

After being gone for three months, the first day back in the Kraig Lab was like jumping onto a bike after a long hiatus from exercising; it took me a few days to get back in the swing of things, but, just like riding a bike, I got back into the research routine within a few days. It was very refreshing to be able to focus completely and solely on a research project, especially after the normal lecture-lab schedule of science classes on the block plan. However, Cornell classes still played a part in my research this block. Using my newly-acquired knowledge of cellular metabolism from biochemistry in block 3, I was able to act the expert in our discussions of metabolism as it relates to my research project from this summer.

For the month of December, I continued the research I had begun with Dr. Kraig during the summer, localizing oxidative stress to specific cell types following spreading depression in hippocampal slice cultures. The first few days in lab were spent reviewing what had already been done. Over the summer, I worked to optimize the protocol for using CellROX, a new fluorescent oxidative stress marker; working with Dr. Kraig, we determined which fixative gave the lowest background fluorescence, whether to use sectioned or whole slice cultures, and whether to incubate with CellROX pre- or post-spreading depression treatment. I also optimized immunohistochemical staining protocols in order to fluorescently label the different cell types in the slice cultures. Immunohistochemical staining relies on a primary antibody to recognize certain regions specific to a certain cell type; a fluorescently-tagged secondary antibody is then used to mark regions tagged by the primary antibody, which allows cells to be visualized. Once these protocols had been optimized, we moved on to answer our research question. To localize oxidative stress to specific cell types, spreading depression was induced in hippocampal slice cultures which were then incubated overnight in CellROX. After fixing the cultures, whole

slices were immunohistochemically stained and mounted on slides. Confocal microscopy was used to visualize double-labeled (marked with CellROX and immunohistochemical fluorescence) cells.

With a clear idea of what had been accomplished, we spent this block working to tie up loose ends in the project. First, we finished imaging the double-labeled, slide-mounted cultures from the summer using confocal microscopy. Working with one of the graduate students in the Kraig Lab, together we then developed a protocol to quantify the relative intensity of CellROX fluorescence in double-labeled cells using the confocal images. We also looked at the overall, tissue-level increase in CellROX fluorescence following spreading depression, began comparing the relative increase in fluorescence when different numbers of spreading depression were triggered, and began to establish a timeline for oxidative stress increase following spreading depression. While we made considerable progress over the block, I was only able to see the confocal imaging step to completion. This block was another reminder that science takes time, and I wished I could stay for another month or two to see the rest of the project to completion.

Despite the feeling of disappointment in not seeing everything completed by the end of the month, I am very satisfied with how much I have learned during my time in the Kraig Laboratory. For example, I actually taught a graduate student from another lab how to do immunohistochemical staining I had optimized over the summer; it is really rewarding to realize that not only did I learn new techniques, but that I know them well enough to teach them to someone else.

In addition, this block I also got a sneak peek into the paper writing/journal submission process. I had no idea some of the rigorous expectations published journals have. In planning an experiment, one might even need to have a certain journal in mind, because different journals

can have very different requirements. The writing process I was privy to this block was also more rigorous than that which I've used for some of the science papers I've written at Cornell. At Cornell, because of time constraints, I could stop at a certain point and decide that whatever hypotheses I came up with were good enough. However, in writing a manuscript for publication, experimentation must continue until all there is reasonable support for one hypothesis or another.

Finally, I also got to know the University of Chicago, the Hyde Park neighborhood, and the city of Chicago itself a bit better. I got to practice being independent; I was able to cook for myself again, something I missed this summer. After this month, I also decided to apply to the University of Chicago MD/PhD program this coming summer.

This block, not only did I get the opportunity to continue research for my honors thesis and for publication, which will be extremely beneficial when I apply to MD/PhD programs, but I also made valuable connections with Cornell alumni.