

**THE EFFECTS OF SODIUM BUTYRATE (NaB) AND MALTREATMENT EARLY IN
POSTNATAL DEVELOPMENT ON DNA METHYLATION IN THE HIPPOCAMPUS AND
AMYGDALA OF ADULT MALE RATS**

by

Isabella Archer

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Master of Science in Neuroscience

Spring 2019

© 2019 Isabella Archer
All Rights Reserved

**THE EFFECTS OF SODIUM BUTYRATE (NaB) AND MALTREATMENT EARLY IN
POSTNATAL DEVELOPMENT ON DNA METHYLATION IN THE HIPPOCAMPUS AND
AMYGDALA OF ADULT MALE RATS**

by

Isabella Archer

Approved: _____
Tania Roth, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

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

Approved: _____
John Pelesko, Ph.D.
Interim Dean of the College of Arts and Sciences

Approved: _____
Douglas J. Doren, Ph.D.
Interim Vice Provost for Graduate and Professional Education

ACKNOWLEDGMENTS

First and foremost, I would like to thank Almighty God for making this possible for me.

I would like to thank Dr. Tania Roth for giving me such a great opportunity and being an ideal mentor to me. From the day I joined your class in Fall 2016, you have been very supportive, and you had so much faith in me. Your interest in student success was what motivated me to want to be a part of your lab and I must say, that is the best decision I have ever made. I had the opportunity to know you more and see what an incredible human being you are. Unbeknownst to you, whenever I doubted myself, you gave me the self-confidence I needed. Through this year, you have been such a great mentor, and dealt with me in a such a way that no one has ever before. I couldn't have asked for anyone to be my supervisor other than you.

Next, I would like to thank my committee members, Dr. Eric Roth and Dr. Jeffery Rosen. I am so glad you both agreed to be on my committee and mentor me. I really appreciate all the time and commitment you have invested in me and I am glad to have met awesome professors like yourselves. I would also like to thank the Psychology and Brain sciences administration, especially Dawn Butler and Shirley Wilson for dealing with my numerous questions. To the staff, I would like to say thank you for all the support.

Next, I would like to thank all the graduate students in the department for welcoming me and making feel at home and giving me a lot of help. I would like to thank the Roth lab, for dealing with me and always offering help when needed. I would especially like to thank Samantha Keller, for guiding throughout my undergraduate years and Tiffany Doherty for guiding me through my quest to achieve my masters.

I would like to thank and acknowledge the funding that supported this research from The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD; 1R01HD087509).

Finally, I would like to thank my family and friends for all the support given to me. This would not have been possible if I didn't have really supportive family and friends. Believing in me alone was enough to get me through and you all did more than that.

I am forever grateful to everyone who journeyed with me. Your efforts will never be forgotten.

TABLE OF CONTENT

LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	viii
Chapter	
1 INTRODUCTION	1
2 METHODS	4
Caregiving Manipulations	4
Drug Preparation and Administration	5
Methylation Assays on Adult Brain Tissue	5
Statistical Analysis	6
3 RESULTS	7
Caregiving Behaviors and Infant Responses	7
Methylation Patterns in Amygdala	9
Methylation Patterns in Dorsal Hippocampus	11
Methylation Patterns in Ventral Hippocampus	13
4 DISCUSSION	15
REFERENCES	19
Appendix	
A IACUC APPROVAL	22

LIST OF TABLES

Table 1 Average percent occurrence of individual caregiving behaviors directed to
 infants across the 7 days 8

LIST OF FIGURES

Figure 1	Overall maternal caregiving behaviors (A) and ultrasonic pup vocalization (B)	8
Figure 2	Average percent methylation of <i>bdnf</i> I (A), IV (B), and IX (C) DNA in the adult amygdala	10
Figure 3	Average percent methylation of <i>bdnf</i> I (A), IV (B), and IX (C) DNA in the adult dorsal hippocampus	12
Figure 4	Average percent methylation of <i>bdnf</i> I (A), IV (B), and IX (C) DNA in the adult ventral hippocampus	14

ABSTRACT

Early life stress, especially childhood maltreatment, increases the risk for cognitive dysfunction and mental problems later in life. Studies have sought to identify how these phenotypes come about by examining structural and functional changes in various brain regions. Recent work has also focused on identifying whether changes in DNA methylation, an epigenetic modification that helps regulate gene transcription important for the structural and functional capacity of the brain, is also responsible for phenotypic outcomes. Using a rat model, we investigated whether maltreatment leaves long-term epigenetic modifications on DNA associated with the *brain-derived neurotrophic factor (bdnf)* gene in the adult amygdala and hippocampus and whether administering an epigenome modifying drug (sodium butyrate, NaB) at the time of maltreatment prevents these epigenetic modifications. We focused on the *bdnf* gene as it is critical for structural and functional capacity of the brain and known to be sensitive to early life stress. During first post-natal week, infant male rats were either administered NaB or a vehicle solution and exposed to either maltreatment or nurturing maternal care. We then assessed DNA methylation after pups reached adulthood. We replicate observations we made in a previous report that exposure to maltreatment alters *bdnf* DNA methylation in the amygdala and hippocampus. We also find concurrent administration of NaB with maltreatment capable of changing patterns of methylation. These results provide further empirical support that DNA methylation modifications are biological consequences of experiences within the caregiving environment and highlight the ability to potentially intervene and change epigenetic trajectories.

