PREVENTATIVE EFFECTS OF VALPROIC ACID ON OUTCOMES ASSOCIATED WITH CAREGIVER MALTREATMENT

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Honors Bachelor of Science in Neuroscience with Distinction

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ABSTRACT

Early-life experiences play a significant role in influencing individual development. Importantly, early-life stress can negatively impact the developing brain, producing changes that may lead to psychopathy later in life. These changes can be induced epigenetically through DNA methylation. Previous work in our lab has shown that there is increased DNA methylation in the prefrontal cortex (PFC) of rats exposed to maltreatment in infancy. One way to prevent this is through the use of epigenome-modifying drugs. This project was aimed at investigating the preventative effects of valproic acid (VPA) on maltreatment-induced DNA methylation. Using a rodent model of early-life adversity, we investigated the effects of a 200, 400, or 600 mg/kg dose of VPA on DNA methylation at *Bdnf* exon IX and globally across the PFC. Once a successful dose of VPA was found, we additionally investigated the long-term effects of this drug on future caregiving behavior. Results show that VPA was unsuccessful at preventing this increase in methylation at *Bdnf* exon IX across all doses tested. However, the 400 mg/kg dose of VPA was successful in lowering methylation globally across the PFC. These findings indicate that VPA does have an epigenome-modifying effect but does not specifically influence *Bdnf* exon IX methylation in the PFC at the doses tested. Global methylation results indicate that VPA may have broader, genome-wide effects on methylation. Further, dams who received a 400 mg/kg dose of VPA in infancy exhibited a greater amount of aversive caregiving behaviors than those who received saline vehicle, independent of their own early-life environment. Taken together, these data provide further insight into the complex relationship between epigenetics and developmental outcomes.

Chapter 1

INTRODUCTION

1.1 Early-life adversity

Our experiences in early life play a fundamental role in our developmental trajectory. Because the brain is changing so rapidly during this period of time, these experiences are particularly impactful in the etiology of behavior and disease. Our epigenome, coupled with these early-life experiences, help shape behavioral outcomes throughout our lives (Lester et al., 2011; Hochberg et al., 2011; Fraser-Mustard, 2010; Loman & Gunnar, 2010). Indeed, while nature versus nurture has been a classic debate in developmental psychology, a more accurate argument for the biological basis of behavior examines how nature *interacts* with nurture, that is, how our epigenome is influenced by our environment and experiences (Weaver, 2014; Murgatroyd & Spengler, 2011; Goldberg, Allis, & Bernstein, 2007). Understanding how early-life experiences affect our lifelong behavior is crucial for informing research in developmental psychology, and specifically, in the context of psychological disorders.

One type of experience that has profound effects on the brain is early-life adversity, which can include physical, emotional, or sexual abuse as well as physical or emotional neglect (Duffy, McLaughlin, & Green, 2018). The effects of these stressful experiences can be both detrimental and long-lasting, influencing lifelong behavior and even affecting future generations (Maccari et al., 2014). Caregiver maltreatment, one type of early-life adversity, includes less nurturing care and more abusive or neglectful behaviors by a parent or caregiver (Tarantola, 2018). In 2014,

the WHO reported that almost one in four adults worldwide were victims of maltreatment in childhood, making this an extremely prevalent issue (Tarantola, 2018).

There is a strong link between exposure to maltreatment and adverse behavioral outcomes, such as psychological or developmental disorders. Victims of maltreatment are more likely to have reduced cognitive performance, a heightened responsivity to stress, and impaired social development (Kundakovic & Champagne, 2015; Cicchetti & Toth, 2005; Johnson et al., 1999). Exposure to maltreatment has also been associated with increased risk of developing psychological disorders, such as depression and anxiety (Li, D'Arcy, & Meng, 2016; Cicchetti & Toth, 2005; Lee & Hoaken, 2007; Gibb, Butler, & Beck, 2003). This link between maltreatment and adverse behavioral outcomes is thought to be mediated by epigenetic changes in the brain (Kundakovic & Champagne, 2015; Romens et al., 2014; Lutz & Turecki, 2014). These changes occur molecularly as a result of our environment and influence the expression of our genes (Kundakovic & Champagne, 2015; Day & Sweatt, 2011; Murgatroyd et al., 2009; Lubin, Roth, & Sweatt, 2008). Recent research in this field has allowed a greater understanding of how early-life experiences can shape behavior and provides important insight into many psychopathologies.

1.2 Epigenetics

The field of epigenetics has been growing rapidly since it was first defined by Conrad Waddington in the 1940s (Deans & Maggert, 2015). The term epigenetics, which translates literally to "above genetics," describes how the environment and genetic factors interact to produce a unique phenotype (DuPont, Armant, & Brenner, 2009). Epigenetic modifications do not alter the DNA sequence itself, but rather, can

either increase or decrease the expression of a certain gene (Weinhold, 2006). These modifications can be the result of positive or negative environmental experiences, such as environmental enrichment (Zhang et al., 2018; Kuzumaki et al., 2011) or stress (Silberman, Acosta, & Zorilla Zubilete, 2016; Roth et al., 2009). Epigenetic modifications can occur through mechanisms such as histone modifications or DNA methylation, the latter of which has been most extensively studied in the context of psychology (Bird, 2002).

1.3 DNA methylation

DNA methylation is the process by which a methyl group is added to the 5'-position on a cytosine nucleotide of a DNA molecule. These methyl groups are added by a DNA methyltransferase enzyme (DNMT), usually at a CpG site, a place on DNA sequence where a cytosine is followed by a guanine (Moore, Le, & Guoping, 2013). CpG sites are often located in gene promoter regions, and methylation of these sites is generally associated with suppressed transcription and decreased gene expression. DNA methylation can interfere with transcription mechanisms by physically blocking the sections of the DNA sequence that transcription proteins target or by recruiting co-repressors such as histone deacetylases (HDACs) (Bird, 2002).

Aberrant patterns of DNA methylation have been implicated in many psychological disorders, such as depression (Chen et al., 2017; Fuchikami et al., 2011), posttraumatic stress disorder (Kuan et al., 2017; Roth et al., 2011), and schizophrenia (Grayson & Guidotti, 2013; Connor & Akbarian, 2008). DNA methylation has additionally been studied in the context of early-life stress and maltreatment, as a potential mechanism for how adverse experiences influence future behavior (Lewis & Olive, 2014; McGowan & Szyf, 2010; Szyf, Weaver, & Meaney,

2007). Thus, further research involving DNA methylation will allow us to better understand how early-life adversity interacts with our genes on a molecular basis.

1.4 Histone modifications

Histone modifications are another type of epigenetic mechanism and include histone acetylation and deacetylation. DNA is wrapped around complexes of histones, the proteins that allow DNA to be packed into chromatin and chromosomes (Eberharter & Becker, 2002). Gene expression is influenced depending on how tightly the DNA is wrapped around these histone complexes. Histone acetylation is the process by which an acetyl group is added to a histone tail by a histone acetyltransferase (HAT), generally resulting in increased gene expression (Clayton, Hazzalin, & Mahadevan, 2006). The addition of an acetyl group allows the DNA to become looser around the histone, making the DNA more accessible for transcription factors and consequently, increasing transcription (Clayton, Hazzalin, & Mahadevan, 2006). The opposite process can also occur; histone deacetylation is the process of removing an acetyl group from a histone tail by a histone deacetylase (HDAC). This results in decreased transcription and gene expression (Ng & Bird, 2000).

