# Laidlaw Reflective Report Summer 1 2023

**What was the overall structure and methodology of your research project as it relates to your experience of conducting research?**

My research project consisted of three stages which overlapped both conceptually and in time. The first stage revolved around an in depth reading and analysis of the literature surrounding astrocytes, senescence, and Alzheimer's disease. This is a relatively new line of research and as such, there are few papers that specifically focus on these three topics together. I also began to investigate the theory underlying immunohistochemistry. Through this, I started to understand the mechanism and importance of each stage of the experiment. I needed to grasp these concepts in order to determine why the stain didn't work and to know what aspects of each step in the protocol to change in an attempt to see the stain more clearly.

The next stage of my project focused on creating a protocol for Senescence Associated Beta-Galactosidase (SA-β-Gal) and p16 immunohistochemical staining. Immunohistochemical staining is a technique used to visualise specific proteins or antigens within tissue samples. Each step of the experimental procedure required modification to optimise the immunohistochemical protocol and achieve the highest quality staining for both proteins. There was a lot of modularity in the design and variability in the results I obtained from the staining. A sharp contrast between the tissue background and the stained protein had to be established.

The final stage of my project concentrated on creating a protocol for Congo red staining. Congo red staining is a histological technique used to detect amyloid deposits in brain and kidney tissue. It binds to amyloid proteins which enables the visualisation of the distribution and extent of amyloid pathology in brain samples.

**What were the final findings of your project?**

I achieved my goals for each of the three stages of my research project. I completed a short literature review, finalised a protocol for β-Gal but not p16 immunohistochemistry, and finalised a protocol for Congo red staining. I can now see that senescent astrocytes are clustered around amyloid plaques.

My first attempts with β-Gal and p16 had contrasting outcomes. The signal-to-noise ratio from the tissues that were stained for Beta-Gal was extremely high and it was exceedingly difficult to observe the stain. On the other hand, the signal from the p16 stained tissue was extremely low and almost imperceptible. After repeated optimisations, I created a finalised protocol for β-Gal immunohistochemical staining.

The finalised protocol for SA-β-Gal is:

- **Antigen Retrieval** – 50 mM Sodium Citrate (pH 6.2) at 83°C for 10 minutes total (5 minutes + 5 minutes). Approx. 1 ml per well.
- **3 Washes** - with 0.05% TBS-Tween for 5 minutes each
- **Quench** – 1 ml of 0.3% H2O2 for 20 minutes
- **3 Washes** - with 0.05% TBS-Tween for 5 minutes each
- **Block** – 500ul of 5% NGS for 1 hour
- **3 Washes** - with 0.05% TBS-Tween for 5 minutes each
- **Primary Antibody** –250ul of 1:1200 Primary with 0.3% Triton and 5% NGS. Incubated at 4°C for 43hrs 15 mins.
- **3 Washes** - with 0.05% TBS-Tween for 5 minutes each
- **Secondary Antibody** – 250ul of 1:500 secondary (chicken) in TBS for 2 hrs
- **3 Washes** - with 0.05% TBS-Tween for 5 minutes each
- **DAB** - at 1x conc. for 3 mins on half of the tissue. At 0.5x conc. for 5 mins on the other half.
- **Dehydration** - Place slides in increasing concentrations of Ethanol (70% -> 85% -> 95% -> 100% -> 100%) for 5 minutes each. Place slides in Xylene for 5 minutes.
- **DPX and Coverslip**

As shown in Fig. 1 and Fig. 2, the objects stained have the morphology of astrocytes. This is in line with our hypothesis that the majority of senescent cells would be astrocytes.