Chapter 1

INTRODUCTION

Early life stress, especially childhood maltreatment, increases the risk for maladaptive behavioral outcomes, including cognitive dysfunction and psychiatric disorder (Lansford et al, 2002; McCrory et al, 2011). For example, studies have shown that exposure to trauma during early development increases the risk for post-traumatic stress disorder (PTSD) (Breslau et al, 1999) and often renders individuals with heightened stress responsivity (Silberman et al, 2016). Over the years, as one way to envision how these phenotypes arise studies have often examined underlying structural and functional changes in various brain regions. Early stress is often associated with increases in amygdala and decreases in hippocampal volumes (McCrory et al, 2011; McEwen 1999). There are also functional changes, such that in the amygdala by way of example, humans and non-human animals have heightened stress reactivity and neural responses to threat (Fareri and Tottenham, 2016; Tottenham and Sheridan, 2010).

The early caregiving environment may produce structural and functional changes in the amygdala and hippocampus through changes in DNA methylation, an epigenetic process through which methyl groups are added to cytosines (McGowan and Roth 2015). For the *bdnf* gene, increased DNA methylation is typically associated with decreased *bdnf* gene expression (Blaze and Roth, 2013; Roth et al, 2009). Epidemiological studies have begun to show DNA methylation changes in the human

genome reflective of early life experiences. For example, a study looking at epigenetic regulation of the glucocorticoid receptor gene (*NR3C1*) in postmortem human hippocampal tissue shows a link between childhood abuse, decreased levels of glucocorticoid receptor mRNA, and increased methylation of DNA associated with a promoter region of the gene (McGowan et al, 2009). Further, another study shows a relationship between childhood maltreatment and higher *bdnf* methylation present in blood (Perroud et al, 2013).

As there are often confounds in human studies making it difficult to confidently associate maltreatment with an epigenetic and behavioral outcome, here we utilized a rodent model to better be able to link the experience of maltreatment with an epigenetic change in the brain. The model used is a variation used in the Baram (Ivy et al, 2008) and Sullivan (Roth & Sullivan, 2005) laboratories which exploits the idea that, deprivation of resource can produce aberrant caregiving behaviors which in turn can affect neural mechanisms and behavior of offspring (Roth et al, 2014). Previous findings using this model have shown DNA methylation modifications in the amygdala and hippocampus in the candidate gene *bdnf* (Roth et al, 2014). The *bdnf* gene is a gene that codes for a protein involved in neural plasticity and neurogenesis and is involved in life-long cognitive and emotional processes (Nina, 2014; Wei et al, 2014) and experiences epigenetic changes due to early life stress (Kordi-Tamandani et al 2012; Roth et al 2009; Roth et al 2014). Furthermore, there has been associations between aberrant methylation of the *bdnf* gene and various psychiatric disorders associated with early life

adversity (Kang et al, 2013; Keller et al, 2010; Kordi-Tamandani et al 2012; Perroud et al, 2013).

Here we look to replicate methylation observations in our previous study (Roth et al 2014) and to extend these by looking at whether NaB can prevent methylation associated with maltreatment. NaB is a histone deacetylase inhibitor that can decrease DNA methylation (Sarker et al, 2011; Wei et al, 2014). NaB is known to down-regulate DNA methyltransferase 1 (DNMT1), an enzyme that catalyzes DNA methylation (Sarker et al, 2011). In a study done by Wei et al (2014) in adult rats, NaB produced both functional methylation changes in the *bdnf* gene and behavior (antidepressant-like behaviors). Developmental work is just beginning to show that histone deacetylase inhibitors may be useful in preventing epigenetic alterations if administered to animals prior to adverse experiences (Burenkova, Aleksandrova, & Zarayskaya, 2019; Kao et al., 2012; A. Sarkar et al., 2014). This gives investigators a unique way to determine whether preventing an epigenetic change prevents an aberrant behavioral outcome. Here we therefore seek to investigate whether NaB delivered at the time of maltreatment prevents methylation we normally see in maltreated-animals when adult.

Chapter 2

METHODS

Caregiving Manipulations

Long-Evan rat mothers and pups (24 litter in total, 9-12 pups per litter) were used for this experiment. Rats and pups were housed in polypropylene cages with ample bedding and *ad libitum* access to food and water in a light- and temperature-controlled room (12-hour light/dark cycle with lights on at 6:00 am). All experimental procedures were done during the light cycle. Dams were bred in the lab and allowed to have one litter before they were used in experimental paradigm, alleviating potential confounds associated with being a first-time mother. On postnatal day (PN) 1, infant Long-Evan male pups were either assigned to spend time with a stressed dam with limited nesting material in a novel environment (maltreatment condition, MAL) or to remain in their home cage to receive nurturing care (normal care condition, NMC). Pups in the maltreatment condition were exposed to their caregiving condition for 30 minutes daily from PN1-7 (e.g. Roth et al, 2009; Roth et al, 2014). Both conditions were video-taped, and a subset of recordings (randomly chosen) were later assessed to observe caregiving behavior. Both conditions were also audio recorded for 40 kHz ultrasonic vocalizations (distress measures) and a subset of recordings were listened to at a later time.

