University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Williamson Forename: Sarah

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2. Supervisor:

   Surname: Edgar Forename: Julia

   E-mail address: Julia.Edgar@glasgow.ac.uk

3. Research Project Report

   3.1 **Project Title (maximum 20 words):** Oligodendroglial-mediated axonal support during different axonal activity conditions.

   3.2 **Project Lay Summary (copied from application):** The myelin sheath is the insulation that surrounds nerve cell axons (wires) and speeds relay of messages from one nerve to another. We have shown that the myelin sheath also 'feeds' the axon. In multiple sclerosis (MS), the myelin sheath gets injured and eventually degenerates. More importantly, the underlying axon often gets damaged too, and this eventually leads to permanent neurological disability. We have evidence to support the possibility that axons get damaged in MS because the injured myelin sheath is unable to 'feed' them. We will

examine how inflammatory factors that are present in MS lesions might be involved. **Due to unexpected difficulties, we did not undertake the original study, but carried out a modification** focusing on the 'feeding' process hypothesised to occur between the 'myelinic channel', and the axon, in response to different axonal activity conditions.

Start Date: 1/06/16 **Finish Date:** 29/07/16

3.3 Original project aims and objectives (100 words max):

Hypothesis: Inflammatory factors, including tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) that are present in multiple sclerosis lesions, perturb the structure and/or function of the myelinic channel.

Aims: To visualize the myelinic channel in a cell culture model of central nervous system (CNS) myelin and quantify structural changes in the channel in response to inflammatory factors, over time.

3.4 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

Parent mice were genotyped by polymerase chain reaction (PCR) of genomic DNA and gel electrophoresis were used in order to identify transgenic Plp1CreERT2::'stop'-floxed td-tomato::CNP-PEX-EOS2 mice for breeding. Embryos in which oligodendroglial cells were labelled with tdTomato protein and peroxisomes with photoconvertible mEOS2 protein, were used to establish myelinating cultures. I was trained in health and safety when using carcinogenic ethidium bromide to visualise the PCR products. I was taught how to dissect embryonic mouse spinal cord; receiving training in micro-dissection and sterile technique. I was taught how to dissociate and plate spinal cord cells onto

coverslips and subsequently culture and feed them under sterile conditions. Following 24 days in vitro hydroxyamoxifen was added to cultures for 1 hour, minus those in the negative control, and the following day my supervisor fixed a selection of hydroxytamoxifen positive cover slips to represent time point 0 (T0). Some of the remaining cover slips (controls) were left untreated and fixed after 5 minutes (T5) or 1 hour (T1h) whilst the others were subjected to either one of the 3 conditions –increased potassium, increased glutamate, tetrodotoxin (TTX)- and fixed at T5 and T1h. I was also taught how to perform immunocytochemistry against 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) which allowed non tdTomato fluorescent cells to be easily visualised. I was trained to use an Olympus BX51 wide field epifluorescence microscope up to x60 oil immersion objective lens, and Micromanager 1.4.22 Software to perform~~ed~~ unbiased image capture of oligodendrocyte extensions and peroxisomes. I was also trained in using Image J software to add scale bars and count the peroxisomes contained within the myelinic channel.

3.5 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

We tested the hypothesis that neural activity increases numbers of peroxisomes in the myelinic channel by investigating the impact of different neuronal activity conditions on peroxisome numbers in the myelinic channel. The tamoxifen negative condition was used to test leaky transgene expression, and as expected, no td-tomato positive cells were identified. After immunocytochemistry labelling of CNP across all conditions and time points -allowing oligodendrocyte cell bodies and extensions to be visualised in the tamoxifen negative control - peroxisome counts were comparable to that seen in the tamoxifen induced control; figures 1a and b show a decline in peroxisome count over time to an end point of around half the initial count in both conditions. The results

obtained from the other 3 conditions (see figures 1c-e) show different trends; In the myelinated culture subjected to increased potassium levels to increase neural activity, 5 minutes of treatment showed little impact on peroxisome count in the channel relative to T0, with a decrease relative to T0 observed following 1 hour of treatment. The culture subjected to TTX to block neural activity displayed a substantial drop in peroxisome count at T5 following which a slight increase was seen at T1h, and cells exposed to increased glutamate levels, to increase neural activity, showed an increase relative to T0 at T5, following which a decrease to roughly T0 levels was seen at T1h. Peroxisome count peaked in the glutamate condition at T5, with an average of ~7 peroxisomes per 20um of myelinic channel, with the lowest count occurring at T5 in the TTX condition showing an average of ~2 peroxisomes per length. No inferential statistics were run as results over time were not truly independent.