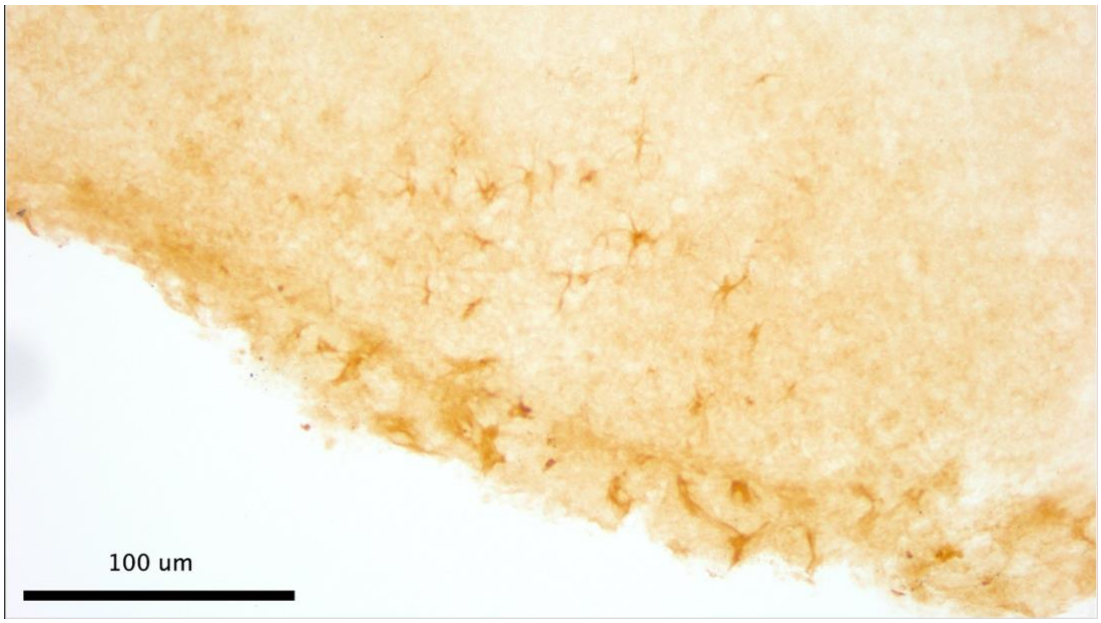


Figure 1: Visualisation of Senescent Astrocytes via β-Galactosidase Immunohistochemistry in an APP/PS1 mouse (20x Magnification)

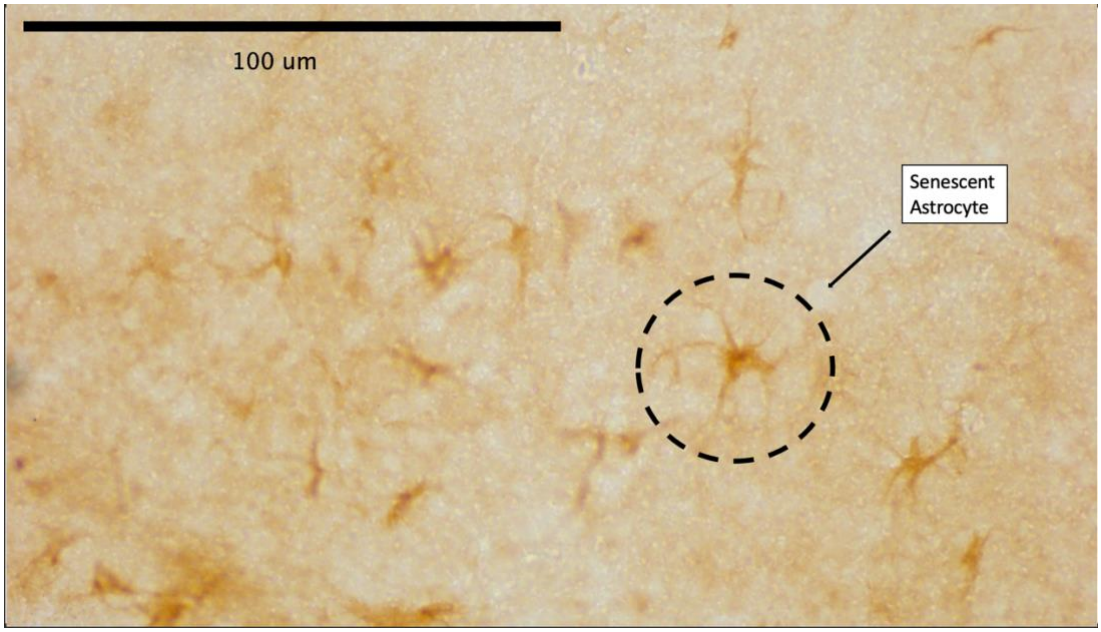


Figure 2: Visualisation of Senescent Astrocytes via β-Gal Immunohistochemistry in an APP/PS1 mouse (40x Magnification)

