstrate low levels of LG toward their own offspring (Champagne, 2008; Francis, Diorio, Liu, & Meaney, 1999). Work utilizing natural variations in LG maternal behavior found epigenetic modulation of the *ERα* gene within the MPOA of dams. Specifically, low-LG mothers showed decreased *ERα* gene expression within the MPOA relative to high-LG mothers (Champagne, et al., 2006). This effect was transmitted to female offspring, but cross-fostering these offspring with a high-LG mother rescued *ERα* expression, showing that mother-infant interactions early in life are critical for MPOA development (Champagne, Weaver, Diorio, Sharma, & Meaney, 2003). Social enrichment postweaning also enhanced LG behaviors in low-LG female offspring (Champagne & Meaney, 2007). The variability of *ERα* expression and transmission of LG behaviors to offspring is mediated by DNA methylation, as low-LG caregivers demonstrate higher methylation of the *ERα1b*

promoter (Champagne, et al., 2006). The MPOA is a sexually dimorphic region critical in maternal behavior (Champagne, et al., 2006; Alison S. Fleming, et al., 1983; A. S. Fleming & Walsh, 1994; Numan & Stolzenberg, 2009; Pedersen, et al., 1994; Pereira & Morrell, 2011) and estrogen is a transcription factor with known protective effects (Lee & McEwen, 2001). Estrogen interacts with histone acetylation, suggesting a route for estrogen to affect expression of many genes (Gagnidze, Weil, Faustino, Schaafsma, & Pfaff, 2013; Kim, et al., 2001). Estrogen levels also affect sexual behaviors, allowing for epigenetic alterations to have effects on mating capabilities of females (Gagnidze, et al., 2013; Pfaff, 1989).

It is well established that the maternal behavior directed toward male versus female offspring differs, as dams spend more time licking their male pups than their female pups (Celia L. Moore, 1984; C. L. Moore & Chadwick-Dias, 1986; C. L. Moore & Morelli, 1979). Because of the sex-specific nature of maternal care, altering the sex composition of litters changes pup-directed maternal behavior (Alleva, Caprioli, & Laviola, 1989). The resulting alterations in maternal behavior have lifelong effects on the brain and behavior of these offspring (Alleva, Caprioli, & Laviola, 1986; Cirulli, Adriani, & Laviola, 1997; Laviola & Terranova, 1998). Within the *Oprm1* gene promoter, which encodes for the μ-opioid receptor, it was discovered that female rats raised in female-only litters demonstrated higher levels of methylation within the hippocampus as compared to females who belonged to mixed litters (i.e. litters containing both male and female offspring) (Hao, Huang, Nielsen, & Kosten,

2011). No effects on DNA methylation of *Oprm1*were found within the nucleus accumbens, suggesting that this effect is brain-region specific (Hao, et al., 2011). The μ-opioid receptor is critical for mother-infant relationships, and thus, modulation of this receptor within attachment and maternal behavior circuitry could have critical implications for the maternal behavior of these offspring (Nelson & Panksepp, 1998). Another study that manipulated litter sex composition found hypermethylation of the hippocampal *GR* gene in adolescent female rats from female-only litters (Kosten, Huang, & Nielsen, 2014). Because females receive less LG than their male counterparts, this corroborates other data with regard to lower LG behavior and enhanced DNA methylation of the *GR* gene in offspring (Weaver, et al., 2004).

Our lab and others have studied the effects of aversive rearing experiences on the *Bdnf* gene (Figure 2). The medial prefrontal cortex (mPFC) is a region critical for cognitive and memory processes and has been implicated in several neuropsychiatric disorders (Broadbelt, Byne, & Jones, 2002; Chai et al., 2011; Joel, Weiner, & Feldon, 1997; Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004; Vertes, 2006). Additionally, lesions to the mPFC disrupt maternal behaviors such as pup retrieval and pup licking (Afonso, et al., 2007; Febo, et al., 2010). In a model of early-life maltreatment whereby rat pups are exposed to 30-minute bouts of caregiver maltreatment (frequent stepping on, dropping, dragging, actively avoiding and rough handling) daily for the first postnatal week, variability in gene expression and DNA methylation can be detected in developing and adult females (Blaze, Scheuing, &

Roth, 2013; Doherty, Forster, & Roth, 2016; Roth, Lubin, Funk, & Sweatt, 2009; Roth, Matt, Chen, & Blaze, 2014), and further, maltreated-females grow up to mistreat their own offspring (Roth, et al., 2009).

Within the whole PFC, DNA methylation of the *Bdnf* gene was enhanced across the lifespan (during infant, adolescent, and adult time points) in maltreated animals, which corresponded with decreased *Bdnf* expression in adult females (Roth, et al., 2009). Within the mPFC, female pups subjected to maltreatment displayed a transient decrease in DNA methylation at the *Reelin* gene, which was no longer present in adolescence or adulthood (Blaze, et al., 2013). However, these females showed decreased gene expression of *Reelin* in adulthood, signifying that although DNA methylation was no longer different, these developmental experiences resulted in differential expression of *Reelin* (Blaze, et al., 2013). Adult females also displayed decreased methylation of *Bdnf* exon I but increased methylation of *Bdnf* exon IV in adulthood (Blaze, et al., 2013). Gadd45b, which plays a role in DNA demethylation (Ma, Guo, Ming, & Song, 2009; Ma et al., 2009), was the only epigenetic regulator significantly altered (lower mRNA levels) within the mPFC of adult females (Blaze & Roth, 2013), thus the mechanism (or mechanisms) underlying the maltreatment-induced alterations in female gene expression and DNA methylation remains to be elucidated.

Using the same maltreatment regimen, female-specific modulations were also detected within the amygdala. The amygdala is a region involved in maternal behavior

(A. S. Fleming, et al., 1980; A. S. Fleming & Walsh, 1994; Numan, et al., 1993) and amygdalar pathways are particularly involved in maternal aggression (Ferris et al., 1992; Lubin, Elliott, Black, & Johns, 2003; Sheehan, Paul, Amaral, Numan, & Numan, 2001). Female rats that were maltreated in infancy displayed reduced expression of the oxytocin receptor gene in infancy and adolescence (Hill, Warren, & Roth, 2014), a gene important for maternal behavior (i.e. higher oxytocin receptor levels are associated with more maternally responsive females) (Champagne, Diorio, Sharma, & Meaney, 2001; Champagne, et al., 2003; Francis, Champagne, & Meaney, 2000). During adolescence, enhanced DNA methylation of the *Bdnf* gene (Doherty, et al., 2016), and decreased *Bdnf* gene expression and increased Neuropeptide Y (*NPY*) gene expression (Hill, et al., 2014) were found. Contrary to adolescent gene expression, *Bdnf* gene expression was enhanced in females in adulthood (Hill, et al., 2014) and this paralleled lower methylation levels (Roth, et al., 2014). These results further illustrate the transient and dynamic nature of epigenetic changes resulting from caregiver experiences. It is currently unclear what mechanism (or mechanisms) could underlie these changes. To further probe these effects of early-life experience and ascertain the way by which these epigenetic modifications could alter maternal behavioral outcomes, an important factor in future research will be parsing apart the nuclei within the amygdala that are functionally distinct and differentially contribute to maternal behavior (Numan, et al., 1993).

Finally, restricted access to a caregiver can also have long-lasting epigenetic and behavioral consequences for female offspring (Kalinichev, Easterling, Plotsky, & Holtzman, 2002). In a mouse model of maternal separation, adolescent female C57BL/6J mice that experienced daily separation from their mother from postnatal days (PND) 1 through PND 14 demonstrated decreased *Bdnf* gene expression and enhanced *GR* methylation in the hippocampus (Kundakovic, Lim, Gudsnuk, & Champagne, 2013). Interestingly, in Balb/cJ mice that were exposed to this same manipulation, *Bdnf* expression was enhanced within the PFC and increased levels of *Bdnf* exon IX methylation within the hippocampus were found (Kundakovic, Lim, et al., 2013). Altogether, data highlight the brain region-dependent nature of epigenetic modifications in females in response to different rearing environments.

Exposure to maltreatment in infancy (PND 1-7)

Methylation status of *Bdnf* gene in adulthood

mPFC	Amygdala	dHPC	vHPC
↓ Exon I ↑ Exon IV	↓ Exon IV	No change	↑ Exon I

Figure 1.2 Epigenetic modulation of the *Bdnf* gene by early-life stress. Exposure to caregiver maltreatment in infancy has lifelong implications on the methylation status of the *Bdnf* gene. This figure summarizes the changes in DNA methylation of exons I and IV which are present in adulthood in female animals that were exposed to caregiver maltreatment in the first week of life. Abbreviations: mPFC= medial prefrontal cortex; dHPC= dorsal hippocampus; vHPC= ventral hippocampus; PND=postnatal day. (Figure from Keller and Roth, 2016).

.

1.6 Generational Transmission of Epigenetic Modifications and Phenotypes

Quality of maternal behavior is passed from mother to female offspring (Champagne, et al., 2006; Francis, et al., 2000; Francis, et al., 1999; Liu, et al., 1997; Roth, et al., 2009; Weaver, et al., 2004). The transmission of LG behaviors from mother to female offspring is mediated by rearing experience, as cross-fostering offspring to high-LG mothers is sufficient to enhance LG levels (Francis, et al., 1999; Weaver, et al., 2004). Additionally, LG behaviors are enhanced by social experiences post-weaning (Champagne & Meaney, 2007). In a multigenerational stress design where three generations of rats were exposed to restraint and swim stressors, changes in antepartum behavior were found to be altered across generations (Ward, et al., 2013). Specifically, tail chasing behavior prior to parturition varied as a consequence of multiple generations of stress exposure. The first generation exposed to the stressor did not show behavioral variations, however, the second and third generation of stress-exposed females showed a reduction in tail chasing behavior, with the third generation showing the most severe decrease in tail chasing prevalence (Ward, et al., 2013). In addition to programming of antepartum and maternal behavior, stress responsivity is likewise transmitted from parent to offspring (Zaidan & Gaisler-Salomon, 2015; Zaidan, et al., 2013).

Another line of generational transmission comes from studies in rats using the endocrine disruptor vinclozolin, which is known to produce pregnancy abnormalities

and kidney disease (Nilsson, Anway, Stanfield, & Skinner, 2008). In addition, vinclozolin when limited to F0 exposure and then using male offspring to generate successive generations, alters expression levels of over 1000 hippocampal and 100 amygdala genes in F3 generation females, with concomitant increases in anxiety-like behavior (Skinner, Anway, Savenkova, Gore, & Crews, 2008). In our own research where we found enhanced levels of DNA methylation of the *Bdnf* gene in the PFC and hippocampus in female rats with a history of maltreatment, we found this same change in the next generation of offspring (Roth, et al., 2009). Interestingly, cross-fostering pups of dams that experienced maltreatment in infancy was not sufficient to completely rescue DNA methylation levels (Roth, et al., 2009). This might indicate that these epigenetic marks were heritable (i.e. the associated epigenetic marks were transmitted through the germline as a result of environmental experiences). Prepartum behavior however was different in females that had been exposed to maltreatment such that previously maltreated dams displayed more anxiety-related behaviors during the last three days of pregnancy (Roth, et al., 2009). Thus, it is uncertain whether the biological effects ascertained in our model were due to a compromised gestational environment (i.e. maternal state during gestation) or that the epigenetic marks were passed through the germline. Regardless of the mode of transmission in our study or others highlighted here, together data indicate that stress exposure in females has behavioral and epigenetic consequences for her offspring and grand-offspring. More research is certainly warranted in the areas of behaviorally-mediated vs. germ-line-mediated inheritance.

1.7 Interventions to Alter the Female Epigenome

The inherently malleable epigenome may be a target of therapeutic or behavioral intervention, and many studies have shown this to be true (e.g (Roth, et al., 2009; Szyf, 2009; Weaver et al., 2005)). However, sex differences exist under basal conditions in levels of various epigenetic regulators, and these sex differences in epigenetic regulators contribute to sex differences in behavior (e.g. (Auger, Jessen, & Edelmann, 2011; Nugent, et al., 2015)). For example, within the amygdala, mRNA levels of *Dnmt3a*, *MeCP2* (Kolodkin & Auger, 2011), and Gadd45b (Kigar, Chang, Hayne, Karls, & Auger, 2016) are higher in developing females as compared to males. Sex differences are also found in baseline levels of posttranslational histone modifications and DNA methylation throughout other regions of the brain including the cortex, hypothalamus, and BNST/POA (Schwarz, Nugent, & McCarthy, 2010; Shen et al., 2015; Tsai, Grant, & Rissman, 2009). This suggests that experiments manipulating these molecules may see divergent effects between the sexes. Additionally, levels of these regulators are dynamic and levels between the sexes differ across developmental time periods, so assessing the efficacy of administration of epigenetic regulators across the lifespan is of importance (Kurian, Forbes-Lorman, & Auger, 2007).

There are some data to support the notion that drugs that manipulate the epigenome can have positive effects on the female brain. For example, administration of a DNMT inhibitor in adulthood rescued aberrant PFC DNA methylation patterns

resulting from exposure to caregiver maltreatment (Roth, et al., 2009). This suggests that modulating DNA methylation profiles could be utilized to normalize consequences of early-life stress, even when the intervention occurs in adulthood. Similarly, epigenetic modifications resulting from inhibition of HDACs have beneficial effects, although very few of the studies that have been conducted included female subjects. Sodium butyrate decreased depressive-like behaviors in mice, and this effect was further enhanced by co-administration with the antidepressant fluoxetine (Schroeder, et al., 2007). In a rat model of neonatal maternal separation, adult females exposed to separation from PND2-9 demonstrated a reduced fear-potentiated startle response, which corresponded with increased serum estradiol and decreased histone methylation in the frontal cortex (Kao et al., 2012). Treatment with the HDACi valproic acid, but not the DNMT inhibitor 5-aza-2'-deoxycytidine, prior to daily maternal separation reversed this decrease in fear-potentiated startle behavior and histone methylation (Kao, et al., 2012). As the DNMT and HDAC inhibitors employed in these studies lack target specificity (i.e. many gene loci would be presumed to be affected) and can produce off-target effects, there is a strong need to explore strategies that enable select epigenetic modifications.

Maternal diet has strong modulatory effects on the epigenome, health, and behavioral outcomes of her offspring. Supplementing maternal diet with folic acid, a methyl donor, ameliorated aberrant epigenetic profiles in mouse offspring induced by exposure to BPA (Dolinoy, et al., 2007). Methyl donor supplementation also rescued

alterations in DNA methylation and behavior resulting from exposure to high fat diet during gestation. Specifically, when a high fat diet was paired with methyl donor supplementation, the global hypomethylation typically induced by gestational exposure to high fat diet was eliminated in the PFC of female rat offspring (Carlin, George, & Reyes, 2013). This treatment also ameliorated the enhancement of μ-opioid receptor mRNA in the nucleus accumbens and PFC, showing dietary supplementation is capable of rescuing both global and gene specific aberrations induced by gestational exposure to high fat diet (Carlin, et al., 2013). Further, methyl donor supplementation rescued the high fat diet preference and reduced locomotor activity observed in offspring of dams that consumed a high fat diet during pregnancy (Carlin, et al., 2013). Such epigenetic alterations could contribute to differences in processing rewarding stimuli. Because maternal behavior is a motivated behavior and pup-interactions elicit a reward response in dams (Ferris et al., 2005; Gaffori & Le Moal, 1979; Hansen, Harthon, Wallin, Löfberg, & Svensson, 1991), aberrant reward processing could contribute to deficient maternal behavior towards offspring. Taken together, these data highlight the ability of the maternal diet to regulate the epigenome of offspring and the therapeutic potential for dietary supplementation during the prenatal/early postnatal period.

Environmental interventions similarly rescue LG behavior in females that received low levels of LG in infancy. As previously mentioned, social enrichment post-weaning enhances levels of LG behavior in females that received low LG in

infancy. This coincided with enhanced oxytocin receptor binding and exploratory behavior as measured by the open-field test (Champagne & Meaney, 2007). This suggests that social interactions for the female beyond those occurring in infancy have behavioral and neurobiological implications, and such manipulations are capable of modulating behavioral trajectories. Natural variation in maternal care also has implications for the development of reward systems, with adult offspring of low-LG dams exhibiting a blunted increase in the dopamine signal within the nucleus accumbens in response to licking and grooming pups. Administration of the selective dopamine re-uptake inhibitor GBR 12909 brought the dopamine signal generated by pup interactions up to that of high-LG offspring (Champagne, et al., 2004). Thus, drugs that manipulate dopamine systems could have positive effects for maternal care-associated deficits in reward processing, and future studies could examine potential epigenetic underpinnings of this drug action and efficacy.

1.8 Concluding Remarks

We have highlighted data demonstrating that environmental factors throughout development modify the female epigenome and create epigenetic marks within brain regions critical for maternal behavior. Epigenetic marks can be both long-lasting and dynamic (i.e. some continue to transpire over the course of the lifespan or can be modified). Future research is needed to better ascertain the effects of environmental and psychosocial perturbations on the female epigenome, as well as motherhood itself.

These data are important to have in hand, as their understanding has implications for interventions for negligent/abusive maternal care as well as for neuropsychiatric disorders such as postpartum depression, which is estimated to occur in about 15% of pregnancies and could potentially have epigenetic underpinnings (Kaminsky & Payne, 2014; O'Hara & McCabe, 2013).

Acknowledgments

Work on this article was supported by a grant from The National Institute of General Medical Sciences (1P20GM103653).

REFERENCES

Adlakha, Y. K., & Saini, N. (2014). Brain microRNAs and insights into biological functions and therapeutic potential of brain enriched miRNA-128. [journal article]. *Molecular Cancer, 13*(1), 1-18. doi: 10.1186/1476-4598-13-33

Afonso, V. M., Sison, M., Lovic, V., & Fleming, A. S. (2007). Medial prefrontal cortex lesions in the female rat affect sexual and maternal behavior and their sequential organization. *Behavioral Neuroscience, 121*(3), 515-526. doi: 10.1037/0735-7044.121.3.515

Alleva, E., Caprioli, A., & Laviola, G. (1986). Postnatal social environment affects morphine analgesia in male mice. *Physiology & Behavior, 36*(4), 779-781.

Alleva, E., Caprioli, A., & Laviola, G. (1989). Litter gender composition affects maternal behavior of the primiparous mouse dam (Mus musculus). *Journal of Comparative Psychology, 103*(1), 83-87.

Auger, A. P., Jessen, H. M., & Edelmann, M. N. (2011). Epigenetic organization of brain sex differences and juvenile social play behavior. *Hormones and Behavior, 59*(3), 358-363.

Barrett, J., & Fleming, A. S. (2011). Annual research review: all mothers are not created equal: neural and psychobiological perspectives on mothering and the importance of individual differences. *Journal of Child Psychology and Psychiatry, 52*(4), 368-397. doi: 10.1111/j.1469-7610.2010.02306.x

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell, 116*(2), 281-297. doi: 10.1016/s0092-8674(04)00045-5

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience and Biobehavioral Reviews, 35*(3), 565-572. doi: 10.1016/j.neubiorev.2010.07.002

Benoit, J. D., Rakic, P., & Frick, K. M. (2015). Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behavioural Brain Research, 281*, 1-8.

Blaze, J., & Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International Journal of Developmental Neuroscience, 31*(8), 804-810. doi: 10.1016/j.ijdevneu.2013.10.001

Blaze, J., Scheuing, L., & Roth, T. L. (2013). Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to maltreatment or nurturing care during infancy. *Developmental Neuroscience, 35*(4), 306-316. doi: 10.1159/000350716

Bosch, O. J., Pförtsch, J., Beiderbeck, D. I., Landgraf, R., & Neumann, I. D. (2010). Maternal behaviour is associated with vasopressin release in the medial preoptic area and bed nucleus of the stria terminalis in the rat. *Journal of Neuroendocrinology, 22*(5), 420-429. doi: 10.1111/j.1365-2826.2010.01984.x

Boulle, F., Pawluski, J. L., Homberg, J. R., Machiels, B., Kroeze, Y., Kumar, N., . . . van den Hove, D. L. (2016). Developmental fluoxetine exposure increases

behavioral despair and alters epigenetic regulation of the hippocampal BDNF gene in adult female offspring. *Hormones and Behavior, 80*, 47-57.

Broadbelt, K., Byne, W., & Jones, L. B. (2002). Evidence for a decrease in basilar dendrites of pyramidal cells in schizophrenic medial prefrontal cortex. *Schizophrenia Research, 58*(1), 75-81.

Brunelli, S. A., Shair, H., & Hofer, M. A. (1994). Hypothermic vocalizations of rats pups (Rattus norvegicus) elicit and direct maternal search behavior. *Journal of Comparative Psychology, 108*(3), 298-303.

Brunelli, S. A., Shindledecker, R. D., & Hofer, M. A. (1989). Early experience and maternal behavior in rats. *Developmental Psychobiology, 22*(3), 295-314. doi: 10.1002/dev.420220308

Calafat, A. M., Ye, X., Wong, L.-Y., Reidy, J. A., & Needham, L. L. (2008). Exposure of the US population to Bisphenol A and 4-tertiary-Octylphenol: 2003-2004. *Environmental Health Perspectives*, 39-44.

Carlin, J., George, R., & Reyes, T. M. (2013). Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology. *PLoS ONE, 8*(5).

Chahrour, M., Jung, S. Y., Shaw, C., Zhou, X., Wong, S. T., Qin, J., & Zoghbi, H. Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science, 320*(5880), 1224-1229.

Chai, X. J., Whitfield-Gabrieli, S., Shinn, A. K., Gabrieli, J. D., Castañón, A. N., McCarthy, J. M., . . . Öngür, D. (2011). Abnormal medial prefrontal cortex

resting-state connectivity in bipolar disorder and schizophrenia. *Neuropsychopharmacology, 36*(10), 2009-2017.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology, 29*(3), 386-397.

Champagne, F. A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., & Meaney, M. J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *The Journal of Neuroscience, 24*(17), 4113-4123.

Champagne, F. A., Diorio, J., Sharma, S., & Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences, 98*(22), 12736-12741.

Champagne, F. A., & Meaney, M. J. (2007). Transgenerational effects of social environment on variations in maternal care and behavioral response to novelty. *Behavioral Neuroscience, 121*(6), 1353.

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. [Research Support, Non-U.S. Gov't]. *Endocrinology, 147*(6), 2909-2915. doi: 10.1210/en.2005-1119

Champagne, F. A., Weaver, I. C., Diorio, J., Sharma, S., & Meaney, M. J. (2003). Natural variations in maternal care are associated with estrogen receptor alpha

expression and estrogen sensitivity in the medial preoptic area. *Endocrinology, 144*(11), 4720-4724. doi: 10.1210/en.2003-0564

Chen, K., & Rajewsky, N. (2007). The evolution of gene regulation by transcription factors and microRNAs. *Nature Reviews Genetics, 8*(2), 93-103.

Cirulli, F., Adriani, W., & Laviola, G. (1997). Sexual segregation in infant mice: behavioural and neuroendocrine responses to d-amphetamine administration. *Psychopharmacology, 134*(2), 140-152.

Consiglio, A. R., & Lucion, A. B. (1996). Lesion of hypothalamic paraventricular nucleus and maternal aggressive behavior in female rats. *Physiology & Behavior, 59*(4–5), 591-596. doi:

Della Seta, D., Minder, I., Dessì-Fulgheri, F., & Farabollini, F. (2005). Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. *Brain Research Bulletin, 65*(3), 255-260.

Doherty, T. S., Forster, A., & Roth, T. L. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural Brain Research, 298*(Pt A), 55-61. doi: 10.1016/j.bbr.2015.05.028

Dolinoy, D. C., Huang, D., & Jirtle, R. L. (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proceedings of the National Academy of Sciences, 104*(32), 13056-13061.

Dulac, C., O'Connell, L. A., & Wu, Z. (2014). Neural control of maternal and paternal behaviors. *Science, 345*(6198), 765-770.

Febo, M., Felix-Ortiz, A. C., & Johnson, T. R. (2010). Inactivation or inhibition of neuronal activity in the medial prefrontal cortex largely reduces pup retrieval and grouping in maternal rats. *Brain Research, 1325*, 77-88.

Ferris, C. F., Foote, K. B., Meltser, H. M., Plenby, M. G., Smith, K. L., & Insel, T. R. (1992). Oxytocin in the amygdala facilitates maternal aggression. *Annals of the New York Academy of Sciences, 652*(1), 456-457.

Ferris, C. F., Kulkarni, P., Sullivan, J. M., Harder, J. A., Messenger, T. L., & Febo, M. (2005). Pup suckling is more rewarding than cocaine: evidence from functional magnetic resonance imaging and three-dimensional computational analysis. *The Journal of Neuroscience, 25*(1), 149-156.

Fleming, A. S., Miceli, M., & Moretto, D. (1983). Lesions of the medial preoptic area prevent the facilitation of maternal behavior produced by amygdala lesions. *Physiology & Behavior, 31*(4), 503-510.

Fleming, A. S., Vaccarino, F., & Luebke, C. (1980). Amygdaloid inhibition of maternal behavior in the nulliparous female rat. *Physiology & Behavior, 25*(5), 731-743.

Fleming, A. S., & Walsh, C. (1994). Neuropsychology of maternal behavior in the rat: c-fos expression during mother-litter interactions. *Psychoneuroendocrinology, 19*(5–7), 429-443.

Francis, D. D., Champagne, F. A., & Meaney, M. J. (2000). Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Journal of Neuroendocrinology, 12*(12), 1145-1148.