Pups were then left undisturbed (aside from weekly cage changes) in the home cage until weaning (PN21-23), at which time they were pair-housed with a subject from the same group and allowed to mature to PN90. Rats were anesthetized and brains were

harvested and stored at -80°C until processing. All procedures were approved by the University of Delaware Animal Care and Use Committee prior to beginning the experiment.

Drug Preparation and Administration

Prior to caregiving manipulations, NaB (400 mg/kg) was dissolved in a 5% sucrose solution and delivered orally to pups with a pipette (vehicle animals were given only the 5% sucrose solution in ultra-pure water), based on their current weight.

Methylation Assays on Adult Brain Tissue

Brains were sliced with a cryostat at 100 microns and then dissected on dry ice using stereotaxic coordinates (George and Watson, 2007) to retrieve the dorsal hippocampus, ventral hippocampus, and amygdala (central and basolateral nuclei were extracted together to yield sufficient DNA for downstream analyses). DNA was then extracted from these brain regions (Qiagen AllPrep DNA kit), bisulfite converted (Qiagen EpiTect Bisulfite), and underwent direct bisulfite sequencing (BSP) as previously described to measure DNA methylation levels (Parrish et al 2012; Roth et al 2014). Bisulfite sequencing allows for the estimation of methylation at individual cytosine sites (Parrish et al 2012). Bisulfite-treated samples were amplified by primer sets targeting *bdnf* exons I, IV, and IX as used in our previous study (Roth et al 2014). BSP samples were sent to University of Delaware DNA Sequencing and Genotyping Center and sequenced using reverse primers. Average methylation levels across a targeted *bdnf*

region were determined using Chromas software as previously described (Parrish et al 2012; Roth et al 2009, 2014).

Statistical Analysis

Overall type of caregiving behavior (aversive or nurturing) for the two infant conditions (MAL and NMC) was analyzed using a two-way ANOVA with Tukey-Kramer post-hoc tests. Individual types of caregiving behaviors (i.e. step on, drop, nurse) and pup ultrasonic vocalizations were analyzed using unpaired t-tests. BSP data for each exon (averaged across individual cytosine-guanine [CG] dinucleotide sites, as there were no site-specific changes responsible for methylation changes previously reported, Roth et al 2014) and brain region were analyzed using two-way ANOVAs and Tukey-Kramer post-hoc tests when appropriate. For all analyses, differences were said to be statistically different when $p \leq 0.05$. For comparison purposes to previous research, non-significant trends at $p < 0.1$ are also reported.

Chapter 3

RESULTS

Caregiving Behaviors and Infant Responses

Consistent with our previous reports (e.g. Roth et al, 2009; 2014), overall caregiving behaviors (Fig. 1A) show that there is a main effect of type of behavior displayed [$F(1, 16) = 49.31, p < 0.001$] and an interaction of type of behavior and infant condition, [$F(1, 16) = 73.3, p < 0.001$] such that the maltreatment condition is less nurturing and more aversive than the normal maternal condition (multiple comparisons tests, all p 's < 0.001). Within the maltreatment condition (Table 1), dams exhibited higher proportions of specific aversive caregiving behaviors (pup dropping, actively avoiding, and rough handling) and less nurturing caregiving behaviors (pup licking/grooming and crouching over/nursing). Pups also responded differently to the caregiving conditions (Fig. 1B), as pups emitted significantly more ultrasonic vocalizations in the maltreatment condition ($t_8 = 4.65, df=8, p < 0.01$).



Figure 1 Overall maternal caregiving behaviors (A) and ultrasonic pup vocalizations (B) averaged across the 7-day paradigm. *** p 's < 0.001 for MAL aversive versus NMC aversive and MAL nurturing versus NMC nurturing; *** p < 0.001 for NMC aversive versus NMC nurturing in panel A. ** p < 0.01 MAL versus NMC in panel B. n = 5 litters/group; error bars = SEM; NMC = normal maternal care, MAL = maltreatment.

Table 1 Average percent occurrence of individual caregiving behaviors directed toward infants across the 7 days.

	Normal	Maltreatment
Adverse caregiving behaviors		
Step on	4.3%	1.9%
Drop ($t_8 = 2.48$)	2.7%*	7.5%
Drag	4.9%	5.2%
Avoid ($t_8 = 4.32$)	1.3%**	9.7%
Roughly handle ($t_8 = 5.48$)	8.7%***	24.2%
Nurturing caregiving behaviors		
Lick/groom ($t_8 = 2.78$)	34.9%*	21.5%
Hover/nurse ($t_8 = 2.65$)	43.1%*	30.0%

* p < 0.05, ** p < 0.01, *** p < 0.001 normal versus maltreatment

Methylation Patterns in Amygdala

DNA methylation patterns were measured to assess the effects of the two caregiving environments and/or NaB. A two-way ANOVA showed there was a main effect of infant condition (Fig. 2A) such that maltreatment produced significantly less methylation [$F(1, 45) = 4.72, p=0.04$] of *bdnf* exon I. There was no main effect of drug [$F(1, 45) = 1.48, p=0.23$] nor an infant condition x drug interaction [$F(1, 45) = 0.13, p=0.72$] on methylation of *bdnf* exon I.