As shown in Figure 3, the attempts to stain senescent astrocyte nuclei were unsuccessful, which deviated from both established literature and conventional laboratory protocols. This lack of success may stem from several factors, including tissue degradation, potential ineffectiveness of the primary antibody in binding, or protein damage incurred during the experimental process.


Figure 3: Unsuccessful Senescent Astrocyte Labelling Attempt using p16 Immunohistochemistry in an APP/PS1 mouse (10x Magnification)

The completed Congo Red stain protocol is:

- If the slides are cover-slipped: Soak slides in Xylene overnight. Remove coverslip and wash briefly in Xylene.
- Rehydrate tissue: Place slides in Xylene for 5 minutes then decreasing concentrations of Ethanol (100%-> 100% -> 95% -> 85% -> 70%) for 5 minutes each.
- Place slides in Congo Red Solution for 15 minutes.
- Dip slides (approx. 2 seconds) in the first well of Deionised Water 3 times to wash away excess Congo Red.
- Dip slides (approx. 2 seconds) in the second well of Deionised Water 3 times wash away excess Congo Red.
- Place slides in Alkaline Alcohol for 30 minutes to differentiate stain.
- Place slides in Tap Water for 1 minute.

- Dehydrate tissue: Place slides in increasing concentrations of Ethanol (70% -> 85% -> 95% -> 100% -> 100%) for 5 minutes each. Place slides in Xylene for 5 minutes.
- Coverslip slides using DPX.

As shown in Fig. 4, microglia are clustered around plaques. IBA1 immunohistochemistry was used to stain microglia and Congo Red was used to stain amyloid plaques. This provides evidence of inflammation around plaques.