Francis, D. D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286*(5442), 1155-1158.

Gaffori, O., & Le Moal, M. (1979). Disruption of maternal behavior and appearance of cannibalism after ventral mesencephalic tegmentum lesions. *Physiology & Behavior, 23*(2), 317-323.

Gagnidze, K., Weil, Z. M., Faustino, L. C., Schaafsma, S. M., & Pfaff, D. W. (2013). Early histone modifications in the ventromedial hypothalamus and preoptic area following oestradiol administration. *Journal of Neuroendocrinology, 25*(10), 939-955.

Ghahramani, N. M., Ngun, T. C., Chen, P.-Y., Tian, Y., Krishnan, S., Muir, S., . . . Forger, N. G. (2014). The effects of perinatal testosterone exposure on the DNA methylome of the mouse brain are late-emerging. *Biology of Sex Differences, 5*(8).

Giovenardi, M., Padoin, M. J., Cadore, L. P., & Lucion, A. B. (1998). Hypothalamic paraventricular nucleus modulates maternal aggression in rats: effects of ibotenic acid lesion and oxytocin antisense. *Physiology & Behavior, 63*(3), 351-359.

Gonzalez, A., Lovic, V., Ward, G., Wainwright, P., & Fleming, A. (2001). Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Developmental Psychobiology, 38*(1), 11-32.

Gore, A. C., Walker, D. M., Zama, A. M., Armenti, A. E., & Uzumcu, M. (2011). Early life exposure to endocrine-disrupting chemicals causes lifelong molecular reprogramming of the hypothalamus and premature reproductive aging. *Molecular Endocrinology, 25*(12), 2157-2168.

Grant, P. A. (2001). A tale of histone modifications. *Genome Biology, 2*(4), 0003.0001-0006.

Guy, J., Cheval, H., Selfridge, J., & Bird, A. (2011). The role of MeCP2 in the brain. *Annual Review of Cell and Developmental Biology, 27*, 631-652.

Hansen, S., Harthon, C., Wallin, E., Löfberg, L., & Svensson, K. (1991). Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness. *Behavioral Neuroscience, 105*(4), 588.

Hao, Y., Huang, W., Nielsen, D. A., & Kosten, T. A. (2011). Litter gender composition and sex affect maternal behavior and DNA methylation levels of the oprm1 gene in rat offspring. *Frontiers in Psychiatry, 2*, 21. doi: 10.3389/fpsyt.2011.00021

He, L., & Hannon, G. J. (2004). MicroRNAs: small RNAs with a big role in gene regulation. *Nature Reviews Genetics, 5*(7), 522-531.

Hill, K. T., Warren, M., & Roth, T. L. (2014). The influence of infant-caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural Brain Research, 272*, 175-180.

Hodes, G. E., Pfau, M. L., Purushothaman, I., Ahn, H. F., Golden, S. A., Christoffel, D. J., . . . Flanigan, M. E. (2015). Sex differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. *The Journal of Neuroscience, 35*(50), 16362-16376.

Insel, T. R., & Harbaugh, C. R. (1989). Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. *Physiology & Behavior, 45*(5), 1033-1041. doi:

Jessen, H. M., & Auger, A. P. (2011). Sex differences in epigenetic mechanisms may underlie risk and resilience for mental health disorders. *Epigenetics, 6*(7), 857-861.

Joel, D., Weiner, I., & Feldon, J. (1997). Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analog of the Wisconsin Card Sorting Test, but do not disrupt latent inhibition: implications for animal models of schizophrenia. *Behavioural Brain Research, 85*(2), 187-201.

Kalinichev, M., Easterling, K. W., Plotsky, P. M., & Holtzman, S. G. (2002). Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long–Evans rats. *Pharmacology Biochemistry and Behavior, 73*(1), 131-140.

Kaminsky, Z., & Payne, J. (2014). Seeing the future: epigenetic biomarkers of postpartum depression. *Neuropsychopharmacology, 39*(1), 234.

Kao, G.-S., Cheng, L.-Y., Chen, L.-H., Tzeng, W.-Y., Cherng, C. G., Su, C.-C., . . . Yu, L. (2012). Neonatal isolation decreases cued fear conditioning and frontal

cortical histone 3 lysine 9 methylation in adult female rats. *European Journal of Pharmacology, 697*(1), 65-72.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*(6), 593-602.

Kigar, S. L., Chang, L., Hayne, M. R., Karls, N. T., & Auger, A. P. (2016). Sex differences in Gadd45b expression and methylation in the developing rodent amygdala. *Brain Research, 1642*, 461-466.

Kim, M. Y., Hsiao, S. J., & Kraus, W. L. (2001). A role for coactivators and histone acetylation in estrogen receptor α-mediated transcription initiation. *The EMBO Journal, 20*(21), 6084-6094.

Kimble, D. P., Rogers, L., & Hendrickson, C. W. (1967). Hippocampal lesions disrupt maternal, not sexual, behavior in the albino rat. *Journal of Comparative and Physiological Psychology, 63*(3), 401.

Klampfl, S. M., Brunton, P. J., Bayerl, D. S., & Bosch, O. J. (2014). Hypoactivation of CRF receptors, predominantly type 2, in the medial-posterior BNST is vital for adequate maternal behavior in lactating rats. *The Journal of Neuroscience, 34*(29), 9665-9676. doi: 10.1523/jneurosci.4220-13.2014

Klein, S. L., Schiebinger, L., Stefanick, M. L., Cahill, L., Danska, J., de Vries, G. J., . . . Woodruff, T. K. (2015). Opinion: sex inclusion in basic research drives

discovery. *Proceedings of the National Academy of Sciences, 112*(17), 5257-5258.

Kolodkin, M., & Auger, A. (2011). Sex difference in the expression of DNA methyltransferase 3a in the rat amygdala during development. *Journal of Neuroendocrinology, 23*(7), 577-583.

Kosten, T. A., Huang, W., & Nielsen, D. A. (2014). Sex and litter effects on anxiety and DNA methylation levels of stress and neurotrophin genes in adolescent rats. *Developmental Psychobiology, 56*(3), 392-406. doi: 10.1002/dev.21106

Kristal, M. B. (2009). The biopsychology of maternal behavior in nonhuman mammals. *ILAR Journal, 50*(1), 51-63. doi: 10.1093/ilar.50.1.51

Kundakovic, M., Gudsnuk, K., Franks, B., Madrid, J., Miller, R. L., Perera, F. P., & Champagne, F. A. (2013). Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. *Proc Natl Acad Sci U S A, 110*(24), 9956-9961.

Kundakovic, M., Lim, S., Gudsnuk, K., & Champagne, F. A. (2013). Sex-specific and strain-dependent effects of early life adversity on behavioral and epigenetic outcomes. *Front Psychiatry, 4*, 78. doi: 10.3389/fpsyt.2013.00078

Kurian, J. R., Forbes-Lorman, R. M., & Auger, A. P. (2007). Sex difference in mecp2 expression during a critical period of rat brain development. *Epigenetics, 2*(3), 173-178.

Laviola, G., & Terranova, M. L. (1998). The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. *Neuroscience and Biobehavioral Reviews, 23*(2), 197-213.

Lee, S. J., & McEwen, B. S. (2001). Neurotrophic and neuroprotective actions of estrogens and their therapeutic implications. *Annual Review of Pharmacology and Toxicology, 41*(1), 569-591.

Levenson, J. M., Roth, T. L., Lubin, F. D., Miller, C. A., Huang, I.-C., Desai, P., . . . Sweatt, J. D. (2006). Evidence that DNA (Cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. *Journal of Biological Chemistry, 281*(23), 15763-15773. doi: 10.1074/jbc.M511767200

Li, B., Carey, M., & Workman, J. L. (2007). The role of chromatin during transcription. *Cell, 128*(4), 707-719.

Li, M., & Fleming, A. S. (2003a). Differential involvement of nucleus accumbens shell and core subregions in maternal memory in postpartum female rats. *Behavioral Neuroscience, 117*(3), 426.

Li, M., & Fleming, A. S. (2003b). The nucleus accumbens shell is critical for normal expression of pup-retrieval in postpartum female rats. *Behavioural Brain Research, 145*(1), 99-111.

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., . . . Ngo, Q.-M. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature, 462*(7271), 315-322.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277*(5332), 1659-1662.

Lonstein, J. S., Wagner, C. K., & De Vries, G. J. (1999). Comparison of the "nursing" and other parental behaviors of nulliparous and lactating female rats. *Hormones and Behavior, 36*(3), 242-251. doi:

Lubin, D. A., Elliott, J. C., Black, M. C., & Johns, J. M. (2003). An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. *Behavioral Neuroscience, 117*(2), 195-201.

Lutz, P.-E., & Turecki, G. (2014). DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience, 264*, 142-156.

Ma, D. K., Guo, J. U., Ming, G. L., & Song, H. (2009). DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. *Cell Cycle, 8*(10), 1526-1531.

Ma, D. K., Jang, M. H., Guo, J. U., Kitabatake, Y., Chang, M. L., Pow-Anpongkul, N., . . . Song, H. (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science, 323*(5917), 1074-1077. doi: 10.1126/science.1166859

Ma, D. K., Marchetto, M. C., Guo, J. U., Ming, G.-l., Gage, F. H., & Song, H. (2010). Epigenetic choreographers of neurogenesis in the adult mammalian brain. *Nature Neuroscience, 13*(11), 1338-1344.

McCarthy, M. M., Auger, A. P., Bale, T. L., De Vries, G. J., Dunn, G. A., Forger, N. G., . . . Wilson, M. E. (2009). The epigenetics of sex differences in the brain. *The Journal of Neuroscience, 29*(41), 12815-12823. doi: 10.1523/JNEUROSCI.3331-09.2009

McCarthy, M. M., & Nugent, B. (2013). Epigenetic contributions to hormonally-mediated sexual differentiation of the brain. *Journal of Neuroendocrinology, 25*(11), 1133-1140.

McGowan, P. O., Suderman, M., Sasaki, A., Huang, T., Hallett, M., Meaney, M. J., & Szyf, M. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS ONE, 6*(2), e14739.

Melzer, D., Rice, N. E., Lewis, C., Henley, W. E., & Galloway, T. S. (2010). Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. *PLoS ONE, 5*(1), e8673.

Midoro-Horiuti, T., Tiwari, R., Watson, C. S., & Goldblum, R. M. (2010). Maternal bisphenol a exposure promotes the development of experimental asthma in mouse pups. *Environmental Health Perspectives, 118*(2), 273.

Millar, C. B., & Grunstein, M. (2006). Genome-wide patterns of histone modifications in yeast. *Nature Reviews Molecular Cell Biology, 7*(9), 657-666.

Moore, C. L. (1984). Maternal contributions to the development of masculine sexual behavior in laboratory rats. *Developmental Psychobiology, 17*(4), 347-356. doi: 10.1002/dev.420170403

Moore, C. L., & Chadwick-Dias, A.-M. (1986). Behavioral responses of infant rats to maternal licking: variations with age and sex. *Developmental Psychobiology, 19*(5), 427-438.

Moore, C. L., & Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. [.]. *Journal of Comparative and Physiological Psychology, 93*(4), 677-684.

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology, 38*(1), 23-38. doi: 10.1038/npp.2012.112

Moretto, D., Paclik, L., & Fleming, A. (1986). The effects of early rearing environments on maternal behavior in adult female rats. *Developmental Psychobiology, 19*(6), 581-591. doi: 10.1002/dev.420190609

Nelson, E. E., & Panksepp, J. (1998). Brain substrates of infant-mother attachment: contributions of opioids, oxytocin, and norepinephrine. *Neuroscience and Biobehavioral Reviews, 22*(3), 437-452.

Nilsson, E. E., Anway, M. D., Stanfield, J., & Skinner, M. K. (2008). Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction, 135*(5), 713-721. doi: 10.1530/rep-07-0542

Nugent, B. M., Wright, C. L., Shetty, A. C., Hodes, G. E., Lenz, K. M., Mahurkar, A., . . . McCarthy, M. M. (2015). Brain feminization requires active repression of masculinization via DNA methylation. *Nature Neuroscience.*

Numan, M., & Numan, M. J. (1997). Projection sites of medial preoptic area and ventral bed nucleus of the stria terminalis neurons that express Fos during maternal behavior in female rats. *Journal of Neuroendocrinology, 9*(5), 369-384.

Numan, M., Numan, M. J., & English, J. B. (1993). Excitotoxic amino acid injections into the medial amygdala facilitate maternal behavior in virgin female rats. [Research Support, Non-U.S. Gov't]. *Hormones and Behavior, 27*(1), 56-81. doi: 10.1006/hbeh.1993.1005

Numan, M., & Stolzenberg, D. S. (2009). Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Frontiers in Neuroendocrinology, 30*(1), 46-64.

Numan, M., & Woodside, B. (2010). Maternity: neural mechanisms, motivational processes, and physiological adaptations. *Behavioral Neuroscience, 124*(6), 715.

O'Hara, M. W., & McCabe, J. E. (2013). Postpartum depression: current status and future directions. *Annual Review of Clinical Psychology, 9*, 379-407.

Palanza, P. L., Howdeshell, K. L., Parmigiani, S., & vom Saal, F. S. (2002). Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environmental Health Perspectives, 110*(Suppl 3), 415.

Panagiotidou, E., Zerva, S., Mitsiou, D. J., Alexis, M. N., & Kitraki, E. (2014). Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats. *Journal of Endocrinology, 220*(3), 207-218.

Pawluski, J. L., & Galea, L. A. M. (2007). Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience, 149*(1), 53-67.

Pawluski, J. L., Lambert, K. G., & Kinsley, C. H. (2016). Neuroplasticity in the maternal hippocampus: Relation to cognition and effects of repeated stress. *Hormones and Behavior, 77*, 86-97. doi:

Pedersen, C. A., Caldwell, J. D., Walker, C., Ayers, G., & Mason, G. A. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behavioral Neuroscience, 108*(6), 1163.

Pena, C. J., Monk, C., & Champagne, F. A. (2012). Epigenetic effects of prenatal stress on 11 beta-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PLoS ONE, 7*(6), e39791.

Pereira, M., & Morrell, J. I. (2011). Functional mapping of the neural circuitry of rat maternal motivation: effects of site-specific transient neural inactivation. *Journal of Neuroendocrinology, 23*(11), 1020-1035.

Pfaff, D. W. (1989). Features of a hormone-driven defined neural circuit for a mammalian behavior. Principles illustrated, neuroendocrine syllogisms, and multiplicative steroid effects. *Annals of the New York Academy of Sciences, 563*, 131-147.

Plotsky, P., & Meaney, M. (1993). Early, postnatal experience alters hypothalamic corticotropin-relesing factor (CRF) mRNA, median eminence CRF content and

stress-induced release in adult rats. *Brain Research Molecular Brain Research, 18*(3), 195-200.

Provencal, N., & Binder, E. B. (2015). The neurobiological effects of stress as contributors to psychiatric disorders: focus on epigenetics. *Current Opinion in Neurobiology, 30*, 31-37.

Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S., Talsness, C. E., . . . vom Saal, F. S. (2007). In vivo effects of Bisphenol A in laboratory rodent studies. *Reproductive Toxicology, 24*(2), 199-224.

Ridderinkhof, K. R., Ullsperger, M., Crone, E. A., & Nieuwenhuis, S. (2004). The role of the medial frontal cortex in cognitive control. *Science, 306*(5695), 443-447.

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry, 65*(9), 760-769. doi: 10.1016/j.biopsych.2008.11.028

Roth, T. L., Matt, S., Chen, K., & Blaze, J. (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the homecage. *Developmental Psychobiology, 56*(8), 1755-1763.

Roth, T. L., & Sweatt, J. D. (2011). Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormones and Behavior, 59*(3), 315-320. doi: 10.1016/j.yhbeh.2010.05.005

Schroeder, F. A., Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biological Psychiatry, 62*(1), 55-64.

Schwarz, J. M., Nugent, B. M., & McCarthy, M. M. (2010). Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. *Endocrinology, 151*(10), 4871-4881.

Sheehan, T., Paul, M., Amaral, E., Numan, M. J., & Numan, M. (2001). Evidence that the medial amygdala projects to the anterior/ventromedial hypothalamic nuclei to inhibit maternal behavior in rats. *Neuroscience, 106*(2), 341-356.

Shen, E. Y., Ahern, T. H., Cheung, I., Straubhaar, J., Dincer, A., Houston, I., . . . Forger, N. G. (2015). Epigenetics and sex differences in the brain: a genome-wide comparison of histone-3 lysine-4 trimethylation (H3K4me3) in male and female mice. *Experimental Neurology, 268*, 21-29.

Skinner, M. K., & Anway, M. D. (2005). Seminiferous cord formation and germ-cell programming: epigenetic transgenerational actions of endocrine disruptors. *Annals of the New York Academy of Sciences, 1061*(1), 18-32. doi: 10.1196/annals.1336.004

Skinner, M. K., Anway, M. D., Savenkova, M. I., Gore, A. C., & Crews, D. (2008). Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS ONE, 3*(11), e3745. doi: 10.1371/journal.pone.0003745

St-Cyr, S., & McGowan, P. O. (2015). Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in Behavioral Neuroscience, 9,* 145. doi: 10.3389/fnbeh.2015.00145

Stanton, M. E., & Levine, S. (1990). Inhibition of infant glucocorticoid stress response: Specific role of maternal cues. *Developmental Psychobiology, 23*(5), 411-426. doi: 10.1002/dev.420230504

Stern, J. M. (1986). Licking, touching, and suckling: contact stimulation and maternal psychobiology in rats and women. *Annals of the New York Academy of Sciences, 474*(1), 95-107. doi: 10.1111/j.1749-6632.1986.tb28001.x

Stern, J. M., & Lonstein, J. S. (2001). Neural mediation of nursing and related behaviors. *Progress in Brain Resarch, 133*, 263-278.

Sterrenburg, L., Gaszner, B., Boerrigter, J., Santbergen, L., Bramini, M., Elliott, E., . . . Kozicz, T. (2011). Chronic stress induces sex-specific alterations in methylation and expression of corticotropin-releasing factor gene in the rat. *PLoS ONE, 6*(11), e28128.

Sterrenburg, L., Gaszner, B., Boerrigter, J., Santbergen, L., Bramini, M., Roubos, E. W., . . . Kozicz, T. (2012). Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor–producing neurons in the rat paraventricular nucleus, central amygdala, and bed nucleus of the stria terminalis. *Journal of Neuroscience Research, 90*(1), 179-192.

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: the role of experience and epigenetic mechanisms. *Hormones and Behavior, 77*, 204-210.

Stolzenberg, D. S., Stevens, J. S., & Rissman, E. F. (2012). Experience-facilitated improvements in pup retrieval; evidence for an epigenetic effect. *Hormones and Behavior, 62*(2), 128-135.

Stolzenberg, D. S., Stevens, J. S., & Rissman, E. F. (2014). Histone deacetylase inhibition induces long-lasting changes in maternal behavior and gene expression in female mice. *Endocrinology, 155*(9), 3674-3683.

Stroud, H., Do, T., Du, J., Zhong, X., Feng, S., Johnson, L., . . . Jacobsen, S. E. (2014). Non-CG methylation patterns shape the epigenetic landscape in Arabidopsis. *Nature Structural & Molecular Biology, 21*(1), 64-72.

Szyf, M. (2009). Epigenetics, DNA methylation, and chromatin modifying drugs. *Annual Review of Pharmacology and Toxicology, 49*, 243-263.

Tamura, Y., Kunugi, H., Ohashi, J., & Hohjoh, H. (2007). Epigenetic aberration of the human REELIN gene in psychiatric disorders. *Molecular Psychiatry, 12*(6), 593-600.

Tsai, H.-W., Grant, P. A., & Rissman, E. F. (2009). Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics, 4*(1), 47-53.

Valinezhad Orang, A., Safaralizadeh, R., & Kazemzadeh-Bavili, M. (2014). Mechanisms of miRNA-mediated gene regulation from common

downregulation to mRNA-specific upregulation. *International Journal of Genomics, 2014*, 15. doi: 10.1155/2014/970607

Vasudevan, S. (2012). Posttranscriptional upregulation by microRNAs. *Wiley Interdisciplinary Reviews: RNA, 3*(3), 311-330. doi: 10.1002/wrna.121

Vertes, R. P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience, 142*(1), 1-20.

Waddington, C. H. (1940). *Organisers and genes.* : Cambridge University Press.

Ward, I. D., Zucchi, F. C., Robbins, J. C., Falkenberg, E. A., Olson, D. M., Benzies, K., & Metz, G. A. (2013). Transgenerational programming of maternal behaviour by prenatal stress. *BMC Pregnancy and Childbirth, 13*(Suppl 1), S9.

Waterland, R. A., & Jirtle, R. L. (2003). Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Molecular and Cellular Biology, 23*(15), 5293-5300. doi: 10.1128/mcb.23.15.5293-5300.2003

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience, 7*(8), 847-854. doi: 10.1038/nn1276

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., & Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *The Journal of Neuroscience, 25*(47), 11045-11054.

Zaidan, H., & Gaisler-Salomon, I. (2015). Prereproductive stress in adolescent female rats affects behavior and corticosterone levels in second-generation offspring. *Psychoneuroendocrinology, 58,* 120-129.

Zaidan, H., Leshem, M., & Gaisler-Salomon, I. (2013). Prereproductive stress to female rats alters corticotropin releasing factor type 1 expression in ova and behavior and brain corticotropin releasing factor type 1 expression in offspring. *Biological Psychiatry, 74*(9), 680-687.

Zucchi, F. C., Yao, Y., Ward, I. D., Ilnytskyy, Y., Olson, D. M., Benzies, K., . . . Metz, G. A. (2013). Maternal stress induces epigenetic signatures of psychiatric and neurological diseases in the offspring. *PLoS ONE, 8*(2), e56967.

Chapter 2

RESCUE OF MATERNAL BEHAVIOR IN DAMS WITH A HISTORY OF MALTREATMENT VIA PHARMACOLOGICAL MANIPULATION OF DNA METHYLATION

The quality of parental care received during development is known to profoundly influence an individual's phenotype. Maternal behavior is one such behavior shaped by one's own experience with a caregiver. Our lab has previously found that female rats with a history of maltreatment during infancy mistreat their own offspring. One proposed mechanism through which early-life experiences influence maternal behavior is via epigenetic mechanisms. Indeed, our lab has previously identified a number of altered epigenetic patterns throughout the brain of adult female rats with a history of maltreatment. In the current study, we sought to investigate the role of DNA methylation in aberrant maternal behavior in dams with a history of maltreatment. We administered zebularine, a drug known to alter DNA methylation, to dams exposed during infancy to the scarcity-adversity model of low nesting resources, and then characterized the quality of their care towards their offspring in the home cage. First, we replicated our previous finding that dams with a history of maltreatment mistreat their own offspring. Second, we now show that maltreated-

dams treated with intracerebroventricular administration of zebularine showed lower levels of adverse behavior toward their offspring. Third, we found that administration of zebularine disturbed maternal behavior in dams that received nurturing care in infancy such that these animals exhibited enhanced levels of adverse behavior toward offspring relative to their vehicle-treated counterparts. These findings lend support to the hypothesis that epigenetic alterations resulting from maltreatment are causally related to behavioral consequences. Further, our data highlight that early-life experience dictates the implications of epigenome-modifying drugs administered to adult animals, supporting the idea that specific epigenetic patterns underlie specific behavioral outcomes.

2.1 Introduction

Infant experiences with a caregiver have lifelong behavioral consequences and the mechanisms through which these early-life experiences are capable of inducing long-term effects on phenotype continue to be elucidated (Champagne, Francis, Mar, & Meaney, 2003; Doherty, Blaze, Keller, & Roth, 2017; Meaney, 2001; T. L. Roth, Lubin, Funk, & Sweatt, 2009; Weaver et al., 2004). Epigenetic alterations offer one potential mechanism through which experiences in infancy can perpetuate their consequences throughout the lifespan (Anier et al., 2014; Blaze, Asok, & Roth, 2015; Champagne & Curley, 2009; Champagne et al., 2006; Keller & Roth, 2016; Lutz & Turecki, 2014; Maccari, Krugers, Morley-Fletcher, Szyf, & Brunton, 2014; McGowan

& Roth, 2015; C. Murgatroyd et al., 2009; T. L. Roth, et al., 2009; T. L. Roth & Sweatt, 2011; St-Cyr & McGowan, 2015). For example, experiencing adverse maternal care induces both transient and long-term modifications to the epigenome (Blaze, et al., 2015; Blaze & Roth, 2017; Blaze, Scheuing, & Roth, 2013; Doherty, Forster, & Roth, 2016; Labonté et al., 2012; McGowan et al., 2009; Perroud et al., 2011; T. L. Roth, et al., 2009; T. L. Roth, Matt, Chen, & Blaze, 2014). Epigenetic mechanisms, such as DNA methylation and posttranslational histone modifications, are capable of influencing gene expression without altering the underlying genomic sequence. DNA methylation, or the addition of methyl groups to cytosine residues on DNA, typically represses the expression of genes (Jones & Takai, 2001). This often occurs at cytosine residues which are followed by guanine residues, referred to as CG sites (Holliday & Grigg, 1993). These epigenetic modifications can have functional implications by altering levels of gene expression and in turn protein products, in brain regions that control behavior.

