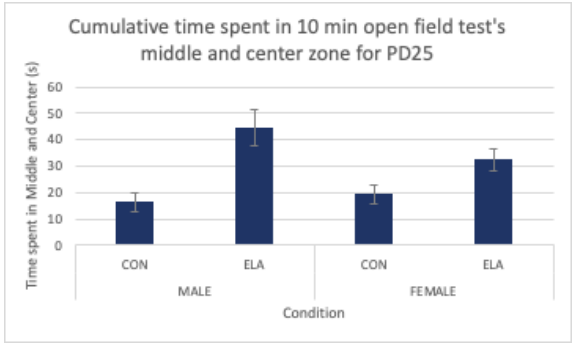
DNA methylation as a possible mechanism for anxiety-like behavior in an early life adversity model
Alissa Chen, Class of 2022

Trauma at a young age has been shown to increase the risk of developing neuropsychiatric disorders [1]. The rat model of maternal separation (MS) is analogous to caregiver deprivation in humans and allows us to systematically study the consequences of early life adversity (ELA). Previous research has shown that ELA changes DNA methylation patterns [7,9] and disrupts parvalbumin (PV) expression in inhibitory PV-containing interneurons in brain regions associated with anxiety [4,5,6]. MS has led to female rats showing increased anxiety-like behavior earlier compared to males [6]. These results are comparable to the increased prevalence of anxiety and depression in women compared to men [1]. However, there is no previous research on the correlation between ELA, PV expression, methylation patterns, and anxiety-like behavior. Consequently, I will be looking at methylation as a possible mechanism for the sex- and age-dependent manner of MS's effects on PV expression and anxiety-like behavior.

Maternal Separation
My study is a 2x2x2 design, where we are looking at rearing condition (ELA, CON), sex (male, female) and age (PD25 (post-natal day 25), PD45) as independent variables. After the birth of a litter of rats, the pups will be separated from their mother for 4 hours a day and placed into individual cups and cages, deprived of littermate and maternal stimulation during this time.
Open Field Test
Rats were placed into an open field, black, opaque Plexiglass 100 cm x 100 cm square arena. Ethovision tracked rats' overall distance traveled, velocity, time spent in center, and frequency of visits to the center zone as a measure of anxiety-like behavior [2].
Elevated Zero Maze
Rats were placed into the elevated zero maze, a circular apparatus made of black, opaque Plexiglass with two open areas with no walls and two closed areas with high walls. Ethovision tracked four-paw entries into/time spent in open and closed areas, number of arm transitions from closed to open areas, and head pokes/dips as a measure of anxiety-like behavior [3].



PD25, or juvenile, male ELA rats spent significantly more time in the middle and center zone area for the 10 minutes of the open field test in comparison to CON male rats. Our findings are supported by another study that showed similar results with PD35, or adolescent, male rats and also implied ELA male rats showed more anxiety-like and impulsive behavior [8]. Similarly, PD25 female ELA rats also spent significantly more time in the middle and center zone area for the 10 minute open field test in comparison to CON female rats. These results could also indicate more impulsive behavior in juvenile female rats. Some of my analyses are still ongoing, and I will continue into the fall as a part of my honors research.

Jennifer Honeycutt
Funded by the Life Sciences Fellowship

References

[1] Albert, P. (2015). Why is depression more prevalent in women? *Journal of Psychiatry & Neuroscience*, *40*(4), 219–221. https://doi.org/10.1503/jpn.150205

[2] Coley, E. J. L., Demaestri, C., Ganguly, P., Honeycutt, J. A., Peterzell, S., Rose, N., Ahmed, N., Holschbach, M., Trivedi, M., & Brenhouse, H. C. (2019). Cross-Generational Transmission of Early Life Stress Effects on HPA Regulators and Bdnf Are Mediated by Sex, Lineage, and Upbringing. *Frontiers in Behavioral Neuroscience*, *13*, 101. https://doi.org/10.3389/fnbeh.2019.00101

[3] Demaestri, C., Brenhouse, H. C., & Honeycutt, J. A. (2019). 22 kHz and 55 kHz ultrasonic vocalizations differentially influence neural and behavioral outcomes: Implications for modeling anxiety via auditory stimuli in the rat. *Behavioural Brain Research*, *360*, 134–145. https://doi.org/10.1016/j.bbr.2018.12.005

[4] Gildawie, K. R., Honeycutt, J. A., & Brenhouse, H. C. (2020). Region-specific Effects of Maternal Separation on Perineuronal Net and Parvalbumin-expressing Interneuron Formation in Male and Female Rats. *Neuroscience*, *428*, 23–37. https://doi.org/10.1016/j.neuroscience.2019.12.010

[5] Grassi-Oliveira, R., Honeycutt, J. A., Holland, F. H., Ganguly, P., & Brenhouse, H. C. (2016). Cognitive impairment effects of early life stress in adolescents can be predicted with early biomarkers: Impacts of sex, experience, and cytokines. *Psychoneuroendocrinology*, *71*, 19–30. https://doi.org/10.1016/j.psyneuen.2016.04.016

[6] Holland, F. H., Ganguly, P., Potter, D. N., Chartoff, E. H., & Brenhouse, H. C. (2014). Early life stress disrupts social behavior and prefrontal cortex parvalbumin interneurons at an earlier time-point in females than in males. *Neuroscience Letters*, *566*, 131–136. https://doi.org/10.1016/j.neulet.2014.02.023

[7] Jiang, S., Kamei, N., Bolton, J. L., Ma, X., Stern, H. S., Baram, T. Z., & Mortazavi, A. (2019). Intra-individual methylomics detects the impact of early-life adversity. *Life Science Alliance*, *2*(2), e201800204. https://doi.org/10.26508/lsa.201800204

[8] Kim, H.-B., Yoo, J.-Y., Yoo, S.-Y., Suh, S. W., Lee, S., Park, J. H., Lee, J.-H., Baik, T.-K., Kim, H.-S., & Woo, R.-S. (2020). Early-life stress induces EAAC1 expression reduction and attention-deficit and depressive behaviors in adolescent rats. *Cell Death Discovery*, *6*(1), 73. https://doi.org/10.1038/s41420-020-00308-9

[9] Thaweethee-Sukjai, B., Suttajit, S., Thanoi, S., Dalton, C. F., Reynolds, G. P., & Nudmamud-Thanoi, S. (2019). Parvalbumin Promoter Methylation Altered in Major Depressive Disorder. *International Journal of Medical Sciences*, *16*(9), 1207–1214. https://doi.org/10.7150/ijms.36131