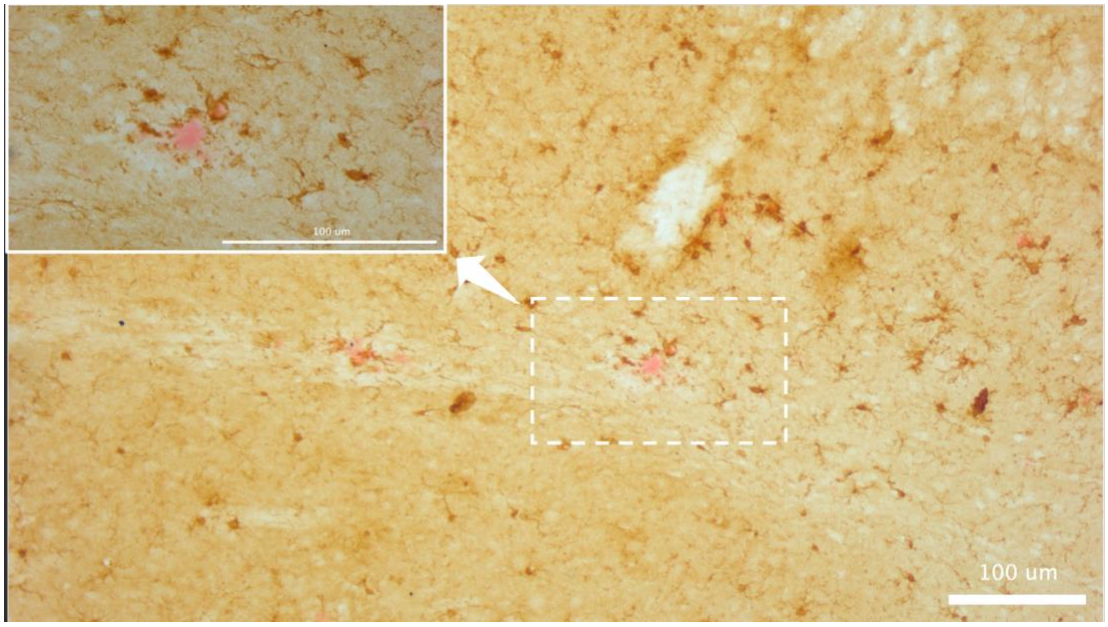


Figure 4: Dual Visualisation of Microglia using IBA1 Immunohistochemistry and Amyloid plaques with Congo Red Staining (10x and 40x Magnification)

During my time in the lab, I also engaged in another experiment focusing on the immunohistochemical staining of microglia nuclei using Pu.1. Figure 5 presents a visual representation of the successfully stained tissue samples.

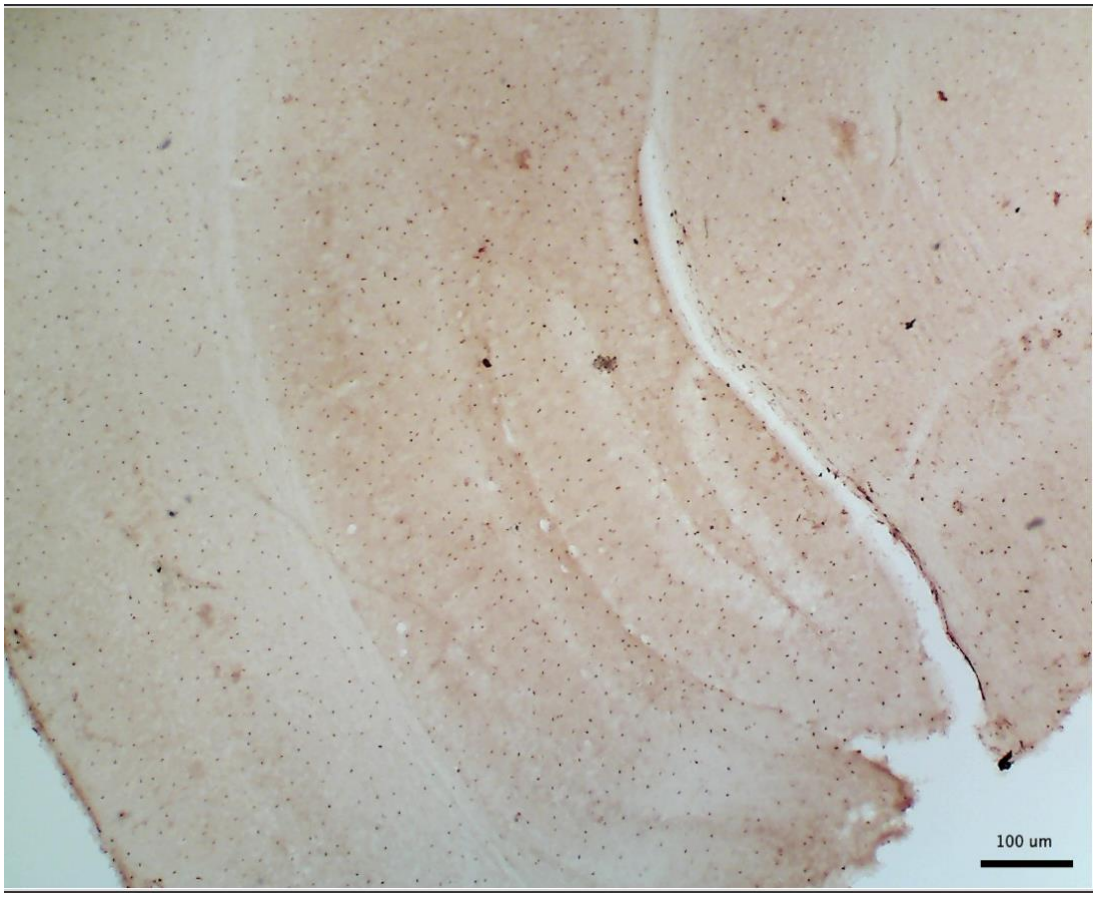


Figure 5: Visualisation of Microglia Nuclei Using Pu.1 Immunohistochemistry in an APP/PS1 mouse (10x Magnification)