Maternal behavior is a complex behavior requiring the recruitment of multiple brain regions including the nucleus accumbens (NAC) (Li & Fleming, 2003a, 2003b; Olazabal & Young, 2006), bed nucleus of the stria terminalis (BNST) (Lonstein & De Vries, 2000; Numan & Numan, 1996; Numan & Numan, 1995, 1997; Numan, Numan, Marzella, & Palumbo, 1998; Perrin, Meurisse, & Lévy, 2007), ventral tegmental area (VTA) (Hansen, Harthon, Wallin, Löfberg, & Svensson, 1991; Hernandez-Gonzalez, Navarro-Meza, Prieto-Beracoechea, & Guevara, 2005; Numan & Numan, 1995;

Pedersen, Caldwell, Walker, Ayers, & Mason, 1994), prefrontal cortex (PFC) (V. M. Afonso, Sison, Lovic, & Fleming, 2007; Hernandez-Gonzalez, et al., 2005; Sabihi, Dong, Durosko, & Leuner, 2014), amygdala (Ferris et al., 1992; Fleming, Vaccarino, & Luebke, 1980; D. A. Lubin, Elliott, Black, & Johns, 2003; Numan, Numan, & English, 1993; Sheehan, Paul, Amaral, Numan, & Numan, 2001), and medial preoptic area (MPOA) (V. M. Afonso, et al., 2007; Numan & Numan, 1997; Numan, et al., 1998; Numan & Stolzenberg, 2009; Numan & Woodside, 2010). In this circuit, hormones including estrogen act on the MPOA to stimulate maternal behavior (Numan, Rosenblatt, & Komisaruk, 1977; Numan & Stolzenberg, 2009). The MPOA is then primed to become active in response to pup stimuli. The PFC and amygdala are involved in processing sensory information, such as pup scent, which elicit maternal responsiveness (Numan, 2012). The MPOA in turn projects to the VTA, which provides dopaminergic input to the NAC. This projection is important for the rewarding component of pup interactions (Veronica M Afonso, King, Chatterjee, & Fleming, 2009). Dysregulation within this circuitry can lead to altered or impaired maternal responsiveness (Numan, 2012), and epigenetic modifications within this circuit is one potential mechanism through which dysregulation could occur. Indeed, experience-driven alterations in DNA methylation in maternal circuitry can influence maternal behavior via altered functioning in these brain regions (Champagne, et al., 2006; Razin & Riggs, 1980; Stolzenberg & Champagne, 2016).

Using the scarcity-adversity model of low nesting resources, a validated rodent model of caregiver maltreatment (Walker et al., 2017), our lab has previously identified altered gene expression and DNA methylation in some of the brain regions controlling maternal behavior (Blaze & Roth, 2013; Blaze, et al., 2013; T. L. Roth, et al., 2009). Coinciding with altered patterns of DNA methylation, our lab has likewise found aberrant maternal behavior in females subjected to maltreatment (T. L. Roth, et al., 2009), consistent with studies in humans finding disrupted maternal behavior (e.g. increased hostility and reduced warmth toward children, impaired mother-child bonding, and increased used of physical punishment) in women that experienced childhood abuse (Bailey, DeOliveira, Wolfe, Evans, & Hartwick, 2012; Banyard, 1997; Cort, Toth, Cerulli, & Rogosch, 2011; Cross et al., 2016; Muzik et al., 2013; Roberts, O'Connor, Dunn, Golding, & Team, 2004). Maternal behavior is an intergenerational behavior, as the quality of maternal care a female experiences influences the quality of care she will give her own offspring (Champagne, 2008; Cort, et al., 2011; Francis, Champagne, & Meaney, 2000; Francis, Diorio, Liu, & Meaney, 1999; T. L. Roth, et al., 2009). Therefore, it is important to establish the neurobiological underpinning of aberrant maternal behavior and explore treatments that can improve maternal behavior to prevent the perpetuation of poor maternal care across generations.

Previous work from our lab has validated the ability for the epigenome-altering drug, zebularine, to reverse maltreatment-induced DNA methylation and expression of

the brain-derived neurotrophic factor (*Bdnf*) gene in the adult PFC (T. L. Roth, et al., 2009). Based upon this, in the current study, we sought to assess the ability of zebularine to rectify consequences of maltreatment on maternal behavior when administered to adult dams.

2.2 Methods

2.2.1 Subjects

All animal procedures were conducted following approval by the University of Delaware Institutional Animal Care and Use committee using NIH established guidelines. This study utilized Long-Evans rats that were bred in house. Dams were maintained on a 12 hour light/dark cycle and were given *ad libitum* access to food and water. Postnatal day (PN) 0 was classified as the day of parturition. Figure 1 provides an approximate timeline of experimental procedures performed in this study.

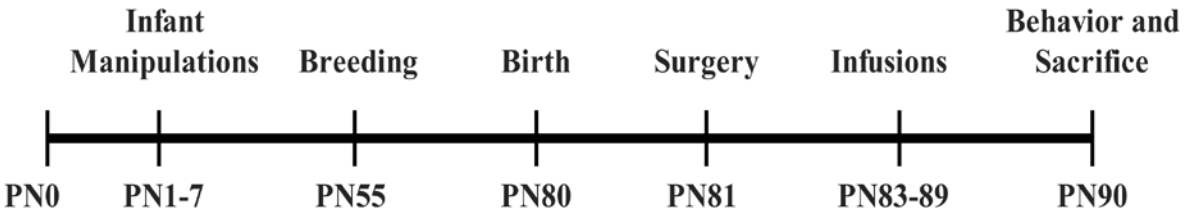

Figure 2.1 This figure depicts an approximate timeline of experimental procedures.

2.2.2 Caregiving Manipulations

Rodent pups were exposed to the scarcity adversity model of low nesting resources for 30 minutes per day from PN 1-7 (Blaze, et al., 2013; Doherty, et al., 2017; Doherty, et al., 2016; Hill, Warren, & Roth, 2014; T. L. Roth, et al., 2009; T. L. Roth, et al., 2014; Walker, et al., 2017). This model employs a within litter design whereby 1/3 of the litter is dedicated to the maltreatment condition, 1/3 of the litter is dedicated to the cross-foster care condition, and 1/3 of the litter receives normal maternal care. For the maltreatment condition, pups were exposed to another dam with limited nesting resources in a novel environment. Dams were matched for postpartum age and diet to the biological mom of the experimental litter, as pups are unable to distinguish between their biological dam and a diet-matched dam (Leon, 1975). In the cross-foster care condition, pups were also exposed to another dam in a novel environment. However, this dam had been given ample nesting resources and is familiar with the environment (i.e. had habituated for one hour). In the normal maternal care condition, the pups were marked, weighed, and subsequently returned to the home cage with their biological mother. Maternal behavior in each of these conditions was recorded. A subset of 5 of the 13 litters from which experimental subjects were taken was scored to confirm the replicability of this model (i.e. increased levels of adverse behavior by the caregiver during the maltreatment condition relative to the two control conditions). Videos were coded for adverse

(roughly handling, dropping, dragging, stepping on, or actively avoiding pups) behaviors in five-minute time bins.

At the time of weaning, male and female offspring were separated and only female offspring were utilized for the duration of the study. Males were used for other experiments in our laboratory. Female subjects were placed into cages of two or three animals from the same infant condition. When female rodents exposed to these infant manipulations reached adulthood (around PN55), they were be bred with naïve breeder males and permitted to give birth. After the female had successfully bred with the male (i.e. a sperm plug was found), animals were single-housed and remained undisturbed until one day following parturition.

2.2.3 Stereotaxic Surgery and Drug Infusions

One day after parturition, stereotaxic surgery was performed to implant a cannula into the left lateral ventricle following a protocol similar to one used previously by our lab (T. L. Roth, et al., 2009). To induce anesthesia, dams were placed in an induction chamber containing 5% isoflurane in oxygen. Once anesthesia was induced, animals were administered 2 mL of sterile saline and .03 mg/kg buprenorphine. The dam was then placed into a stereotaxic frame. Anesthesia was maintained using 2-3% isoflurane in oxygen and a stainless steel guide cannula (22 gauge, 8 mm length, Plastics One Inc., Roanoke, VA) was implanted into the left lateral ventricle (1.5 mm posterior, 2.0mm lateral, and 3.0 mm ventral relative to

bregma). At the time of surgery, cannula placement was verified using gravitational saline letdown as has been done in previous reports (Asok, Schulkin, & Rosen, 2016). A dummy cannula extending 1mm beyond the guide cannula was inserted into the guide cannula upon cessation of surgery to prevent cannula blockage. While the dam was undergoing surgery, her pups were left in the home cage on a heating pad and monitored. To examine the presence of the Trivers–Willard effect, which predicts that females with a history of stress will have a higher female:male pup ratio, pups were sexed and counted (Trivers & Willard, 1973). Dams were allowed one day of recovery after surgery during which they were left undisturbed and monitored to ensure appropriate recovery (e.g. maintaining weight and grooming properly).

Following recovery, daily infusions of zebularine or vehicle were performed. Zebularine is a cytosine analog known to incorporate into DNA and consequently alters DNA methylation (Champion et al., 2010). This drug is known to alter levels of DNA methylation when administered to adult rats (F. D. Lubin, Roth, & Sweatt, 2008; E. D. Roth et al., 2015; T. L. Roth, et al., 2009). We selected a drug dose and treatment regimen shown to reverse aberrant DNA methylation and gene expression levels (T. L. Roth, et al., 2009). Specifically, zebularine (600 ng/µl in 10% DMSO, 2 µl volume, infusion rate of 1µl/min) was administered once daily for seven days. Vehicle was comprised of 10% DMSO in sterile saline.

2.2.4 Adult Behavior

A 30 minute behavioral recording was collected 24 hours following the final infusion, as this is the same time point we have observed an effect of zebularine on methylation and gene expression (T. L. Roth, et al., 2009). Recordings were later coded offline for adverse (roughly handling, dropping, dragging, stepping on, and avoiding the pups) maternal behaviors by scorers blind to experimental conditions. Because a previous report from our lab found enhanced levels of adverse behaviors performed toward offspring in dams with a history of maltreatment (T. L. Roth, et al., 2009), the total number of adverse behaviors conducted throughout the 30 minute recording were tallied. This method of recording each bout of a behavior has been used by others to probe for differences in maternal care in dams with a history of early-life stress (C. A. Murgatroyd & Nephew, 2013), providing more resolution that can be lost when collapsing behaviors across time bins (as we have commonly done).

2.2.5 Statistical Analyses

Behavioral data collected from the infant manipulations were analyzed using a one-way ANOVA. Behavioral data from dams previously exposed to infant manipulations (i.e. total number of adverse maternal behaviors in the 30 minute recording) were analyzed using two-way ANOVAs. T-tests were used for post-hoc analyses to further probe statistically significant effects, with Bonferroni corrections applied when necessary.

2.3 Results

2.3.1 Infant Manipulations

A one-way ANOVA performed on adverse behaviors observed across our infant manipulations revealed a main effect of infant condition ($F_{(2,12)} = 20.17$, $p = .0001$; Figure 2). Post-hoc comparisons showed that animals in the maltreatment condition experienced significantly more adverse behaviors relative to the normal care ($p = .0013$) and cross foster care ($p = .0002$) conditions. We did not find any differences in the levels of adverse care between the cross-foster and normal care conditions ($p = .4145$). These results are consistent with previous reports employing the scarcity-adversity model of low nesting resources (Blaze, et al., 2013; Doherty, et al., 2017; T. L. Roth, et al., 2009). These data validate the efficacy of our model to experimentally induce an adverse caregiving environment.

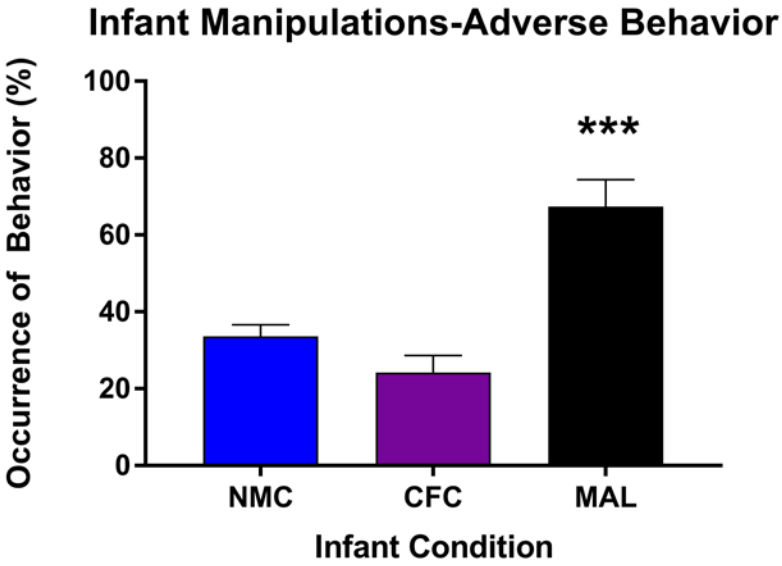

Figure 2.2 Pups in the maltreatment condition incurred more adverse behaviors from the caregiver relative to pups placed in the cross-foster and normal maternal care conditions. n=5 litters; *** denotes $p < .001$.

2.3.2 Trivers–Willard Effect

We did not find a significant difference in the female:male pup ratio ($F_{(2,65)}$ = 1.34, p = .2691) or litter size ($F_{(2,65)}$ = .5636, p = .5719) as a result of infant condition (Figure 3). These data indicate that within the scarcity-adversity model of low nesting resources, dams with a history of maltreatment do not show altered litter compositions compared to control dams.

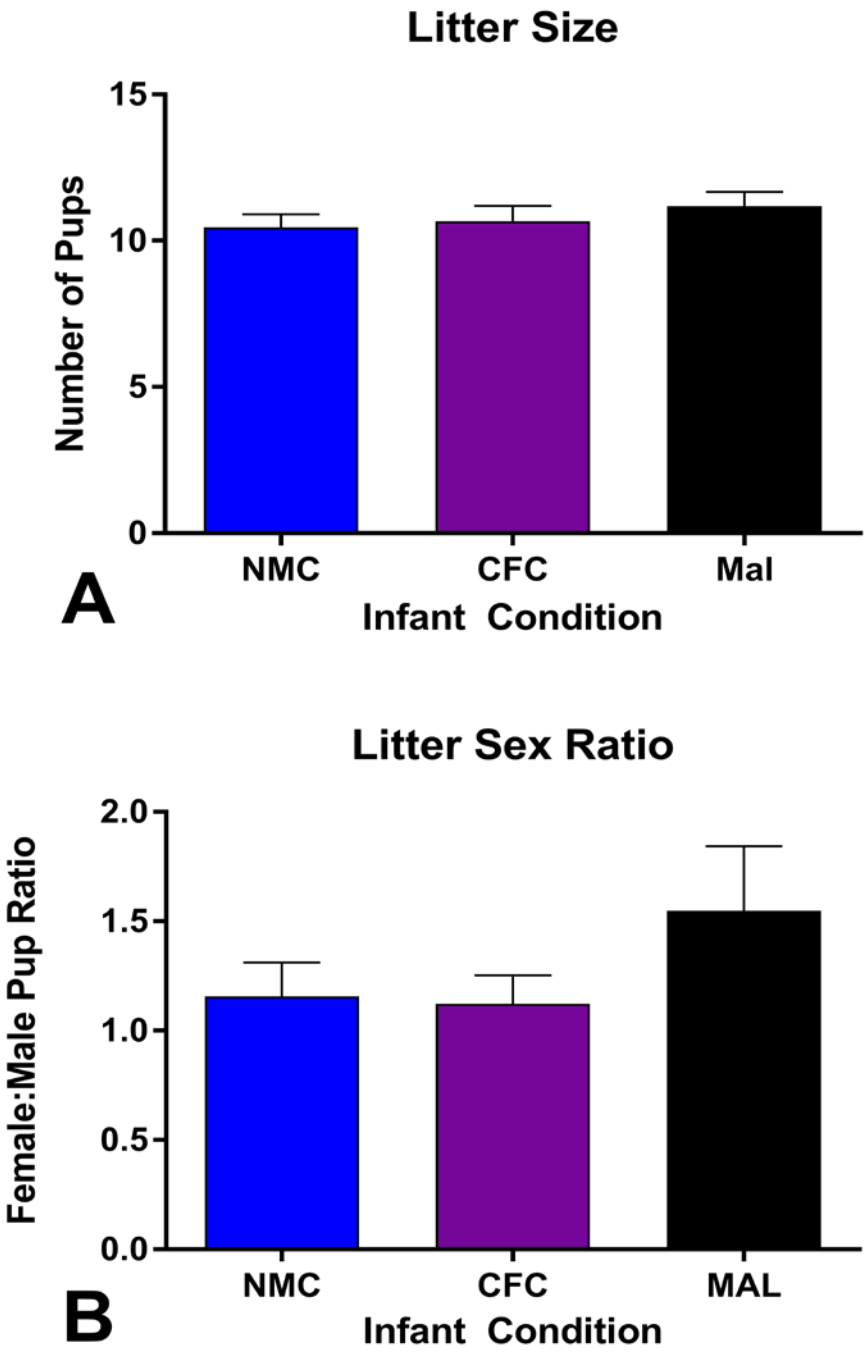

Figure 2.3 No differences were found in litter size (A) or the female: male pup ratio (B) as a result of infant caregiver condition. n=22-24/group.

2.3.3 Adult Maternal Behavior

No significant differences in maternal behavior were found between subjects in the cross-foster and normal maternal care vehicle groups ($t_{(19)} = 0.5541$, $p = .5927$) nor the cross-foster and normal maternal care zebularine groups ($t_{(21)} = 1.071$, $p = .2962$), therefore the nurturing care vehicle groups were collapsed and the nurturing care zebularine groups were collapsed to increase statistical power. A two-way ANOVA performed on the number of adverse behaviors performed by dams demonstrated an infant condition and drug treatment interaction ($F_{(1,60)} = 8.036$, $p = .0062$). Consistent with our previous finding (T. L. Roth, et al., 2009), post-hoc analyses revealed that females with a history of maltreatment (i.e. maltreatment-vehicle group) performed more aversive behaviors toward their offspring relative to animals with a history of nurturing care (i.e. nurturing-vehicle group) ($t_{(29)} = 2.315$, $p = .0279$). There was a significant difference between the maltreatment group administered zebularine versus the vehicle-treated maltreatment group, suggesting that zebularine rescued maltreatment-induced aberrations in maternal behavior ($t_{(18)} = 2.466$, $p = .0239$).

Treatment with zebularine disrupted maternal care in females without a history of maltreatment (i.e. controls), such that there was a marginally significant difference between drug-treated animals and their vehicle-treated counterparts ($t_{(42)} = 2.006$, $p = .0513$). Drug-treated controls showed more adverse behaviors. No significant differences were found between the zebularine-treated dams with a history of nurturing care versus the maltreated dams given vehicle ($p = .8927$), or the vehicle-

treated nurturing care group versus the zebularine-treated maltreatment group (p = .1332). Taken together, our data indicate that zebularine normalizes maternal behavior in dams with a history of maltreatment while disturbing maternal behavior in dams with a history of nurturing care in infancy (Figure 4).

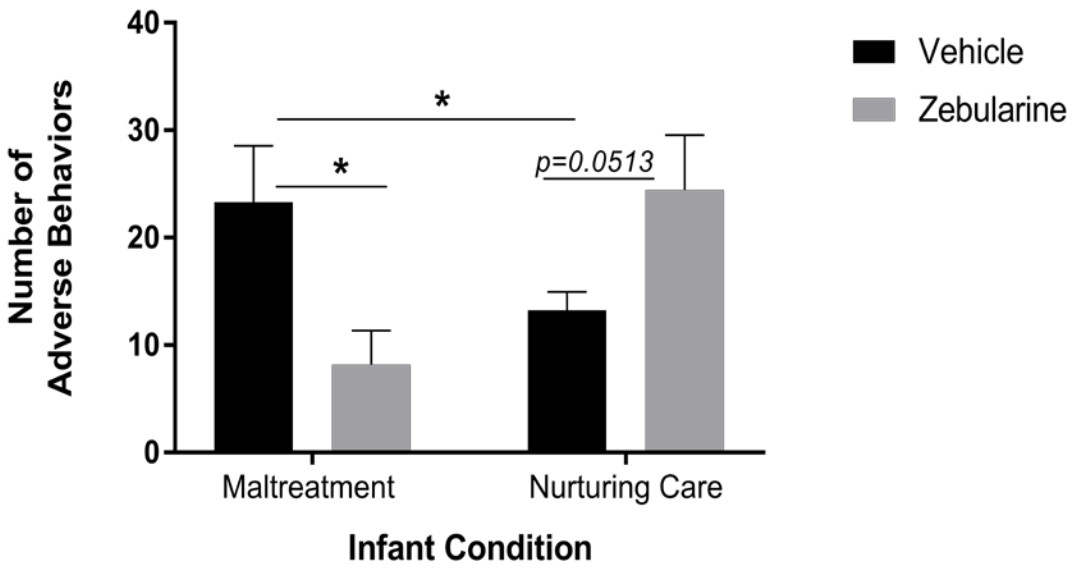

Figure 2.4 Animals with a history of maltreatment exhibited more adverse behaviors toward their pups as compared to dams without a history of maltreatment. Treatment with zebularine significantly reduced levels of adverse behavior exhibited toward offspring in previously maltreated dams. Zebularine treatment disturbed behavior in dams without a history of maltreatment such that drug-treated dams exhibited higher levels of adverse behavior toward offspring relative to vehicle-treated controls. n=10-23/group; * denotes $p < .05$.

2.4 Discussion

We replicated our previous finding that dams with a history of maltreatment mistreat their own offspring (T. L. Roth, et al., 2009). Further, we found that daily administration of zebularine at a dose previously shown to rescue aberrant DNA methylation and gene expression (T. L. Roth, et al., 2009) normalized maternal behavior in maltreated dams. Interestingly, this drug disturbed maternal behavior in animals without of a history of maltreatment such that these animals displayed enhanced levels of adverse behaviors toward offspring. These data suggest that the effects of zebularine are specific to caregiving history, as the drug elicited opposite effects in animals with a history of maltreatment relative to animals with no history of maltreatment.

While it may seem perplexing that zebularine had contrasting effects on dams dependent on their early-life history, our lab has previously identified a number of differences within the epigenome resulting from exposure to our maltreatment paradigm (Blaze, et al., 2015; Blaze & Roth, 2013; Blaze & Roth, 2017; Blaze, et al., 2013; Doherty, et al., 2016; T. L. Roth, et al., 2009; T. L. Roth, et al., 2014). These differences are widespread; we have discovered maltreatment-induced changes in methylation in the PFC (Blaze & Roth, 2017; Blaze, et al., 2013; T. L. Roth, et al., 2009), amygdala (T. L. Roth, et al., 2014), and hippocampus (T. L. Roth, et al., 2014). Thus, it seems plausible that the divergent effects of the drug could be a result of the

existing epigenetic differences in animals with different caregiving histories. Presumably zebularine reduced aberrant methylation in dams with a history of maltreatment, and this in turn normalized their maternal behavior. On the other hand, zebularine presumably disrupted normative methylation patterns in dams without a history of maltreatment which, in turn, produced disruptions in their maternal behavior. Indeed several studies have previously found behavioral disruptions in non-stressed animals administered zebularine (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010; F. D. Lubin, et al., 2008; E. D. Roth, et al., 2015). A necessary next step for us will be to examine DNA methylation and gene expression patterns in both sets of females to better elucidate the effects of our zebularine treatment regimen. These results nonetheless hint heavily at the causal nature of the relationship between the epigenome and behavioral phenotypes. Our data also argue that future work is warranted to discern the conditions under which zebularine has beneficial as opposed to harmful impacts on behavior.

There is a precedent in the literature for other epigenome-modifying drugs to alter maternal responsiveness in female rodents. In a maternal sensitization paradigm, female mice treated with a histone deacetylase inhibitor showed increased maternal responsiveness toward pups (Stolzenberg, Stevens, & Rissman, 2012, 2014). In this study, the expression of genes known to be involved in maternal behavior, including estrogen receptor beta (ERβ) was altered for 30 days following the sensitization paradigm (Stolzenberg, et al., 2014), suggesting that the facilitatory effects of this

drug on maternal behavior can be long-lasting. Additionally, maternal behavior has been improved by administration of drugs whose main target is not the epigenome, such as those that alter reward circuitry. For example, increasing levels of dopamine pharmacologically was found to increase levels of licking and grooming toward offspring in dams that received low levels of licking and grooming as infants (Champagne et al., 2004). However, to the knowledge of the authors this is the first time that an epigenome-modifying drug has been utilized to rectify maltreatment-induced aberrations in maternal behavior in adult animals.

Experiencing different types of early-life stress elicits diverse biological and behavioral outcomes (Dong et al., 2015; Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011; Schmidt, Wang, & Meijer, 2011; St-Cyr & McGowan, 2015). It is unknown if behavioral consequences resulting from other types of early-life stress, such as maternal separation or prenatal stress, could be rectified by altering DNA methylation. Additionally, our study focused on a female-specific behavior, and as such it is unclear if this drug would likewise ameliorate behavioral consequences of maltreatment in male subjects. For example, male rats subjected to the scarcity-adversity model of low nesting resources demonstrate deficits in fear extinction that are not observed in females exposed to the model (Doherty, et al., 2017). Sex differences exist throughout the epigenome (McCarthy et al., 2009; Nugent et al., 2015; Schwarz, Nugent, & McCarthy, 2010) and in levels of epigenetic regulators (Kolodkin & Auger, 2011; Kurian, Forbes-Lorman, & Auger, 2007), so it is possible

that epigenome-modifying treatments would not be equally effective in male and female subjects. It should be noted however that previous work from our lab established an ability for zebularine administered at the same dose as this study to rescue maltreatment-induced DNA methylation and gene expression in the PFC of both female and male subjects, which would suggest that zebularine would likewise be efficacious for behavioral deficits elicited by maltreatment in males (T. L. Roth, et al., 2009). While we examined behavior at a time point when maltreatment-induced DNA methylation is known to be rescued by zebularine treatment (i.e. 24 hours after a week of daily infusions), looking at the ability for zebularine to change behavior over the course of the seven day infusion regimen would also be an interesting future direction.