Dysregulation in normal histone acetylation or deacetylation patterns has also been linked to numerous psychological disorders, including depression, schizophrenia, and bipolar disorder (Nestler et al., 2016; Machado-Vieira, Ibrahim, & Zarate, 2011; Gavin & Sharma, 2009). Further research has additionally investigated the interplay between DNA methylation and histone modifications, suggesting that these epigenetic mechanisms interact and may work together to influence our genetic expression as a result of environmental changes (Vaissière, Sawan, & Herceg, 2008; Miller, Campbell, & Sweatt, 2007; Dobosy & Selker, 2001).

1.5 Epigenome-modifying drugs

Pharmacological substances that target the epigenome have been an area of increasing focus in current research (Day, 2014). While many of these epigenome-modifying drugs have been studied as therapeutic agents for cancer, recent work has shown their potential for use in treating neurological and psychological disorders, such as Parkinson's disease, Rett syndrome, depression, and anxiety. (Heerboth et al., 2014; Grayson, Kundakovic, & Sharma, 2010; Abel & Zukin, 2008). Generally, epigenome-modifying drugs inhibit the enzymes that are associated with DNA methylation or histone acetylation (Grayson, Kundakovic, & Sharma, 2010). Histone deacetylases (HDACs) are one example of these enzymes, which act by removing acetyl groups from histone proteins. This leads to a closed chromatin structure, which is less favorable for transcription and results in decreased gene expression (Szyf, 2009). One class of drugs, called histone deacetylase inhibitors (HDACi), prevents these HDACs from removing acetyl groups; this leads to an open chromatin structure that is more favorable for transcription, resulting in increased gene expression (Szyf, 2009).

One HDACi that has been of increasing focus in the field of epigenetics is valproic acid (VPA). While VPA has been used primarily as an antiepileptic drug, it also functions in epigenetic regulation as an HDACi (Monti, Polazzi, & Contestabile, 2009). VPA mainly affects molecular pathways responsible for neuroprotection, synaptic plasticity, and neuronal survival, making this drug an important focus for developmental epigenetics research (Monti, Polazzi, & Contestabile, 2009). In addition to acting directly on HDACs, VPA can also cause changes in DNA methylation. The processes of histone acetylation and DNA methylation interact closely to influence genetic expression. Indeed, even when an HDACi directly influences histone acetylation, this can influence DNA methylation as well (Dong et

al., 2007). It has been suggested that VPA may either directly or indirectly influence DNA demethylation, possibly via modifications of specific histones that modulate DNA demethylation (Milutinovic et al., 2007; Detich, Bovenzi, & Szyf, 2003). VPA's functional significance in neural development and synaptic plasticity, as well as its role as an HDACi, makes this drug an important tool for aiding our understanding of how the environment influences our behavior.

Previous work with HDACis has shown that the changes in histone acetylation caused by these drugs can lead to behavioral effects, including positive changes in cognitive processes such as contextual memory, spatial memory, and fear extinction (Gräff & Tsai, 2013). One study suggests hippocampal delivery of an HDACi can enhance fear extinction in mice, informing potential treatments for psychiatric disorders such as posttraumatic stress disorder (Lattal, Barret, & Wood 2007). Other studies have focused on the effects of HDACis on memory and these findings show promise for treatments against cognitive dysfunctions such as Alzheimer's disease (Gräff & Tsai, 2013). However, further research of the exact mechanisms by which certain HDACis interact with our epigenome is necessary in order to better understand their therapeutic potential in psychological disorders.

1.6 Brain-derived neurotrophic factor gene

While many genes can be affected by early-life adversity, one gene of particular interest is brain-derived neurotrophic factor (*Bdnf*). *Bdnf* plays important roles in neurogenesis, neuron growth, neuron plasticity, and neuron survival (Miranda et al., 2019; Binder & Scharfman, 2004), thus making it an important target in understanding the developmental trajectory of the brain. Furthermore, expression of *Bdnf* is incredibly susceptible to environmental influences, and changes in its

expression have been linked to changes in behavior and certain psychopathologies (Roth & Sweatt, 2011; Boulle et al., 2012). For example, one study investigated a polymorphic variant of *Bdnf*, finding that when variant mice were placed in a stressful setting, they displayed increased anxiety-like behaviors (Chen et al., 2007). Moreover, another study found that in chronically stressed rats, exercise was able to increase levels of *Bdnf* and decrease depressive-like behavior (Marais, Stein, & Daniels, 2009).

In our lab, we have extensively studied the relationship between exposure to early-life stress and *Bdnf* DNA methylation. Previous work has established that exposure to maltreatment in infancy leads to increased DNA methylation and consequently, decreased expression of the *Bdnf* gene in the prefrontal cortex (PFC) (Doherty et al., 2019; Blaze, Scheuing, & Roth, 2013; Roth et al., 2009). These epigenetic changes in *Bdnf* were further observed in the offspring of female dams who were exposed to maltreatment via our scarcity-adversity paradigm, indicating the perpetuating impact of these experiences on this gene (Roth et al., 2009). Thus, *Bdnf*'s important developmental functions combined with its sensitivity to environmental influences make this gene an important focus of our current work to further understand the impact of early-life stress on the brain.

1.7 A rodent model of early-life adversity

We utilize a rodent model in our lab to examine the effects of early-life stress on the epigenome. The use of an animal model allows us to efficiently and effectively manipulate early-life conditions, which is a challenge to control in human work (Phillips & Roth, 2019). We use a scarcity-adversity model to examine how rat pups respond, both behaviorally and epigenetically, to differing types of caregiving behavior (Roth et al., 2009; Ivy et al., 2008). In our experimental design, a dam is

placed in an environment where she is deprived of bedding, her main nesting resource, and given inadequate habituation time. This induces stress in the dam, resulting in her exhibiting more aversive behaviors towards her pups, such as stepping on, actively avoiding, or rough handling. In comparison, a dam that is placed in an environment with adequate nesting resources will exhibit more nurturing behaviors towards her pups, such as licking and grooming, hovering, and nursing. This model allows us to manipulate caregiver behavior such that we are able to study the effects of maternal maltreatment early in life.

Other models of early-life stress can include maternal separation or isolation, however, depriving a dam of her resources allows us to induce stress in pups with their mother present (Ivy et al., 2008). In human cases of abuse and parental stress, the parent is often present, but exhibiting abnormal and fragmented behavior toward the child (Whipple & Webster-Stratton, 1991). Consequently, our animal model of early-life stress may have more translational implications to similar research in humans.

Previous work in our lab has specifically focused on the epigenetic effects of maternal maltreatment induced by our paradigm. Exposure to aberrant caregiving for just seven days during infancy has been shown to lead to an increase in DNA methylation and thus, decreased expression of the *Bdnf* gene in the PFC; these methylation changes have also been shown to persist into adulthood and in future generations (Roth et al., 2009). Thus, there are long-term, epigenetic effects that are implicated through adversity in early life that can perpetuate to offspring. Additionally, work with this paradigm has investigated behavioral outcomes, suggesting fear conditioning, fear extinction, maternal behavior, and depressive and anxiety-like behaviors are affected by our early-life experiences (Doherty et al., 2017;

Blaze et al., 2015; Roth et al., 2009). These differential behavioral outcomes, as well as changes in *Bdnf* DNA methylation and gene expression, provide empirical evidence for just some of the many effects early-life adversity can have on our brain and developmental trajectory.

