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

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2. Supervisor:

Surname:	Hughes	Forename:	David I
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3. Research Project Report

3.1 **Project Title (8 words)**: Neuroanatomical features of identified spinal dorsal horn neurons

3.2 **Project Lay Summary (copied from application)**: Our ability to distinguish innocuous tactile sensations from those that induce pain is of critical importance to our daily survival. However, in some cases of chronic pain the capacity to discriminate between these sensory inputs may be lost. Sensory information is relayed from the periphery to the brain via the spinal cord. This project will study the normal connectivity of sensory fibres with functionally-defined neurons in the spinal cord as subtle changes to this basic circuitry in chronic pain states may underlie the resultant altered states of sensory perception.

3.3 Six weeks of work between a start date of 03.06.13 and an end date of 30.08.13

3.4 **Original project aims and objectives (71 words)**: This project aims to determine the frequency of excitatory inputs from defined origins on to electrophysiologically-defined excitatory interneurons using neuroanatomical techniques. We will use this data to form a comparison with parallel studies looking at excitatory inputs on to identified inhibitory interneuron populations. These experiments will improve our understanding of the complex circuitry of the spinal dorsal horn and ultimately will help develop more effective treatments to manage chronic pain states.

3.5 **Methodology: Summarise and include reference to training received in research methods etc. (238 words)**: This project used tissue from *in vitro* electrophysiological experiments where individual dorsal horn interneurons had been recorded from and labelled using whole-cell patch-clamp techniques. The tissue slices (400µm parasagittal slices of mouse spinal cord) were first scanned using a confocal microscope (Leica LSM 710) to determine the morphology of recorded cells and then re-sectioned into 60µm-thick sections. Individual sections containing large portions of a labelled cell's dendritic arbor were selected for subsequent immunocytochemistry to label the axon terminals of excitatory interneurons and modality-specific primary afferents. The sections were first incubated in a cocktail of primary antibodies to VGLUT2 (a marker of terminals from excitatory interneurons), VGLUT1 (a marker of central terminals from myelinated afferent fibres that mediate innocuous tactile sensations), and a combination of both isolectin B4 (IB4) and calcitonin gene-related peptide (CGRP) to distinguish pain-mediating C-fibres. These were then incubated in a cocktail of species-specific secondary antibodies conjugated to Alexa 488, Dylite 649 and Pacific Blue. Selected areas containing the dendritic arbor of labelled cells were then re-scanned on a confocal microscope, and the image stacks were used to plot the distribution of VGLUT1, VGLUT2 and IB4/CGRP inputs on to the recorded cells using Neurolucida for Confocal. Extensive training was received in histology, tissue processing, immunocytochemistry, confocal microscopy and data analysis. Examples of confocal image stacks illustrating contacts from VGLUT2, CGRP and IB4, and VGLUT1 terminals on to dendrites from recorded neurons are shown in Appendix 1.

3.6 **Results: Summarise key findings (81 words). Please include any relevant tables or images as an appendix to this report**: Five Calretinin eGFP islet cells were scanned and analysed. The total length of the dendrites from these cells varied from 1125.7 µm to 5360.3 µm. Most contacts plotted on to the dendritic arbor of these cells were from VGLUT2 expressing terminals (ranging from 58.1 - 74.2% of the total number of contacts), with a significant portion also from CGRP/IB4 expressing terminals (20.8 - 41.4%), but relatively sparse inputs from VGLUT1 expressing terminals (0.5 - 7.1%). For all data see Appendix 2.