Having established a protocol which enabled me to visualise plaques, I then could examine senescent astrocytes were clustered around the plaques. In Figures 6 and 7, the observed cellular morphology resembles that of astrocytes, congregated in close proximity to amyloid plaques.

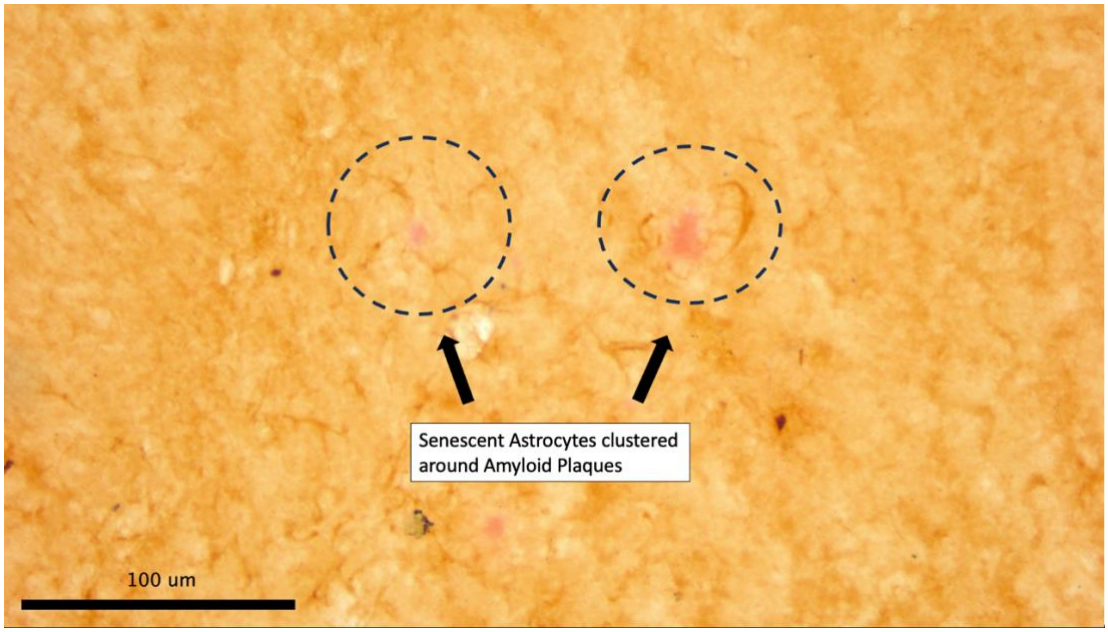


Figure 6: Dual Visualisation of Senescent Astrocytes using β-Gal Immunohistochemistry and Amyloid Plaques using Congo Red Staining in an APP/PS1 mouse (20x Magnification)