1.8 The current study

Previous research in our lab has focused on the effects of other epigenome-modifying drugs, including sodium butyrate and zebularine (Doherty et al., 2019; Keller et al., 2019; Keller et al., 2018), on DNA methylation levels associated with caregiver maltreatment. Building upon this work, this study was focused on VPA, an HDACi that increases gene expression by inhibiting the removal of acetyl groups from histone tails. Previous work has shown HDACi's successful at preventing negative outcomes associated with early-life adversity (Burenkova et al., 2019; Kao et al., 2012). Thus, the first aim of this study was to investigate the preventative effects of three doses of VPA (200 mg/kg, 400 mg/kg, and 600 mg/kg) on maltreatment-induced DNA methylation. We sought to find a dose of VPA that was sufficient in lowering *Bdnf* exon IX and global methylation in the PFC in pups exposed to maltreatment dams in our scarcity-adversity paradigm.

We additionally wanted to explore the long-term effects of these epigenetic changes, investigating if these methylation changes affected future caregiving behavior. Thus, the second aim of this study investigated the effects of our chosen dose of VPA in adulthood. We specifically wanted to explore the caregiving behavior of dams who were exposed to maltreatment concurrent with VPA treatment in infancy, to examine the long-term effects of HDACi administration concurrent with early-life experiences.

Chapter 2

METHODS

2.1 Subjects

27 litters of Long Evans rats (7-12 female pups per litter) were generated in our colony from Charles River dams. Colonies were bred in house at the University of Delaware and housed in 18" x 9" x 8" polypropylene cages with ample bedding and access to food and water *ad libitum* in a temperature-controlled colony room. Additionally, our colony room was maintained on a 12-hour light/dark cycle (lights on at 7:00 am and off at 7:00 pm), with experimentation occurring during the light cycle. To prevent confounding variables associated with first-time motherhood, dams were initially bred, and their first litter was not used in experimentation. PN0 was considered the day of birth, and all dams and pups were left undisturbed on this day before beginning experimentation on PN1 and ending on PN7. Prior to experimentation, pups were assigned to one of two caregiving conditions (maltreatment or normal care) and to one of four drug conditions (200 mg/kg VPA, 400 mg/kg VPA, 600 mg/kg VPA, or sterile saline vehicle). All procedures and protocols were approved by the University of Delaware Animal Care and Use Committee prior to experimentation.

2.2 Drug preparation and administration

Before exposure to the caregiving behavior paradigm, pups received their assigned dose of VPA or saline vehicle on each day of experimentation (PN1-7). VPA and saline vehicle doses were prepared directly before experimentation; to prepare VPA, the drug was weighed in increments of 50 g and dissolved in 1 mL of saline solution to obtain the desired dose. Pups were weighed daily and assigned a dose of

either VPA or saline vehicle, which was administered intraperitoneally. Pups were additionally assigned and marked with a number for identification throughout experimentation.

2.3 Caregiving behavior paradigm

We utilized a simplified version of the scarcity-adversity model previously established and used by our laboratory (e.g. Doherty et al., 2017; Doherty, Forster, & Roth, 2016; Blaze, Scheuing, & Roth, 2013; Roth et al., 2009). Using a within litter design, pups were randomly assigned and exposed to one of two caregiving conditions: normal care or maltreatment, for 30 minutes per day during PN1-7. In the normal care condition, pups remained with their biological mother in the home cage, serving as the control group. In the maltreatment condition, pups were placed with a stressed dam in a new cage with limited nesting materials (approximately 100 mL). This resulted in higher instances of adverse behaviors exhibited by the dam, such as dragging, stepping on, or dropping the pups, as demonstrated by previous research in our laboratory (Blaze, Scheuing, & Roth, 2013). This condition was run in an 18” x 12” x 18” black plexi container under red light conditions. Pups were placed back with their biological mother immediately after experimentation.

Caregiving behavior was recorded on video on each day of experimentation, as well as ultrasonic (40 kHz) pup vocalizations. Each 30-minute video session was separated into 5-minute sections, in which nurturing and aversive behaviors were tallied discreetly. Nurturing behaviors included licking and grooming, hovering, and nursing, whereas aversive behaviors included dropping, dragging, stepping on, rough handling, and actively avoiding. Nurturing and adverse behaviors were totaled and averaged across the 7 days of experimentation and across the two observers, then later

used for statistical analysis. Vocalization recordings were separated into 1-minute sections, measured discreetly. Scores were averaged across the 7 days of experimentation and then later used for statistical analysis.

2.4 Biochemical analyses

Pups were euthanized on PN8 after experimentation was complete. PFC tissue was isolated, homogenized, and stored at -80° C. DNA was then extracted using the Qiagen AllPrep DNA/RNA Mini Kit and tested for quality using spectrophotometry using the NanoDrop 2000. The purified DNA samples were then bisulfite converted using the Qiagen Epitect Bisulfite Kit, which converted the unmethylated cytosines to uracil. Samples then underwent methylation-specific real-time PCR to quantify methylation levels at *Bdnf* exon IX. Primers targeting *Bdnf* exon IX and *tubulin,* our reference gene, were used. All reactions were performed in triplicates, with outliers excluded where appropriate. An outlier was identified if a corresponding CQ value deviated by more than 0.8. We confirmed our product using melt curve analysis and gel electrophoresis. Genome-wide methylation levels were also measured using the Epigentek MethylFlash™ Global DNA Methylation Kit colorimetric ELISA.

2.5 Caregiving behavior as an outcome measure

Once an effective dose of VPA was determined to be 400 mg/kg, we proceeded with the second aim of this study, using a total of 8 litters of Long Evans rats generated from naïve Charles River dams, with approximately 4-8 female pups per litter (F0 generation). We repeated the behavioral paradigm described above, with pups assigned to one of two caregiving conditions (maltreatment or normal care). However, for this experiment, pups were assigned to one of only two drug conditions

(400 mg/kg VPA or saline vehicle). Doses of VPA and saline vehicle were prepared and administered as described previously.

After exposure to our behavioral paradigm concurrent with administration of VPA or saline vehicle, the pups in these litters were grown up and weaned at approximately PN21-23, and then bred at PN60. This yielded 41 litters of F1 generation pups, culled to approximately 8 pups per litter, with an even split between the sexes where possible. Caregiving behavior of the F0 generation toward the F1 generation of pups was then recorded at PN1, 4, and 7. The dams were approximately PN80-90 for the F0 generation. This behavioral data was scored as described previously; behaviors were scored continuously, as these dams were more active.

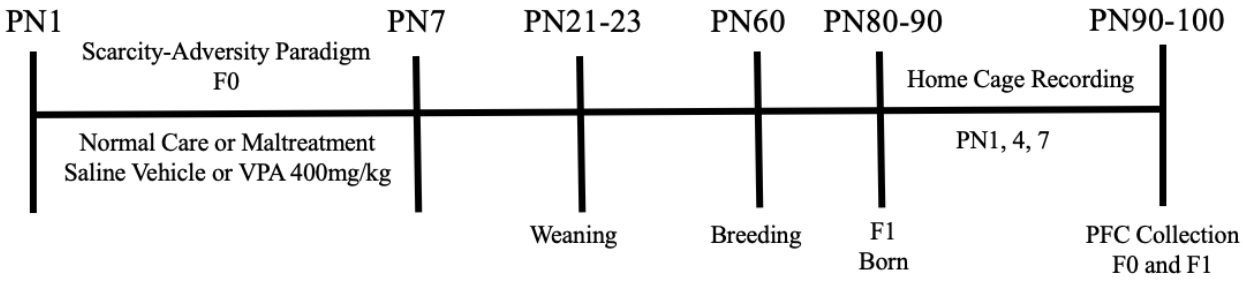


Figure 1. Experimental timeline for the second aim of our study. F0 pups were exposed to the scarcity-adversity paradigm concurrent with either saline vehicle of 400 mg/kg VPA and were then bred at PN60 to yield the F1 generation. The caregiving behavior of the F0 generation toward the F1 pups was recorded and quantified as nurturing or aversive on PN1, 4, and 7 for F1 pups (adapted from Collins, 2020).