3.7 **Discussion (394 words)**: The dorsal horn of the spinal cord is a region of the central nervous system (CNS) that receives direct input from afferent fibres serving a range of sensory modalities, including touch and pain. While much is known about the arborisation of these sensory fibres (Todd, 2010), relatively little is known about the cells they target or the synaptic circuits they are involved in. Work in Dr Hughes' laboratory aims to identify discrete dorsal horn interneuron populations and determine their role in identified spinal circuits. The Hughes laboratory, in collaboration with colleagues in Australia, has recently shown that a population of inhibitory interneurons which express the calcium-binding protein parvalbumin (PV) are responsible for modulating input from afferent fibres responsive to innocuous touch (Hughes *et al*., 2012). These cells, located mainly in laminae II inner and III, have characteristic anatomical and electrophysiological features: they are islet cells that are activated by principally by low threshold mechanoreceptive afferent inputs (LTMs) and show fast-spiking discharge patterns. Work in the Hughes laboratory to identify and define other classes of inhibitory (and also excitatory) interneurons, is on-going. This aim of this project was to use anatomical

techniques to determine the likely source of inputs on to a second class of cells with similar morphology, but different electrophysiological and neurochemical properties. These islet cells, which express the calcium-binding protein calretinin (CR) and also display fast-spiking firing patterns, are most prevalent in lamina II outer and receive a strong excitatory drive from as yet unidentified sources. My findings show that these inputs are likely to be derived primarily from excitatory interneurons, but also from putative nociceptive afferents, whereas inputs from LTMs are rare. While the axons of PV-expressing islet cells are known to modulate the passage of LTM into the spinal dorsal horn, the synaptic targets of CR-expressing islet cells remains to be determined. It is therefore clear that despite having similar morphology and electrophysiological properties, CR and PV islet cells respond to very different modalities of sensory input and are likely to influence activity in different sensory circuits. As PV-expressing islet cells are activated by, and in turn inhibit, sensory inputs from LTMs, it is tempting to speculate that CR-expressing islet cells provide a source of presynaptic inputs to modulate sensory input from nociceptive afferents.

Todd AJ (2010). Nature Reviews Neuroscience 11:823-836

Hughes *et al*., (2012). Journal of Physiology 590:3927-3951

4. **Reflection by the student on the experience and value of the studentship (226 words)**: The experience surpassed my expectations. I have been taught a number of techniques and skills that will be useful in my further studies and a future career, such as histology, tissue processing and confocal microscopy. I have also had the opportunity to observe a range of different research techniques and studies being carried out in the Hughes laboratory and the Spinal Cord Group, which has also been helpful and inspiring. The tasks given to me have been interesting and challenging, and being left to analyse cells by myself has given me confidence in my own abilities. There were always people around whom I could ask for help and advice, and I have felt nothing but welcome. The data I have collected has already been presented in a seminar series at the University of Melbourne, and will be included in a manuscript to be submitted for publication before the end of 2013. This has made me feel useful, further boosting my confidence and also inspired me to continue my studies and aim for a PhD after my undergraduate degree. All in all, the summer project has been challenging and very rewarding. I feel like I'm left with concrete skills and experience that will be helpful in my career, and I also feel more prepared for the final year of my degree and the 4th year research project.

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

   The data I have collected has already been presented in a seminar series at the University of Melbourne, and will be included in a poster to be presented at the Society for Neuroscience meeting in November. This data will also be included in a manuscript to be submitted for publication before the end of 2013.