Our data here lend support to the hypothesis that altered DNA methylation resulting from the early-life experience is causally related to the behavioral deficits observed in these animals. While we would expect *Bdnf* methylation to be normalized in the brain of maltreated dams administered zebularine based on previous findings (T. L. Roth, et al., 2009), future work is needed to establish which genes could be underlying the observed behavioral effects of zebularine and circuit-level alterations responsible for the deficits seen in maternal behavior that zebularine is capable of modulating. Zebularine cannot cross the blood-brain barrier and therefore needs to be administered centrally (Beumer et al., 2006). As a result, this treatment cannot be utilized in humans. However, several environmental factors, including exercise (Boschen, McKeown, Roth, & Klintsova, 2017; Denham, Marques, O'Brien, &

Charchar, 2014; Laker et al., 2014), diet (Carlin, George, & Reyes, 2013; K. Lillycrop, Phillips, Jackson, Hanson, & Burdge, 2006; K. A. Lillycrop, Phillips, Jackson, Hanson, & Burdge, 2005; Moreno Gudiño, Carías Picón, & de Brugada Sauras, 2017), and social interaction (Branchi, Karpova, D'Andrea, Castrén, & Alleva, 2011; Champagne & Curley, 2005; Kuzumaki et al., 2011), all measures that can easily be employed in humans, have been shown to have lasting influences on the epigenome. Thus, zebularine is a useful tool for understanding the relationship between epigenetic and behavioral outcomes of stress, leading to knowledge that can then be leveraged in the development of treatments and/or interventions in human cases of early adversity. This is especially critical given that the impact of maltreatment on the brain and behavior is multigenerational. One example of this comes from a recent study that found reduced cortical gray matter volume in the brains of offspring of women that experienced maltreatment in childhood (Moog et al., 2018). It is important to establish treatments aimed at maternal behavior and the associated neurobiological deficits in human populations in order to improve outcomes for those directly exposed to adversity as well as outcomes for following generations. Further work is needed to determine whether less invasive treatments such as social enrichment, diet, and exercise could have facilitatory effects on maternal behavior through altering the epigenome. The data reported here help to construct the necessary foundation for such efforts.

Overall, the finding that targeting the epigenome was successful in attenuating poor maternal behavior is an exciting step forward in the literature. These data confirm the utility of a rodent model to study the behavioral phenomenon of transgenerational patterns of parenting. They then further provide support for studying the relationship between maltreatment-induced epigenetic modifications and perpetuated patterns of maternal maltreatment of offspring. Finally, they offer insight into the potential of exploiting that relationship to subvert the often tragic outcomes of adversity.

Acknowledgements

For assistance with experiments, we would like to thank Isabella Archer, Shannon Trombley, Anna Nowak, Ashley Atalese, Johanna Chajes, Richard Keller, Nabil Nasir, Derica Nyameke, and Lauren Reich. Funding for this work was provided by a University Doctoral Fellowship awarded to SMK, a University Dissertation Fellowship awarded to TSD, and The Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01HD087509) awarded to TLR.

REFERENCES

Afonso, V. M., King, S., Chatterjee, D., & Fleming, A. S. (2009). Hormones that increase maternal responsiveness affect accumbal dopaminergic responses to pup-and food-stimuli in the female rat. *Hormones and behavior, 56*(1), 11-23.

Afonso, V. M., Sison, M., Lovic, V., & Fleming, A. S. (2007). Medial prefrontal cortex lesions in the female rat affect sexual and maternal behavior and their sequential organization. *Behavioral Neuroscience, 121*(3), 515-526. doi: 10.1037/0735-7044.121.3.515

Anier, K., Malinovskaja, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2010). DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology, 35*(12), 2450-2461.

Anier, K., Malinovskaja, K., Pruus, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2014). Maternal separation is associated with DNA methylation and behavioural changes in adult rats. *European Neuropsychopharmacology, 24*(3), 459-468.

Asok, A., Schulkin, J., & Rosen, J. B. (2016). Corticotropin releasing factor type-1 receptor antagonism in the dorsolateral bed nucleus of the stria terminalis disrupts contextually conditioned fear, but not unconditioned fear to a predator odor. *Psychoneuroendocrinology, 70,* 17-24.

Bailey, H. N., DeOliveira, C. A., Wolfe, V. V., Evans, E. M., & Hartwick, C. (2012). The impact of childhood maltreatment history on parenting: A comparison of

maltreatment types and assessment methods. *Child abuse & neglect, 36*(3), 236-246.

Banyard, V. L. (1997). The impact of childhood sexual abuse and family functioning on four dimensions of women's later parenting. *Child abuse & neglect, 21*(11), 1095-1107.

Beumer, J. H., Joseph, E., Egorin, M. J., Parker, R. S., D'argenio, D. Z., Covey, J. M., & Eiseman, J. L. (2006). A mass balance and disposition study of the DNA methyltransferase inhibitor zebularine (NSC 309132) and three of its metabolites in mice. *Clinical cancer research, 12*(19), 5826-5833.

Blaze, J., Asok, A., & Roth, T. L. (2015). Long-term effects of early-life caregiving experiences on brain-derived neurotrophic factor histone acetylation in the adult rat mPFC. *Stress, 18*(6), 607-615.

Blaze, J., & Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience, 31*(8), 804-810. doi: 10.1016/j.ijdevneu.2013.10.001

Blaze, J., & Roth, T. L. (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and psychopathology, 29*(2), 477-489.

Blaze, J., Scheuing, L., & Roth, T. L. (2013). Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to

maltreatment or nurturing care during infancy. *Developmental Neuroscience, 35*(4), 306-316. doi: 10.1159/000350716

Boschen, K., McKeown, S., Roth, T., & Klintsova, A. (2017). Impact of exercise and a complex environment on hippocampal dendritic morphology, Bdnf gene expression, and DNA methylation in male rat pups neonatally exposed to alcohol. *Developmental neurobiology, 77*(6), 708-725.

Branchi, I., Karpova, N. N., D'Andrea, I., Castrén, E., & Alleva, E. (2011). Epigenetic modifications induced by early enrichment are associated with changes in timing of induction of BDNF expression. *Neuroscience letters, 495*(3), 168-172.

Carlin, J., George, R., & Reyes, T. M. (2013). Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology. *PLoS ONE, 8*(5).

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology, 29*(3), 386-397.

Champagne, F. A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., & Meaney, M. J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. *The Journal of Neuroscience, 24*(17), 4113-4123.

Champagne, F. A., & Curley, J. P. (2005). How social experiences influence the brain. *Current opinion in neurobiology, 15*(6), 704-709.

Champagne, F. A., & Curley, J. P. (2009). Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neuroscience & Biobehavioral Reviews, 33*(4), 593-600.

Champagne, F. A., Francis, D. D., Mar, A., & Meaney, M. J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology & behavior*, *79*(3), 359-371.

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. [Research Support, Non-U.S. Gov't]. *Endocrinology, 147*(6), 2909-2915. doi: 10.1210/en.2005-1119

Champion, C., Guianvarc'h, D., Sénamaud-Beaufort, C., Jurkowska, R. Z., Jeltsch, A., Ponger, L., . . . Guieysse-Peugeot, A.-L. (2010). Mechanistic insights on the inhibition of c5 DNA methyltransferases by zebularine. *PLoS One, 5*(8), e12388.

Cort, N. A., Toth, S. L., Cerulli, C., & Rogosch, F. (2011). Maternal intergenerational transmission of childhood multitype maltreatment. *Journal of Aggression, Maltreatment & Trauma, 20*(1), 20-39.

Cross, D., Kim, Y. J., Vance, L. A., Robinson, G., Jovanovic, T., & Bradley, B. (2016). Maternal Child Sexual Abuse Is Associated With Lower Maternal Warmth Toward Daughters but Not Sons. *Journal of child sexual abuse, 25*(8), 813-826.

Denham, J., Marques, F. Z., O'Brien, B. J., & Charchar, F. J. (2014). Exercise: putting action into our epigenome. *Sports Medicine, 44*(2), 189-209.

Doherty, T. S., Blaze, J., Keller, S. M., & Roth, T. L. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental psychobiology, 59*, 703-714.

Doherty, T. S., Forster, A., & Roth, T. L. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural brain research*. doi: 10.1016/j.bbr.2015.05.028

Dong, E., Dzitoyeva, S. G., Matrisciano, F., Tueting, P., Grayson, D. R., & Guidotti, A. (2015). Brain-derived neurotrophic factor epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice. *Biological psychiatry, 77*(6), 589-596.

Ferris, C. F., Foote, K. B., Meltser, H. M., Plenby, M. G., Smith, K. L., & Insel, T. R. (1992). Oxytocin in the amygdala facilitates maternal aggression. *Annals of the New York Academy of Sciences, 652*(1), 456-457.

Fleming, A. S., Vaccarino, F., & Luebke, C. (1980). Amygdaloid inhibition of maternal behavior in the nulliparous female rat. *Physiology & Behavior, 25*(5), 731-743.

Francis, D. D., Champagne, F. A., & Meaney, M. J. (2000). Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Journal of Neuroendocrinology, 12*(12), 1145-1148.

Francis, D. D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286*(5442), 1155-1158.

Hansen, S., Harthon, C., Wallin, E., Löfberg, L., & Svensson, K. (1991). Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness. *Behavioral Neuroscience, 105*(4), 588.

Hernandez-Gonzalez, M., Navarro-Meza, M., Prieto-Beracoechea, C., & Guevara, M. (2005). Electrical activity of prefrontal cortex and ventral tegmental area during rat maternal behavior. *Behavioural processes, 70*(2), 132-143.

Hill, K. T., Warren, M., & Roth, T. L. (2014). The influence of infant-caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural Brain Research, 272*, 175-180.

Holliday, R., & Grigg, G. (1993). DNA methylation and mutation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 285*(1), 61-67.

Jones, P. A., & Takai, D. (2001). The role of DNA methylation in mammalian epigenetics. *Science, 293*(5532), 1068-1070.

Keller, S. M., & Roth, T. L. (2016). Environmental influences on the female epigenome and behavior. *Environmental epigenetics, 2*(2), 1-10.

Kolodkin, M., & Auger, A. (2011). Sex difference in the expression of DNA methyltransferase 3a in the rat amygdala during development. *Journal of Neuroendocrinology, 23*(7), 577-583.

Kurian, J. R., Forbes-Lorman, R. M., & Auger, A. P. (2007). Sex difference in mecp2 expression during a critical period of rat brain development. *Epigenetics, 2*(3), 173-178.

Kuzumaki, N., Ikegami, D., Tamura, R., Hareyama, N., Imai, S., Narita, M., . . . Ando, T. (2011). Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment. *Hippocampus, 21*(2), 127-132.

Labonté, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., . . . Meaney, M. J. (2012). Genome-wide epigenetic regulation by early-life trauma. *Archives of general psychiatry, 69*(7), 722-731.

Laker, R. C., Lillard, T. S., Okutsu, M., Zhang, M., Hoehn, K. L., Connelly, J. J., & Yan, Z. (2014). Exercise prevents maternal high-fat diet–induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring. *Diabetes, 63*(5), 1605-1611.

Leon, M. (1975). Dietary control of maternal pheromone in the lactating rat. *Physiology & Behavior, 14*(3), 311-319.

Li, M., & Fleming, A. S. (2003a). Differential involvement of nucleus accumbens shell and core subregions in maternal memory in postpartum female rats. *Behavioral Neuroscience, 117*(3), 426.

Li, M., & Fleming, A. S. (2003b). The nucleus accumbens shell is critical for normal expression of pup-retrieval in postpartum female rats. *Behavioural Brain Research, 145*(1), 99-111.

Lillycrop, K., Phillips, E., Jackson, A., Hanson, M., & Burdge, G. (2006). *Dietary protein restriction in the pregnant rat induces altered epigenetic regulation of the glucocorticoid receptor and peroxisomal proliferator-activated receptor alpha in the heart of the offspring which is prevented by folic acid.* Paper presented at the Proceedings of the Nutritional Society.

Lillycrop, K. A., Phillips, E. S., Jackson, A. A., Hanson, M. A., & Burdge, G. C. (2005). Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *The Journal of nutrition, 135*(6), 1382-1386.

Lonstein, J., & De Vries, G. (2000). Maternal behaviour in lactating rats stimulates c-fos in glutamate decarboxylase-synthesizing neurons of the medial preoptic area, ventral bed nucleus of the stria terminalis, and ventrocaudal periaqueductal gray. *Neuroscience, 100*(3), 557-568.

Lubin, D. A., Elliott, J. C., Black, M. C., & Johns, J. M. (2003). An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. *Behavioral Neuroscience, 117*(2), 195-201.

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *The Journal of Neuroscience, 28*(42), 10576-10586.

Lutz, P.-E., & Turecki, G. (2014). DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience, 264*, 142-156.

Maccari, S., Krugers, H., Morley-Fletcher, S., Szyf, M., & Brunton, P. (2014). The consequences of early-life adversity: Neurobiological, behavioural and epigenetic adaptations. *Journal of neuroendocrinology, 26*(10), 707-723.

McCarthy, M. M., Auger, A. P., Bale, T. L., De Vries, G. J., Dunn, G. A., Forger, N. G., . . . Wilson, M. E. (2009). The epigenetics of sex differences in the brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 29*(41), 12815-12823. doi: 10.1523/JNEUROSCI.3331-09.2009

McGowan, P. O., & Roth, T. L. (2015). Epigenetic pathways through which experiences become linked with biology. *Development and psychopathology, 27*(2), 637-648.

McGowan, P. O., Sasaki, A., D'alessio, A. C., Dymov, S., Labonté, B., Szyf, M., . . . Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature neuroscience, 12*(3), 342-348.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual review of neuroscience, 24*(1), 1161-1192.

Moog, N. K., Entringer, S., Rasmussen, J. M., Styner, M., Gilmore, J. H., Kathmann, N., . . . Buss, C. (2018). Intergenerational effect of maternal exposure to

childhood maltreatment on newborn brain anatomy. *Biological psychiatry, 83*(2), 120-127.

Moreno Gudiño, H., Carías Picón, D., & de Brugada Sauras, I. (2017). Dietary choline during periadolescence attenuates cognitive damage caused by neonatal maternal separation in male rats. *Nutritional neuroscience, 20*(6), 327-335.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., . . . Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature neuroscience, 12*(12), 1559-1566.

Murgatroyd, C. A., & Nephew, B. C. (2013). Effects of early life social stress on maternal behavior and neuroendocrinology. *Psychoneuroendocrinology, 38*(2), 219-228.

Muzik, M., Bocknek, E. L., Broderick, A., Richardson, P., Rosenblum, K. L., Thelen, K., & Seng, J. S. (2013). Mother–infant bonding impairment across the first 6 months postpartum: The primacy of psychopathology in women with childhood abuse and neglect histories. *Archives of women's mental health, 16*(1), 29-38.

Mychasiuk, R., Ilnytskyy, S., Kovalchuk, O., Kolb, B., & Gibb, R. (2011). Intensity matters: brain, behaviour and the epigenome of prenatally stressed rats. *Neuroscience, 180*, 105-110.

Nugent, B. M., Wright, C. L., Shetty, A. C., Hodes, G. E., Lenz, K. M., Mahurkar, A., . . . McCarthy, M. M. (2015). Brain feminization requires active repression of masculinization via DNA methylation. *Nature Neuroscience*.

Numan, M. (2012). Maternal behavior: neural circuits, stimulus valence, and motivational processes. *Parenting, 12*(2-3), 105-114.

Numan, M., & Numan, M. (1996). A lesion and neuroanatomical tract-tracing analysis of the role of the bed nucleus of the stria terminalis in retrieval behavior and other aspects of maternal responsiveness in rats. *Developmental psychobiology, 29*(1), 23-51.

Numan, M., & Numan, M. J. (1995). Importance of pup-related sensory inputs and maternal performance for the expression of Fos-like immunoreactivity in the preoptic area and ventral bed nucleus of the stria terminalis of postpartum rats. *Behavioral neuroscience, 109*(1), 135.

Numan, M., & Numan, M. J. (1997). Projection sites of medial preoptic area and ventral bed nucleus of the stria terminalis neurons that express Fos during maternal behavior in female rats. *Journal of Neuroendocrinology, 9*(5), 369-384.

Numan, M., Numan, M. J., & English, J. B. (1993). Excitotoxic amino acid injections into the medial amygdala facilitate maternal behavior in virgin female rats. [Research Support, Non-U.S. Gov't]. *Hormones and Behavior, 27*(1), 56-81. doi: 10.1006/hbeh.1993.1005

Numan, M., Numan, M. J., Marzella, S. R., & Palumbo, A. (1998). Expression of c-fos, fos B, and egr-1 in the medial preoptic area and bed nucleus of the stria terminalis during maternal behavior in rats. *Brain research, 792*(2), 348-352.

Numan, M., Rosenblatt, J. S., & Komisaruk, B. R. (1977). Medial preoptic area and onset of maternal behavior in the rat. *Journal of comparative and physiological psychology, 91*(1), 146.

Numan, M., & Stolzenberg, D. S. (2009). Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Frontiers in Neuroendocrinology, 30*(1), 46-64.

Numan, M., & Woodside, B. (2010). Maternity: neural mechanisms, motivational processes, and physiological adaptations. *Behavioral Neuroscience, 124*(6), 715.

Olazabal, D., & Young, L. (2006). Oxytocin receptors in the nucleus accumbens facilitate “spontaneous” maternal behavior in adult female prairie voles. *Neuroscience, 141*(2), 559-568.

Pedersen, C. A., Caldwell, J. D., Walker, C., Ayers, G., & Mason, G. A. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behavioral Neuroscience, 108*(6), 1163.

Perrin, G., Meurisse, M., & Lévy, F. (2007). Inactivation of the medial preoptic area or the bed nucleus of the stria terminalis differentially disrupts maternal behavior in sheep. *Hormones and behavior, 52*(4), 461-473.

Perroud, N., Paoloni-Giacobino, A., Prada, P., Olié, E., Salzmann, A., Nicastro, R., . . . Dieben, K. (2011). Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Translational psychiatry, 1*(12), e59.

Razin, A., & Riggs, A. D. (1980). DNA methylation and gene function. *Science, 210*(4470), 604-610.

Roberts, R., O'Connor, T., Dunn, J., Golding, J., & Team, A. S. (2004). The effects of child sexual abuse in later family life; mental health, parenting and adjustment of offspring. *Child abuse & neglect, 28*(5), 525-545.

Roth, E. D., Roth, T. L., Money, K. M., SenGupta, S., Eason, D. E., & Sweatt, J. D. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics, 2*, 1-8.

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry, 65*(9), 760-769. doi: 10.1016/j.biopsych.2008.11.028

Roth, T. L., Matt, S., Chen, K., & Blaze, J. (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the homecage. *Developmental Psychobiology, 56*(8), 1755-1763.

Roth, T. L., & Sweatt, J. D. (2011). Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormones and Behavior, 59*(3), 315-320. doi: 10.1016/j.yhbeh.2010.05.005

Sabihi, S., Dong, S. M., Durosko, N. E., & Leuner, B. (2014). Oxytocin in the medial prefrontal cortex regulates maternal care, maternal aggression and anxiety during the postpartum period. *Frontiers in behavioral neuroscience, 8*, 258.

Schmidt, M. V., Wang, X.-D., & Meijer, O. C. (2011). Early life stress paradigms in rodents: potential animal models of depression? *Psychopharmacology, 214*(1), 131-140.

Schwarz, J. M., Nugent, B. M., & McCarthy, M. M. (2010). Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. *Endocrinology, 151*(10), 4871-4881.

Sheehan, T., Paul, M., Amaral, E., Numan, M. J., & Numan, M. (2001). Evidence that the medial amygdala projects to the anterior/ventromedial hypothalamic nuclei to inhibit maternal behavior in rats. *Neuroscience, 106*(2), 341-356.

St-Cyr, S., & McGowan, P. O. (2015). Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in Behavioral Neuroscience, 9*, 145. doi: 10.3389/fnbeh.2015.00145

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: the role of experience and epigenetic mechanisms. *Hormones and Behavior, 77*, 204-210.

Stolzenberg, D. S., Stevens, J. S., & Rissman, E. F. (2012). Experience-facilitated improvements in pup retrieval; evidence for an epigenetic effect. *Hormones and Behavior, 62*(2), 128-135.

Stolzenberg, D. S., Stevens, J. S., & Rissman, E. F. (2014). Histone deacetylase inhibition induces long-lasting changes in maternal behavior and gene expression in female mice. *Endocrinology, 155*(9), 3674-3683.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science, 179*(4068), 90-92.

Walker, C.-D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., . . . Sullivan, R. M. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*(just-accepted), 1-63.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience, 7*(8), 847-854. doi: 10.1038/nn1276

Chapter 3

PHARMACOLOGICAL MANIPULATION OF DNA METHYLATION IN ADULT FEMALE RATS RESCUES BEHAVIORAL CONSEQUENCES OF EARLY-LIFE MALTREATMENT

Exposure to adversity early in development alters brain and behavioral trajectories. Data continue to accumulate that epigenetic mechanisms are a mediating factor between early-life adversity and adult behavioral phenotypes. Previous work from our laboratory has shown that female Long-Evans rats exposed to maltreatment during infancy display both aberrant forced swim behavior and patterns of brain DNA methylation in adulthood. Therefore, we examined the possibility of rescuing the aberrant forced swim behavior in maltreated-adult females by administering an epigenome-modifying drug (zebularine) at a dose previously shown to normalize DNA methylation patterns. We found that zebularine normalized behavior in the forced swim test in maltreated females, such that they performed at the levels of controls (females that had been exposed to only nurturing care during infancy). These data help link DNA methylation to an adult phenotype in our maltreatment model, but more broadly provide additional evidence that non-targeted epigenetic manipulations can change behavior associated with early-life adversity.

3.1 Introduction

The period after birth is a sensitive period during which environmental experiences are capable of altering the trajectory of brain development (Greenough, Black, & Wallace, 1987; Knudsen, 2004; Rice & Barone Jr, 2000). Exposure to adversity during this time has lifelong implications for the brain and, consequently, behavior (Blaze et al., 2017; Blaze & Roth, 2013; Blaze, Scheuing, & Roth, 2013; Brunson, Eghbal-Ahmadi, Bender, Chen, & Baram, 2001; Doherty, Blaze, Keller, & Roth, 2017; Fride, Dan, Gavish, & Weinstock, 1985; Heim, Owens, Plotsky, & Nemeroff, 1997; Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 2003; T. L. Roth, Lubin, Funk, & Sweatt, 2009). Caregiver maltreatment is a form of early-life adversity incurred by 10-15% of the population in the United States (Gilbert et al., 2009; Lutz & Turecki, 2014). Individuals with a history of maltreatment are more likely to experience cognitive deficits such as problems with learning and memory (De Bellis, Woolley, & Hooper, 2013; Pears & Fisher, 2005; Rogosch, Cicchetti, & Aber, 1995). Further, exposure to maltreatment confers susceptibility to developing psychiatric disorders including major depression, schizophrenia, and posttraumatic stress disorder (Beers & De Bellis, 2002; Cicchetti & Toth, 2005; Heim & Binder, 2012; Provencal & Binder, 2015; Schenkel, Spaulding, DiLillo, & Silverstein, 2005; Shea, Walsh, MacMillan, & Steiner, 2005).

Our laboratory implements a rodent model of caregiver maltreatment (Blaze, Asok, & Roth, 2015; Blaze & Roth, 2013; Blaze & Roth, 2017; Blaze, et al., 2013; Doherty, et al., 2017; Doherty, Forster, & Roth, 2016) to better understand the consequences of early adversity on brain and behavioral development. Previous work from our lab has shown that female rodents exposed to brief bouts of daily caregiver maltreatment exhibit as adults mild deficits in novel object recognition (NOR) and an increased latency to become immobile in the forced swim test (Doherty, et al., 2017). Interestingly, when animals were tested on these behaviors in adolescence, no differences were observed in behavioral performance between animals with a history of maltreatment relative to animals with a history of nurturing care in infancy (Doherty, et al., 2017).

One way through which these early-life experiences might induce long-term consequences on behavior is via epigenetic alterations (Champagne et al., 2006; Heim & Binder, 2012; Kundakovic, Lim, Gudsnuk, & Champagne, 2013; Lewis & Olive, 2014; McGowan & Roth, 2015; Murgatroyd et al., 2009; T. L. Roth, 2012; T. L. Roth, et al., 2009; Silberman, Acosta, & Zubilete, 2016; Weaver et al., 2004). Epigenetic alterations, which are changes to chromatin that are capable of influencing gene expression without altering the underlying genomic sequence, include DNA methylation and posttranslational histone modifications (Attwood, Yung, & Richardson, 2002; Kouzarides, 2007; Li, Carey, & Workman, 2007). Our lab has uncovered a number of epigenetic alterations throughout the adult brain of female subjects with a history of caregiver maltreatment, including increased DNA

methylation of the brain-derived neurotrophic factor (*Bdnf*) gene in the hippocampus and prefrontal cortex coinciding with reduced methylation in the amygdala (Blaze, et al., 2015; Blaze & Roth, 2013; Blaze & Roth, 2017; Blaze, et al., 2013; Doherty, et al., 2016; T. L. Roth, et al., 2009; T. L. Roth, Matt, Chen, & Blaze, 2014). These same brain regions are recruited for behaviors known to be aberrant as a result of maltreatment (Antunes & Biala, 2012; Blair, 2008; Drevets et al., 1997; Duncan et al., 1986; Duncan, Johnson, & Breese, 1993; Warburton & Brown, 2015; Zierhut et al., 2010), suggesting that these neurobiological changes could be involved in maltreatment-induced phenotypes. However, the extent to which epigenetic modifications resulting from exposure to maltreatment contribute to the altered behavioral trajectories in these animals is unknown. The goal of this study was to determine the ability of some phenotypic outcomes associated with caregiver maltreatment to be rescued by altering adult DNA methylation. Further, a positive finding would lend support to our hypothesis that the epigenetic changes resulting from early-life maltreatment are causally related to phenotypic outcomes.