2.6 Statistical analysis

A two-way ANOVA followed by Sidak's multiple comparisons test was used to compare differences in caregiving behavior (factors: caregiver condition and behavior type). An unpaired t-test was used to compare differences in ultrasonic vocalization occurrences (normal care vs maltreatment). A two-way ANOVA followed by Sidak's multiple comparisons test was used to compare differences in *Bdnf* exon IX methylation (factors: caregiver condition and drug condition). A two-way ANOVA followed by Tukey's multiple comparisons test was used to compare differences in global methylation (factors: caregiver condition and drug condition).

Significance was set at $p<.05$ for all analyses unless otherwise specified. GraphPad Prism was used to perform all statistical tests and to create graphs.

Chapter 3

RESULTS

3.1 Caregiving behavior and pup vocalizations

For the 27 litters used to establish an effective dose of VPA, pups were exposed to one of two caregiving conditions: maltreatment or normal care. Caregiving behavior was recorded as nurturing or aversive. Nurturing behaviors included licking and grooming, hovering, and nursing, while aversive behaviors included dropping, dragging, stepping on, rough handling, and actively avoiding. A two-way ANOVA (Figure 2) revealed a significant difference in percent occurrence of behaviors between nurturing and aversive groups [$F(1,100)=8.751$, $p=0.0039$], as well as a significant interaction [$F(1,100)=126.4$, $p<.0001$]. Post-hoc testing showed that pups in the maltreatment condition experienced a significantly greater proportion of aversive behaviors ($p<.0001$) and a significantly lower proportion of nurturing behaviors ($p<.0001$) compared to pups in the normal care group. Within the maltreatment group, pups experienced a significantly greater proportion of aversive behaviors compared to nurturing behaviors ($p<.0001$). Within the normal care group, pups experienced a significantly greater proportion of nurturing behaviors compared to aversive behaviors ($p<.0001$). These findings are consistent with previous results obtained in our laboratory using this scarcity-adversity model (Doherty et al., 2017; Doherty, Forster, & Roth, 2016; Blaze, Scheuing, & Roth, 2013; Roth et al., 2009).

Pup responses to their respective caregiving conditions were also recorded as 40 kHz ultrasonic vocalizations. An unpaired t-test (Figure 3) showed a significant difference in occurrence of ultrasonic vocalizations between pups in the maltreatment condition and those in the normal care condition [$t(49)=8.767$, $p<.0001$], with

maltreatment pups emitting a significantly greater proportion of these vocalizations compared to normal care pups, indicating that maltreatment pups experienced more distress. These findings are consistent with previous vocalization results obtained in our laboratory using this scarcity-adversity model (Doherty, Forster, & Roth, 2016; Blaze, Scheuing, & Roth, 2013; Roth et al., 2009).

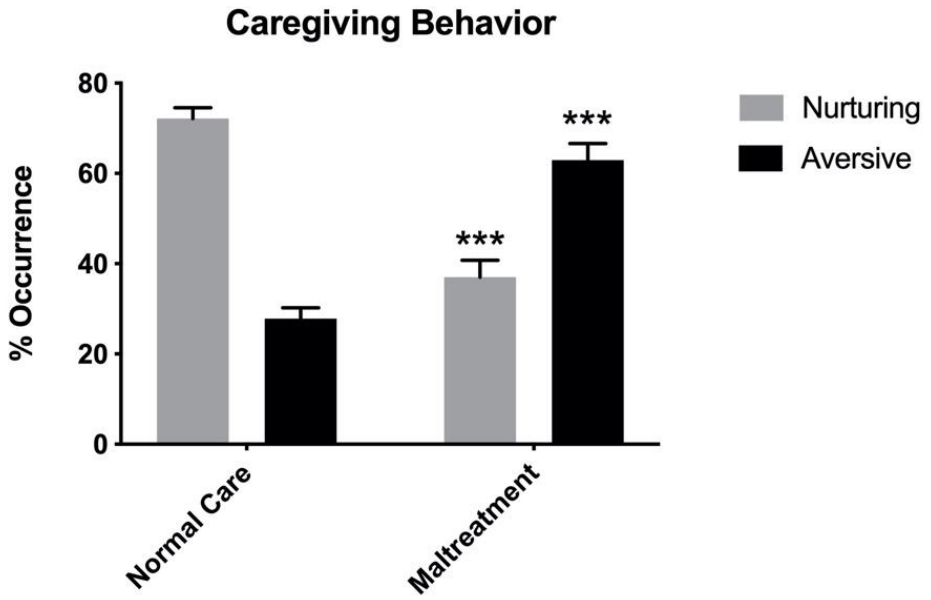


Figure 2. Percent occurrence of nurturing and aversive behavior across the two caregiving conditions: normal care and maltreatment. Pups in the maltreatment condition received a significantly greater proportion of aversive behaviors (***p<.0001) and a significantly lower proportion of nurturing behaviors (***p<.0001) compared to pups in the normal care group. n=27 litters, 4-12 per group. Error bars represent SEM.

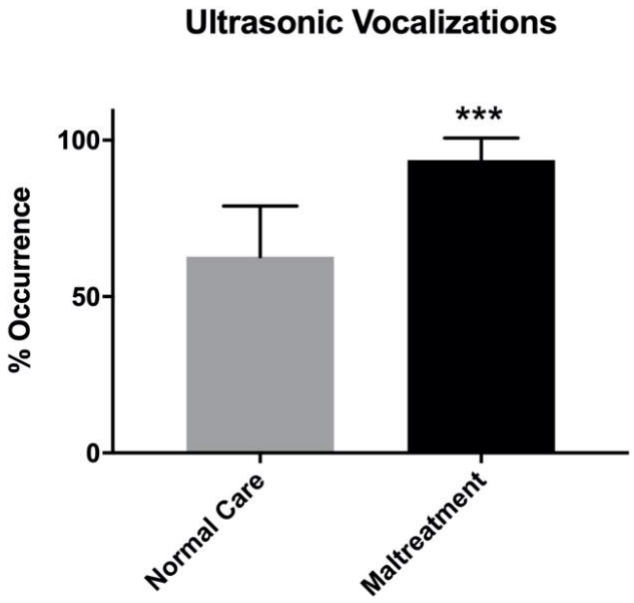


Figure 3. Percent occurrence of 40 kHz ultrasonic vocalizations across the two caregiving conditions: normal care and maltreatment. Pups in the maltreatment condition emitted a significantly greater proportion of these vocalizations (***p<.0001) compared to pups in the normal care group. n=27 litters, 4-12 per group. Error bars represent SEM.

3.2 *Bdnf* exon IX and global methylation

Previous work in our lab has shown that exposure to our early-life stress paradigm can result in increases in DNA methylation at *Bdnf* exon IX in the PFC (Roth et al., 2009). To determine which dose of VPA successfully prevents these methylation changes, PFC tissue was collected at PN8, and DNA methylation levels were quantified at *Bdnf* exon IX. A two-way ANOVA (Figure 4) revealed significant differences in methylation between pups in the normal care condition and pups in the maltreatment condition, such that pups in the maltreatment condition had significantly more methylation of *Bdnf* exon IX in the prefrontal cortex compared to pups in the

normal care condition [$F(1,79)=8.557$, $p=0.0045$]. No significant differences in methylation were found between drug conditions [$F(3,79)=0.9145$, $p=0.4379$]. Although these data indicate that VPA was not effective at preventing an increase in DNA methylation, we do replicate previous findings in our lab (Doherty et al., 2019; Roth et al., 2009), that pups exposed to the maltreatment condition have significantly higher levels of methylation at *Bdnf* exon IX in the PFC compared to pups exposed to the normal care condition.