A two-way ANOVA showed no infant condition x drug interaction [$F(1, 33) = 0.001, p=0.97$], nor main effect of infant condition [$F(1, 33) = 0.29, p=0.59$] or drug [$F(1, 33) = 1.72, p=0.2$] on methylation of *bdnf* exon IV within the amygdala (Fig. 2B). Even though there was a not significant effect of infant condition on amygdala *bdnf* IX DNA methylation (Fig. 2C), there was a non-significant trend of increased methylation with exposure to maltreatment [$F(1, 39) = 3.76, p = 0.06$]. There was no main effect of drug [$F(1, 39) = 0.38, p=0.54$] nor an infant condition x drug interaction [$F(1, 39) = 1.93, p=0.17$] on *bdnf* IX methylation.

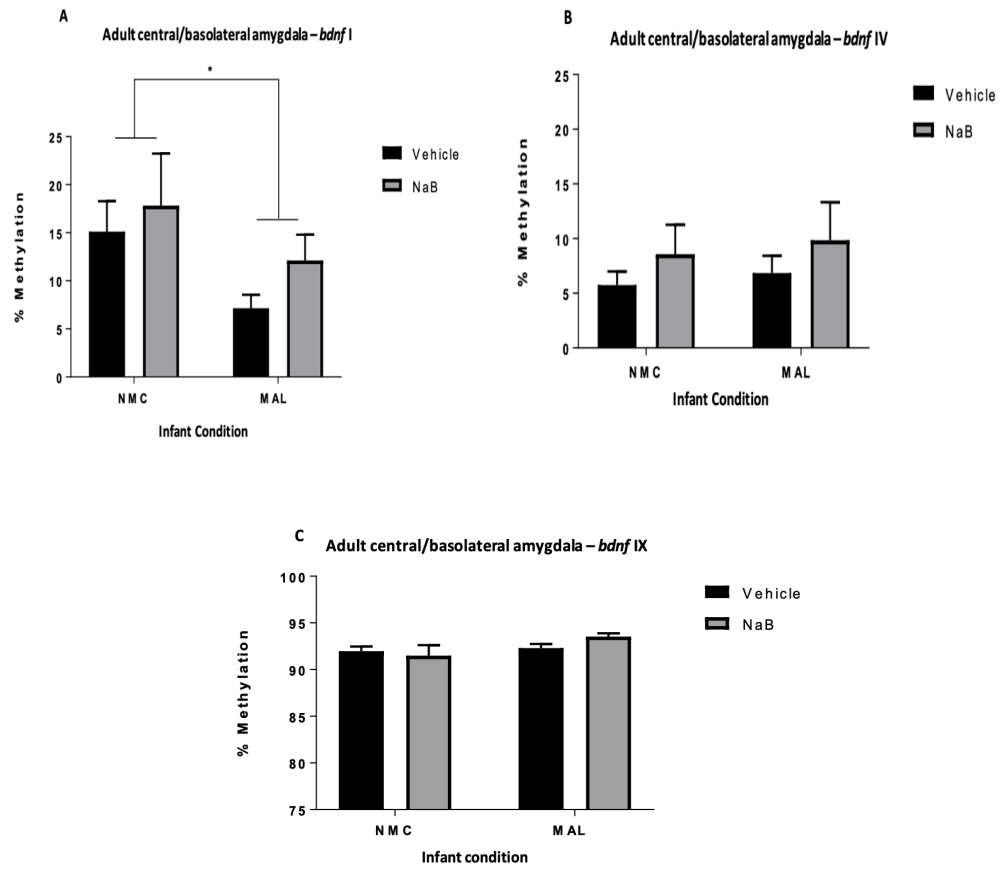


Figure 2 Average percent methylation of *bdnf I* (A), *IV* (B), and *IX* (C) DNA in the adult amygdala. Data presented are an estimation of average methylation levels across the 10 (*bdnf I*), 12 (*bdnf IV*), and 11 (*bdnf IX*) CG sites amplified. * $p < 0.05$ for NMC versus MAL in panel A. $n = 10-14$ /group; error bars = SEM; NMC = normal

Methylation Patterns in Dorsal Hippocampus

In the dorsal hippocampus, using a two-way ANOVA, a non-significant trend [$F(1, 43) = 3.73, p = 0.06$] suggests a decrease in methylation in *bdnf* I with exposure to maltreatment (Fig. 3A). There was no main effect of drug [$F(1, 43) = 0.13, p=0.72$] nor an infant condition x drug interaction [$F(1, 43) = 0.12, p=0.73$] on methylation of exon I. Likewise, a two-way ANOVA on dorsal hippocampus *bdnf* IV methylation showed a non-significant trend of maltreatment on methylation [$F(1, 38) = 2.69, p = 0.1$] (Fig. 3B). There was no main effect of drug [$F(1, 38) = 0.07, p=0.8$] nor an interaction between infant condition and drug [$F(1, 38) = 0.14, p=0.71$].

A two-way ANOVA showed a significant main effect of infant condition, with maltreatment increasing methylation of *bdnf* IX [$F(1, 45) = 21.46, p < 0.001$]. Further, it also showed a significant main effect of drug [$F(1, 45) = 5.46, p=0.02$] as well as an infant-condition x drug interaction [$F(1, 45) = 39.76, p < 0.001$] on IX methylation (Fig. 3C). Tukey post-hoc tests revealed significantly less methylation in maltreated-subjects that received NaB than vehicle counterparts ($p=0.02$), though their methylation was still higher than vehicle-treated controls ($p<0.001$). There was more methylation in controls that received NaB than their vehicle counterparts ($p < 0.001$), reaching similar levels as present in the maltreated-vehicle subjects ($p=0.41$ versus MAL vehicle and $p=0.65$ versus MAL NaB).