Although limited, data exist showing that manipulating the adult epigenome has the capacity to rescue outcomes of stress in both rodents and human populations (T. L. Roth, et al., 2009; Weaver, et al., 2004). For example, previous work from our lab demonstrated the capability of administration of zebularine (at the same dose used in this study), a drug known to modify DNA methylation, to normalize *Bdnf* DNA methylation and gene expression in the prefrontal cortex of animals with a history of maltreatment (T. L. Roth, et al., 2009). Further, in clinical studies individuals

benefitting from pharmaceutical or therapeutic interventions demonstrate a normalization of epigenetic marks (Lopez et al., 2013; Perroud et al., 2013). The current study aimed to expand upon previous findings from our lab and explore the implications of manipulating DNA methylation on behavioral outcomes of maltreatment in adult subjects. Because the phenotypes under investigation were found in female, but not male, subjects exposed to caregiver maltreatment (Doherty, et al., 2017), only female subjects were utilized in the current study.

3.2 Methods

3.2.1 Subjects and Infant Manipulations

Long-Evans rats bred in-house were employed in this study. Animals were given *ad libitum* access to food and water and maintained on a 12-hour light/dark cycle. All animal procedures were performed with approval from the University of Delaware Institutional Animal Care and Use committee following NIH established guidelines. Day of parturition was deemed postnatal day (PN) 0.

The scarcity-adversity model of low nesting resources was used as previously described (Blaze & Roth, 2017; Doherty, et al., 2017; Doherty, et al., 2016; T. L. Roth, et al., 2009; Walker et al., 2017). These manipulations were performed for 30 minutes daily from PN1-7. Briefly, this model uses a within litter design whereby 1/3 of the litter is exposed to maltreatment outside of the home cage, 1/3 of the litter is cross-fostered to a nurturing dam outside of the home cage, and the remaining 1/3 of

the litter remains in the home cage with the biological dam. All dams were matched in postpartum age and diet, as it has been demonstrated that pups cannot distinguish between dams fed the same diet (Leon, 1975). For the maltreatment condition, the dam was given limited nesting resources in an unfamiliar environment to care for the infant rats. The cross-foster dam was given ample nesting resources and given one hour to habituate to the chamber prior to receiving infant rats. Videos of these infant manipulations were recorded. To confirm the efficacy of this model, behavioral videos of the infant manipulations from five (selected using a random number generator) of the 14 litters from which experimental subjects were collected were coded for nurturing (i.e. nursing, licking and grooming the pups) and adverse (i.e. actively avoiding, roughly handling, stepping on, dropping, and dragging the pups) behaviors by trained scorers (89.5% inter-rater reliability). Each occurrence of a behavior was coded in five-minute time bins.

At the time of weaning, female rats were placed into cages of two or three animals from the same infant condition. Only female offspring were used for behavioral testing in this study. Male subjects were utilized in each of the infant conditions and remained with the dam as part of the litter until weaning to eliminate the possibility of same-sex litter composition altering maternal behavior (Hao, Huang, Nielsen, & Kosten, 2011; Kosten, Huang, & Nielsen, 2014; Moore & Morelli, 1979).

3.2.2 Stereotaxic Surgery

Between PN70-80, stereotaxic surgery was performed. Anesthesia was induced using 5% isoflurane in oxygen. Following induction of anesthesia, animals were administered 2 mL of sterile saline and .03 mg/kg buprenorphine. Animals were subsequently placed into a stereotaxic frame and anesthesia was maintained using 2-3% isoflurane in oxygen. A stainless steel guide cannula (22 gauge, 8 mm length, Plastics One Inc., Roanoke, VA) was implanted into the left lateral ventricle using the following coordinates: 1.5 mm posterior, 2.0mm lateral, and 3.0 mm ventral relative to bregma. At the time of surgery, cannula placement was verified using gravitational saline let-down as has been done in other reports (Asok, Schulkin, & Rosen, 2016). Once surgery was completed, a dummy cannula extending 1mm beyond the guide cannula was inserted into the guide cannula. Animals were single-housed following surgery to avoid injury. In a subset of animals, cannula tracks were also visually confirmed postmortem after slicing brains in a cryostat at -12º C.

3.2.3 Drug Administration

After one day of recovery from stereotaxic surgery, intracerebroventricular (ICV) infusion of either zebularine or vehicle began as has been previously described (T. L. Roth, et al., 2009). Zebularine, which is a cytosine analog, incorporates into DNA and prevents DNA methyltransferases (DNMTs) from adding methyl groups to DNA (Champion et al., 2010; Gnyszka, JASTRZĘBSKI, & Flis, 2013). This drug has

previously been demonstrated to alter levels of DNA methylation in the brains of adult animals (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010; Lubin, Roth, & Sweatt, 2008; E. D. Roth et al., 2015; T. L. Roth, et al., 2009). Zebularine (Sigma Aldrich) was dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with sterile saline such that the solution was comprised of 10% DMSO. Zebularine was administered via an infusion cannula (28 gauge) attached to PE20 tubing at a dose of 600 ng/µl delivered at the rate of 1 µl per minute. This dose was selected because it has been previously demonstrated to reverse maltreatment-induced DNA methylation of the *Bdnf* gene (T. L. Roth, et al., 2009). An equivalent amount of vehicle (10% DMSO in sterile saline) was administered at the same rate. Zebularine or a vehicle solution was administered once daily for seven days.

3.2.4 Adult Behavior

One day after the final drug or vehicle infusion, behavioral testing commenced. Animals were run through a battery of behavioral tests including open-field, NOR, and forced swim testing following protocols previously implemented by our lab and others (Arakawa, 2003; Castagné, Moser, Roux, & Porsolt, 2011; Doherty, et al., 2017; Oliveira, Hawk, Abel, & Havekes, 2010) and briefly described below. A timeline of experimental procedures can be seen in Figure 1. Testing was conducted in a room with white noise under red light. A camera placed on the ceiling above the behavioral apparatuses and Any-maze software (Stoelting Co., Wood Dale, IL) were used to

record all behavioral procedures. After behavioral testing, a subset of rats were sacrificed and their brains were harvested for slicing. Post-mortem vaginal lavages were performed to determine estrous cycle stage for all females.

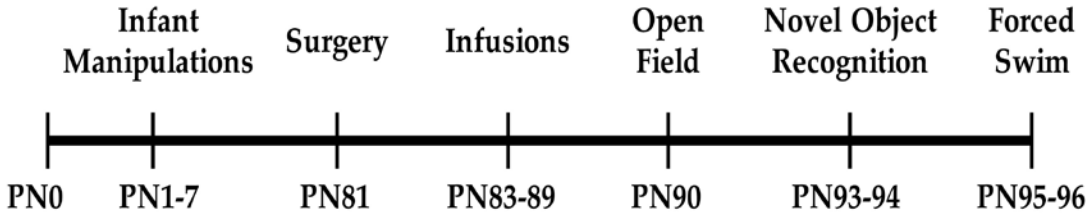

Figure 3.1 Timeline of experimental procedures implemented in this study.

3.2.5 Open-field Testing

Rats were placed into a circular arena (84 cm diameter × 36 cm height) for 10 minutes. Any-maze software (Stoelting Inc.) was used to score behavior in open-field testing. Time spent in the center of the field, number of entries into the center, and distance traveled were all recorded. The apparatus was cleaned using 70% ethanol in between subjects.

3.2.6 Novel Object Recognition

Novel object testing occurred in the same apparatus utilized for open-field testing. Animals were habituated to this chamber for an additional two days following open-field testing. Subjects were exposed to two identical objects for 15 minutes. The total time the rat spent investigating the objects was scored. Binder clips and conical tubes were used as objects. Total time spent exploring the items was recorded. Twenty-four hours later, rats were exposed to one item from the previous day in addition to a novel item. Objects used and their placement in the apparatus were counterbalanced across experimental subjects. To quantify the amount of time the rats spent exploring the novel object, a ratio was computed whereby the total time spent exploring the novel object was divided by the total time spent exploring both the novel and familiar objects. Behavior was recorded using Any-maze software and later scored offline by trained scorers blind to experimental groups.

3.2.7 Forced Swim

One day after NOR, rats were placed into a bucket (29 cm diameter × 48 cm height) of 25ºC water and given 15 minutes to swim. Once rats were removed from the water, they were dried using a microfiber cloth and their cage was placed on a heating pad under a heat lamp until they were completely dry. Twenty-four hours later, animals were returned to the water bucket for a five-minute test. Behavior was recorded using Any-maze software and later scored offline by trained scorers blind to experimental conditions. Time spent immobile, which was defined as performing only the motions necessary to keep the head above water, and latency until the first bout of immobility were coded. Two animals were removed from the data set for neglecting to demonstrate immobility during the forced swim test (1 normal maternal care vehicle subject and 1 cross-foster care zebularine subject).

3.2.8 Statistical Analyses

A chi-squared analysis revealed that there were no significant differences in estrus cycle (i.e. estrus, diestrus day one, diestrus day two, and proestrus) status across experimental groups ($\chi 2$ (15, N= 71) = 20.8, p= .1688). Two-way ANOVAs (levels: infant condition, drug or vehicle) were used for analyzing behavioral data. For all analyses, $p < .05$ was used to denote statistical significance. T-tests were used for post-hoc analyses and Bonferroni corrections were applied where necessary.

3.3 Results

3.3.1 Infant Manipulations

Two-way ANOVAs performed on nurturing and aversive care observed across our infant conditions revealed a main effect of caregiving behavior ($F_{(2,24)} = 67.74$, $p < .0001$) and a significant interaction of caregiving behavior and infant manipulation condition ($F_{(1,24)} = 51.72$, $p < .0001$), which is consistent with findings from other reports using the form of the scarcity-adversity model (Blaze & Roth, 2013; Doherty, et al., 2017; Doherty, et al., 2016; Hill, Warren, & Roth, 2014; T. L. Roth, et al., 2009; T. L. Roth, et al., 2014). As illustrated in Figure 2, post-hoc analyses revealed that significantly more adverse behaviors were observed in the maltreatment condition relative to the cross-foster ($p < .0001$) and normal maternal ($p < .0001$) care conditions, while more nurturing behaviors were performed in the cross-foster ($p < .0001$) and normal maternal care ($p = .0002$) conditions relative to the maltreatment condition. No differences in nurturing ($p = .4797$) or adverse care ($p = .9242$) were observed between the cross-foster and normal care conditions. More nurturing care was observed relative to adverse care in both normal care ($p < .0001$) and cross-foster care ($p < .0001$) conditions, while more adverse care was observed relative to nurturing care in the maltreatment condition ($p = .0003$).

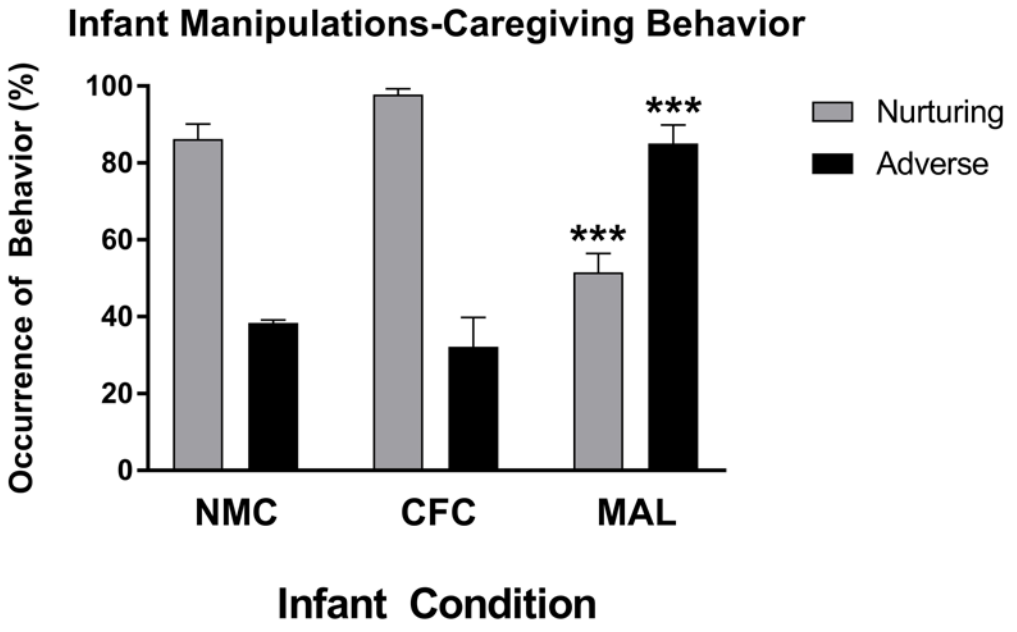

Figure 3.2 Infant rats exposed to the maltreatment condition received more adverse and less nurturing behavior relative to infant rats assigned to the cross-foster and normal maternal care conditions. NMC, normal maternal care; CFC, cross-foster care; MAL, maltreatment. n= 5 dams; *** denotes $p < .0001$.

3.3.2 Open-field Testing

No statistically significant effects of infant caregiver condition or drug treatment were observed in the open-field test on any of the measures recorded including entries into the center (Figure 3a), time spent in the center (Figure 3b), or distance traveled (Figure 3c) within the behavioral apparatus (p's > .05). These results suggest that locomotor behavior was not altered as a result of infant caregiver or drug treatment condition.

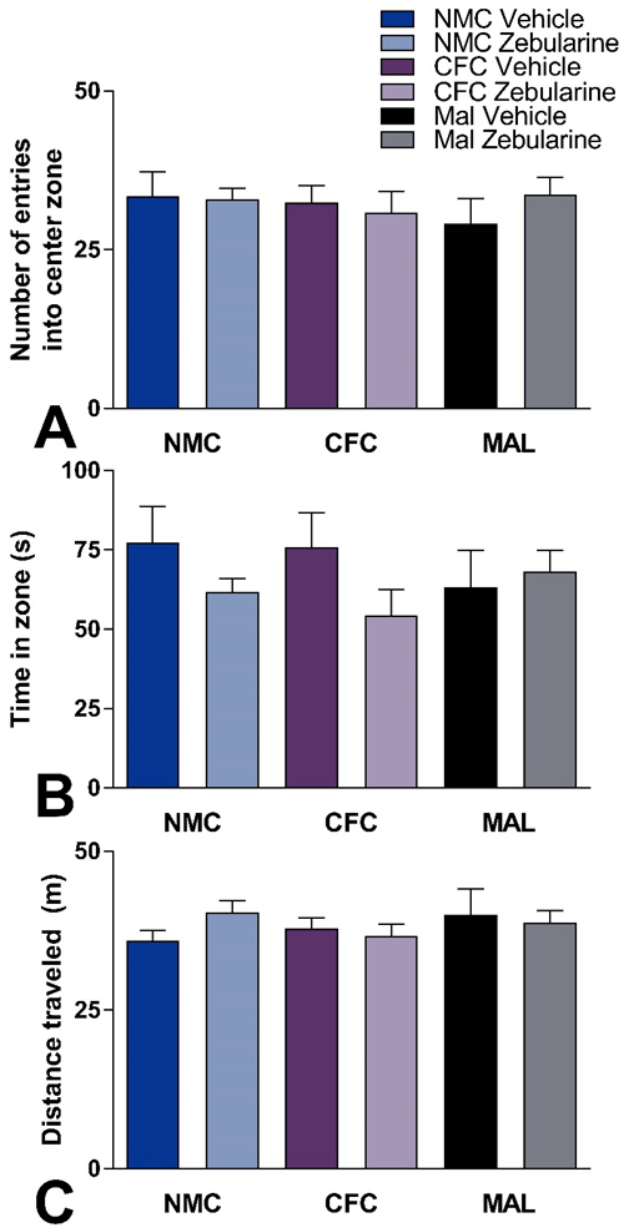

Figure 3.3 No differences were found as a result of infant condition or drug treatment on number of entries into the center zone (A), time spent in zone (B), or distance traveled (C) in the open-field test. NMC, normal maternal care; CFC, cross-foster care; MAL, maltreatment. n=11-13/group.

3.3.3 Novel Object Recognition

Neither infant caregiver condition nor drug treatment had a statistically significant effect on total time spent exploring the objects during habituation (Figure 4a, p's > .05). There was likewise no significant effect of drug treatment or infant caregiver condition on the novel-to-familiar object ratio (Figure 4b, p's > .05). As demonstrated by t-tests performed relative to chance (i.e. 50% exploration time with the novel object), subjects from all conditions were able to perform NOR (p's < .05).

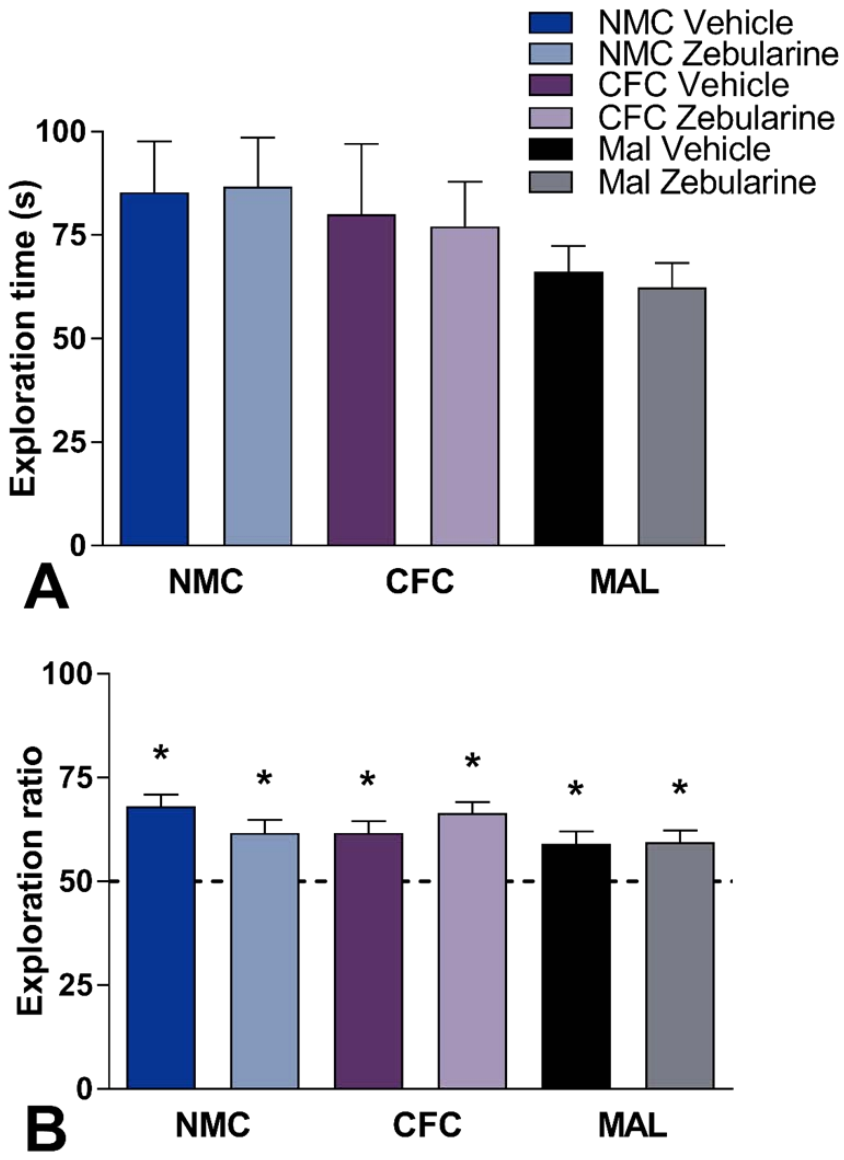

Figure 3.4 We did not find an effect of infant caregiver history or zebularine treatment on time spent investigating objects (A) or novel-object recognition testing (B). Subjects from all conditions were able to perform NOR. Dashed line at 50% indicates chance performance. NMC, normal maternal care; CFC, cross-foster care; MAL, maltreatment. n=11-13/group; * denotes $p < .05$.

3.3.4 Forced Swim

A significant interaction was found between drug treatment and infant condition ($F_{(2,64)}$ =8.234, p =.0007) on the latency to immobility in the forced swim test. Post-hoc analyses revealed that the maltreatment group administered vehicle was significantly different from the normal (p = .0027) and cross-foster care (p = .0146) vehicle-treated control groups (Figure 5a). Further, the zebularine-treated animals with a history of maltreatment were significantly different from their vehicle-treated counterparts (p = .0022). This suggests that animals with maltreatment history display altered behavior in the forced swim test and zebularine administration in adulthood was able to normalize this behavior measure. No statistically significant differences were found as a result of drug treatment or infant condition on total time spent immobile (Figure 5b, p's > .05).

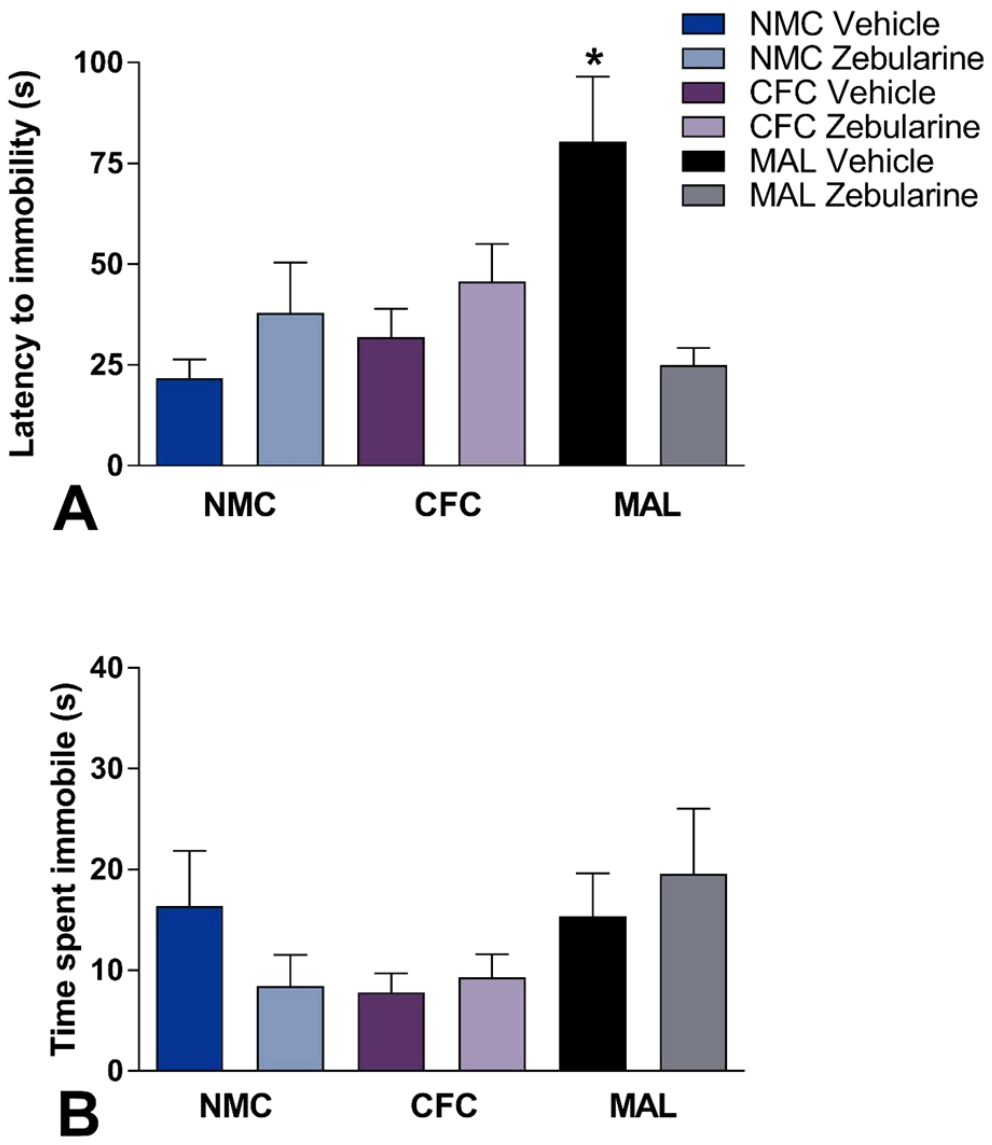

Figure 3.5 Female rats with a history of maltreatment demonstrated an increased latency to become immobile in the forced swim test (A). Treatment with zebularine normalized this behavior, but did not alter behavior in females with a history of nurturing maternal care. No differences were found as a result of infant manipulation or drug treatment on total time spent immobile (B). NMC, normal maternal care; CFC, cross-foster care; MAL, maltreatment. n=11-13/group; * denotes p =.0007.