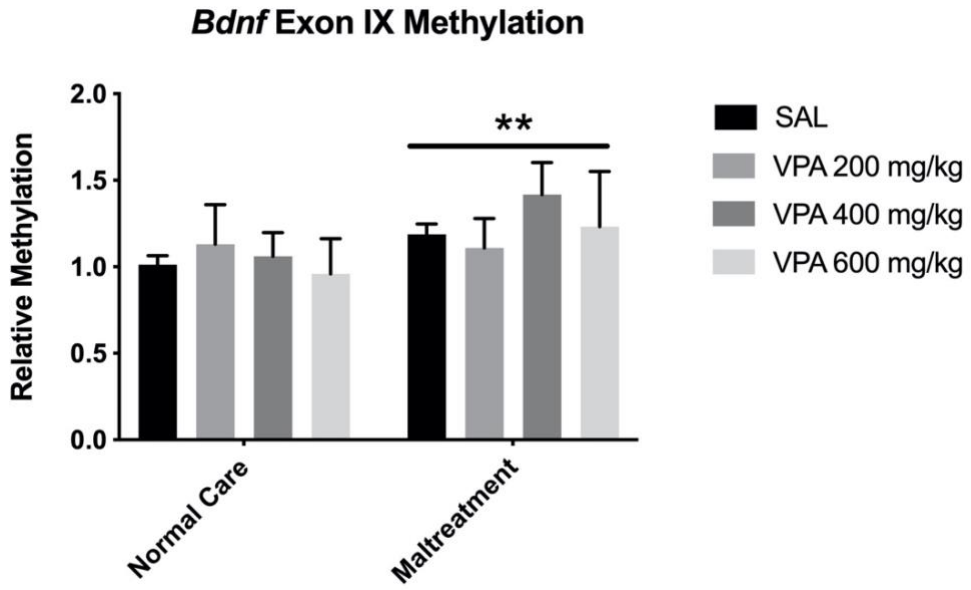


Figure 4. Relative levels of *Bdnf* exon IX methylation for the two caregiving conditions and four drug conditions. Pups in the maltreatment condition had significantly more methylation of *Bdnf* exon IX in the prefrontal cortex (**$p<.01$) compared to pups in the normal care group. No significant differences in methylation were found between drug conditions. n=27 litters, 8-16 per group. Error bars represent SEM.

To assess if VPA was able to change methylation states more broadly on a genome-wide scale, global methylation levels across the whole PFC were additionally measured using the same tissue. A two-way ANOVA (Figure 5) revealed no significant differences in global methylation between pups in the normal care condition and pups in the maltreatment condition [$F(1,74)=2.199$, $p=0.1423$]. Significant differences in global methylation were found between drug conditions [$F(3,74)=14.03$, $p<.0001$], indicating a main effect of drug. Post-hoc analysis showed that pups receiving a 400 mg/kg dose of VPA in the normal care condition had significantly lower global methylation levels ($p=.0070$) compared to pups receiving saline in the normal care condition. Additionally, pups receiving a 400 mg/kg dose of VPA in the maltreatment condition had marginally lower global methylation levels ($p=.054$) compared to pups receiving saline in the maltreatment condition.

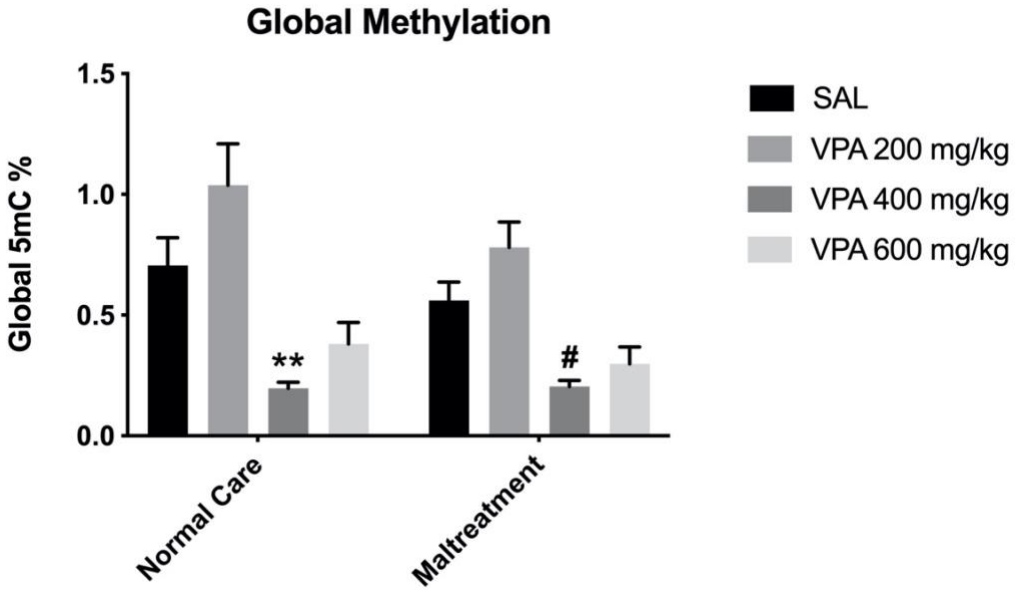


Figure 5. Global methylation levels for the two caregiving conditions and four drug conditions. Pups receiving a 400 mg/kg dose of VPA in the normal care condition had significantly lower global methylation levels (**p<.01) compared to pups receiving saline in the normal care condition. Pups receiving a 400 mg/kg dose of VPA in the maltreatment condition had marginally lower global methylations (#p=.054) compared to pups receiving saline in the maltreatment condition. n=27 litters, 8-16 per group. Error bars represent SEM.

3.3 F0 caregiving behavior toward F1 pups

After establishing an effective dose of VPA, 8 litters of female pups were run through our scarcity-adversity paradigm concurrent with administration of either saline vehicle or 400 mg/kg VPA. These females (F0 generation) were bred at PN60, yielding 41 litters of F1 generation pups. The caregiving behavior of the F0 generation toward the F1 pups within their home cage (with abundant nesting material) at PN1, 4, and 7 was recorded and quantified as nurturing or aversive, as described previously. A two-way ANOVA (Figure 6) revealed no significant differences in occurrence of F0

aversive behavior between caregiving conditions [$F(1,37)=0.09516$, $p=0.7595$]. However, significant differences in occurrence of F0 aversive behavior were found between drug conditions [$F(1,37)=7.701$, $p=0.0086$], such that F0 dams who received 400 mg/kg of VPA exhibited a greater amount of aversive behaviors than those who received saline vehicle.