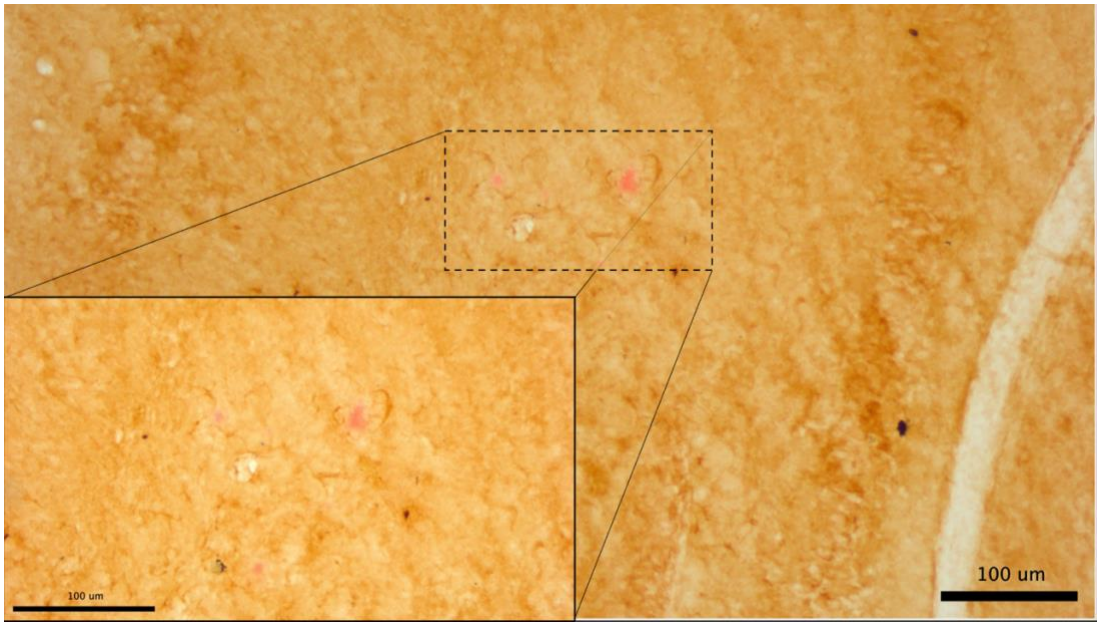


Figure 7: Dual-Labelling of Senescent Astrocytes via β-Gal Immunohistochemistry and Amyloid Plaques using Congo Red Staining in an APP/PS1 mouse (10x and 20x Magnification)

**What were the significant achievements and challenges of the research project experience?**

The biggest achievements for me throughout my time in the lab this summer were learning the complex theory underlying immunohistochemical staining, mastering the techniques necessary for its completion and completing a full optimisation for beta-galactosidase on mouse brain tissue in an eight-week period. Other than this, I'm proud that I was able to become independent in the lab. I learned how to keep proper records of my experiments, maintain a lab book and how to analyse papers thoroughly. These are transferable skills that will be of significant use over the next years.

Along with this protocol for β-Gal, I also finalised a procedure for Congo Red staining. This was something that had never been attempted in the lab I was working in before. I was able to produce a protocol for use in the lab.

The biggest challenge I faced in terms of lab work was mastering one of the last stages of the experiment. This step involved transferring the 40µm thick slices of mouse brain tissue from its well onto a slide using a fine paintbrush. This was quite difficult on a technical level and required good fine motor control. I struggled with this as the tissue would easily fold or tear. Overcoming this hurdle required a combination of patience and practice.

**How does your finished project compare to your original proposal and objectives?**

The optimisation phase of the β-Gal immunohistochemistry process presented its fair share of setbacks and challenges. These hiccups, though frustrating at times, offered valuable learning experiences. One notable consequence of these setbacks was the decision to forgo PCR analysis, a decision driven by the pressing constraints of time.

Given the complexities and intricacies involved in PCR analysis, it became apparent that dedicating the necessary time and resources to achieve accurate results in this area would have been impractical. Instead, the research focus was shifted primarily to the immunohistochemistry aspect, where the optimisation process was already demanding substantial attention and effort. This decision, while influenced by time pressure, also

underlined the importance of adaptability in research. It taught me that in the face of unforeseen challenges, it is sometimes necessary to reassess priorities and allocate resources where they are most needed to ensure the successful completion of a project.

**What is the next step for this research in your view?**

Currently these results are solely based on morphology, the next step would be to run a double-label immunohistochemistry with β-Gal and GFAP, which is an astrocytic marker. This would confirm that the senescent cells we are seeing are astrocytes.

As stated in my proposal, it would be beneficial to conduct PCR on senescent astrocytes to study gene expression in these cells. The results from these PCRs could be paired with the results from the immunohistochemical experiments to form a more complete picture of senescent astrocytes in APP/PS1 mice.