3.6 Discussion (500 words max):

The results suggest that oligodendroglial cells respond to axonal activity, and hence, energy requirements by increasing transport of peroxisomes into the myelinic channel which extends along the myelinated axon. For each condition a decrease in peroxisome count was seen from T0 to T5 with the exception of the glutamate culture, and a noticeable decrease was seen from 5 minutes to 1 hour with the exception of the TTX condition which remained stable over this time period. This may suggest that the cultures response to the condition occurs within the first 5 minutes, with peroxisome release decreasing or halting soon after as, potentially, peroxisomes are actively transported through the channel during the remainder of the hour. This is supported by the results from the two control conditions which both show a gradual decrease in peroxisome count within the channel over time. In future experiments the cultures will be assessed over

more time points to determine whether this trend continues. When looking at each condition individually, most of the results were as expected; increased glutamate led to an initial increase in peroxisome count, whereas TTX administration lead to a decrease in peroxisome count within the channel. This is a reflection of what potentially may be a communication existing between axon and oligodendroglial cell, which allows the glial cell to reliably support the axon under high and low activity states. The increased potassium condition gave unexpected results, with the culture showing little to no change in peroxisome count after 5 minutes of exposure. Further biological repeats would be required to determine the reliability of these results, and given more time these would have been performed. Peroxisomes are an indirect way of measuring this transport system however subsequent experiments may look into labelling compounds related directly with energy transfer to the axon such as glucose and lactate. A further limitation lies in the poor quality of the myelinated cultures used causing difficulty in visualising cell morphology at times and as such issues arose in distinguishing the myelin extension from background fluorescence. This will have reduced reliability of the results, which given more time would have been negated slightly with further biological repeats.

This research ties-in with work looking at axonal degeneration, the source of many pathophysiological dysfunctions in diseases such as multiple sclerosis (MS). Recent work has provided evidence that a system independent from demyelination which causes degeneration of axons (Nikic et al 2011) with a possible theory being it involves transport of glycolytic products between oligodendroglial cells and axons. This is supported by the presence of lactate transporters on the border between axon and oligodendroglial cell (Rinholm et al 2011) as well as an apparent motor protein transport system contained within the myelinic channel (Funfschilling 2010). Our study supports the concept of a communication existing between oligodendroglial cells and the axons they ensheath, which allows axons to feedback how much energy they require. As this process may be involved in the pathophysiology of MS, it is important to study the communication and

relationship shared by the cells in order to generate novel therapies and treatments for neurodegenerative diseases.

4. **Reflection by the student on the experience and value of the studentship (300 words max):**

I am extremely grateful for the opportunity to work for the past 2 months in Dr Edgar's lab and have gained a great number of skills, both in and outwith the lab. I had the opportunity to work independently as well as with others. I followed various protocols directly relevant to my project. However, I also got the opportunity to work on other projects occurring in the lab, further expanding my knowledge and experiences. I gained an understanding of unbiased data collection and a greater understanding and respect for health and safety regulations; learning how to ensure both my own safety and the safety of others in a fast-paced and hazard-filled environment. I was welcomed warmly into weekly lab meetings and had the opportunity to present my data in many of them, further extending my confidence in public speaking and gaining a greater understanding of my own work. I also got the chance to listen to and understand other work going on in the lab and in other labs around the world; this was extremely interesting. I am grateful to have had the chance to talk to many PhD students about their experiences in getting a PhD and patiently answering my many questions, allowing me to become more confident in my choices for next year.

5. **Dissemination: (note any presentations/publications submitted/planned from the work):**

No plans yet with data as project was a preliminary study. My supervisor plans on running various biological repeats then potentially working with other conditions and/or time frames.

6. Signatures: Supervisor  Date 25/8/16

Student  Date 25/8/16

References:

Fünfschilling, U., Supplie, L., Mahad, D., Boretius, S., Saab, A., Edgar, J., Brinkmann, B., Kassmann, C., Tzvetanova, I., Möbius, W., Diaz, F., Meijer, D., Suter, U., Hamprecht, B., Sereda, M., Moraes, C., Frahm, J., Goebbels, S. and Nave, K. (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature*.

Rinholm, J., Hamilton, N., Kessaris, N., Richardson, W., Bergersen, L. and Attwell, D. (2011). Regulation of Oligodendrocyte Development and Myelination by Glucose and Lactate. *Journal of Neuroscience*, 31(2), pp.538-548.