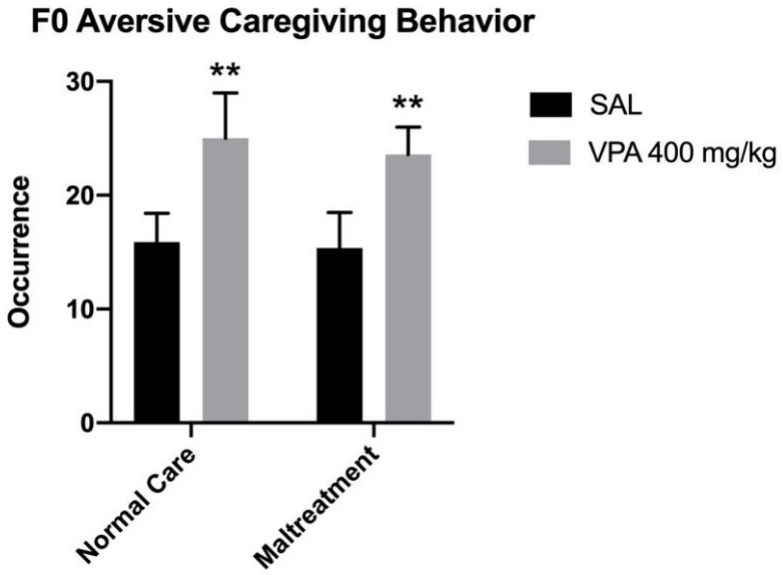


Figure 6. Occurrence of aversive behavior exhibited by F0 dams towards F1 pups. No significant differences in occurrence of F0 aversive behavior were found between caregiving conditions. Significant differences in occurrence of F0 aversive behavior were found between drug conditions (**p<.01), such that F0 dams who received 400 mg/kg of VPA exhibited a greater amount of aversive behaviors than those who received saline vehicle. n=8-12 per group. Error bars represent SEM.

Chapter 4

DISCUSSION

4.1 Treatment with VPA is effective at preventing methylation globally but not at *Bdnf* exon IX

The first aim of this study was to investigate the preventative effects of VPA on maltreatment-induced DNA methylation. Our lab has previously established that exposure to caregiver maltreatment in infancy leads to increases in methylation at *Bdnf* exon IX in the PFC, therefore we wanted to explore if VPA could be used to prevent these changes. To do this, we treated pups with VPA just before exposure to our scarcity-adversity paradigm. By administering VPA concurrent with early-life stress, we were able to assess the dose-response impact of VPA on the epigenome of pups exposed to maltreatment.

We replicated previous work from our lab showing that our scarcity-adversity model of limited nesting resources induces stress in the dam such that it alters her caregiving behavior (Doherty et al., 2017; Doherty, Forster, & Roth, 2016; Blaze, Scheuing, & Roth, 2013; Roth et al., 2009). Our data showed that dams with limited nesting resources exhibited a significantly higher proportion of aversive behaviors and a significantly lower proportion of nurturing behaviors towards pups, when compared to normal care dams. Further, our vocalization data confirmed that this altered caregiving behavior modified behavior of pups. Pups typically emit vocalizations at a 40 kHz frequency when they are experiencing stress (Portfors, 2008). Our data showed pups exposed to maltreatment dams and therefore, a higher proportion of aversive caregiver behaviors, emitted a significantly greater proportion of 40 kHz ultrasonic vocalizations compared to pups exposed to normal care dams. This finding suggests that these pups were indeed experiencing more distress.

Additionally, we were able to replicate previous findings from our lab showing that pups exposed to maltreatment during infancy will have significantly higher levels of DNA methylation at *Bdnf* exon IX in the PFC compared to pups exposed to normal care (Doherty et all, 2019; Roth et al., 2009). However, we did not find any significant differences in methylation in response to VPA, indicating that VPA was not effective in preventing maltreatment-induced methylation at *Bdnf* exon IX at any of the three doses tested. While we expected pups receiving VPA to exhibit lower levels of DNA methylation at *Bdnf* exon IX, other studies involving VPA report exon-specific effects at other exons. One study found that in a model of autism, VPA only effects expression of *Bdnf* exons I, IV, and VI in the fetal brain (Almeida, Roby, & Krueger, 2014), so a potential future direction for this project could explore whether treatment with VPA affects methylation at other *Bdnf* exons or other age points. Additionally, our global methylation data from this study suggests that VPA may have broader, more genome-wide effects, further justifying exploration of other *Bdnf* exons.

Our global methylation findings showed that in the normal care condition, pups receiving a 400 mg/kg dose of VPA had significantly lower global methylation levels compared to pups receiving saline. In the maltreatment condition, these differences in methylation were marginally significant; pups who received a 400 mg/kg dose of VPA had, on average, lower global methylation levels compared to pups who received saline ($p=.054$). These results suggest that a 400 mg/kg dose of VPA is effective in lowering global methylation in the PFC. Further, we found that treatment with 200 mg/kg and 600 mg/kg dose of VPA was not able to successfully lower global methylation levels compared to treatment with saline, suggesting a specific therapeutic window for dosage of this drug.

4.2 Treatment with VPA appears to lead to increased incidence of aversive caregiving behavior in adulthood

The second aim of this study was to investigate the behavioral effects of an effective dose of VPA. After establishing an effective dose of 400 mg/kg VPA, we put pups through our scarcity-adversity paradigm concurrent with either saline or VPA administration during infancy. We then allowed these animals to reach adulthood, bred them, and observed their caregiving behavior towards their own offspring.

Interestingly, dams who received 400 mg/kg dose of VPA in infancy exhibited a greater amount of aversive behaviors than those who received saline vehicle, independent of their own early-life environment. This suggests that there could be an optimal level of global methylation in the brain and that a transient reduction in global methylation can lead to adverse consequences. Indeed, one study found that lower levels of global methylation blocks cellular differentiation (Jackson et al., 2004), thus disturbing this delicate balance of global methylation and affecting important processes downstream that would have implications for behavior.

Additionally, a recent study from our lab similar in design to the current study investigated the effects of zebularine administration on DNA methylation patterns associated with early adversity. Findings showed that administration of zebularine normalized caregiving behavior in dams with a history of maltreatment, but instead disturbed caregiving behavior in dams with no history of maltreatment (Keller, Doherty, & Roth, 2019). These data help illustrate a causal relationship between maltreatment-induced DNA methylation and caregiving behavioral outcomes. Further, another study found that in a rodent model, exposure to mild prenatal stress resulted in *increased* global methylation levels in the PFC, but exposure to high prenatal stress led to *decreased* global methylation. These methylation changes had similar

contrasting behavioral changes as well (Mychasiuk et al., 2011). Altogether, such data highlight the complicated relationship between epigenetic changes and behavioral outcomes, and the need for further experimental studies to elucidate how specific epigenetic patterns underlie specific behavioral outcomes.

Future work on this line of research will focus on analyzing *Bdnf* exon IX and global methylation levels in PFC tissue collected from F0 dams, as well as F1 pups. Based upon our findings, this may inform further investigations into the long-term effects of VPA, including behavioral outcomes. Additional exploration of how VPA affects behavior, such as social interaction and fear conditioning, would provide valuable insight into VPA's potential as a therapy for psychological disorders, such as anxiety and depression. Further, investigating non-pharmacological interventions, such as environmental enrichment or exercise, in the context of early-life stress could inform future prevention and intervention strategies for at-risk children or those who are victims of child abuse.

4.3 Conclusion

Here we show that administration of VPA, concurrent with exposure to maltreatment in infancy, can interact with the epigenome by decreasing levels of global methylation in the PFC. Further, we show that VPA treatment in infancy can have long-term effects on future caregiving behavior, as VPA-treated pups showed increased incidence of aversive behaviors as adult caregivers. Together, data from this study brings us one step closer to understanding how both early-life experiences and alterations in the epigenome can impact behavioral outcomes.

REFERENCES

Abel, T., & Zukin, R. S. (2008). Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Current Opinion in Pharmacology, 8*(1), 57–64.

Almeida, L. E. F., Roby, C. D., & Krueger, B. K. (2014). Increased BDNF expression in fetal brain in the valproic acid model of autism. *Molecular and Cellular Neuroscience, 59*, 57–62.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors, 22*(3), 123-131.

Bird, A. (2002). Epigenetic Memory. *Genes and Development, 16*, 16–21.

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.