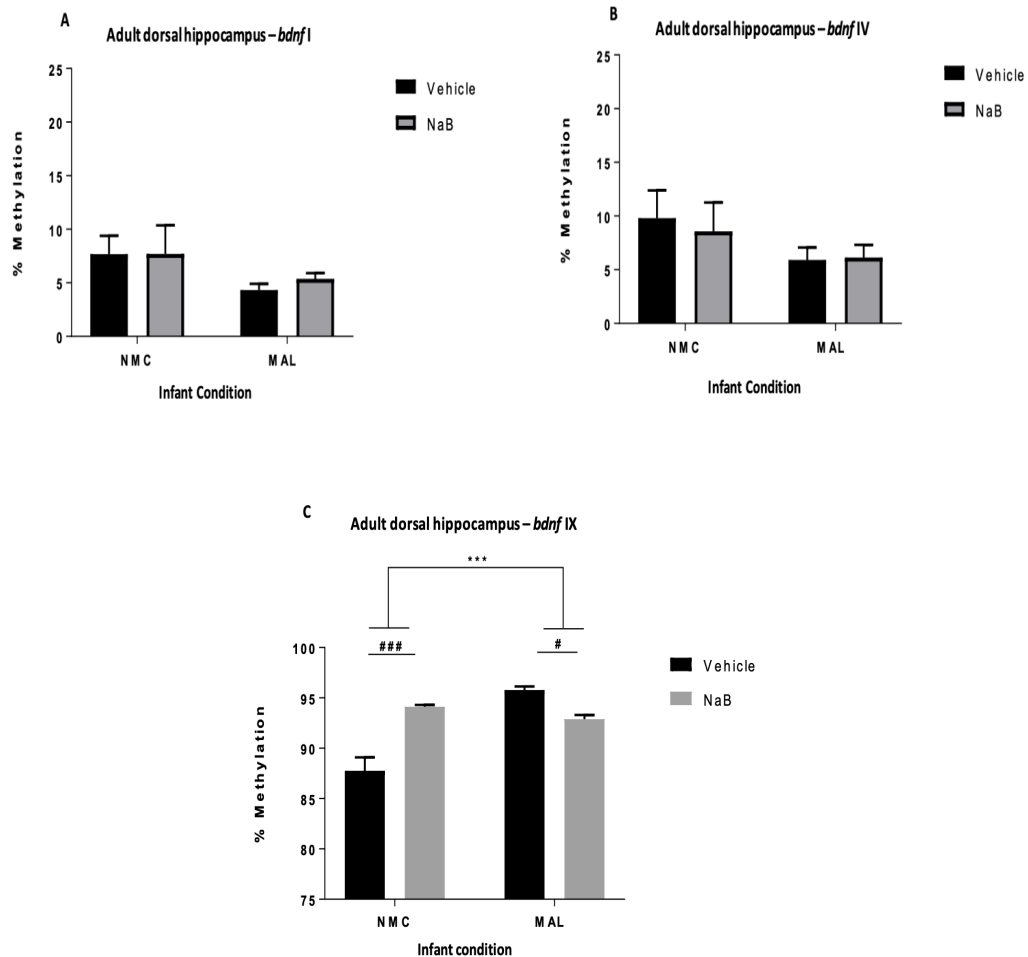


Figure 3 Average percent methylation of *bdnf I* (A), *IV* (B), and *IX* (C) DNA methylation in the dorsal hippocampus. Data presented are estimates of average methylation levels across the 10 (*bdnf I*), 12 (*bdnf IV*), and 11 (*bdnf IX*) CG sites. *** $p < 0.001$ for NMC versus MAL, ### $p < 0.001$ for NMC vehicle versus NMC NaB, and # $p < 0.05$ for MAL vehicle versus MAL NaB in panel C. $n = 10-14$ /group; error bars = SEM; NMC = normal maternal care, MAL = maltreatment; NaB = sodium butyrate.

Methylation Patterns in Ventral Hippocampus

Using a two-way ANOVA, there was a significant main effect of infant condition on ventral hippocampal *bdnf* I, with maltreatment increasing methylation [$F(1, 43) = 5.78, p=0.02$] (Fig.4A). There was also a significant main effect of NaB [$F(1, 43) = 6.36, p=0.02$] such that there was less methylation levels in subjects from both conditions that received NaB. There was no infant condition x drug interaction [$F(1, 43) = 2.08, p=0.16$]

A two-way ANOVA for *bdnf* IV methylation within the ventral hippocampus (Fig. 4B) showed a main effect of infant condition [$F(1, 35) = 5.52, p=0.02$], no main effect of drug [$F(1, 35) = 1.54, p=0.22$], but an infant x drug interaction [$F(1, 35) = 6.65, p=0.01$]. Tukey post-hocs showed NaB lowered methylation in maltreated subjects ($p=0.02$ versus vehicle counterparts). A two-way ANOVA for *bdnf* IX methylation within the ventral hippocampus (Fig. 4C) showed an infant x drug interaction [$F(1, 43) = 17.55, p < 0.001$], with no main effects of infant condition [$F(1, 43) = 0.70, p=0.41$] or drug [$F(1, 43) = 0.58, p=0.45$]. Maltreated-vehicle subjects had significantly higher methylation than vehicle-treated controls ($p=0.003$) and maltreated subjects given NaB ($p=0.004$).