3.4 Discussion

We replicated our previous finding that exposure to adverse caregiving conditions in infancy induces alterations in forced swimming behavior in adult females (Doherty, et al., 2017). Specifically, animals with a history of maltreatment took longer to demonstrate their first bout of immobility relative to animals with a history of nurturing care. The main finding from the current study was that administration of zebularine to maltreated-animals at a dose previously shown to decrease DNA methylation and rescue gene expression (T. L. Roth, et al., 2009) was capable of normalizing this behavior, with no effect on forced swim behavior in animals with a history of nurturing care.

Several interpretations of behavior in the forced swim test have been proposed (De Kloet & Molendijk, 2016; De Pablo, Parra, Segovia, & Guillamón, 1989; Nishimura, Tsuda, Oguchi, Ida, & Tanaka, 1988; R. Porsolt, Bertin, & Jalfre, 1977; R. D. Porsolt, Bertin, & Jalfre, 1978; West, 1990). One interpretation is that animals that learn to go immobile during this inescapable swim stressor are exhibiting an adaptive coping strategy, as immobility would allow for energy conservation (Andolina, Maran, Viscomi, & Puglisi-Allegra, 2015; Campus et al., 2017; De Kloet & Molendijk, 2016; West, 1990). Using this interpretation of the test, our data would suggest that animals with a history of maltreatment are failing to exhibit adaptive coping when faced with a stressful situation. This interpretation would be consistent with other reports finding impairments in coping in individuals with a history of early-life stress (Dich et al.,

2015; Grace, Martin-Gousset, & Angelier, 2017; Wadsworth, 2015). Deficits in the forced swim task may also be reflective of an inability to learn the immobility behavior (De Pablo, et al., 1989). Using this interpretation, our data would suggest that animals with a history of maltreatment exhibit an inability to readily learn this behavior. This interpretation would be consistent with other reports of learning impairments in animals exposed to early adversity (Doherty, et al., 2017; Walker, et al., 2017). A reduction in the latency to immobility in the maltreated-animals that received zebularine could suggest that altering aberrant methylation levels promoted an adaptive coping strategy and/or facilitated learning of immobility behavior. Though this study was not designed to parse out these interpretations, our data nonetheless demonstrate the ability of infusions of zebularine to ameliorate a phenotype associated with exposure to early adversity.

Previous work from our lab found a mild but significant deficit in NOR in adult female animals with a history of maltreatment (Doherty, et al., 2017). While we neglected to replicate the modest deficit in NOR in females with a history of maltreatment, several methodological differences exist between our previous study and the current study that could explain this discrepancy. Due to our drug treatment regimen, animals here received daily handling for one week, whereas in our previous work animals were handled at most three times prior to the behavioral assays. Further, in our previous study, animals were pair-housed, while in the current study animals were single-housed immediately following surgery (to aid in recovery from surgery).

While our maltreated animals did not exhibit a statistically significant deficit in NOR, this group did show a numerically lower novel objet preference and more variability in their performance (M = 59.01, SD = 10.99) relative to vehicle-treated normal care animals (M = 68.16, SD = 8.77). That same reduction in novel object preference and increased variability was observed in our drug-treated normal care animals (M = 61.69, SD = 10.63), as about half the subjects in this group did not exhibit a novel object preference while all of the vehicle-treated normal maternal care animals did exhibit a preference. The lack of NOR performance in some of the zebularine-treated normal maternal care animals is consistent with a previous report finding an impairment in NOR in normal animals after administration of a DNMT inhibitor (Scott, Smith, Barker, Uney, & Warburton, 2017). Zebularine was administered ICV, and thus is acting globally on the epigenome throughout the brain. Because each animal has a different epigenome, it is possible that this drug is exerting divergent effects between animals even within the same infant caregiver group. The effects of zebularine treatment could be similar to the efficacy of drugs like antidepressants, which are only effective in certain individuals or only alleviate some, but not all, of the symptoms of depression (Al-Harbi, 2012; Anderson & Tomenson, 1994; Cipriani et al., 2009; Fournier et al., 2010). Future research is warranted to elucidate the reasons for the variability in NOR performance and the effect, if any, of zebularine treatment and exposure to maltreatment on the ability to perform NOR in individual subjects.

In conclusion, data presented here are consistent with the hypotheses that epigenetic alterations produced by exposure to adverse caregiving conditions play a role in adult phenotypes and that non-targeted epigenetic manipulations can change behavior. The current study is one of few that have investigated the possibility of altering the female epigenome in adulthood (for review see (Keller & Roth, 2016)). While reports have found an effect of zebularine administration on behavior in adult rats (Lubin, et al., 2008; E. D. Roth, et al., 2015), to the knowledge of the authors there are no data on the duration of time that the effects of zebularine administration are maintained. The results of the current study suggest that zebularine is able to impact behavior for at least one week after administration, as the forced swim test was conducted seven days after the final zebularine infusion. While our results suggest that altering brain DNA methylation has implications for behavior, it is unclear which genes are contributing to the observed behavioral effects and whether zebularine may be having any non-specific drug effects, all of which warrant elucidation in future research.

Acknowledgements

We would like to thank Isabella Archer, Shannon Trombley, Anna Nowak, Ashley Atalese, Johanna Chajes, Richard Keller, and Raymond Keller for their help in conducting this study and coding behavioral videos. This work was supported by funding from a University Doctoral Fellowship awarded to SMK, a University Dissertation Fellowship awarded to TSD, and The Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01HD087509) awarded to TLR.

REFERENCES

Al-Harbi, K. S. (2012). Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient preference and adherence*, *6*, 369-388.

Anderson, I., & Tomenson, B. (1994). The efficacy of selective serotonin re-uptake inhibitors in depression: a meta-analysis of studies against tricyclic antidepressants. *Journal of Psychopharmacology*, *8*(4), 238-249.

Andolina, D., Maran, D., Viscomi, M. T., & Puglisi-Allegra, S. (2015). Strain-dependent variations in stress coping behavior are mediated by a 5-HT/GABA interaction within the prefrontal corticolimbic system. *International Journal of Neuropsychopharmacology*, *18*(3), 1-12.

Anier, K., Malinovskaja, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2010). DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology*, *35*(12), 2450-2461.

Antunes, M., & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive processing*, *13*(2), 93-110.

Arakawa, H. (2003). The effects of isolation rearing on open-field behavior in male rats depends on developmental stages. *Developmental psychobiology*, *43*(1), 11-19.

Asok, A., Schulkin, J., & Rosen, J. B. (2016). Corticotropin releasing factor type-1 receptor antagonism in the dorsolateral bed nucleus of the stria terminalis

disrupts contextually conditioned fear, but not unconditioned fear to a predator odor. *Psychoneuroendocrinology, 70*, 17-24.

Attwood, J., Yung, R., & Richardson, B. (2002). DNA methylation and the regulation of gene transcription. *Cellular and Molecular Life Sciences, 59*(2), 241-257.

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *American Journal of Psychiatry, 159*(3), 483-486.

Blair, R. (2008). The amygdala and ventromedial prefrontal cortex: functional contributions and dysfunction in psychopathy. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 363*(1503), 2557-2565.

Blaze, J., Asok, A., Borrelli, K., Tulbert, C., Bollinger, J., Ronca, A. E., & Roth, T. L. (2017). Intrauterine exposure to maternal stress alters Bdnf IV DNA methylation and telomere length in the brain of adult rat offspring. *International Journal of Developmental Neuroscience, 62*, 56-62.

Blaze, J., Asok, A., & Roth, T. L. (2015). Long-term effects of early-life caregiving experiences on brain-derived neurotrophic factor histone acetylation in the adult rat mPFC. *Stress, 18*(6), 607-615.

Blaze, J., & Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International Journal of Developmental Neuroscience, 31*(8), 804-810. doi: 10.1016/j.ijdevneu.2013.10.001

Blaze, J., & Roth, T. L. (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and psychopathology, 29*(2), 477-489.

Blaze, J., Scheuing, L., & Roth, T. L. (2013). Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to maltreatment or nurturing care during infancy. *Developmental neuroscience, 35*(4), 306-316. doi: 10.1159/000350716

Brunson, K. L., Eghbal-Ahmadi, M., Bender, R., Chen, Y., & Baram, T. Z. (2001). Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. *Proceedings of the National Academy of Sciences, 98*(15), 8856-8861.

Campus, P., Canterini, S., Orsini, C., Fiorenza, M. T., Puglisi-Allegra, S., & Cabib, S. (2017). Stress-induced reduction of dorsal striatal D2 dopamine receptors prevents retention of a newly acquired adaptive coping strategy. *Frontiers in pharmacology, 8*, 621.

Castagné, V., Moser, P., Roux, S., & Porsolt, R. D. (2011). Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Neuroscience, 55*(8.10), 1-8.10.

Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial

preoptic area of female offspring. [Research Support, Non-U.S. Gov't]. *Endocrinology, 147*(6), 2909-2915. doi: 10.1210/en.2005-1119

Champion, C., Guianvarc'h, D., Sénamaud-Beaufort, C., Jurkowska, R. Z., Jeltsch, A., Ponger, L., . . . Guieysse-Peugeot, A.-L. (2010). Mechanistic insights on the inhibition of c5 DNA methyltransferases by zebularine. *PLoS One, 5*(8), e12388.

Cicchetti, D., & Toth, S. L. (2005). Child maltreatment. *Annual Review of Clinical Psychology, 1*, 409-438.

Cipriani, A., Furukawa, T. A., Salanti, G., Geddes, J. R., Higgins, J. P., Churchill, R., . . . McGuire, H. (2009). Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. *The lancet, 373*(9665), 746-758.

De Bellis, M. D., Woolley, D. P., & Hooper, S. R. (2013). Neuropsychological findings in pediatric maltreatment: relationship of PTSD, dissociative symptoms, and abuse/neglect indices to neurocognitive outcomes. *Child maltreatment, 18*(3), 171-183.

De Kloet, E., & Molendijk, M. (2016). Coping with the forced swim stressor: towards understanding an adaptive mechanism. *Neural plasticity, 2016*.

De Pablo, J. M., Parra, A., Segovia, S., & Guillamón, A. (1989). Learned immobility explains the behavior of rats in the forced swimming test. *Physiology & Behavior, 46*(2), 229-237.

Dich, N., Hansen, Å. M., Avlund, K., Lund, R., Mortensen, E. L., Bruunsgaard, H., & Rod, N. H. (2015). Early life adversity potentiates the effects of later life stress on cumulative physiological dysregulation. *Anxiety, Stress, & Coping*, *28*(4), 372-390.

Doherty, T. S., Blaze, J., Keller, S. M., & Roth, T. L. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental psychobiology, 59*, 703-714.

Doherty, T. S., Forster, A., & Roth, T. L. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural brain research*. doi: 10.1016/j.bbr.2015.05.028

Drevets, W. C., Price, J. L., Simpson Jr, J. R., Todd, R. D., Reich, T., Vannier, M., & Raichle, M. E. (1997). Subgenual prefrontal cortex abnormalities in mood disorders. *Nature, 386*(6627), 824-827.

Duncan, G. E., Breese, G., Criswell, H., Stumpf, W., Mueller, R., & Covey, J. (1986). Effects of antidepressant drugs injected into the amygdala on behavioral responses of rats in the forced swim test. *Journal of Pharmacology and Experimental Therapeutics, 238*(2), 758-762.

Duncan, G. E., Johnson, K. B., & Breese, G. R. (1993). Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. *Journal of Neuroscience, 13*(9), 3932-3943.

Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C., & Fawcett, J. (2010). Antidepressant drug effects and depression severity: a patient-level meta-analysis. *Jama*, *303*(1), 47-53.

Fride, E., Dan, Y., Gavish, M., & Weinstock, M. (1985). Prenatal stress impairs maternal behavior in a conflict situation and reduces hippocampal benzodiazepine receptors. *Life sciences*, *36*(22), 2103-2109.

Gilbert, R., Widom, C. S., Browne, K., Fergusson, D., Webb, E., & Janson, S. (2009). Burden and consequences of child maltreatment in high-income countries. *The lancet*, *373*(9657), 68-81.

Gnyszka, A., JASTRZĘBSKI, Z., & Flis, S. (2013). DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer research*, *33*(8), 2989-2996.

Grace, J. K., Martin-Gousset, L., & Angelier, F. (2017). Delayed effect of early-life corticosterone treatment on adult anti-predator behavior in a common passerine. *Physiology & Behavior*, *177*, 82-90.

Greenough, W. T., Black, J. E., & Wallace, C. S. (1987). Experience and brain development. *Child development*, *58*, 539-559.

Hao, Y., Huang, W., Nielsen, D. A., & Kosten, T. A. (2011). Litter gender composition and sex affect maternal behavior and DNA methylation levels of the oprm1 gene in rat offspring. *Frontiers in Psychiatry*, *2*, 21. doi: 10.3389/fpsyt.2011.00021

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene–environment interactions, and epigenetics. *Experimental neurology, 233*(1), 102-111.

Heim, C., Owens, M. J., Plotsky, P. M., & Nemeroff, C. B. (1997). Persistent changes in corticotropin-releasing factor systems due to early life stress: relationship to the pathophysiology of major depression and post-traumatic stress disorder. *Psychopharmacology bulletin, 33*(2), 185.

Hill, K. T., Warren, M., & Roth, T. L. (2014). The influence of infant-caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural brain research, 272*, 175-180.

Keller, S. M., & Roth, T. L. (2016). Environmental influences on the female epigenome and behavior. *Environmental epigenetics, 2*(2), 1-10.

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of cognitive neuroscience, 16*(8), 1412-1425.

Kosten, T. A., Huang, W., & Nielsen, D. A. (2014). Sex and litter effects on anxiety and DNA methylation levels of stress and neurotrophin genes in adolescent rats. *Developmental psychobiology, 56*(3), 392-406. doi: 10.1002/dev.21106

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell, 128*(4), 693-705.

Kundakovic, M., Lim, S., Gudsnuk, K., & Champagne, F. A. (2013). Sex-specific and strain-dependent effects of early life adversity on behavioral and epigenetic outcomes. *Frontiers in Psychiatry, 4*, 78. doi: 10.3389/fpsyt.2013.00078

Leon, M. (1975). Dietary control of maternal pheromone in the lactating rat. *Physiology & Behavior, 14*(3), 311-319.

Lewis, C. R., & Olive, M. F. (2014). Early life stress interactions with the epigenome: potential mechanisms driving vulnerability towards psychiatric illness. *Behavioural pharmacology, 25*(5 0 6), 341.

Li, B., Carey, M., & Workman, J. L. (2007). The role of chromatin during transcription. *Cell, 128*(4), 707-719.

Lopez, J., Mamdani, F., Beaulieu, M., Yang, J., Berlim, M., Ernst, C., & Turecki, G. (2013). Epigenetic regulation of BDNF expression according to antidepressant response. *Molecular psychiatry, 18*(4), 398.

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *Journal of Neuroscience, 28*(42), 10576-10586.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*(6), 434.

Lutz, P.-E., & Turecki, G. (2014). DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience, 264*, 142-156.

McEwen, B. S. (2003). Early life influences on life-long patterns of behavior and health. *Developmental Disabilities Research Reviews, 9*(3), 149-154.

McGowan, P. O., & Roth, T. L. (2015). Epigenetic pathways through which experiences become linked with biology. *Development and psychopathology, 27*(2), 637-648.

Moore, C. L., & Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. [.]. *Journal of comparative and physiological psychology, 93*(4), 677-684.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., . . . Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature neuroscience, 12*(12), 1559-1566.

Nishimura, H., Tsuda, A., Oguchi, M., Ida, Y., & Tanaka, M. (1988). Is immobility of rats in the forced swim test "behavioral despair?". *Physiology & Behavior, 42*(1), 93-95.

Oliveira, A. M., Hawk, J. D., Abel, T., & Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learning & memory, 17*(3), 155-160.

Pears, K., & Fisher, P. A. (2005). Developmental, cognitive, and neuropsychological functioning in preschool-aged foster children: Associations with prior maltreatment and placement history. *Journal of Developmental & Behavioral Pediatrics, 26*(2), 112-122.

Perroud, N., Salzmann, A., Prada, P., Nicastro, R., Hoeppli, M., Furrer, S., . . . Malafosse, A. (2013). Response to psychotherapy in borderline personality

disorder and methylation status of the BDNF gene. *Translational psychiatry, 3*(1), e207.

Porsolt, R., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives internationales de pharmacodynamie et de therapie*, *229*(2), 327-336.

Porsolt, R. D., Bertin, A., & Jalfre, M. (1978). "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. *European journal of pharmacology*, *51*(3), 291-294.

Provencal, N., & Binder, E. B. (2015). The neurobiological effects of stress as contributors to psychiatric disorders: focus on epigenetics. *Current opinion in neurobiology*, *30*, 31-37.

Rice, D., & Barone Jr, S. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental health perspectives*, *108*(Suppl 3), 511.

Rogosch, F. A., Cicchetti, D., & Aber, J. L. (1995). The role of child maltreatment in early deviations in cognitive and affective processing abilities and later peer relationship problems. *Development and psychopathology*, *7*(4), 591-609.

Roth, E. D., Roth, T. L., Money, K. M., SenGupta, S., Eason, D. E., & Sweatt, J. D. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics*, *2*, 1-8.

Roth, T. L. (2012). Epigenetics of neurobiology and behavior during development and adulthood. *Developmental psychobiology*, *54*(6), 590-597.

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological psychiatry, 65*(9), 760-769. doi: 10.1016/j.biopsych.2008.11.028

Roth, T. L., Matt, S., Chen, K., & Blaze, J. (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the homecage. *Developmental psychobiology, 56*(8), 1755-1763.

Schenkel, L. S., Spaulding, W. D., DiLillo, D., & Silverstein, S. M. (2005). Histories of childhood maltreatment in schizophrenia: relationships with premorbid functioning, symptomatology, and cognitive deficits. *Schizophrenia research, 76*(2), 273-286.

Scott, H., Smith, A. E., Barker, G. R., Uney, J. B., & Warburton, E. C. (2017). Contrasting roles for DNA methyltransferases and histone deacetylases in single-item and associative recognition memory. *Neuroepigenetics, 9*, 1-9.

Shea, A., Walsh, C., MacMillan, H., & Steiner, M. (2005). Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females. *Psychoneuroendocrinology, 30*(2), 162-178.

Silberman, D. M., Acosta, G. B., & Zubilete, M. A. Z. (2016). Long-term effects of early life stress exposure: role of epigenetic mechanisms. *Pharmacological research, 109*, 64-73.

Wadsworth, M. E. (2015). Development of maladaptive coping: A functional adaptation to chronic, uncontrollable stress. *Child development perspectives, 9*(2), 96-100.

Walker, C.-D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., . . . Sullivan, R. M. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress, 20*(5), 421-448.

Warburton, E., & Brown, M. (2015). Neural circuitry for rat recognition memory. *Behavioural brain research, 285*, 131-139.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience, 7*(8), 847-854. doi: 10.1038/nn1276

West, A. P. (1990). Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test. *Progress in neuro-psychopharmacology & biological psychiatry, 14*(6), 863-877.

Zierhut, K., Bogerts, B., Schott, B., Fenker, D., Walter, M., Albrecht, D., . . . Düzel, E. (2010). The role of hippocampus dysfunction in deficient memory encoding and positive symptoms in schizophrenia. *Psychiatry Research: Neuroimaging, 183*(3), 187-194.

Chapter 4

SEX DIFFERENCES IN CAREGIVING IN THE SCARCITY-ADVERSITY MODEL OF LOW NESTING RESOURCES OUTSIDE THE HOME CAGE

The effects of exposure to developmental stress often diverge for males and females. Using the scarcity-adversity model of low nesting resources outside the home cage, our lab has discovered sex differences in both behavioral and epigenetic consequences of repeated exposure to caregiver maltreatment. For the measures we have performed to date, we have found more consequences for females. The reasons underlying this sex disparity are unknown. In the current experiment, we aimed to discern the quality of maternal care received by male and female pups in our model. As we have previously found more behavioral and epigenetic consequences in females, we hypothesized that females receive more adverse care compared to their male littermates. Our hypothesis was supported; in our maltreatment condition, we found that female pups received more adverse care than males. This sex difference in adverse care was not present in our two control conditions (cross-foster and normal maternal care). These data lend support to the notion that one reason females in our model incur more behavioral and epigenetic consequences is a result of greater mistreatment by the dam.

4.1 Introduction

Animal models of early adversity are invaluable in investigating outcomes and mechanisms of developmental stress, as these models afford the ability to conduct experiments that would not be possible to perform in humans. A number of animal models exist (Walker et al., 2017), and similar to data from human populations (Carmen, Rieker, & Mills, 1984; MacMillan et al., 2001; Stein, Golding, Siegel, Burnam, & Sorenson, 1988), sex differences are often found in outcomes associated with developmental stress (Bath et al., 2017; Benoit, Rakic, & Frick, 2015; Blaze et al., 2017; Blaze & Roth, 2017; Darnaudery & Maccari, 2008; Doherty, Blaze, Keller, & Roth, 2017; Francis, Young, Meaney, & Insel, 2002; Mueller & Bale, 2008; Richardson, Zorrilla, Mandyam, & Rivier, 2006; Roth, Matt, Chen, & Blaze, 2014; St-Cyr & McGowan, 2015; Viveros, Díaz, Mateos, Rodríguez, & Chowen, 2010; Zuena et al., 2008). Our lab utilizes the scarcity-adversity model of low nesting resources outside the home cage, which capitalizes on resource deprivation to experimentally induce caregiver maltreatment (Blaze & Roth, 2013; Blaze, Scheuing, & Roth, 2013; Doherty, et al., 2017; Roth, Lubin, Funk, & Sweatt, 2009; Roth, et al., 2014; Walker, et al., 2017). Using this model, we have uncovered a number of sex-specific outcomes of maltreatment in the domains of both neurobiology and behavior, with females typically exhibiting more consequences relative to male subjects (Blaze & Roth, 2013; Blaze & Roth, 2017; Doherty, et al., 2017; Roth, et al., 2014). For example, in the realm of behavior, female rats exposed to the scarcity-adversity model exhibit altered

behavior on the novel object recognition and forced-swim tests not present in maltreated males, while males show deficits in fear extinction that are not exhibited by females subjected to maltreatment (Doherty, et al., 2017). Following exposure to maltreatment, animals of both sexes exhibit increased DNA methylation of the brain-derived neurotrophic factor (*Bdnf*) gene in whole prefrontal cortex as well as altered levels of various epigenetic regulators in the medial prefrontal cortex (mPFC) (Blaze & Roth, 2013; Roth, et al., 2009). In the dorsal hippocampus, males but not females have reduced *Bdnf* methylation, while both males and females showed reduced *Bdnf* methylation in the amygdala (Roth, et al., 2014). Female, but not male, rats subjected to maltreatment show increased DNA methylation of the *Bdnf* gene that is concomitant with reduced histone 3 lysine 9/14 acetylation at the *Bdnf* exon *IV* promoter in the mPFC (Blaze, Asok, & Roth, 2015; Blaze & Roth, 2017).

Other animal models that differ in timing and severity of early adversity have too found sex differences. Many have reported male subjects to be more susceptible to the effects of developmental stress, especially gestational stress, as compared to their female counterparts (Loi et al., 2017; Mueller & Bale, 2008; Viveros, et al., 2010; Walker, et al., 2017; Zuena, et al., 2008). For example, a model of prenatal stress found that adult males subjected to prenatal stress exhibited increased depressive-like and anxiety-like behaviors coinciding with reduced corticotropin-releasing factor (*Crf*) gene DNA methylation in the hypothalamus, none of which were found in females exposed to the same model (Mueller & Bale, 2008).

In rodent species commonly used in studies of early adversity, the mother provides all nurturance. However, she does so differently to males and females. For example, dams spend more time licking the anogenital regions of male pups (C. L. Moore, 1985; C. L. Moore & Morelli, 1979). Anogenital licking is critical for the survival of pups, as pups cannot urinate or defecate unless they receive anogenital licking. This behavior has important implications for the programming of stress systems, as glucocorticoid receptor (*GR*) gene DNA methylation and expression are under the influence of nurturance received during infancy (Caldji et al., 1998; Francis, Diorio, Liu, & Meaney, 1999; Weaver et al., 2004). Further, sex differences in nurturance has been shown to mediate sex differences in methylation at the estrogen receptor alpha (ERα) promoter, (Kurian, Olesen, & Auger, 2010) a gene with importance in the development of sex-specific behaviors (Ogawa, Lubahn, Korach, & Pfaff, 1997; Ogawa, Taylor, Lubahn, Korach, & Pfaff, 1996; Patchev, Gotz, & Rohde, 2004).

Because we have observed sex differences in brain and behavioral outcomes in our model of caregiver maltreatment, this raises the possibility that males and females are experiencing different care during our experimental manipulations. Sex differences in adverse behavior received by males and females during our maltreatment paradigm could explain the propensity for females subjected to our model to present more consequences of maltreatment despite the tendency for female rats to show resilience to stress at the age during which the manipulations are performed (i.e. postnatal days (PN) 1-7) (Walker, et al., 2017). Though we have always quantified levels of adverse

care in our model, we have never done so with reference to sex of offspring. In the current study, we now quantify adverse behavior directed toward male versus female pups.

4.2 Methods

4.2.1 Subjects

This study utilized Long-Evans rats that were bred in house. Dams were maintained on a 12 hour light/dark cycle and were allowed *ad libitum* access to food and water. A total of ten experimental litters were implemented in this study. Day of parturition was deemed PN0. Litters were culled to 12 pups on PN1. All experimental procedures were approved by the University of Delaware Institutional Animal Care and Use Committee following guidelines established by the NIH.