Boulle, F., Van Den Hove, D. L. A., Jakob, S. B., Rutten, B. P., Hamon, M., Van Os, J., Lesch, K-P, Lanfumey, L., Steinbusch, H. W., Kenis, G. (2012). Epigenetic regulation of the BDNF gene: Implications for psychiatric disorders. *Molecular Psychiatry, 17*(6), 584–596.

Burenkova, O.V., Aleksandrova, E. A., & Zarayskaya, I. Y. (2019). Effects of early life stress and HDAC inhibition on maternal behavior in mice. *Behavioral Neuroscience,* 133(1), 39-49.

Cicchetti, D., & Toth, S. (2005). Child Maltreatment. *Annual Reviews of Clinical Psychology*, 409–438.

Clayton, A. L., Hazzalin, C. A., & Mahadevan, L. C. (2006). Enhanced Histone Acetylation and Transcription: A Dynamic Perspective. *Molecular Cell, 23*(3), 289–296.

Chen, Z-Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C-J., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L., Lee, F. S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*(5796), 140–143.

Chen, D., Meng, L., Pei, F., Zheng, Y., & Leng, J. (2017). A review of DNA methylation in depression. *Journal of Clinical Neuroscience, 43*, 39–46.

Collins, N. (2020). Rescuing intergenerational transmission of aberrant DNA methylation associated with early life maltreatment. Presented at the University of Delaware's Department of Psychological and Brain Sciences Brown Bag Presentation, Newark, DE.

Connor, C. M., & Akbarian, S. (2008). DNA methylation changes in schizophrenia and bipolar disorder. *Epigenetics*, *3*(2), 55–58.

Day, J. J. (2014). New approaches to manipulates the epigenome. *Dialogues in Clinical Neuroscience*, 16(3), 345-357.

Day, J. J., & Sweatt, D. J. (2011). Epigenetic mechanisms in cognition. *Neuron*, *70*(5), 813–829.

Deans, C., & Maggert, K. A. (2015). What do you mean, "Epigenetic"? *Genetics*, *199*(4), 887–896.

Detich, N., Bovenzi, V., & Szyf, M. (2003). Valproate induces replication-independent active DNA demethylation. *Journal of Biological Chemistry*, *278*(30), 27586–27592.

Dobosy, J. R., & Selker, E. U. (2001). Emerging connections between DNA methylation and histone acetylation. *Cellular and Molecular Life Sciences*. *58*, 721–727.

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., Chajes, J. R., Reich, L., Duffy, H. B. D., & Roth, T. L. (2019). Preventing epigenetic traces of caregiver maltreatment: A role for HDAC inhibition. *International Journal of Developmental Neuroscience*, *78*(April), 178–184.

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. *Behavioral Brain Research*, *298*, 55–61.

Dong, E., Guidotti, A., Grayson, D. R., & Costa, E. (2007). Histone hyperacetylation induces demethylation of reelin and 67-kDa glutamic acid decarboxylase promoters. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(11), 4676–4681.

Duffy, K. A., McLaughlin, K. A., Green, P. A. (2018). Early life adversity and health-risk behaviors: proposed psychological and neural mechanisms. *The Annals of the New York Academy of Science, 1428*(1), 151–169.

Dupont, C., Armant, D. R., & Brenner, C. A. (2009). Epigenetics: Definition, mechanisms and clinical perspective. *Seminars in Reproductive Medicine*, *27*(5), 351–357.

Eberharter, A., & Becker, P. B. (2002). Histone acetylation: A switch between repressive and permissive chromatin. Second in review on chromatin dynamics. *EMBO Reports*, *3*(3), 224–229.

Fraser-Mustard, J. (2017). Early Brain Development and Human Development. *Encyclopedia on Early Childhood Development*, 1–4.

Fuchikami, M., Morinobu, S., Segawa, M., Okamoto, Y., Yamawaki, S., Ozaki, N., … Terao, T. (2011). DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS ONE*, *6*(8), 4–10.

Gavin, D. P., & Sharma, R. P. (2010). Histone modifications, DNA methylation, and Schizophrenia. *Neuroscience and Biobehavioral Reviews*, *34*(6), 882–888.

Gibb, B. E., Butler, A. C., & Beck, J. S. (2003). Childhood abuse, depression, and anxiety in adult psychiatric outpatients. *Depression and Anxiety*, *17*(4), 226-228.

Goldberg, A. D., Allis, C. D., & Bernstein, E. (2007). Epigenetics: A Landscape Takes Shape. *Cell*, *128*(4), 635–638.

Gräff, J., & Tsai, L.-H. (2013). The Potential of HDAC Inhibitors as Cognitive Enhancers. *Annual Review of Pharmacology and Toxicology*, *53*(1), 311–330.

Grayson, D. R., & Guidotti, A. (2013). The dynamics of DNA methylation in schizophrenia and related psychiatric disorders. *Neuropsychopharmacology*, *38*(1), 138–166.

Grayson, D. R., Kundakovia, M., & Sharma, R. P. (2010). Is There a Future for Histone Deacetylase Inhibitors in the Pharmacotherapy of Psychiatric Disorders? *Molecular Pharmacology, 77*(2), 126-135.

Heerboth, S., Lapinska, K., Snyder, N., Leary, M., Rollinson, S., & Sarkar, S. (2014). Use of epigenetic drugs in disease: An overview. *Genetics and Epigenetics, 1*(6), 9–19.

Hochberg, Z., Feil, R., Constancia, M., Fraga, M., Junien, C., Carel, J. C., Boileau, Y., Le Bouc, C. L., Deal, K., Lillycrop, R, Scharfmann, A., Sheppard, M., Skinner, M., Szyf, R., Waterland, A., Waxman, D. J., Whitelaw, E., Ong, K., Albertsson-Wikland, K. (2011). Child health, developmental plasticity, and epigenetic programming. *Endocrine Reviews*, *32*(2), 159–224.

Hui Ng, H., & Bird, A. (2000). Histone deacetylases: Silencers for hire. *Trends in Biochemical Sciences*, *25*(3), 121–126.

Ivy, A. S., Brunson, K. L., Sandman, C., & Baram, T. Z. (2008). Dysfunctional Nurturing Behavior in Rat Dams with Limited Access to Nesting Material: A Clinically Relevant Model for Early-Life Stress. *Neuroscience*, *154*(3), 1132–1142.

Jackson, M., Krassowska, A., Gilbert, N., Chevassut, T., Forrester, L., Ansell, J., & Ramsahoye, B. (2004). Severe Global DNA Hypomethylation Blocks Differentiation and Induces Histone Hyperacetylation in Embryonic Stem Cells. *Molecular and Cellular Biology*, *24*(20), 8862–8871.

Johnson, J. G., Cohen, P., Brown, J., Smailes, E. M., & Bernstein, D. P. (2009). Childhood maltreatment increases risk for personality disorders during early adulthood. *Archives of General Psychiatry*, *56*, 600-606.

Kao, G. S., Cheng, L.Y., Chen, L. H., Tzeng, W. Y., Cherng, C. G., Su, C. C., Wang, C. Y., Yu, L. (2012). Neonatal isolation decreases cues fear conditioning and frontal cortical histone 3 lysine 9 methylation in adult female rats. *European Journal of Pharmacology*, 697(1-3), 65-72.

Keller, S. M., Doherty, T. S., & Roth, T. L. (2018). Pharmacological manipulation of DNA methylation in adult female rats normalizes behavioral consequences of early-life maltreatment. *Frontiers in Behavioral Neuroscience*, *12*(June), 1–9.

Keller, S. M., Doherty, T. S., & Roth, T. L. (2019). Pharmacological manipulation of DNA methylation normalizes maternal behavior, DNA methylation, and gene expression in dams with a history of maltreatment. *Scientific Reports*, *9*(1), 1–13.