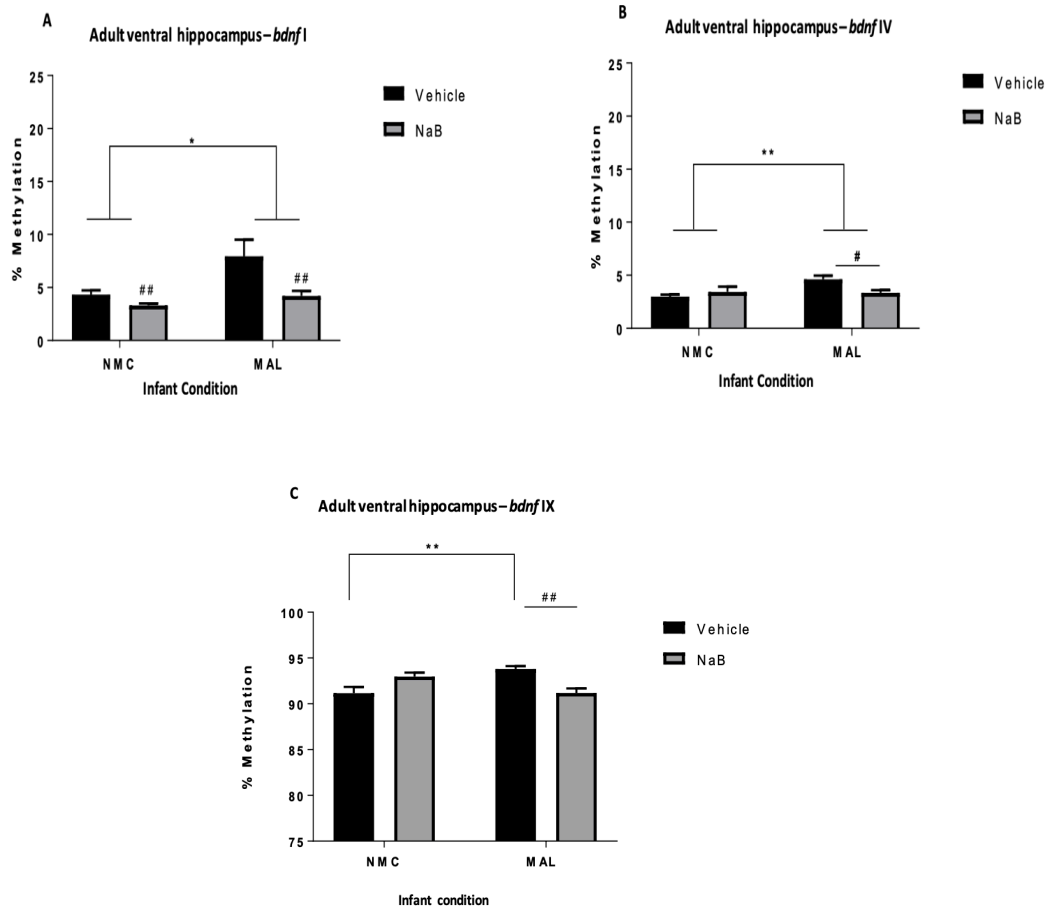


Figure 4 Average percent methylation of *bdnfI* (A), IV (B), IX (C) DNA in the adult ventral hippocampus. Data presented are average methylation across the 10 (*bdnfI*), 12 (*bdnfIV*), and 11 (*bdnfIX*) CG sites. * $p < 0.05$ for NMC versus MAL and ^{##} $p < 0.01$ for NaB versus vehicle in panel A. ^{**} $p < 0.01$ for NMC versus MAL and [#] $p < 0.05$ for MAL vehicle versus MAL NaB in panel B. ^{**} $p < 0.01$ in NMC vehicle versus MAL vehicle and [#] $p < 0.01$ MAL vehicle versus MAL NaB. $n = 10-14$ /group; error bars = SEM; NMC = normal maternal care, MAL = maltreatment; NaB = sodium butyrate.

Chapter 4

DISCUSSION

In this thesis, we used a rat model in which rats were exposed at infancy to maltreatment from a caregiver after being administered either NaB or a vehicle solution. This was done to explore the hypothesis that epigenetic modulations such as DNA methylation are affected by maltreatment. Additionally, we wanted to explore the hypothesis that administering NaB prevents the maltreatment effect on DNA methylation.

Observations of caregiving behaviors during sessions showed that infants in the maltreatment condition experienced higher aversive behaviors, including being dropped, actively avoided, and roughly handled. Infants in the normal care condition were subjected to higher amounts of nurturing behaviors, which included more crouching/nursing, and pup licking/grooming. Ultrasonic vocalizations suggest that pups in the maltreatment condition experienced more adversity, as they had more vocalization emissions. Overall, these findings are in line with those initially reported with this model (e.g. Roth et al, 2009; Roth et al 2014). In our lab, we usually include a third set of caregiver manipulations (cross-foster care); we decided not to include that set of caregiver manipulation in our study to decrease the amount of animals needed for this project because previous studies rarely show a significant difference between normal maternal care and cross-foster care (Roth et al 2014).

