Emma Noel
Advised by: Dr. Jennifer Honeycutt
Program in Neuroscience, Bowdoin College, Brunswick, ME
Grua/O'Connell Research Fellowship

Early life adversity (ELA), such as exposure to abuse or neglect in childhood, can result in adverse behavioral and neurological responses in brain areas connected to emotional functioning (Brown et. al, 2019). Individuals that experience ELA have been found to exhibit higher rates of anxiety and depression later in life (Krugers et. al 2017). Thus, there is a growing need to identify biological pathways and potential biomarkers involved in maladaptive responses to ELA. DNA methylation is a post-natal epigenetic modification implicated in gene expression, via the regulation of transcription (Szyf et. al 2013). My project utilized ELA in a Sprague Dawley rat model to explore later life outcomes on behavior and neurology. Specifically, I looked at the potential of DNA methylation as a biomarker for anxiety-like behavior. Analysis of brain regions will continue into the summer, and this project will culminate in an honors thesis and presentation at the Society for Neuroscience (Sfn) next summer.

First, the rats were introduced to the maternal separation paradigm, which is a translational model of caregiver deprivation, such as in an orphanage or child detainment at the U.S.-Mexico Border (Cohodes et. al, 2021). Following the maternal separation paradigm, we conducted two behavioral assays to test for anxiety-like behavior, the Open Field Test (OFT) and Elevated Zero Maze (EZM). The behavioral results show increased anxiety-like behavior dependent on age, suggesting there is a critical period in which ELA can alter later life behavior. After behavioral testing, we saved three brain regions from one brain hemisphere for immunohistochemistry (IHC) testing and dissected the other hemisphere for ELISA testing (Figure 1). We will utilize ELISA assays to assess and characterize global patterns of DNA methylation in the three brain regions isolated: Prefrontal Cortex (PFC), Bed Nucleus of the Stria Terminalis (BNST), and Basolateral Amygdala (BLA) and immunohistochemistry to analyze the co- localization of DNA methylation markers with markers for different neural subtypes.

With the help of the Grua/O'Connell Fellowship, we were able to purchase primary and secondary antibodies, as well as mounting media for IHC analysis. This semester, we began IHC analysis on the PFC, and preliminary results suggest an age and sex-dependent increase in 5-hydroxymethylcytosine (5-hmc) in cells expressing parvalbumin, which is a protein implicated in anxiety related pathways. These results suggest that DNA methylation increases for males and decreases for females exposed to ELA across development, but not for controls. Additional analysis of PFC and the brain regions detailed in Figure 1 is necessary to confirm these results and will occur this summer.

This semester, with the help of BioME funding, we were able to purchase Qiagen DNAeasy Blood and Tissue Purification kits and ELISA 5-hmc and 5-mc assays. We began to homogenize and purify the DNA in the brain samples collected to prepare the tissue to run on the ELISAs. Additionally, I developed a working protocol for effective tissue homogenization to optimize DNA yields, via running the DNA elution process on sample brains in multiple iterations to determine the correct DNA concentrations for ELISA analysis. This summer, I will run the purified DNA on the ELISA kits to assess global methylation rates and I will begin to run immunohistochemistry on the BNST and BLA.

Overall, DNA methylation is an epigenetic modification that is relevant to the production of anxiety and depression following ELA. By characterizing global and brain-region specific differences in DNA methylation with respect to sex and age, critical windows for symptom onset and thus treatment intervention may be discerned. Additionally, by examining the role of DNA methylation with respect to behavior, we may gain further insight into the relationship between genes and the environment, which dominates the conversation surrounding the prognosis and development of psychiatric disorders.

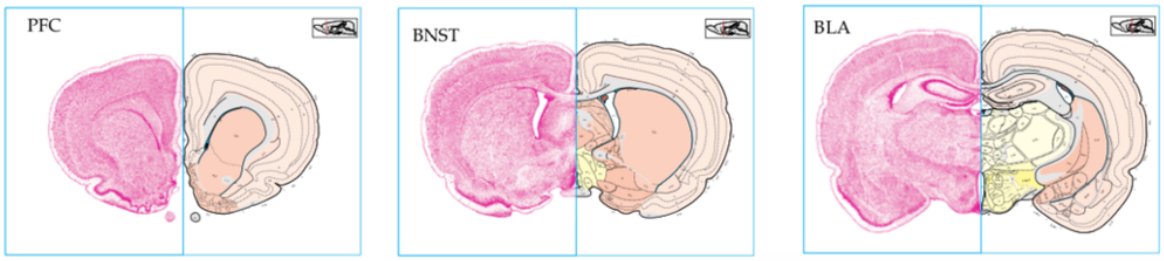

Figure 1. Brain Regions analyzed. Prefrontal Cortex (PFC), Bed Nucleus of the Stria Terminalis (BNST), Basolateral Amygdala (BLA) *Swanson, Larry. "Brain Maps Atlases." Larry Swanson, 2004.*

References

Brown, Amanda, Laura M. Fiori, and Gustavo Turecki. “Bridging Basic and Clinical Research in Early Life Adversity, DNA Methylation, and Major Depressive Disorder.” *Frontiers in Genetics* 10 (2019): 229. https://doi.org/10.3389/fgene.2019.00229.

Cohodes, Emily M., Sahana Kribakaran, Paola Odriozola, Sarah Bakirci, Sarah McCauley, H. R. Hodges, Lucinda M. Sisk, Sadie J. Zacharek, and Dylan G. Gee. “Migration-Related Trauma and Mental Health among Migrant Children Emigrating from Mexico and Central America to the United States: Effects on Developmental Neurobiology and Implications for Policy.” *Developmental Psychology*

Krugers, Harm J., J. Marit Arp, Hui Xiong, Sofia Kanatsou, Sylvie L. Lesuis, Aniko Korosi, Marian Joels, and Paul J. Lucassen. 2017. “Early Life Adversity: Lasting Consequences for Emotional Learning.” *SI:Stressors in Animals* 6 (February): 14–21.

Szyf, Moshe. “DNA Methylation, Behavior and Early Life Adversity.” *Epigenetics: Development, Diseases and Memories* 40, no. 7 (July 20, 2013): 331–38.