**Sex-Specific Impact of Prenatal Stress on Parvalbumin Expression and Anxiety-Like Behavior**
**Lindsay Golan, 2027**

**Introduction**: Parvalbumin (PV) interneurons are one of the primary inhibitory neurons in the central nervous system, and are notable due to their ability to quickly reset after an action potential (Heizmann, 1984). They are key in preventing overexcitation of pyramidal cells, which is connected to the development of numerous negative affective disorders such as depression and anxiety (Chen et al., 2019)(Ferguson & Gao 2018). Exposure to chronic stress and release of glucocorticoids (e.g. corticosterone, cortisol) is associated with decreased parvalbumin expression (Hoogendoorn et al., 2017). A possible mechanism for this decrease is DNA methylation, where a methyl group binds to DNA to prevent transcription of these parvalbumin proteins (Cao-Lei et al., 2020)(Razin et al., 1986). Previous studies using postnatal stress paradigms, such as Early Life Adversity (ELA), have shown sex-specific changes in PV cells. Female rats showed a significant decrease in PV cells, paired with a decrease in DNA methylation markers associated with PV cell production, whereas male rats showed the opposite effect (Noel et al., 2024). This indicates that females are possibly more susceptible to stress and calls for sex-specific treatments. This is important when considering that women are more likely to develop depression (Salk et al., 2017), PTSD (Olff, 2017), and anxiety disorders (Kessler et al., 2012).

**Project Objectives:** This experiment aims to determine if the timing of stress impacts PV expression and DNA methylation. Maternal stress hormones crossing the placenta can invoke epigenetic changes within the fetus (Castro-Quintas et al., 2025). By using a prenatal stress paradigm, we intend to explore if in-utero exposure to stress hormones will have a different effect compared to postnatal stress paradigms. This project is important because if effects differ, it will contribute to a growing literature that allows for tailoring treatment to meet individual needs.

**Methods:** Prenatal stress was conducted on four timed-pregnant dams, during the equivalent of their second trimester, for seven days. They were placed in a clear restraint tube that restricted movement for forty minutes, three times a day (spaced two hours apart). The rat pups were born, and at post-natal day 21 (p21), they were separated from their mothers and reached adolescence. At p27, they were acclimated to the behavioral testing room, and the next day the Open Field Test (OFT) was administered. This test measures anxious behavior by playing distressed rat USVs, and measuring how much time the rat spends in the center of the field as well as tracking the rat's movement. If they spend more time in the center where they are exposed, they are generally considered less anxious. On p29, transcardial perfusions were performed using 0.9% saline followed by 4% paraformaldehyde to fix the brains. Brains were extracted and then preserved in 4% paraformaldehyde, and subsequently in a 30% sucrose solution for cryoprotection. The project will continue through fall with behavioral analysis and immunohistochemistry (IHC) of the brains. IHC is where the brains are stained with antibodies to visualize PV cells and DNA methylation markers using immunohistochemistry. We anticipate increased anxiety-like behavior for the prenatal stress animals compared to controls via decreased center entries in the OFT. Moreover, we expect to see decreased PV cells and increased DNA methylation markers, with a greater significance in these results for female animals.

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