Characterizing the Effect of Early Life Adversity on Sex-specific Behavioral and Epigenetic Outcomes in Rats over Development

Emma Noel, Class of 2023

Early life adversity (ELA), such as exposure to childhood abuse, neglect, or other forms of trauma may result in maladaptive behavioral and neurological responses in brain regions associated with emotion (Brown et. al, 2019), and an increased risk for mental illness later in life. Largely, the nature of such biological pathways remains unknown. However, studies have suggested that epigenetic factors, via alterations in DNA methylation patterns over development following ELA, may play a crucial role in later life increases in anxiety and depression related disorders (Brown et. al, 2019; Szyf, 2013). DNA methylation represses gene expression in response to environmental stimuli over development (Szyf, 2013), thus possibility acting as a culprit of abnormal behavioral and neurological functioning later in life. To address the neurological effects of ELA, my project leveraged this information in a rat model to explore patterns of DNA methylation over development across control and ELA animals in brain regions associated with anxiety. I also utilized two behavioral assays to assess anxiety-like behavior. Additionally, sex-specific patterns emerge following ELA, leading to early-brain maturation in females compared to males (Honeycutt et. al, 2020). Thus, I used both female and male rats to assess the sex-specific outcomes of ELA over development. Overall, we collected behavioral and neurological data for two developmental timepoints this summer and will further analyze that data in the fall.

My project utilized two developmental timepoints, postnatal day (PD) 25 (juvenile) and PD45 (late adolescence) in both female and male rats to assess the differential reaction to ELA over development. We introduced the rats to ELA in the form of maternal separation, which is a translational model for caregiver deprivation, such as in an orphanage or child detainment at the U.S.-Mexico Border (Cohode et. al, 2021). The pups were separated from PD2 to PD21, at which point they were weaned until tested. This summer we ran behavior, utilizing the Open Field Test (OFT), where rats showing less willingness to enter the center of the field are thought to have more anxiety-like behavior, and the Elevated Zero Maze (EZM), where anxiety-like behavior is characterized by time spent in the closed versus open arms of the test. We hypothesized that rats exposed to ELA would display more anxiety-like behavior, with females showing an earlier increase in anxiety-like behavior than males. Preliminary data shows that at PD25, male ELA rats displayed less anxiety-like behavior (n=10) in the OFT compared to male control rats (n=8), with no significant difference in anxiety-like behavior for female rats. While this was unexpected, we are exploring variables that could have led to this effect. At PD45, both male ELA rats (n=4) and female ELA rats (n=2) spent more time in the thigmotaxis zone than control rats, suggesting more anxiety-like behavior. Further analysis must be done before these results are confirmed.

Following behavioral testing, we took brains and dissected one half to obtain samples of Prefrontal Cortex (PFC), Bed Nucleus of the Stria Terminalis (BNST), and Basolateral Amygdala (BLA), which are all regions involved in anxiety. We saved the additional hemisphere for immunohistochemistry (IHC) analysis in the fall. We will also use ELISA assays in the fall to analyze the dissected brain regions for global patterns of DNA methylation and to analyze blood sample corticosterone (a stress hormone) levels. The additional hemisphere will be sliced for the same brain regions and stained for methylation markers 5mc and 5hmc as well as parvalbumin (PV) proteins, using triple staining IHC. PV is a calcium-binding protein that is implicated in the disruption of neural pathways related to anxiety. We chose these two methods as ELISA can pinpoint global methylation within the specific regions mentioned, whereas IHC can show colocalization of DNA methylation markers and PV-containing interneurons. Overlap between PV-containing interneurons and methylation markers would suggest that there is an alteration in gene expression within this area. Overall, this summer I utilized the maternal separation paradigm to explore sex-specific, developmental outcomes of ELA on behavior and gene expression to further characterize early life biomarkers for mental illness.

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