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: **Espie** Forename: **Megan**

   E-mail address: **2175046E@student.gla.ac.uk**

2. Supervisor

   Surname: **Todd** Forename: **Andrew**

   E-mail address: **andrew.todd@glasgow.ac.uk**

3. Research Project Report

   3.1 Project Title (maximum 20 words):

Loss of presynaptic inhibition as a contributing factor in neuropathic pain

   3.2 Project Lay Summary (copied from application):

Neuropathic pain following peripheral nerve injury represents a major unmet clinical need, and development of new treatments requires improved understanding of pain processing within the spinal cord. We have recently identified a population of nerve cells that can block the activity of pain-transmitting sensory fibres by a mechanism known as presynaptic inhibition. We also have preliminary evidence that some of these cells are lost following nerve injury. This project will involve a quantitative analysis of spinal cord cells to confirm that there is cell death, and to test whether this is selective for these inhibitory nerve cells.

3.3 Start Date: **12/06/17** Finish Date: **08/09/17**

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

The main aims of this project are to quantify the loss of Rorb islet cells from the superficial dorsal horn following peripheral nerve injury, and to test the hypothesis that cell death is restricted to these neurons. Since neuropathic pain after nerve injury is triggered by activity in nearby, intact peripheral nerves, we will also determine the extent to which axons of individual Rorb islet cells project into regions of dorsal horn that are innervated by adjacent nerves.

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

Genetically modified mice with the genotype Rorb^{CreERT2};Ai9 were treated with tamoxifen between postnatal days 16-18. The administration of tamoxifen resulted in the nuclear translocation of Cre, excision of a STOP cassette and expression of tdTomato in Rorb-positive cells. The mice then underwent sciatic nerve transection and survived for 4 weeks. This allowed assessment of whether there is loss of calretinin-expressing inhibitory interneurons from the superficial dorsal horn following peripheral nerve injury. The mice were then anaesthetised and perfused with fixative, allowing their lumbar spinal cords to be removed. The spinal cord segments were cut into transverse segments using a vibrating-blade microtome before undergoing multiple-labelling immunofluorescence staining to reveal NeuN (expressed by all neurons), PAX2 (expressed by all inhibitory interneurons) and calretinin, which is expressed by a specific subset of inhibitory interneurons that presynaptically inhibit nociceptive C fibres. They were counterstained with DAPI to reveal cell nuclei. The sections were scanned using a confocal microscope. Before analysis began the microscope images were flipped so that I was blinded to which side of the spinal cord was ipsilateral to the nerve injury. Both sides of the spinal cord were examined and the results compared.
Quantitative analysis was performed using the optical dissector method on these sections. Reference and look up sections were set between 10 and 20 µm and all neurons for which the bottom surface of the nucleus lay between these were included. The Pax2 and calretinin channels were then used to identify inhibitory interneurons that were calretinin-positive or calretinin-negative.

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

NeuN-positive cells (neurons) were present throughout the dorsal horn, and were particularly numerous in the superficial laminae (I-II). A subset of these cells were Pax2-immunoreactive, and were therefore identified as inhibitory interneurons. Calretinin expression was largely restricted to the superficial dorsal horn, and most calretinin-immunoreactive cells were Pax2-negative (i.e.

excitatory neurons). As reported previously, a small population of cells that were immunoreactive for both calretinin and Pax2 was present in laminae I-II.

Quantitative analysis showed that the proportion of lamina I-II neurons that were inhibitory was 24.1% on the side ipsilateral to the nerve injury, and 24.4% on the contralateral side. Calretinin^{+}/Pax2^{+} cells accounted for 5.6% and 5.5% of all neurons on ipsilateral and contralateral sides, respectively.

