Optimizing my Summer Internship

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In the summer of 2017, I participated as a research fellow at the Rosalind Franklin University of Medicine and Science (RFUMS). This position entailed working in the laboratory of Dr. Amiel Rosenkranz (Cell and Molecular Pharmacology), who is known for his extensive research into the relationship between the behavior and the neuroscience of the rat basolateral amygdala (BLA). I worked alongside Dr. Jaime Vantrease as well as LFC alumni Hannah Samberg and Brittany Avonts.

The work over the summer focused on how a neuronal ion channel might be linked to anxiety disorders in humans, using Sprague Dawley rats as the model organism. The channel we were interested in was the small-conductance calcium-activated potassium channel SK2, found on the cell membrane in neurons. SK2 is an important ion channel in central nervous system neurons because it regulates neuronal activity by decreasing and controlling the firing rate of neurons. SK2 was important to our laboratory because increased neuronal activity in the BLA has been linked to increased levels of anxiety. The idea is that if SK2 channels can regulate neuronal activity, then SK2 may be linked to anxiety disorders. We decided to look for sex differences in SK2 expression in the rat BLA, because prior research has revealed sex differences in anxiety disorder rates. It was hypothesized that females have lower levels of SK2 protein then males, as females have a higher incidence of anxiety disorders.

Using the western blot (WB) technique, Dr. Vantrease had already established that there are sex differences in SK2 protein expression in the rat BLA (with females having lower levels of the channel as hypothesized). She wanted to take her experimentation a step further, leading to my work at RFUMS. I conducted immunohistochemical (IHC) staining for SK2 protein on rat BLA sections. My work was not aimed at quantifying SK2 levelsbut instead focused on optimizing the IHC protocol, which would later be used to test our hypothesis. Some variables I examined in the IHC include whether perfusion with or without glutaraldehyde, as well as the concentration of primary antibody and fluorescent antibody, leads to better staining. Perfusion is a method we used to preserve the rat brains before obtaining BLA sections, and glutaraldehyde is a fixation agent commonly used to preserve tissues during perfusion. In an IHC, the primary antibody binds to an antigen (in this case SK2), and the fluorescent antibody is directly or indirectly bound to the primary antibody. Using the IHC technique allows researchers to visualize the location of the antigen. We found that for our particular IHC protocol, perfusing with glutaraldehyde leads to better staining, as do higher concentrations of both the primary and fluorescent antibodies. With this optimized IHC protocol, my laboratory could examine the characteristics of SK2 distribution in the rat BLA, ideally leading to a greater understanding of how SK2 impacts anxiety disorders.

My work and experience at RFUMS was invaluable to my scientific career as an undergraduate student. Importantly, I learned that it is best to ensure that the protocol is optimized, well thought-out, and accurate before conducting an experiment. There is always an incredible number of variables to consider in any protocol, and it is difficult to find the right balance among them when experimenting. Protocol optimization is especially important when working with live animals or expensive reagents, as an inefficient protocol may cause undue suffering and waste.