Nikić, I., Merkler, D., Sorbara, C., Brinkoetter, M., Kreutzfeldt, M., Bareyre, F., Brück, W., Bishop, D., Misgeld, T. and Kerschensteiner, M. (2011). A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nature Medicine*, 17(4), pp.495-499.

Appendix:

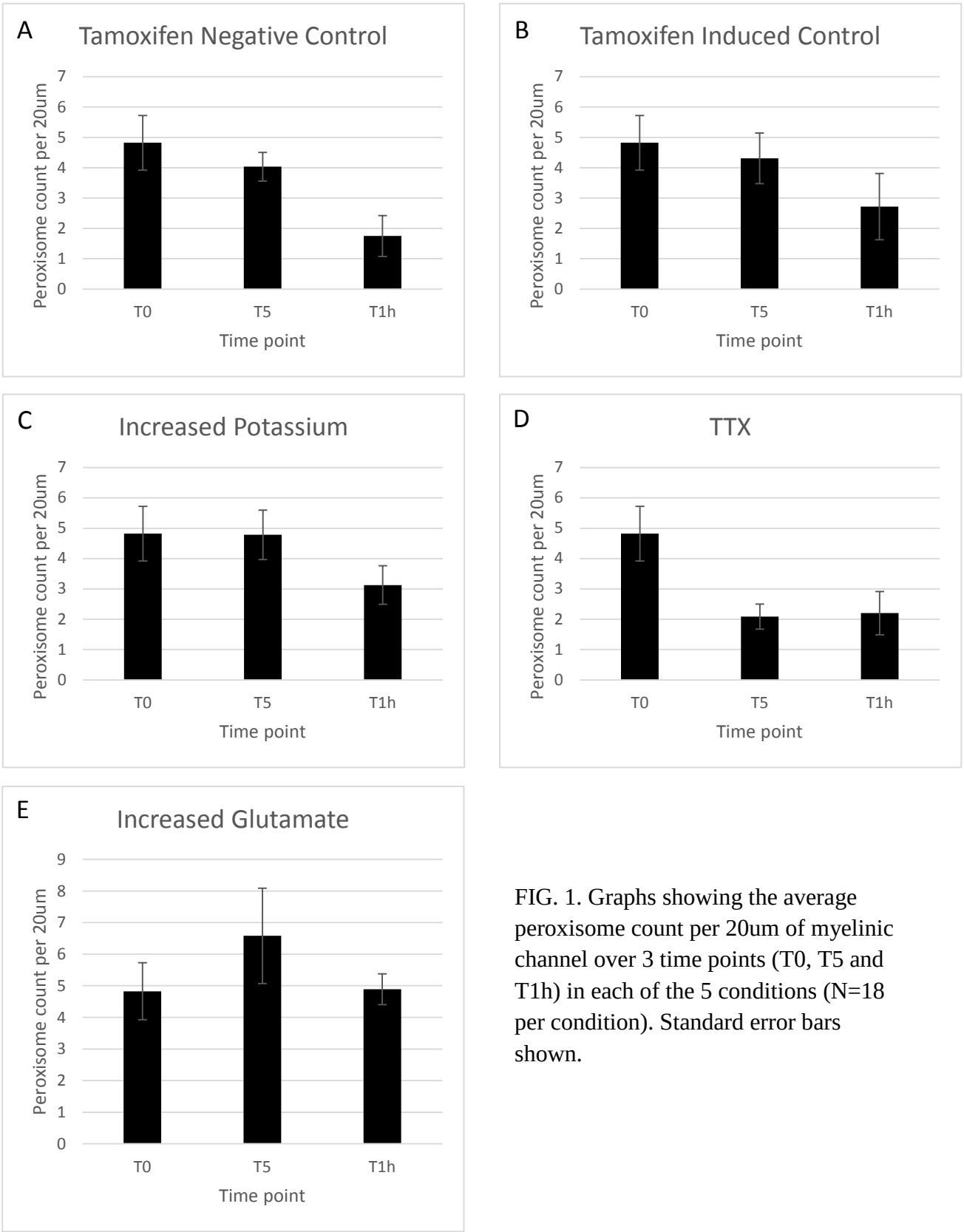

FIG. 1. Graphs showing the average peroxisome count per 20um of myelinic channel over 3 time points (T0, T5 and T1h) in each of the 5 conditions (N=18 per condition). Standard error bars shown.