4.2.2 Caregiving Manipulations

The scarcity-adversity model of low nesting resources outside the home cage was used, as has been done previously by our laboratory (Blaze & Roth, 2013; Blaze & Roth, 2017; Doherty, et al., 2017; Doherty, Forster, & Roth, 2016; Hill, Warren, & Roth, 2014; Roth, et al., 2009; Roth, et al., 2014; Walker, et al., 2017). This model employs a within litter design such that 1/3 of the litter remains with the biological dam in the home cage, 1/3 of the litter is placed with a cross-foster dam in a novel environment, and 1/3 of the litter is exposed to maltreatment in a novel environment.

To induce maltreatment, a dam (matched in postpartum age and diet to the pups' biological mother) is given inadequate nesting resources (i.e. 100 mL of wood shavings) and insufficient time to habituate to the experimental chamber. This manipulation causes the dam to become stressed and, in turn, perform adverse behaviors (i.e. dragging, dropping, stepping on, and roughly handling pups) toward the pups. The cross-foster condition also employs a dam (likewise matched in postpartum age and diet) in an experimental chamber; however, this dam has been given adequate resources to build her nest and at least one hour to habituate to her environment. All dams were matched in postpartum age and diet, as data suggest that pups cannot distinguish between their biological mother and age-matched dams fed the same diet (Leon, 1975). One male and one female pup were assigned to each of the three infant conditions. To distinguish the male and female pups from one another, all the pups of one sex (either male or female) were marked along the back and hind legs using a nontoxic marker. The sex chosen to be colored was counterbalanced across litters. Pups were exposed to these manipulations for 30 minutes daily for the first week of postnatal life. The remaining six pups in the litter were placed on a heating pad located in a separate room for the 30 minute manipulations and were not subjected to any of the infant conditions. Upon completion of the daily manipulations, all 12 pups were returned to the biological dam in the home cage.

To ensure consistency of effects reported in our prior publications, trained scorers without reference to pup sex scored infant manipulations for nurturing

(licking, grooming, nursing) and adverse (dragging, dropping, stepping on, and roughly handling pups) behaviors in five-minute time bins. To probe for differences in adverse care received between the sexes, the total number of adverse behaviors directed toward males and females was recorded and a percentage of adverse behaviors directed toward each sex was computed for each day of the scarcity-adversity model. Daily percentages of adverse behaviors received by each sex taken from each of the seven days of the paradigm were then averaged. Because the aim of this study was to examine sex-specific differences in maltreatment and due to methodological limitations producing an inability to visualize pups under the dam during bouts of nurturance, sex differences in nurturing care were not recorded.

4.2.3 Statistical Analyses

Two-way ANOVAs were conducted to analyze nurturing and adverse behaviors performed by dams during the infant manipulations. T-tests were conducted to probe sex differences in adverse behaviors sustained between the sexes. Bonferroni corrections were applied where necessary. For all analyses, $p < .05$ was used to indicate statistical significance.

4.3 Results

4.3.1 Caregiver Manipulations

A two-way ANOVA conducted on caregiving behavior observed across the infant manipulations revealed an interaction between caregiving behavior and infant condition ($F_{(2,54)} = 84.28$, $p < .0001$) and a main effect of caregiver behavior ($F_{(1,54)} = 41.83$, $p < .0001$). Post-hoc comparisons demonstrated that pups placed in the maltreatment condition incurred more adverse behavior as compared with the cross-foster ($p < .0001$) and normal maternal care ($p < .0001$) control conditions. Further, fewer nurturing behaviors were observed in the maltreatment condition as compared to the cross-foster ($p < .0001$) and normal maternal ($p < .0001$) care conditions. Significantly more adverse relative to nurturing behaviors were performed by dams in the maltreatment condition ($p < .0001$). We did not find any differences in nurturing ($p = .164$) or adverse behavior ($p = .3822$) received by pups in the normal maternal care and cross-foster care conditions. Consistent with other reports using this model (Blaze, et al., 2015; Blaze & Roth, 2013; Blaze & Roth, 2017; Blaze, et al., 2013; Doherty, et al., 2017; Doherty, et al., 2016; Roth, et al., 2009; Roth, et al., 2014), these analyses demonstrate that pups placed in the maltreatment conditions receive more adverse and less nurturing behavior relative to pups in the two control conditions.

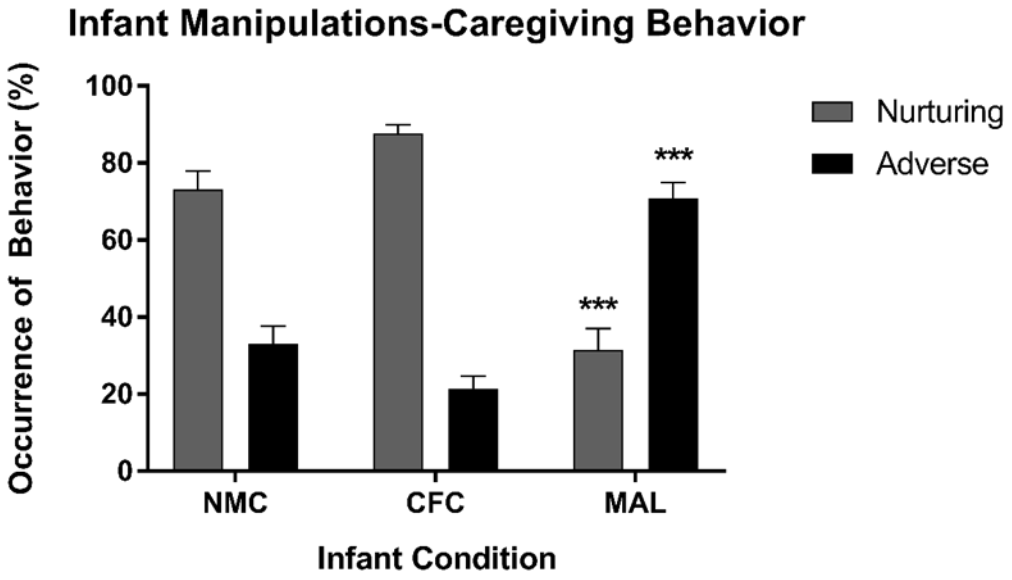

Figure 4.1 Similar to our previous reports where adverse behaviors directed at both sexes were combined, pups in the maltreatment condition experienced more adversity and less nurturance. n=10 litters; *** denotes $p < .001$.

4.3.2 Sex Differences in Caregiving

T-tests performed on the number of adverse behaviors directed toward each sex revealed a significant difference between males and females in the maltreatment condition, with females receiving more adverse care than males ($t_{(18)} = 4.454$, $p = .0003$). No differences were found in levels of adverse care directed toward pups of each sex in the cross-foster ($t_{(14)} = 0.4337$, $p = .6711$) or normal maternal ($t_{(16)} = 1.486$, $p = .1568$) care conditions. These data indicate that female pups receive more adverse care exclusively in the maltreatment condition, where adverse behaviors directed by the dam are more numerous.

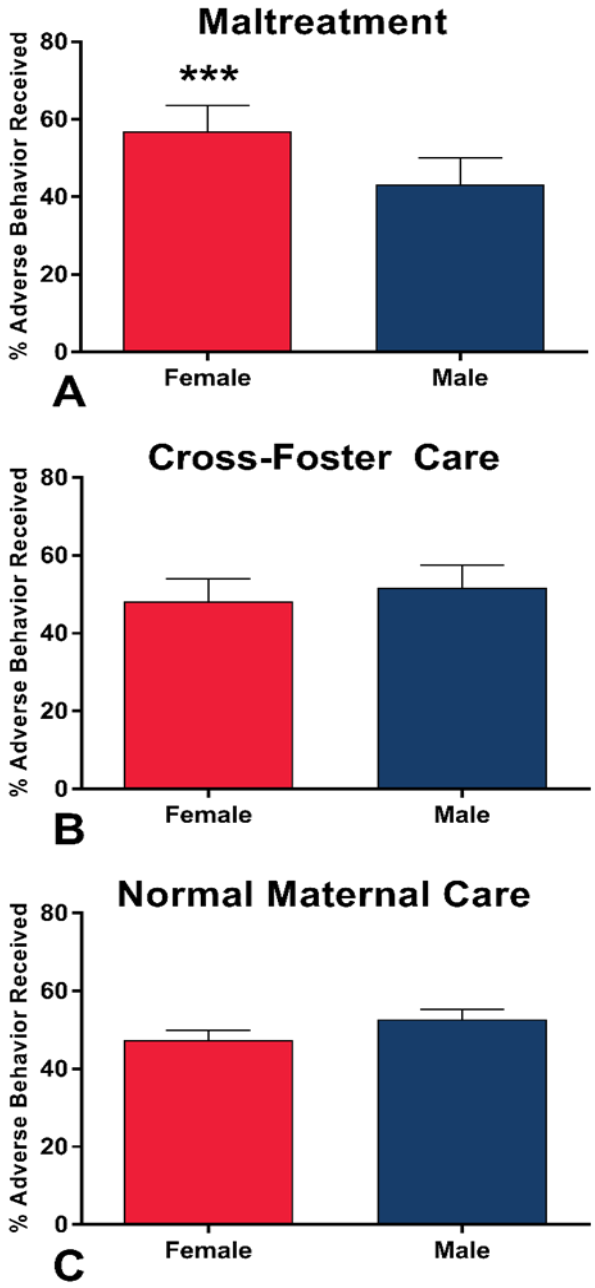

Figure 4.2 In the maltreatment condition, female pups received more adverse care from the dam relative to their male littermates (A). No sex differences in adverse care were detected in the cross-foster (B) or normal maternal (C) care conditions. n=10 litters; *** denotes $p < .001$.

4.4 Discussion

We found that dams direct more adverse behaviors toward female pups as compared to their male littermates within the maltreatment condition of the scarcity-adversity model of low nesting resources outside the home cage. Interestingly, these sex differences were only observed in the maltreatment condition, where the dam performs more adverse behaviors toward the pups; no sex differences in adverse behavior were found in the cross-foster or normal maternal care conditions, conditions where dams perform few adverse behaviors. These data lend support to our hypothesis that females subjected to maltreatment in our rodent model exhibit more consequences of maltreatment relative to their male counterparts because they experience greater maltreatment.

Though it may seem surprising that the sex-specificity of adverse care would vary across infant caregiver conditions in our model, there is precedent for sex-differences in parental care during times of stress or unfavorable environmental conditions. For example, dams have been shown to reject their male offspring during times of food restriction, favoring the survival of their female offspring (McClure, 1981). The Trivers-Willard effect, whereby female mammals shift their offspring composition to contain more female relative to male offspring during times of stress, also predicts greater parental investment in females by parents in stressful conditions (Trivers & Willard, 1973). Further, mothers of low-socioeconomic status have been

found to produce more nutritious breast milk when feeding daughters as compared to sons (Fujita et al., 2012).

Why might female pups incur more adverse behaviors in our maltreatment condition? Dams can distinguish the sex of their pups by odor (C. L. Moore, 1981) and the hormonal status of pups also impacts the amount of anogenital licking the dam will provide to individual pups (C. L. Moore, 1982). This discrimination of pup sex results in male pups receiving more licking and grooming relative to their female littermates (C. L. Moore & Morelli, 1979), and could also contribute to sex differences in adverse behavior received by the pups. As another possible explanation, maternal caregiving toward pups could be sex-biased because of the behavior of the pups (Alleva, Caprioli, & Laviola, 1989; C. L. Moore & Chadwick-Dias, 1986). Male pups exhibit leg extension earlier and with a longer duration after the start of anogenital licking as compared to female pups (C. L. Moore & Chadwick-Dias, 1986). Because this behavior helps to recycle resources back to the dam, it is advantageous for the dam to focus anogenital licking on the male pups. Another potential explanation could involve the increased locomotor activity observed in female as opposed to male rodents (Rosenfeld, 2017). We speculate that enhanced locomotor activity typical of female rodents could place the female pup in contact with the dam more frequently, which in turn results in more adverse behaviors directed toward the female pup.

Ultrasonic vocalizations (USVs), which are a distress call elicited by pups to encourage attention from the dam (Noirot, 1966, 1972), are produced more loudly and

of a longer duration by male versus female pups (Naito & Tonoue, 1987). We have previously found that within the scarcity-adversity model, more USVs are emitted by pups (without reference to sex) in the maltreatment condition relative to the nurturing care conditions (Blaze, et al., 2013; Roth, et al., 2014). These vocalizations trigger maternal responsiveness, as dams increase nest building, pup retrieval, and pup licking in response to pup USVs (Brouette-Lahlou, Vernet-Maury, & Vigouroux, 1992; Brunelli, Shair, & Hofer, 1994; Noirot, 1974). In addition to eliciting maternal responsiveness, vocalizations also dampen levels of maternal aggression and rough handling toward pups (Ihnat, White, & Barfield, 1995). Increased USVs from male pups could contribute to the sex-disparity in maternal behavior observed in maltreated pups in our study. While sex differences in pup odor or pup behavior could underlie the sex differences in caregiver behavior observed in our study, future research is warranted to elucidate the reason underlying this disparity in adverse care directed toward the sexes.

It is important to explore sex and gender differences in caregiving because the quality of parental care received during development has lifelong implications on health and behavior (Banyard, 1997; Doherty, et al., 2017; Liu et al., 1997; Mallers, Charles, Neupert, & Almeida, 2010; Pears & Fisher, 2005; Roberts, O'Connor, Dunn, Golding, & Team, 2004; Roth, et al., 2009; Shaw, Krause, Chatters, Connell, & Ingersoll-Dayton, 2004). Experiencing neglect or abuse in childhood is associated with poor mental health outcomes in adulthood (Beers & De Bellis, 2002; De Bellis et

al., 2002; De Bellis, Woolley, & Hooper, 2013; Edwards, Holden, Felitti, & Anda, 2003; Kendall-Tackett, 2002; MacMillan, et al., 2001; Shea, Walsh, MacMillan, & Steiner, 2005; Wexler, Lyons, Lyons, & Mazure, 1997). Neurobiological perturbations resulting from early-life stress are thought to mediate the relationship between childhood maltreatment and these outcomes (Bremner et al., 2003; Cirulli et al., 2009; De Bellis, et al., 2013; Tarullo & Gunnar, 2006; Teicher et al., 2003). Importantly, the outcomes of incurring childhood maltreatment are often gender or sex-specific (MacMillan, et al., 2001; Weiss, Longhurst, & Mazure, 1999). For example, females subjected to childhood abuse are more likely to experience depression than males (Carmen, et al., 1984; MacMillan, et al., 2001; Stein, et al., 1988). Females are more likely to experience sexual abuse than males (Finkelhor, 1987; Maikovich-Fong & Jaffee, 2010; Silverman, Reinherz, & Giaconia, 1996), and experiencing sexual abuse has been proposed to make females particularly susceptible to developing psychiatric disorders (Cutler & Nolen-Hoeksema, 1991; Weiss, et al., 1999). Further, this is consistent with reports of enhanced levels of depression and anxiety disorder diagnoses in females (Kendler et al., 2010; Kessler, 2003; Sheikh, Leskin, & Klein, 2003; Tolin & Foa, 2006).

Gender differences in parenting occur in humans. For example, boys are more likely to receive harsh discipline by their parents relative to girls (McKee et al., 2007; Straus & Stewart, 1999) while mothers use more emotional talk (i.e. use more emotion words) when reading to their daughters (Aznar & Tenenbaum, 2015). Women provide more affectionate touch (e.g. hugging, stroking, kissing, cuddles) to their sons relative

to their daughters (Fausto-Sterling, Crews, Sung, García-Coll, & Seifer, 2015). Mothers provide more negative feedback toward their daughters relative to their sons when observing their children completing a task, and offer sons more positive feedback as compared to their daughters (Alessandri & Lewis, 1996). Women subjected to maltreatment during childhood show disrupted care toward their own offspring and, consistent with our findings, stressed mothers have been shown to provide poorer care to their daughters (Cross et al., 2016; DiLillo & Damashek, 2003). For example, women who incurred sexual abuse during childhood exhibited reduced warmth toward their daughters, but not toward their sons (Cross, et al., 2016). Together, these data suggest that in humans, females may receive less nurturance, particularly when the mother has experienced abuse.

While this study added to the existing literature highlighting sex differences in maternal care, the sex-specific nature of paternal care cannot be established because male rats do not typically contribute to parenting (Bales & Saltzman, 2016). In humans, differences in caregiving provided by father to their sons versus daughters have been established. Boys, as compared to girls, are more likely to be the target of aggression by abusive fathers (Jouriles & Norwood, 1995). A recent study found that fathers interacted differently with their sons and daughters, engaging in more rough and tumble play with their sons while singing more to their daughters (Mascaro, Rentscher, Hackett, Mehl, & Rilling, 2017). These behavioral differences corresponded with different patterns of brain activation in response to their child's facial expressions (Mascaro, et al., 2017), suggesting a biological underpinning of

these gender differences in caregiving. It is unclear how a two-parent dynamic could contribute to outcomes of caregiver experience in human populations. It is important to note that many studies examining sex-specific parenting behaviors do so under normal conditions, while under the current study we only found sex-differences in maternally-directed behavior during times of maternal resource deprivation.

In summary, we found that dams direct more adverse behaviors toward female as compared to male pups during times of stress. Additional work is needed to establish the consequences and functional implications of this sex-specific direction of adverse caregiving. This study highlights the importance of examining the contribution of maternal care received when interpreting sex-specific outcomes. Female pups receiving more adverse care coupled with less nurturing care (i.e. licking and grooming) relative to male pups could underlie sex differences in outcomes of stress found across other models of early-life stress.

Acknowledgements

We would like to thank Isabella Archer and Shannon Trombley for their help scoring behavioral videos. Funding for this work was provided by a University Doctoral Fellowship awarded to SMK and The Eunice Kennedy Shriver National Institute of Child Health and Human Development (R01HD087509) awarded to TLR.

REFERENCES

Alessandri, S. M., & Lewis, M. (1996). Differences in pride and shame in maltreated and nonmaltreated preschoolers. *Child development, 67*(4), 1857-1869.

Alleva, E., Caprioli, A., & Laviola, G. (1989). Litter gender composition affects maternal behavior of the primiparous mouse dam (Mus musculus). *Journal of comparative psychology, 103*(1), 83-87.

Aznar, A., & Tenenbaum, H. R. (2015). Gender and age differences in parent–child emotion talk. *British Journal of Developmental Psychology, 33*(1), 148-155.

Bales, K. L., & Saltzman, W. (2016). Fathering in rodents: neurobiological substrates and consequences for offspring. *Hormones and behavior, 77*, 249-259.

Banyard, V. L. (1997). The impact of childhood sexual abuse and family functioning on four dimensions of women's later parenting. *Child abuse & neglect, 21*(11), 1095-1107.

Bath, K. G., Nitenson, A. S., Lichtman, E., Lopez, C., Chen, W., Gallo, M., . . . Manzano-Nieves, G. (2017). Early life stress leads to developmental and sex selective effects on performance in a novel object placement task. *Neurobiology of stress, 7*, 57-67.

Beers, S. R., & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. *American Journal of Psychiatry, 159*(3), 483-486.

Benoit, J. D., Rakic, P., & Frick, K. M. (2015). Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behavioural brain research, 281,* 1-8.

Blaze, J., Asok, A., Borrelli, K., Tulbert, C., Bollinger, J., Ronca, A. E., & Roth, T. L. (2017). Intrauterine exposure to maternal stress alters Bdnf IV DNA methylation and telomere length in the brain of adult rat offspring. *International Journal of Developmental Neuroscience*.

Blaze, J., Asok, A., & Roth, T. L. (2015). Long-term effects of early-life caregiving experiences on brain-derived neurotrophic factor histone acetylation in the adult rat mPFC. *Stress, 18*(6), 607-615.

Blaze, J., & Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience, 31*(8), 804-810. doi: 10.1016/j.ijdevneu.2013.10.001

Blaze, J., & Roth, T. L. (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and psychopathology, 29*(2), 477-489.

Blaze, J., Scheuing, L., & Roth, T. L. (2013). Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to

maltreatment or nurturing care during infancy. *Developmental neuroscience, 35*(4), 306-316. doi: 10.1159/000350716

Bremner, D. J., Vythilingam, M., Vermetten, E., Southwick, S. M., McGlashan, T., Nazeer, A., . . . Garg, P. K. (2003). MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *American Journal of Psychiatry*.

Brouette-Lahlou, I., Vernet-Maury, E., & Vigouroux, M. (1992). Role of pups' ultrasonic calls in a particular maternal behavior in Wistar rat: pups' anogenital licking. *Behavioural brain research, 50*(1-2), 147-154.

Brunelli, S. A., Shair, H. N., & Hofer, M. A. (1994). Hypothermic vocalizations of rat pups (Rattus norvegicus) and direct maternal search behavior. *Journal of Comparative Psychology, 108*(3), 298.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences, 95*(9), 5335-5340.

Carmen, E. H., Rieker, P. P., & Mills, T. (1984). Victims of violence and psychiatric illness *The Gender Gap in Psychotherapy* (pp. 199-211): Springer.

Cirulli, F., Francia, N., Berry, A., Aloe, L., Alleva, E., & Suomi, S. J. (2009). Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates. *Neuroscience & Biobehavioral Reviews, 33*(4), 573-585.

Cross, D., Kim, Y. J., Vance, L. A., Robinson, G., Jovanovic, T., & Bradley, B. (2016). Maternal Child Sexual Abuse Is Associated With Lower Maternal Warmth Toward Daughters but Not Sons. *Journal of child sexual abuse, 25*(8), 813-826.

Cutler, S. E., & Nolen-Hoeksema, S. (1991). Accounting for sex differences in depression through female victimization: Childhood sexual abuse. *Sex roles, 24*(7-8), 425-438.

Darnaudery, M., & Maccari, S. (2008). Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain research reviews, 57*(2), 571-585.

De Bellis, M. D., Keshavan, M. S., Shifflett, H., Iyengar, S., Beers, S. R., Hall, J., & Moritz, G. (2002). Brain structures in pediatric maltreatment-related posttraumatic stress disorder: a sociodemographically matched study. *Biological psychiatry, 52*(11), 1066-1078.

De Bellis, M. D., Woolley, D. P., & Hooper, S. R. (2013). Neuropsychological findings in pediatric maltreatment: relationship of PTSD, dissociative symptoms, and abuse/neglect indices to neurocognitive outcomes. *Child maltreatment, 18*(3), 171-183.

DiLillo, D., & Damashek, A. (2003). Parenting characteristics of women reporting a history of childhood sexual abuse. *Child maltreatment, 8*(4), 319-333.

Doherty, T. S., Blaze, J., Keller, S. M., & Roth, T. L. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental psychobiology, 59*, 703-714.

Doherty, T. S., Forster, A., & Roth, T. L. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural brain research*. doi: 10.1016/j.bbr.2015.05.028

Edwards, V. J., Holden, G. W., Felitti, V. J., & Anda, R. F. (2003). Relationship between multiple forms of childhood maltreatment and adult mental health in community respondents: results from the adverse childhood experiences study. *American Journal of Psychiatry, 160*(8), 1453-1460.

Fausto-Sterling, A., Crews, D., Sung, J., García-Coll, C., & Seifer, R. (2015). Multimodal sex-related differences in infant and in infant-directed maternal behaviors during months three through twelve of development. *Developmental psychology, 51*(10), 1351.

Finkelhor, D. (1987). The sexual abuse of children: Current research reviewed. *Psychiatric Annals, 17*(4), 233-241.

Francis, D. D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science, 286*(5442), 1155-1158.

Francis, D. D., Young, L. J., Meaney, M., & Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and

vasopressin (V1a) receptors: gender differences. *Journal of neuroendocrinology*, *14*(5), 349-353.

Fujita, M., Roth, E., Lo, Y. J., Hurst, C., Vollner, J., & Kendell, A. (2012). In poor families, mothers' milk is richer for daughters than sons: A test of Trivers–Willard hypothesis in agropastoral settlements in Northern Kenya. *American Journal of Physical Anthropology*, *149*(1), 52-59.

Hill, K. T., Warren, M., & Roth, T. L. (2014). The influence of infant-caregiver experiences on amygdala Bdnf, OXTr, and NPY expression in developing and adult male and female rats. *Behavioural brain research*, *272*, 175-180.

Ihnat, R., White, N. R., & Barfield, R. J. (1995). Pup's broadband vocalizations and maternal behavior in the rat. *Behavioural processes*, *33*(3), 257-271.

Jouriles, E. N., & Norwood, W. D. (1995). Physical aggression toward boys and girls in families characterized by the battering of women. *Journal of family Psychology*, *9*(1), 69.

Kendall-Tackett, K. (2002). The health effects of childhood abuse: four pathways by which abuse can influence health. *Child abuse & neglect*, *26*(6), 715-729.

Kendler, K. S., Kessler, R. C., Walters, E. E., MacLean, C., Neale, M. C., Heath, A. C., & Eaves, L. J. (2010). Stressful life events, genetic liability, and onset of an episode of major depression in women. *Focus*, *8*(3), 459-470.

Kessler, R. C. (2003). Epidemiology of women and depression. *Journal of affective disorders*, *74*(1), 5-13.

Kurian, J. R., Olesen, K. M., & Auger, A. P. (2010). Sex differences in epigenetic regulation of the estrogen receptor-α promoter within the developing preoptic area. *Endocrinology, 151*(5), 2297-2305.

