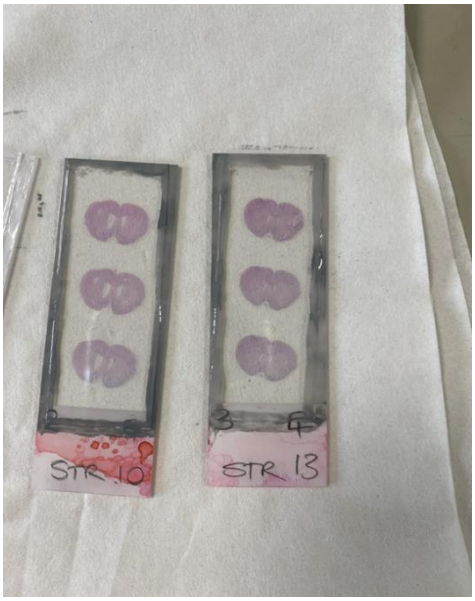
The second year of my undergraduate biological sciences degree at Durham University was challenging to say the least, with laboratory practical's cancelled and a complete transition to online learning it became more difficult to engage with academics and other students in scientific discussions like I would in a practical session or after a lecture throughout my first year. As a result, I was really keen to find a summer research internship to help me gain some more experience and explore further whether research is a career I want to pursue in the future.

Finding summer placements to help boost your CV is a challenge for university students and voluntary or unpaid opportunities outside of a commutable distance from home may not be a financially affordable option for many. I was struggling to find an opportunity I was really passionate about and so I got in contact with Dr Paul Chazot, who taught an interesting series of lectures on neuroscience as part of a physiology module I had taken in my second year, which I thoroughly enjoyed, and asked if there were any opportunities to complete a summer internship in his lab. Paul kindly offered me a project and directed me to the websites of several societies offering funding schemes/awards to undergraduate students, including the British Pharmacological Society Vacation Studentship Award. To apply I sent in my CV, official academic transcript, personal statement, explaining what I hoped to gain from the internship, and an outline of the proposed research project. I was thrilled to learn a few months later that I was one of only 9 students in the country to be awarded a Vacation Studentship, providing a generous stipend to support my living costs throughout my 6 weeks in the lab at Durham University.

My project was well suited to my interests in ageing and disease and involved researching a new therapeutic strategy for Parkinson's disease, centred around establishing the neuroprotective effects of a novel retinoic acid receptor (RAR-modulator) drug in the 6-OHDA lesioned rat model of Parkinson's disease by staining for specific biomarkers in brain slices taken from animals treated with or without the drug via immunohistochemistry and immunofluorescence. The first week of my internship was dedicated to exploring the literature to gain a greater understanding of the pathology of Parkinson's disease, why retinoic acid signalling is a viable target for treatment and potential biomarkers to stain for in relation to four primary areas of interest including dopaminergic neurons (via tyrosine hydroxylase and MAP-2), neuroinflammation (via GFAP, Iba1, CD200), AMPA receptors (via AMPA GluR2) and stress granules (via TIA-1 and G3BP1). In between reading papers and compiling my research into a summary report, I also got the opportunity to help Paul's PhD student Lydia, who I grew to be great friends with throughout my time in the lab, with her project investigating photobiomodulation as a treatment for Covid-19, using *Drosophila* as a model organism. This involved helping to prepare fly food and maintaining fly stocks in addition to learning how to conduct a *Drosophila* brain dissection, which was exciting as this showed me some new techniques and challenges associated with a completely different model organism to the one my internship project was based on.



Photograph of my H&E stained slides. All my experiments were conducted blind and so I didn't know which tissue sections came from treated or untreated individuals, which was exciting to try and work out!

Before I jumped into probing the tissue sections with antibodies, I first completed some simple haematoxylin and eosin staining on several tissue sections from the striatum and substantia nigra, two brain areas heavily implicated in Parkinson's disease, which gave me a great

opportunity to test my deparaffinisation and rehydration protocol, in addition to familiarising myself with the brain anatomy when I visualised the slides under the microscope.

I became particularly interested in GFAP and the non-motor symptoms of Parkinson's disease after reading that elevated GFAP levels, as a result of astrogliosis, has been linked to the decrease in orexin (hypocretin) levels, which is a neuropeptide implicated in regulating the sleep-wake cycle, contributing to the sleep problems experienced by people living with Parkinson's disease. I therefore started my first immunohistochemistry experiments by staining for GFAP. Once the tissue sections had been deparaffinised and rehydrated by running through xylene and a graded ethanol series, I blocked the non-specific binding sites by incubating with normal horse serum, as this was the same species in which the secondary antibody was raised. The primary GFAP antibody, diluted in blocking solution, was incubated overnight in the cold room before incubating with the biotinylated secondary antibody and reagent from the Vectastain ABC HRP kit I was using. To cause the colour change reaction I added DAB, before allowing the slides to dry and mounting/coverslipping.

After an initial look under the microscope there was only minimal staining of astrocytes so I repeated the experiment with a commercial GFAP antibody and with the addition of heat mediated antigen retrieval, to break the methylene bridges formed during the tissue fixation procedure to better expose the antigenic sites for increased antibody binding. I expanded to also stain for tyrosine hydroxylase, the enzyme involved in the rate limiting step of dopamine synthesis, via enzymatic detection with horseradish peroxidase, along with MAP-2 and G3BP1 via a fluorescently labelled secondary antibody, providing me with an opportunity to learn how to use a fluorescent microscope, which was so exciting as I love microscopy! My most notable result was with fluorescent detection of MAP-2 and despite slightly higher background staining than I would have liked, I managed to get some decent photos under the microscope of the brain slices, which I also counterstained with DAPI to stain the nuclei blue. I also got to present my project at a lab meeting, which I enjoyed not only because I got to speak about a project I am so interested in but also because I got to hear about different PhD student project from around the globe, including Nigeria and the USA. Alongside working in the labs, I also went to meet up with the local Durham Parkinson's community group, who I have volunteered with previously, on their trip to the botanical gardens and I was so grateful for the opportunity to learn more about life with Parkinson's disease from the lovely people there.

A photograph of me pipetting a primary antibody, diluted in blocking solution, onto the brain slices in the cold room, ready for overnight incubation.

Overall, I thoroughly enjoyed my time in the neuroscience lab and developed a range of immunostaining skills, in addition to a glimpse into what working with flies entails! After these 6 weeks, I feel much more confident in the lab and well prepared for postgraduate study, in which I am 99.9% sure I will continue down the path of neurodegenerative disease research, with a special focus on Parkinson's disease. I would like to thank the British Pharmacological Society again for providing me with the vacation studentship award and of course Paul for being such a lovely and interesting supervisor who helped make my summer internship such a great experience.