Characterizing the Effect of Early Life Adversity on Sex-specific Behavior and DNA Methylation Patterns over Development

Emma Noel, 2023

Early life adversity (ELA), such as exposure to childhood abuse, neglect, or other forms of trauma can have neurological and behavioral consequences, including an increased risk for the development of psychiatric disorders later in life (Brown et al. 2019). Indeed, the match/mismatch hypothesis shows that adaptive coping strategies in youth can translate to maladaptive patterns as a child's environment changes (Schmidt, 2011). Moreover, mechanisms that were once biologically helpful can manifest adversely as hyperreactive or blunted stress hormonal responses or an elevated heart rate, all of which result in negative health outcomes (Hoffman et al. 2014). While it is well-known that both genetics and the environment work together to shape these changes, the biochemical pathways underlying these changes have yet to be clearly defined. Increasing evidence points to epigenetic alterations, such as DNA methylation, as key and stable regulators of gene expression, especially in certain neural subtypes (Szyf et al. 2013). DNA methylation is the addition of a methyl group to gene, that when in the promoter region, can result in shunted gene expression (Moore et al. 2013). Thus, DNA methylation may contribute to ELA-induced variability through the alteration of gene expression and subsequent protein production. GABAergic parvalbumin (PV) containing cells are implicated in the excitation:inhibition (E:I) balance in the brain, through orchestrating long-range inhibitory signals, and studies have demonstrated variation in PV amount following ELA. Since PV cells are sensitive to ELA and show sex-related outcomes that are linked to affective processing, and therefore these neurons may be uniquely vulnerable to methylation outcomes.

My project investigated the degree to which changes in DNA methylation, particularly in PV cells, contribute to sex-specific and developmental differences seen in ELA. Sprague Dawley rats were introduced to the maternal separation paradigm, which translationally models caregiver deprivation, similar to an orphanage. Last summer, the rats were evaluated at two developmental timepoints (postnatal day (P)25 or P45) for anxiety-like behavior, and blood for corticosterone (stress hormone) analysis and brain tissue from the Prefrontal Cortex (PFC), Bed Nucleus of the Stria Terminalis (BNST), and Basolateral Amygdala (BLA) was saved for global methylation and PV-specific analyses. This year, I developed and optimized a protocol for the purification of DNA from rat brain tissue using the Qiagen DNAeasy Blood and Tissue Purification kits and utilized this kit and protocol to purify DNA from 240 tissue samples this summer. The DNA from PFC samples was run on a 5-mC methylation ELISA, to quantify global changes in DNA methylation. We found no significant changes in PFC global methylation dependent on condition, but in P25 (juvenile) ELA males compared to juvenile control males, there were lower global levels of 5-mC methylation which trended towards significance. Interestingly, preliminary immunohistochemistry (IHC) results show that ELA males exhibited a significant increase in 5-mC intensity in the PFC in PV cells over development compared to control males, suggesting that changes in methylation level are specific to neural subtype. We also ran a corticosterone ELISA and found significantly lower basal levels of stress hormone for juvenile ELA females compared to males, suggesting a sex and rearing condition specific blunted corticosterone response. Both the 5-mC methylation and corticosterone findings show sex-dependent responses, which reiterates the importance of studying both sexes.

This year, we will continue this study and run global 5-mC ELISAs on the BNST and BLA, as well as IHC analysis for every brain area, analyzing for intensity of 5-mC and colocalization of 5-mC with PV-containing interneurons. These experiments will help get a greater picture of methylation as it relates to specific neural subtypes, and once this is complete, we will send out relevant samples for pyrosequencing, a DNA methylation assay, that will quantify methylation percentages on promoter regions.

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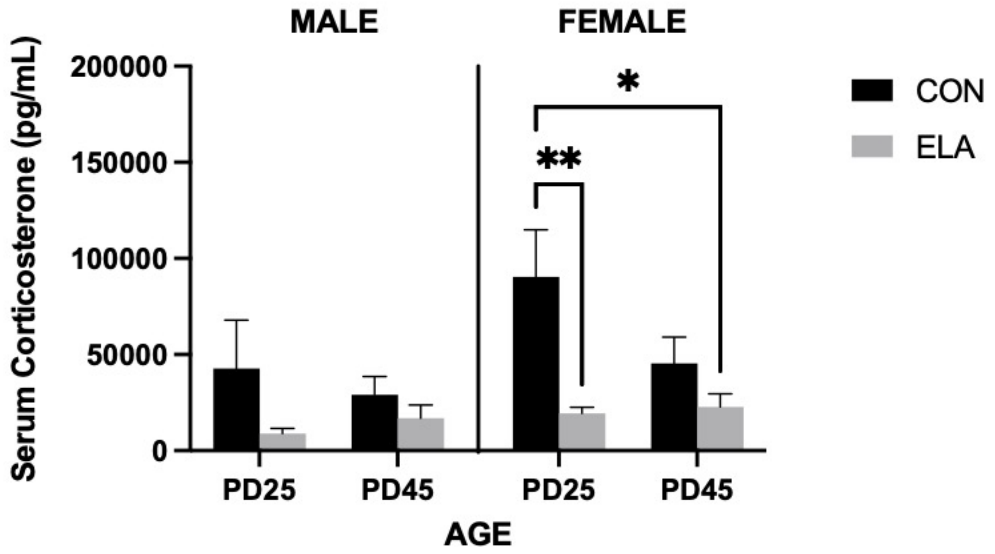

Figure 1. Serum Corticosterone levels present blunted CORT reactivity for P25 ELA females compared to control animals.

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