Our biological findings significant to maltreatment revealed that there is lower methylation (*bdnf* exon I) in the amygdala and higher methylation (*bdnf* I and exon IV) in

the ventral hippocampus of adult male rats. In one of our recent studies, we likewise found lower methylation at exon I in the amygdala and higher methylation at exon IV in the ventral hippocampus and (Roth et al, 2014). Findings with non-significant trends suggested lower methylation in *bdnf* exons I and IV in the dorsal hippocampus; significantly lower methylation for these loci were found in our earlier study (Roth et al, 2014). In our previous report there was a significant decrease in methylation in *bdnf* exon IV within the amygdala associated with maltreatment, but here we did not see this effect. Further, there was significantly higher methylation at *bdnf* exon I in the ventral hippocampus in the cohort in the current study, an effect not observed in our previous report (Roth et al, 2014).

In our previous report, we did not examine methylation at *bdnf* exon IX. Here, our findings showed a non-significant trend such that maltreated-subjects had higher methylation in their amygdala than their normal maternal care counterparts. In the dorsal and ventral hippocampus, there was higher methylation in *bdnf* exon IX with exposure to maltreatment. Altogether, these findings are in agreement with other reports where environmental experiences can produce complex DNA methylation patterns that vary across the *bdnf* gene and brain regions (Kundakovic et al., 2013; Roth et al 2014).

Administering NaB lowered methylation patterns in *bdnf* exons I and IV in the ventral hippocampus to the extent that methylation patterns were decreased to control levels. With administration of NaB, methylation of *bdnf* exon IX in both the dorsal and ventral hippocampus were decreased in maltreated subjects, though methylation in the

dorsal hippocampus did not reach control levels. Interestingly, we also found that NaB increased methylation levels in the normal care condition in *bdnf* IX in the dorsal hippocampus. These results provide further empirical support for the ability of NaB to affect methylation patterns of the *bdnf* gene (Wei et al, 2014). These findings also show that NaB can produce effects that vary across gene loci and brain regions (Stertz et al, 2013) which can also differ based upon infant experience.

There were several limitations in our current study. These include a low sample size that likely limited statistical power for some effects. In our previous study (Roth et al 2014) subjects did not receive any type of stimulation whereas here subjects experienced daily handling for drug delivery and then drug delivery itself. Such variables may have influenced methylation, thus producing some differences in methylation observations between the two studies. Here we did not assess mRNA levels which can be very useful in interpreting the functional consequences of DNA methylation. Studies however continue to associate DNA methylation with gene expression, showing correlations at base line levels of gene expression (i.e. in the absence of any stimulus) as well as in when there are transcriptional responses following presentation of stimuli (Baker-Anderson et al, 2013; Charour et al, 2008). Whole-genome and candidate gene approaches both help reveal causal relationships between the quality of caregiving and DNA methylation (Jensen & Champagne, 2012; Roth, 2012; Szyf & Bick, 2013); but whole-genome approaches provide more detail in relationships regarding gene networks, which may help us better link maltreatment-induced changes in DNA methylation to behavior.

In conclusion, the data collected as part of this thesis highlight the effect of early adverse experiences on amygdala and hippocampal DNA methylation. In addition, the NaB data hint at the possibility of epigenetic interventions to prevent maltreatment-induced epigenetic alterations, which will be a useful strategy in future research to determine if preventing the epigenetic changes likewise prevents the appearance of maladaptive outcomes typically seen with maltreatment in this model. Altogether, this work can help better establish a causal relationship between maltreatment, DNA methylation, and behavioral outcome.

REFERENCES

- 1) Baker-Andresen, D., Ratnu, V. S., & Bredy, T. W. (2013). Dynamic DNA methylation: A prime candidate for genomic metaplasticity and behavioral adaptation. *Trends in Neurosciences*, 36(1), 3–13.
- 2) Breslau, N., Chilcoat, H. D., Kessler, R. C., & Davis, G. C. (1999). Previous exposure to trauma and PTSD effects of subsequent trauma: Results from the Detroit Area Survey of Trauma. *American Journal of Psychiatry*, 156(6), 902-907. doi:10.1176/ajp.156.6.902
- 3) 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.
- 4) Fareri, D. S., & Tottenham, N. (2016). Effects of early life stress on amygdala and striatal development. *Developmental Cognitive Neuroscience*, 19, 233-247. doi:10.1016/j.dcn.2016.04.005
- 5) 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.
- 6) Jensen, C., & Champagne, F. (2012). Epigenetic and neuro- developmental perspectives on variation in parenting behavior. *Parenting, Science and Practice*, 12(2–3), 202–211.
- 7) Kang, H., Kim, J., Lee, J., Kim, S., Bae, K., Kim, S., . . . Yoon, J. (2013). BDNF promoter methylation and suicidal behavior in depressive patients. *Journal of Affective Disorders*, 151(2), 679-685. doi:10.1016/j.jad.2013.08.001
- 8) 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.
- 9) Keller, S., Sarchiapone, M., Zarrilli, F., Videtic, A., Ferraro, A., Carli, V, ... Chiariotti, L. (2010). Increased bdnf promoter methylation in the wernicke area of suicide subjects. *Archives of General Psychiatry*, 67(3), 258–267.
- 10) Kordi-Tamandani, D. M., Sahranavard, R., & Torkamanzei, A. (2012). DNA methylation and expression profiles of the brain-derived neurotrophic factor (BDNF) and dopamine transporter (DAT1) genes in patients with schizophrenia.