Kuan, P. F., Waszczuk, M. A., Kotov, R., Marsit, C. J., Guffanti, G., Gonzalez, A., Yang, X., Koenen, K., Bromet, E., Luft, B. J. (2017). An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Translational Psychiatry*, *7*(6), e1158.

Kundakovic, M., & Champagne, F. A. (2015). Early-life experience, Epigenetics, and the developing brain. *Neuropsychopharmacology*, *40*(1), 141–153.

Kuzumaki, N., Ikegami, D., Tamura, R., Hareyama, N., Imai, S., Narita, M., Torigoe, K., Niikura, K., Takeshima, H., Ando, T., Igarashi, K., Kanno, J., Ushijima, T., Suzuki, T., Narita, M. (2011). Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment. *Hippocampus*, *21*(2), 127–132.

Lattal, K. M., Barrett, R. M., & Wood, M. A. (2007). Systemic or Intrahippocampal Delivery of Histone Deacetylase Inhibitors Facilitates Fear Extinction. *Behavioral Neuroscience*, *121*(5), 1125–1131.

Lee, V., & Hoaken, P. N. S. (2007). Cognition, emotion, and neurobiological development: Mediating the relation between maltreatment and aggression. *Child Maltreatment*, *12*(3), 281–298.

Lester, B. M., Tronick, E., Nestler, E., Abel, T., Kosofsky, B., Kuzawa, C. W., Marsit, C. J., Maze, I., Meaney, M. J., Monteggia, L. M., Reul, J. M. H. M., Skuse, D. H., Sweatt, J. D., Wood, M. A. (2011). Behavioral epigenetics. *Annals of the New York Academy of Sciences*, *1226*(1), 14–33.

Lewis, C. R., & Olive, M. F. (2014). Early life stress interactions with the epigenome: potential mechanisms driving vulnerability towards psychiatric illness. *Behavioral Pharmacology*. *25*(506), 341–351.

Li, M., D'Arcy, C., & Meng, X. (2016). Maltreatment in childhood substantially increases the risk of adult depression and anxiety in prospective cohort studies: Systematic review, meta-analysis, and proportional attributable fractions. *Psychological Medicine*, *46*(4), 717–730.

Loman, M. M., & Gunnar, M. R. (2010). Early experience and the development of stress reactivity and regulation in children. *Neuroscience and Biobehavioral Reviews*, *34*(6), 867–876.

Lubin, F. D., Roth, T. L., & Sweatt, D. J. (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. J., Morley-Fletcher, S., Szyf, M., & Brunton, P. J. (2014). The consequences of early-life adversity: Neurobiological, behavioural and epigenetic adaptations. *Journal of Neuroendocrinology*, *26*(10), 707–723.

Machado-Vieira, R., Ibrahim, L., & Zarate, C. A. (2011). Histone Deacetylases and Mood Disorders: Epigenetic Programming in Gene-Environment Interactions. *CNS Neuroscience and Therapeutics*, *17*(6), 699–704.

Marias, L., Stein, D., Daniels, W.M.U. (2009). Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. *Metabolic Brain Disease, 24*, 587–597.

McGowan, P. O., Szyf, M. (2010). The epigenetics of social adversity in early life: Implications for mental health outcomes. *Neurobiology of Disease, 39*, 66-72.

Miller, C. A., Campbell, S. L., & Sweatt, J. D. (2008). DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. *Neurobiology of Learning and Memory*, *89*(4), 599–603.

Milutinovic, S., Detich, N., & Szyf, M. (2007). Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. *Carcinogenesis*, *28*(3), 560–571.

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Frontiers in Cellular Neuroscience*, *13*(August), 1–25.

Monti, B., Polazzi, E., & Contestabile, A. (2010). Biochemical, Molecular and Epigenetic Mechanisms of Valproic Acid Neuroprotection. *Current Molecular Pharmacology*, *2*(1), 95–109.

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology*, *38*(1), 23–38.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., Holsboer, F., Wotjak, C. T., Almeida, O. F. X., Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature Neuroscience*, *12*(12), 1559–1566.

Murgatroyd, C., & Spengler, D. (2011). Epigenetics of early child development. *Frontiers in Psychiatry*, *2*(APR), 1–15.

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.

Nestler, E. J., Peña, C. J., Kundakovic, M., Mitchell, A., & Akbarian, S. (2016). Epigenetic Basis of Mental Illness. *Neuroscientist*, *22*(5), 447–463.

Phillips, N. L. H., & Roth, T. L. (2019). Animal models and their contribution to our understanding of the relationship between environments, epigenetic modifications, and behavior. *Genes*, *10*(1).

Portfors, C. V. (2007). Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science*, *46*(1), 28–34.

Romens, S. E., Mcdonald, J., Svaren, J., & Pollak, S. D. (2015). Associations Between Early Life Stress and Gene Methylation in Children. *Child Development*, *86*(1), 303–309.

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, 760-769.

Roth, T. L., & Sweatt, J. D. (2012). Epigenetic marking of the BDNF gene by early-life adverse experiences. *Hormonal Behavior*. *59*(3), 315–320.

Roth, T. L., Zoladz, P. R., Sweatt, J. D., & Diamond, D. M. (2011). Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. *Journal of Psychiatric Research*, *45*(7), 919–926.

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

Szyf, M. (2009). Epigenetics, DNA Methylation, and Chromatin Modifying Drugs. *Annual Review of Pharmacology and Toxicology. 49*, 243–265.

Szyf, M., Weaver, I., & Meaney, M. (2007). Maternal care, the epigenome and phenotypic differences in behavior. *Reproductive Toxicology*, *24*(1), 9–19.

Tarantola, D. (2018). Child maltreatment: Daunting and universally prevalent. *American Journal of Public Health*, *108*(9), 1119–1120.

Vaissière, T., Sawan, C., & Herceg, Z. (2008). Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutation Research - Reviews in Mutation Research*, *659*(1–2), 40–48.

Weaver, I. C. G. (2014). Integrating early life experience, gene expression, brain development, and emergent phenotypes: Unraveling the thread of nature via nurture. In *Advances in Genetics* (Vol. 86).

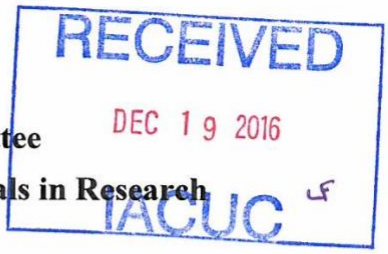
Weinhold, B. (2006). Epigenetics: the science of change. *Environmental Health Perspectives.*, *114*(3), 160–167.

Whipple, E. E., & Webster-Stratton, C. (1991). The role of parental stress in physically abusive families. *Child Abuse and Neglect*, *15*(3), 279–291.

Zhang, T. Y., Keown, C. L., Wen, X., Li, J., Vousden, D. A., Anacker, C., Bhattacharyya, U., Ryan, R., Diorio, J., O'Toole, N., Lerch, J. P., Mukamel, E. A., Meaney, M. J. (2018). Environmental enrichment increases transcriptional and epigenetic differentiation between mouse dorsal and ventral dentate gyrus. *Nature Communications*, *9*(1), 1–11.

Appendix A

UNIVERSITY OF DELAWARE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



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: Glen Tellis, DVM

Date of Approval: 1/30/2017

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University of Delaware
Institutional Animal Care and Use Committee
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: 1216-2020-0	← **(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: 11-18-19	

Official Use Only

IACUC Approval Signature: [signature], DVM

Date of Approval: 2.1.20