Leon, M. (1975). Dietary control of maternal pheromone in the lactating rat. *Physiology & Behavior, 14*(3), 311-319.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277*(5332), 1659-1662.

Loi, M., Mossink, J., Meerhoff, G., Den Blaauwen, J., Lucassen, P., & Joëls, M. (2017). Effects of early-life stress on cognitive function and hippocampal structure in female rodents. *Neuroscience, 342*, 101-119.

MacMillan, H. L., Fleming, J. E., Streiner, D. L., Lin, E., Boyle, M. H., Jamieson, E., . . . Beardslee, W. R. (2001). Childhood abuse and lifetime psychopathology in a community sample. *American Journal of Psychiatry, 158*(11), 1878-1883.

Maikovich-Fong, A. K., & Jaffee, S. R. (2010). Sex differences in childhood sexual abuse characteristics and victims' emotional and behavioral problems: Findings from a national sample of youth. *Child abuse & neglect, 34*(6), 429-437.

Mallers, M. H., Charles, S. T., Neupert, S. D., & Almeida, D. M. (2010). Perceptions of childhood relationships with mother and father: Daily emotional and stressor experiences in adulthood. *Developmental psychology, 46*(6), 1651.

Mascaro, J. S., Rentscher, K. E., Hackett, P. D., Mehl, M. R., & Rilling, J. K. (2017). Child gender influences paternal behavior, language, and brain function. *Behavioral neuroscience, 131*(3), 262.

McClure, P. A. (1981). Sex-biased litter reduction in food-restricted wood rats (Neotoma floridana). *Science, 211*(4486), 1058-1060.

McKee, L., Roland, E., Coffelt, N., Olson, A. L., Forehand, R., Massari, C., . . . Zens, M. S. (2007). Harsh discipline and child problem behaviors: The roles of positive parenting and gender. *Journal of Family Violence, 22*(4), 187-196.

Moore, C. (1982). Maternal behavior of rats is affected by hormonal condition of pups. *Journal of comparative and physiological psychology, 96*(1), 123.

Moore, C. L. (1981). An olfactory basis for maternal discrimination of sex of offspring in rats (Rattus norvegicus). *Animal behaviour, 29*(2), 383-386.

Moore, C. L. (1985). Sex differences in urinary odors produced by young laboratory rats (Rattus norvegicus). *Journal of Comparative Psychology, 99*(3), 336.

Moore, C. L., & Chadwick-Dias, A.-M. (1986). Behavioral responses of infant rats to maternal licking: variations with age and sex. *Developmental psychobiology, 19*(5), 427-438.

Moore, C. L., & Morelli, G. A. (1979). Mother rats interact differently with male and female offspring. [.]. *Journal of comparative and physiological psychology, 93*(4), 677-684.

Mueller, B. R., & Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *Journal of Neuroscience, 28*(36), 9055-9065.

Naito, H., & Tonoue, T. (1987). Sex difference in ultrasound distress call by rat pups. *Behavioural brain research, 25*(1), 13-21.

Noirot, E. (1966). Ultra-sounds in young rodents. I. Changes with age in albino mice. *Animal behaviour, 14*(4), 459-462.

Noirot, E. (1972). Ultrasounds and maternal behavior in small rodents. *Developmental psychobiology, 5*(4), 371-387.

Noirot, E. (1974). Nest-building by the virgin female mouse exposed to ultrasound from inaccessible pups. *Animal behaviour, 22*(2), 410-420.

Ogawa, S., Lubahn, D. B., Korach, K. S., & Pfaff, D. W. (1997). Behavioral effects of estrogen receptor gene disruption in male mice. *Proceedings of the National Academy of Sciences, 94*(4), 1476-1481.

Ogawa, S., Taylor, J. A., Lubahn, D. B., Korach, K. S., & Pfaff, D. W. (1996). Reversal of sex roles in genetic female mice by disruption of estrogen receptor gene. *Neuroendocrinology, 64*(6), 467-470.

Patchev, A. V., Gotz, F., & Rohde, W. (2004). Differential role of estrogen receptor isoforms in sex-specific brain organization. *The FASEB journal, 18*(13), 1568-1570.

Pears, K., & Fisher, P. A. (2005). Developmental, cognitive, and neuropsychological functioning in preschool-aged foster children: Associations with prior

maltreatment and placement history. *Journal of Developmental & Behavioral Pediatrics, 26*(2), 112-122.

Richardson, H. N., Zorrilla, E. P., Mandyam, C. D., & Rivier, C. L. (2006). Exposure to repetitive versus varied stress during prenatal development generates two distinct anxiogenic and neuroendocrine profiles in adulthood. *Endocrinology, 147*(5), 2506-2517.

Roberts, R., O'Connor, T., Dunn, J., Golding, J., & Team, A. S. (2004). The effects of child sexual abuse in later family life; mental health, parenting and adjustment of offspring. *Child abuse & neglect, 28*(5), 525-545.

Rosenfeld, C. S. (2017). Sex-dependent differences in voluntary physical activity. *Journal of neuroscience research, 95*(1-2), 279-290.

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological psychiatry, 65*(9), 760-769. doi: 10.1016/j.biopsych.2008.11.028

Roth, T. L., Matt, S., Chen, K., & Blaze, J. (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the homecage. *Developmental psychobiology, 56*(8), 1755-1763.

Shaw, B. A., Krause, N., Chatters, L. M., Connell, C. M., & Ingersoll-Dayton, B. (2004). Emotional support from parents early in life, aging, and health. *Psychology and aging, 19*(1), 4.

Shea, A., Walsh, C., MacMillan, H., & Steiner, M. (2005). Child maltreatment and HPA axis dysregulation: relationship to major depressive disorder and post traumatic stress disorder in females. *Psychoneuroendocrinology, 30*(2), 162-178.

Sheikh, J., Leskin, G., & Klein, D. (2003). Gender Differences in Panic Disorder: Findings From the National Comorbidity Survey. *Year Book of Psychiatry & Applied Mental Health, 2003*(1), 212-213.

Silverman, A. B., Reinherz, H. Z., & Giaconia, R. M. (1996). The long-term sequelae of child and adolescent abuse: A longitudinal community study. *Child abuse & neglect, 20*(8), 709-723.

St-Cyr, S., & McGowan, P. O. (2015). Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in behavioral neuroscience, 9*, 145. doi: 10.3389/fnbeh.2015.00145

Stein, J. A., Golding, J. M., Siegel, J. M., Burnam, M. A., & Sorenson, S. B. (1988). Long-term psychological sequelae of child sexual abuse: The Los Angeles Epidemiologic Catchment Area study.

Straus, M. A., & Stewart, J. H. (1999). Corporal punishment by American parents: National data on prevalence, chronicity, severity, and duration, in relation to child and family characteristics. *Clinical child and family psychology review, 2*(2), 55-70.

Tarullo, A. R., & Gunnar, M. R. (2006). Child maltreatment and the developing HPA axis. *Hormones and behavior, 50*(4), 632-639.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience & Biobehavioral Reviews, 27*(1), 33-44.

Tolin, D. F., & Foa, E. B. (2006). Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychological bulletin, 132*(6), 959.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science, 179*(4068), 90-92.

Viveros, M.-P., Díaz, F., Mateos, B., Rodríguez, N., & Chowen, J. A. (2010). Maternal deprivation induces a rapid decline in circulating leptin levels and sexually dimorphic modifications in hypothalamic trophic factors and cell turnover. *Hormones and behavior, 57*(4-5), 405-414.

Walker, C.-D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., . . . Sullivan, R. M. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*(just-accepted), 1-63.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience, 7*(8), 847-854. doi: 10.1038/nn1276

Weiss, E. L., Longhurst, J. G., & Mazure, C. M. (1999). Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. *American Journal of Psychiatry, 156*(6), 816-828.

Wexler, B. E., Lyons, L., Lyons, H., & Mazure, C. M. (1997). Physical and sexual abuse during childhood and development of psychiatric illnesses during adulthood. *The Journal of nervous and mental disease, 185*(8), 522-524.

Zuena, A. R., Mairesse, J., Casolini, P., Cinque, C., Alemà, G. S., Morley-Fletcher, S., . . . Catalani, A. (2008). Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. *PLoS One, 3*(5), e2170.

Chapter 5

CONCLUSIONS, IMPLICATIONS, AND FUTURE DIRECTIONS

This dissertation contributes to the growing body of empirical literature demonstrating epigenetic alterations as a mediating factor between early-life maltreatment and later life behavioral consequences. Data from this dissertation highlight the capacity for the consequences of early-life caregiver maltreatment to be rectified in adulthood via administration of a drug known to alter DNA methylation. These data are important, as they show the plasticity of the epigenome even outside of sensitive periods of development can be capitalized on to alter behavior. While epigenome-altering drugs have been utilized in male subjects (Weaver et al., 2004; Weaver et al., 2005), we show for the first time a beneficial effect of zebularine in modifying behavioral outcomes associated with early-life adversity in female subjects. Further, we show for the first time the sex-specific nature of adverse caregiving in a rodent model of caregiver maltreatment, as we found during times of caregiver maltreatment female pups sustain increased rates of adverse behavior relative to male pups.

While the purpose of the experiments conducted in chapters 2 and 3 was to establish the capacity for a drug known to alter maltreatment-induced DNA methylation to rectify behavioral consequences of maltreatment, questions still exist regarding the mechanisms through which the drug is having beneficial effects. In these

experiments, zebularine was administered intracerebroventricular (ICV) and therefore circulated throughout the entire brain, so it is unclear which brain regions were altered by zebularine administration. The behaviors found to be altered by drug treatment (i.e. forced swim and maternal behavior) require circuit-level interactions for appropriate performance (Choi et al., 2013; De Kloet & Molendijk, 2016; Numan, 2012; Numan & Stolzenberg, 2009). Therefore, cellular and circuit level (i.e. functional interactions between cells and brain regions) changes resulting in behavioral improvements in zebularine-treated animals with a maltreatment history warrants further investigation.

The rat genome contains roughly 24,000 genes (Shimoyama et al., 2017). More work is certainly warranted to understand the genes that zebularine is acting on and the specific nature of changes in gene expression (and consequently protein products) induced by the drug treatment, and any non-specific effects the drug may have. Other work utilizing a maternal sensitization paradigm found an epigenome-altering drug to be effective in increasing maternal responsiveness in nulliparous female mice. This coincided with altered gene expression in the medial preoptic area (MPOA), a brain region critical in maternal behavior (Numan, 2012; Numan & Numan, 1995, 1997; Numan, Rosenblatt, & Komisaruk, 1977; Numan & Stolzenberg, 2009). Genes found to be upregulated included estrogen receptor beta (ERβ), oxytocin receptor, and cyclic AMP response element-binding protein. This would suggest that these same genes within the MPOA could be underlying the behavioral effects obtained in chapter 2. As for the ability for zebularine to rescue aberrant forced swim behavior in maltreated females as detailed in chapter 3, methylation of the brain-derived neurotrophic factor (*Bdnf*) gene has been implicated in behavioral abnormalities on the forced swim test associated with early stress (Jin et al., 2017; Seo et al., 2016). Previous work has

established altered methylation patterns of *Bdnf* in animals subjected to the scarcity-adversity model (Blaze & Roth, 2017; T. L. Roth, Lubin, Funk, & Sweatt, 2009; T. L. Roth, Matt, Chen, & Blaze, 2014), and the ability for maltreatment-induced methylation to be normalized with zebularine (T. L. Roth, et al., 2009), making this a plausible mechanism for behavioral improvement in our study. However, it seems plausible that other genes and brain regions would likewise be altered via zebularine treatment and play a role in the behavioral rescue. Genome-wide analyses would be useful in determining the full extent of changes in gene expression induced by zebularine.

We found that zebularine disrupted maternal behavior in animals with no history of maltreatment, consistent with other reports finding behavioral disruption after zebularine treatment in normal animals (i.e. animals never exposed to any stress) (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010; Lubin, Roth, & Sweatt, 2008; E. D. Roth et al., 2015). The effects this drug is having that causes a behavioral disruption in normal animals also warrants investigation, as the deleterious effect the drug has in normal animals could be a result of disruptions in a different set of genes relative to the changes which occurred in the zebularine-treated maltreatment group that showed a beneficial effect of drug treatment. While zebularine disturbed maternal behavior in control animals, we did not find any statistically significant differences in the nurturing care groups given zebularine on any of the other behaviors measured (open-field, novel object recognition, and forced swim test). It is unclear why some behaviors would be disturbed by zebularine treatment but others would remain unchanged. However, this suggests the effects zebularine is having are specific (despite the drug being administered globally), so it is likely zebularine is changing

methylation at some gene loci but not others. Further, because these behavioral tasks recruit different brain circuits, this suggests that zebularine is affecting function in certain brain circuits, but not others.

Once specific genes and brain regions underlying the observed behavioral effects were discovered, targeted genome-editing techniques such as CRISPR/Cas9 (Day, 2014; Hsu, Lander, & Zhang, 2014; Ran et al., 2013) could be utilized to offer more concrete evidence for a causal link between epigenome and phenotype in response to maltreatment. While zebularine cannot currently be used in humans to modify behavior, understanding the biological impact this drug is having could ultimately provide the knowledge required to develop DNA methylation-targeted therapeutics (either behavioral or pharmacological) that could be used to ameliorate symptoms of maltreatment exposure in human females.

The experiments in chapters 2 and 3 used a seven day drug regimen because seven days of drug administration had been previously shown in a report to normalize DNA methylation and gene expression after early adversity (T. L. Roth, et al., 2009). However, it is unclear if seven days of drug treatment would be required to see behavioral improvement, or if behavior would change over time while the animals were experiencing the drug regimen. It is also unknown how long after the final infusion the efficacious (or deleterious) impacts of zebularine would last, as in this dissertation behavior was only measured at a time point when maltreatment-induced DNA methylation is known to be rescued by zebularine (i.e. 24 hours after the final infusion). Further, the maternal brain changes over time (Kim et al., 2010), so this naturally occurring neural change could interact with drug efficacy. An interesting future direction would include allowing dams to give birth to a second litter of pups

and exploring whether improved maternal behavior in zebularine-treated dams with a history of maltreatment or disrupted maternal behavior in zebularine-treated dams without a history of maltreatment would persist.

Previous work has found DNA methylation resulting from maltreatment to be transmitted to the next generation (T. L. Roth, et al., 2009). Due to methodological limitations including separating pups from the dam while the dam was undergoing surgery and administering pain medication (buprenorphine) to the dam that could have been transmitted to the offspring through maternal milk, we were unable to utilize the brains of offspring. Future work should be designed to determine whether zebularine treatment in dams would be sufficient to prevent the transmission of DNA methylation from mother to offspring. Should zebularine be sufficient to prevent transmission of epigenetic marks associated with maltreatment from parent to offspring, a timeline of when the drug would need to be administered (preconception, during gestation, postpartum) would also be worthwhile. Previous work showed that maltreated dams had increased levels of anxiety-like behaviors in the days leading up to parturition (T. L. Roth, et al., 2009), suggesting that the drug would need to be administered preconception to prevent transmission of epigenetic marks to offspring. However, further work is needed to empirically test this prediction.

Results from chapter 4 showed that female subjects in the maltreatment condition of the scarcity-adversity model incur enhanced levels of adverse care relative to their male littermates. Reasons underlying this sex-discrepancy are unclear, but sex differences in pup behavior or odor could be at play (Moore, 1981; Naito & Tonoue, 1987; Rosenfeld, 2017). This effect was context-specific; caregivers only exhibited female bias in adverse caregiving within the maltreatment condition of the

scarcity adversity model. Are there circuit level changes in the brains of dams during the stressful conditions that underlie this sex-difference in caregiving? More work is needed to understand the reasons for and conditions under which female pups receive enhanced levels of adverse caregiving. Nonetheless, these data contribute evidence to the hypothesis that female subjects exhibit more consequences of maltreatment due to receiving more adverse care than their male counterparts.

We only examined the differences in caregiver behavior from PN 1-7 because this is the age during which the scarcity-adversity model in our laboratory is conducted (Walker et al., 2017). Maternal behavior changes over time with the dam spending less time in contact with pups and exhibiting lower levels of aggression toward intruders as pups age (Giovenardi, Consiglio, Barros, & Lucion, 2000; Grota & Ader, 1969; Orpen & Fleming, 1987). Therefore, it would be interesting to see if this model was conducted for a longer period of time if sex differences in adverse caregiving would be maintained until weaning or were specific to the early postnatal time period. Gender differences in parenting have likewise been observed in human populations (Aznar & Tenenbaum, 2015; Fausto-Sterling, Crews, Sung, García-Coll, & Seifer, 2015; McKee et al., 2007; Straus & Stewart, 1999). In the context of human maltreatment, one study did find that maltreating mothers were harsher toward their daughters relative to sons (Alessandri & Lewis, 1996), suggesting that our data have direct translational relevance.

Another important future direction involves establishing the functional consequences of the increased levels of adverse behavior directed toward females in the outcomes of incurring caregiver maltreatment. For example, it is unclear to what extent this enhanced maltreatment is involved in producing aberrant forced swim

behavior in female, but not male, rats subjected to maltreatment. While females subjected to maltreatment tend to show more consequences of maltreatment, there are some phenotypes, such as deficits in fear extinction that are present in males but not in females (Doherty, Blaze, Keller, & Roth, 2017). Therefore, differences in adverse behavior received between the sexes cannot underlie all the observed sex-disparities in the consequences of maltreatment, and thus additional experiments are needed to more fully understand the reasons underlying sex differences in the outcomes of developmental stress.

REFERENCES

Alessandri, S. M., & Lewis, M. (1996). Differences in pride and shame in maltreated and nonmaltreated preschoolers. *Child development, 67*(4), 1857-1869.

Anier, K., Malinovskaja, K., Aonurm-Helm, A., Zharkovsky, A., & Kalda, A. (2010). DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology, 35*(12), 2450-2461.

Aznar, A., & Tenenbaum, H. R. (2015). Gender and age differences in parent–child emotion talk. *British Journal of Developmental Psychology, 33*(1), 148-155.

Blaze, J., & Roth, T. L. (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and psychopathology, 29*(2), 477-489.

Choi, S. H., Chung, S., Cho, J. H., Cho, Y. H., Kim, J. W., Kim, J. M., . . . Shin, K. H. (2013). Changes in c-Fos expression in the forced swimming test: common and distinct modulation in rat brain by desipramine and citalopram. *The Korean Journal of Physiology & Pharmacology, 17*(4), 321-329.

Day, J. J. (2014). New approaches to manipulating the epigenome. *Dialogues in clinical neuroscience, 16*(3), 345.

De Kloet, E., & Molendijk, M. (2016). Coping with the forced swim stressor: towards understanding an adaptive mechanism. *Neural plasticity, 2016*.

Doherty, T. S., Blaze, J., Keller, S. M., & Roth, T. L. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental psychobiology, 59*, 703-714.

Fausto-Sterling, A., Crews, D., Sung, J., García-Coll, C., & Seifer, R. (2015). Multimodal sex-related differences in infant and in infant-directed maternal behaviors during months three through twelve of development. *Developmental psychology, 51*(10), 1351.

Giovenardi, M., Consiglio, A. R., Barros, H. M. T., & Lucion, A. B. (2000). Pup age and aggressive behavior in lactating rats. *Brazilian journal of medical and biological research, 33*(9), 1083-1088.

Grota, L. J., & Ader, R. (1969). Continuous recording of maternal behaviour in Rattus norvegicus. *Animal behaviour, 17*(4), 722-729.

Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell, 157*(6), 1262-1278.

Jin, H.-J., Pei, L., Li, Y.-N., Zheng, H., Yang, S., Wan, Y., . . . Li, M. (2017). Alleviative effects of fluoxetine on depressive-like behaviors by epigenetic regulation of BDNF gene transcription in mouse model of post-stroke depression. *Scientific reports, 7*(1), 14926.

Kim, P., Leckman, J. F., Mayes, L. C., Feldman, R., Wang, X., & Swain, J. E. (2010). The plasticity of human maternal brain: longitudinal changes in brain anatomy during the early postpartum period. *Behavioral neuroscience, 124*(5), 695.

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *The Journal of Neuroscience, 28*(42), 10576-10586.

McKee, L., Roland, E., Coffelt, N., Olson, A. L., Forehand, R., Massari, C., . . . Zens, M. S. (2007). Harsh discipline and child problem behaviors: The roles of positive parenting and gender. *Journal of Family Violence, 22*(4), 187-196.

Moore, C. L. (1981). An olfactory basis for maternal discrimination of sex of offspring in rats (Rattus norvegicus). *Animal behaviour, 29*(2), 383-386.

Naito, H., & Tonoue, T. (1987). Sex difference in ultrasound distress call by rat pups. *Behavioural brain research, 25*(1), 13-21.

Numan, M. (2012). Maternal behavior: neural circuits, stimulus valence, and motivational processes. *Parenting, 12*(2-3), 105-114.

Numan, M., & Numan, M. J. (1995). Importance of pup-related sensory inputs and maternal performance for the expression of Fos-like immunoreactivity in the preoptic area and ventral bed nucleus of the stria terminalis of postpartum rats. *Behavioral neuroscience, 109*(1), 135.

Numan, M., & Numan, M. J. (1997). Projection sites of medial preoptic area and ventral bed nucleus of the stria terminalis neurons that express Fos during maternal behavior in female rats. *Journal of neuroendocrinology, 9*(5), 369-384.

Numan, M., Rosenblatt, J. S., & Komisaruk, B. R. (1977). Medial preoptic area and onset of maternal behavior in the rat. *Journal of comparative and physiological psychology, 91*(1), 146.

Numan, M., & Stolzenberg, D. S. (2009). Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Frontiers in neuroendocrinology*, *30*(1), 46-64.

Orpen, B. G., & Fleming, A. S. (1987). Experience with pups sustains maternal responding in postpartum rats. *Physiology & Behavior, 40*(1), 47-54.

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature protocols, 8*(11), 2281.

Rosenfeld, C. S. (2017). Sex-dependent differences in voluntary physical activity. *Journal of neuroscience research, 95*(1-2), 279-290.

Roth, E. D., Roth, T. L., Money, K. M., SenGupta, S., Eason, D. E., & Sweatt, J. D. (2015). DNA methylation regulates neurophysiological spatial representation in memory formation. *Neuroepigenetics, 2*, 1-8.

Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological psychiatry, 65*(9), 760-769. doi: 10.1016/j.biopsych.2008.11.028

Roth, T. L., Matt, S., Chen, K., & Blaze, J. (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats

exposed to different caregiving environments outside the homecage. *Developmental psychobiology, 56*(8), 1755-1763.

Seo, M. K., Ly, N. N., Lee, C. H., Cho, H. Y., Choi, C. M., Lee, J. G., . . . Park, S. W. (2016). Early life stress increases stress vulnerability through BDNF gene epigenetic changes in the rat hippocampus. *Neuropharmacology, 105*, 388-397.

Shimoyama, M., Smith, J. R., Bryda, E., Kuramoto, T., Saba, L., & Dwinell, M. (2017). Rat genome and model resources. *ILAR journal, 58*(1), 42-58.

Straus, M. A., & Stewart, J. H. (1999). Corporal punishment by American parents: National data on prevalence, chronicity, severity, and duration, in relation to child and family characteristics. *Clinical child and family psychology review, 2*(2), 55-70.

Walker, C.-D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., . . . Sullivan, R. M. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*(just-accepted), 1-63.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature neuroscience, 7*(8), 847-854. doi: 10.1038/nn1276

Weaver, I. C., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., & Szyf, M. (2005). Reversal of maternal programming of stress responses in

adult offspring through methyl supplementation: altering epigenetic marking later in life. *The Journal of Neuroscience, 25*(47), 11045-11054.

Appendix A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL FORMS

RECEIVED
JAN 2 1 2014
IACUC

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Research and Teaching

Title of Protocol: Epigenetic mechanisms in lifelong changes in genes and behavior associated with adverse caregiving	
AUP Number: 1216-2014-0	**← (4 digits only — if new, leave blank)**
Principal Investigator: Tania L. Roth	
Common Name: Rat (Long Evans Blue Spruce) **Genus Species:** *Rattus norvegicus*	

Pain Category: ***(please mark one)***

USDA PAIN CATEGORY: ***(Note change of categories from previous form)***	
Category	**Description**
☐ **B**	Breeding or holding where NO research is conducted
☐ **C**	Procedure involving momentary or no pain or distress
☐ **D**	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
☒ **E**	Procedure where pain or distress cannot be alleviated, as this would adversely affect the procedures, results or interpretation

Official Use Only

IACUC Approval Signature: Glenn Talken DVM

Date of Approval: 2/1/14

RECEIVED
DEC 19 2016
IACUC

University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Application to use animals in Research
(New and 3-Yr submission)

Title of Protocol: Epigenetic mechanisms in lifelong changes in genes and behavior associated with adverse caregiving
AUP Number: 1216-2017-0 ← **(4 digits only — if new, leave blank)**
Principal Investigator: Tania L. Roth
Common Name (Strain/Breed if Appropriate): Rat (Long Evans Blue Spruce) **Genus Species:** Rattus norvegicus
Date of Submission: 12-19-16

Official Use Only

IACUC Approval Signature: Tania Roth, DVM

Date of Approval: 1/30/2017

Appendix B

PERMISSION FORMS

RightsLink®

Title:	Environmental influences on the female epigenome and behavior
Author:	Keller, Samantha M.; Roth, Tania L.
Publication:	Environmental Epigenetics
Publisher:	Oxford University Press
Date:	2016-07-04

Creative Commons