Another next step is finishing the optimisation of P16 immunohistochemistry. After multiple different conditions, no staining of the tissue was observed. Further optimisation experiments would need to be conducted to find the correct conditions for this protein.

If I were to continue with this line of research, I would extend the study into senolytics. Senolytics allow the selective killing of senescent cells. The effects on behaviour and cognition could be tested in mice with Alzheimer's disease.

**What did you learn about yourself as a researcher?**

My jump into the world of research marked both my initiation into the rigors of academic life and the deep-seated culture that accompanies it. Research is not always a thrilling quest for new knowledge; instead, it often involves the seemingly monotonous repetition of experiments in pursuit of accuracy and optimisation. The need to replicate experiments multiple times to refine and validate results could become tediously repetitive. However, it was precisely through these repetitive tasks that I discovered resilience. Even when faced with errors or experiments gone awry, I was able to continue on.

Independence became another pivotal aspect of my research journey. As I gained experience, I relished the autonomy I had over my research hours and experimental approaches. This independence allowed me to explore creative solutions and adapt to unforeseen challenges. As a researcher, I have discovered that I enjoy the independence that comes with overseeing my own project.

Yet, in the solitude of experimentation, I also discovered the importance of collaboration. Working alongside others in the lab proved to be immensely rewarding. The dynamic exchange of ideas, the shared pursuit of knowledge, and the sense of camaraderie were all important aspects on my research journey.

Looking back, my introduction to the world of research not only tested my resilience but also instilled in me a profound appreciation for the interplay between independence and collaboration in the pursuit of scientific discovery.

**What did you learn about yourself as a leader, and about your perspective on leadership, during the summer?**

Throughout my time in the lab, I realized the significance of self-confidence when faced with doubt and over-thinking. There were moments when I needed to trust in my abilities more, to back myself when faced with challenges, and to believe in my capacity to overcome obstacles. Overthinking led me down rabbit holes of hesitation, making tasks seem more daunting than they were. As my confidence grew in the lab, these moments of hesitation became less frequent but are still something I need to improve on.

Additionally, I grappled with self-motivation on occasion. There were times when the grind of research seemed daunting, and I had to summon my inner drive to stay on track. It underscored the importance of setting personal goals and maintaining a strong sense of purpose to stay motivated.

This journey also afforded me the opportunity to observe a diverse range of leadership styles among my colleagues and mentors. Some took a hands-on approach, guiding and directing at

every fork in the road, while others embraced a more hands-off philosophy, granting autonomy and fostering independence. Witnessing these varying leadership styles broadened my perspective on effective leadership and highlighted the importance of adaptability in different contexts.

**How did your experience compare and contrast to the goals you set out in your PDP?**

At the beginning of summer, I set out five goals in my Personal Development Plan: 1) Make quicker and more effective decisions. 2) Become more organized. 3) Improve focus and avoid task-hopping. 4) Boost confidence in public speaking. 5) Enhance teamwork skills.

Quick decision-making became crucial during this project due to time constraints. I have made progress in this area during my eight weeks in the lab, I still tend to overthink at times. This is something I aim to improve.

My organizational skills were initially lacking, but I had to adapt quickly to meet project demands. Estimating the time experiments would take was a challenge, but I gradually improved my task and time management.

Staying focused during experiments wasn't too difficult but maintaining concentration while reading and analysing astrocyte papers proved challenging. I eventually adopted the "pomodoro" technique to enhance focus, working for 25 minutes and taking 5-minute breaks.

Opportunities to enhance my public speaking skills were limited due to infrequent lab meetings in the summer. I did work on them when discussing my research with my supervisor and ALS group, but there wasn’t an opportunity for feedback. Moving forward, practicing with feedback will be essential for improvement.

Teamwork has been a cornerstone of my experience in the lab, as collaborating with fellow researchers has enriched my understanding and skills. Whether it's brainstorming solutions or assisting other Laidlaw scholars with their projects, the camaraderie within the research community has been both rewarding and educational.