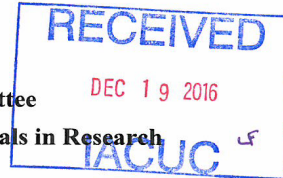
Molecular Biology Reports, 39(12), 10889-10893. doi:10.1007/s11033-012-1986-0

- 11) 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.
- 12) Lansford, J. E., Dodge, K. A., Pettit, G. S., Bates, J. E., Crozier, J., and Kaplow, J. (2002). A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. *Arch. Pediatr. Adolesc. Med.* 156, 824–830.
- 13) McCrory, E., De Brito, S. A., & Viding, E. (2011). The Impact of Childhood Maltreatment: A Review of Neurobiological and Genetic Factors. *Frontiers in Psychiatry*, 2, 48.
- 14) McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* 22, 105–122.
- 15) McGowan, P. O., & Roth, T. L. (2015). Epigenetic pathways through which experiences become linked with biology. *Development and Psychopathology*, 27(02), 637-648.
- 16) 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. doi:10.1038/nn.2270
- 17) Nina N. Karpova, Role of BDNF epigenetics in activity-dependent neuronal plasticity, *Neuropharmacology*, Volume 76, Part C, 2014, Pages 709-718, ISSN 0028-3908.
- 18) Parrish RR, Day JJ, Lubin FD. Direct bisulfite sequencing for examination of DNA methylation with gene and nucleotide resolution from brain tissues. *Curr Protoc Neurosci* 2012;60, 7.24.1–7.24.12.
- 19) 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). doi:10.1038/tp.2012.140

- 20) Roth, T. L. (2012) Epigenetics of neurobiology and behavior during development and adulthood. *Developmental Psychobiology*, 54(6), 590–597.
- 21) Roth, T. L., & Sullivan, R. M. (2005). Memory of early maltreatment: Neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biological Psychiatry*, 57(8), 823–831.
- 22) 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.
- 23) 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.
- 24) Sarkar, A., Chachra, P., Kennedy, P., Pena, C. J., Desouza, L. A., Nestler, E. J., & Vaidya, V. A. (2014). Hippocampal HDAC4 contributes to postnatal fluoxetine-evoked depression-like behavior. *Neuropsychopharmacology*, 39(9), 2221.
- 25) Silberman, D. M., Acosta, G. B., & Zubilete, M. A. (2016). Long-term effects of early life stress exposure: Role of epigenetic mechanisms. *Pharmacological Research*, 109, 64-73. doi:10.1016/j.phrs.2015.12.033
- 26) Stertz, L., Fries, G. R., Aguiar, B. W., Pfaffenseller, B., Valvassori, S. S., Gubert, C., . . . Kapczinski, F. (2013). Histone deacetylase activity and brain-derived neurotrophic factor (BDNF) levels in a pharmacological model of mania. *Revista Brasileira De Psiquiatria*, 36(1), 39-46.
- 27) Szyf, M., & Bick, J. (2013). DNA methylation: A mechanism for embedding early life experiences in the genome. *Child development*, 84(1), 49–57.
- 28) Tottenham, N., & Sheridan, M. A. (2010). A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Frontiers in human neuroscience*, 3, 68. doi:10.3389/neuro.09.068.2009
- 29) Wei, Y. B., Melas, P. A., Wegener, G., Mathe, A. A., & Lavebratt, C. (2014). Antidepressant-like effect of sodium butyrate is associated with an increase in TET1 and in 5-Hydroxymethylation levels in the Bdnf gene. *International Journal of Neuropsychopharmacology*, 18(2).

Appendix A
IACUC APPROVAL

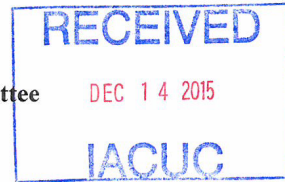
University of Delaware
Institutional Animal Care and Use Committee
Application to Use Animals in Application to use animals in Research
(New and 3-Yr submission)



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: <u><i>Tania L. Roth, DVM</i></u>
Date of Approval: <u>11/30/2017</u>

University of Delaware
 Institutional Animal Care and Use Committee
 Annual Review



Title of Protocol: Epigenetic mechanisms in lifelong changes in genes and behavior associated with adverse caregiving	
AUP Number: 1216-2016-2	← (4 digits only)
Principal Investigator: Tania L. Roth	
Common Name: Rat (Long Evans Blue Spruce)	
Genus Species: Rattus norvegicus	
Pain Category: <i>(please mark one)</i>	
USDA PAIN CATEGORY: <i>(Note change of categories from previous form)</i>	
Category	Description
<input type="checkbox"/> B	Breeding or holding where NO research is conducted
<input type="checkbox"/> C	Procedure involving momentary or no pain or distress
<input type="checkbox"/> D	Procedure where pain or distress is alleviated by appropriate means (analgesics, tranquilizers, euthanasia etc.)
<input checked="" type="checkbox"/> 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:	<u><i>Jim Talbot, DVM</i></u>
Date of Approval:	<u>2/1/16</u>