Optimizing protocols to investigate potential epigenetic mechanisms of early life adversity in prefrontal cortex parvalbumin interneurons

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Early life adversity (ELA) is extremely prevalent in youth, and is linked to a higher likelihood of developing affective disorders later in life. It is characterized by negative environmental factors such as abuse or neglect that often result in maladaptive behavioral changes (Duffy et al., 2018). These changes can also be seen at the molecular level within certain regions of the brain, particularly the prefrontal cortex (PFC). My project was specifically looking at Parvalbumin (PV) interneurons within the PFC to better understand the molecular changes that occur due to ELA (Soares et al., 2020). PV cells are calcium-binding proteins that regulate through GABAergic, inhibitory signaling. They are notably susceptible to ELA as we have seen in past research, there are changes to PV density and function (Nieves et al., 2020). A past study in the Honeycutt Lab found that when subjects are exposed to ELA there are changes to the methylation patterns of PV cells in the PFC, marked by 5-methylcytosine (5-mC) intensity (Noel et al., 2024). However, this study was only able to gather these results from a heterogeneous cell population. The goal of this project was to isolate the PV cells in a homogenous population in hopes of better understanding the mechanisms behind the changes in methylation and PV cell counts.

We used a maternal separation paradigm to induce ELA in a rodent model. After the developmental period, the rats were euthanized, and the neural tissue was dissected. The tissue was dissociated using the gentleMACS Octo Dissociator (Miltenyi Biotec) and then the samples were incubated with a FITC and phycoerythrin antibody, and then an antibody microbead. Using an immunomagnetic column separator, our goal was to isolate solely the PV cells within the sample. To confirm the results of the protocol, a flow cytometer was used. However, due to the complexity and novelness of this procedure we were unable to entirely confirm the success of the cell isolation. This summer our goal has been to optimize the protocol so that it is fully functional and data collection can begin. Nonetheless, our data is promising and shows efficacy within the protocol. We were able to confirm our red blood cell removal step is working which is seen by the elimination of the red fluorescence within the swath (Fig. 1, 2). We were also able to identify a subpopulation of interest after the antibody stain which has potential to be our PV cells (Fig. 3, 4).

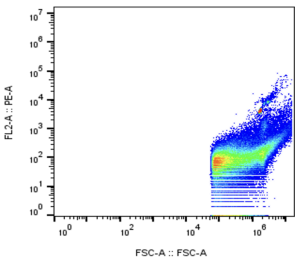

Figure 1: Before RBC removal

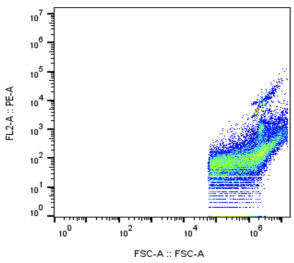

Figure 2: After RBC removal

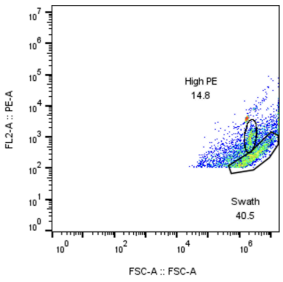

Figure 3: Before antibody stain

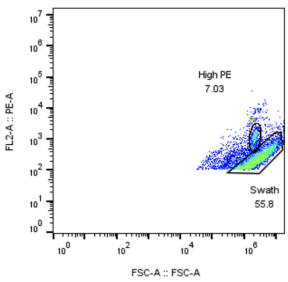

Figure 4: After antibody stain

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