3.7 Discussion (500 words max):

The dorsal horn of the spinal cord is part of the central nervous system that receives input from a number of sensory afferent fibres carrying information about various modalities including touch, pain, temperature and itch. The afferent fibres can be classified into those that are myelinated (A fibres) and those that are unmyelinated (C fibres). Nociceptive C fibres can be divided into two groups; non-peptidergic C fibres, which are mainly associated with the epidermis, and peptidergic C fibres, which innervate deeper skin and other tissues. Mas-related G-protein coupled receptor D is a sensory neuron specific G-protein coupled receptor that has been shown to define a population of non-peptidergic nociceptive C-fibres in the mouse. These C-MrgD afferent fibres convey nociceptive input from the skin. The C-MrgD fibres which enter the dorsal horn of the spinal cord activate interneurons, which in turn drive local circuits to process and modulate the pain before the brain can perceive it. In the dorsal horn of the spinal cord there are nerve cells that can block the pain information that is sent along the C-MrgD fibres by a mechanism called pre-synaptic inhibition. This reduces the ability of the C fibres to activate their target cells. A specific population of cells have been identified as presynaptically inhibiting the C-MrgD afferents via axoaxonic synapses. These cells can be identified by the expression of two proteins: the orphan receptor Rorb and the calcium-binding protein calretinin. These cells belong to a morphological population called islet cells, and this specific subset are therefore known as Rorb islet cells.

Unpublished data (D Hughes, M Mustapa, A Todd) has shown that there is loss of dendritic staining for Rorb islet cells in the dorsal horn after peripheral nerve injury, and the most likely explanation for this would be the death of Rorb islet cells. However, previous studies carried out in the rat have suggested there is no loss of inhibitory interneurons following peripheral nerve injury (Polgár et al 2005) and the findings from this experiment suggest that this is also true for the mouse. Alternative explanations for the loss of dendritic staining could be that for some reason tdTomato expression is switched off in these cells, or that there is shrinkage of their dendritic trees after nerve injury. The latter explanation seems quite likely, because these cells appear to receive much of their excitatory synaptic input from C-MrgD afferents, and these synapses are known to be lost following nerve transection.

In conclusion, the percentage difference of Pax2+ neurons and CR+Pax2+ neurons between the ipsilateral and contralateral sides was not significant, which suggests that there is no loss of Rorb islet cells following sciatic nerve transection. These cells could still contribute to neuropathic pain, because their axons are known to extend outside the mediolateral extent of their dendritic trees, and are therefore likely to extend into the territories of neighbouring peripheral nerves. Loss of input from the damaged nerve may therefore lead to reduced presynaptic inhibition of C-MrgD nociceptive afferents in nearby intact nerves.

(Polgár E, Hughes DI, Arham AZ, Todd AJ (2005) J Neurosci 25:6658-6666).

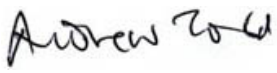
4. Reflection by the student on the experience and value of the studentship (300 words max):

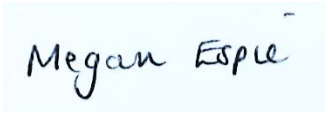
I feel very privileged to have been given the opportunity to complete this summer research project and it has given me an invaluable insight into the world of research and an appreciation for those who choose research as a full time career, having the patience to work on projects lasting a lot longer than just 6 weeks. It was a pleasure working in the Neuroscience lab where the staff explained everything clearly and were happy to answer all my questions. I observed many techniques in the lab including the use of a vibrating-blade microtome to cut sections of the spinal cord, immunofluorescence staining, confocal microscopy and I was taught how to analyse images using the optical dissector method on the computer programme Neurolucida. I also observed viral injections into the spinal cord of mice, perfusion of the mice and dissection of the mice. During my first week in the lab I was given some research papers to read relating to the topic of my research project and this really helped me to get my head around the terminology used and to further educate me about the nervous system and pain processing in the body. Neuroscience is an area that I became very interested in after completing a teaching block in neurology during my second year of medicine and it was great to see other people working in the lab who had previously completed a degree in medicine and are now working towards a PhD. Seeing the paths that they have chosen to follow has encouraged me to incorporate research into my future career and possibly complete an intercalated degree or PhD. I would love to get the opportunity to go back to the lab in the future and would recommend similar research projects to any student given the opportunity.

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

This work will form part of an article to be submitted to a scientific journal.

6. Signatures:

Supervisor  Date 07/09/2017

Student  Date 07/09/2017