Aoife O'Connell
Supervisor: Dr Declan McKernan

Research Project: Investigating the mechanism of alpha synuclein induced degeneration and synaptic protein expression changes in a cell culture model of dopaminergic degeneration.

During the summer of my third year studying BSc Biomedical Science at National University of Galway Ireland, I was given the opportunity to undertake a seven-week research project under the guidance of Dr Declan McKernan. This research experience was invaluable following the challenges of the two previous years where many laboratory practicals were cancelled. I have always had a strong interest in research and was delighted to find a summer project which really appealed to me to help me further decide whether research is a path I would like to continue in the future.

When I first began looking at summer internships, I contacted Dr Declan McKernan to inquire if there were any opportunities to complete a summer internship in his lab. Dr Declan McKernan offered me a position on his Parkinson teams and advised me to apply for funding or awards offered by some societies. I was delighted to learn in May that I had been awarded a Vacation Studentship from the British Pharmaceutical Society. This award helped support me throughout my 7 weeks in the lab and allowed me to fully immerse myself in the experience.

In terms of my research, my focus was investigating the mechanism of alpha synuclein induced degeneration and synaptic protein expression changes in a cell culture model of dopaminergic degeneration. To investigate this a neurons cell culture model, the SHSY5Y cell line, which mimics viral infection was used alongside FNO75, a novel small-molecule inducer of protein oligomerisation. Cell culture was performed throughout the 7 weeks and cells were treated with various combinations of FNO75 and Poly I:C at various time intervals (24/48 hours).

The first 3 weeks of the project involved preparing the various solutions and refining my western blotting skills. My project focused on three main proteins with the western blotting, PSD95, tyrosine hydroxylase and synaptophysin. Preparation of the various solutions was time consuming but offered the opportunity to develop my knowledge of common solutions and their use as part of the procedure. Following this I began the western blotting procedure. This involved protein lysis of samples followed by a Bradford assay. This allowed normalisation of the protein concentration of the samples to ensure a consistent amount of protein was used for all samples. Following this the samples were subject to electrophoresis, then transfer of the proteins on the gel to the membrane. The membrane would then be blocked followed by covering by a primary and secondary antibody. My results, which can be seen in figure 1, included three independent replicates of cells treated at 24 and 48 hours. These samples were exposed to the various proteins of interest (PSD95, TH and Synaptophysin) as well as the endogenous protein, B-Actin, to allow for quantification.

Following completion of the western blots I began my statistical analysis. This involved use of Image Studio to generate OD readings from the membrane. These readings were then transferred to Microsoft Excel where the ratio was calculated using the endogenous protein B-Actin. Subsequently the fold change relative to the control was calculated. Following this I used GraphPad Prism and one way ANOVA to investigate if there was any significant change in comparison to the control for each protein investigated. The results found no significant difference, leading to a null hypothesis. Despite this I think continuation of this project to investigate if an increase in sample size influences statistically significant of the data would be interesting.

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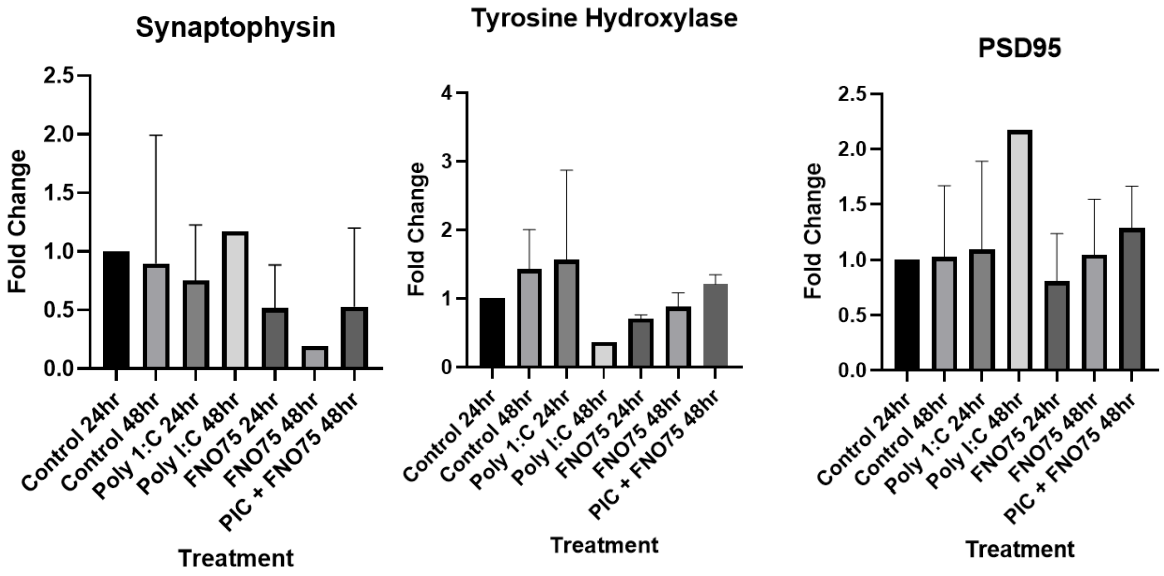

Figure 1- Statistical analysis of protein fold change from three independent sample replicates. Overall no significant changes were found.

Overall, I thoroughly enjoyed my experience during my time in the lab, it allowed me to develop my cell culture and western blotting skills, while also giving me an insight into what research works entails. Throughout project I encountered various challenges including failed blots, incomplete transfers and issues with sample buffers. This provided me with an opportunity to develop my troubleshooting skills as well as many other transferrable skills including effective communication and working independently and as part of a lab group. These skills, as well as my confidence in the lab which grew throughout the 7 weeks, will be invaluable as I complete my thesis next year as well as throughout the rest of my career. I would like to thank the British Pharmaceutical Society for the financial support which allowed me to fully commit to this project, and Dr Declan McKernan, for all his help and support throughout the seven weeks and who was integral in making this project such a positive experience.