6. Signatures:

Supervisor

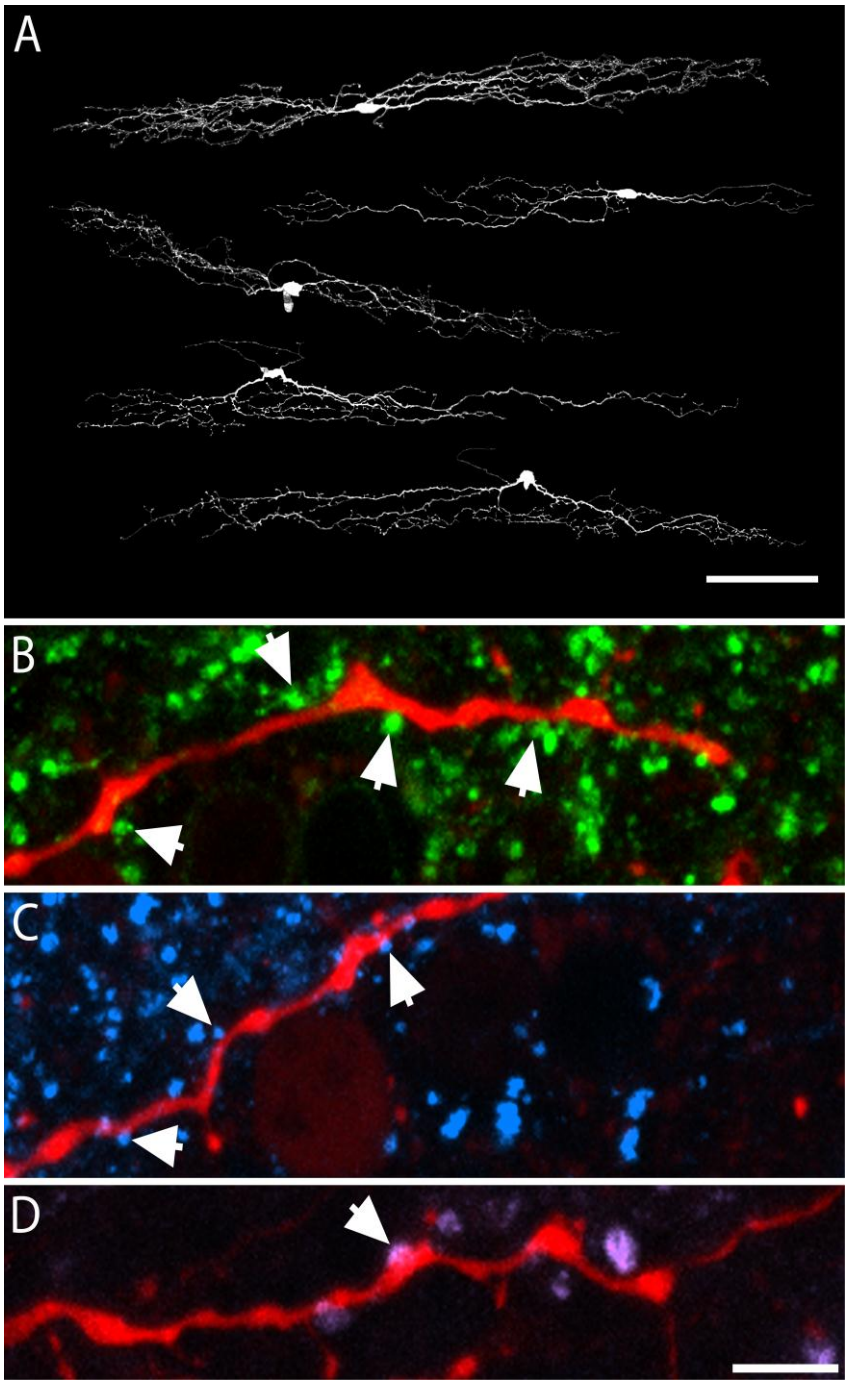
Date: September 24th, 2013.

Student

Date: September 24th, 2013.

Appendix 1

Figure 1. Panel A illustrates the morphological features of CReGFP expressing islet cells analysed during this study. Panel B shows examples of islet cell dendrites (red; B-D) receiving contacts (arrowheads) from axon terminals expressing VGLUT2 (green; B), IB4/CGRP (blue; C) and VGLUT1 (magenta; D). Scale bars: A= 100 µm; B-D = 5µm



Appendix 2.

Table 1. VGLUT2, CGRP/IB4 and VGLUT1 inputs on CR-expressing islet cells.

	No. of inputs	Total length (µm)	Inputs/100µm
VGLUT2	209	3873	5.4
	202	3946.9	5.12
	335	5630.3	5.95
	89	1125.7	7.91
	184	2669.7	6.89
Mean			6.3 (St Dev ± 1.1)
	No. of inputs	**Total length (µm)**	**Inputs/100µm**
CGRP/IB4	104	3873	2.68
	72	3946.9	1.82
	239	5630.3	4.24
	25	1125.7	2.22
	78	2669.7	2.92
Mean			2.8 (St Dev ± 0.9)
	No. of inputs	**Total length (µm)**	**Inputs/100µm**
VGLUT1	6	3864	0.15
	21	3946.9	0.53
	3	5360.3	0.05
	6	1125.7	0.53
	13	2669.7	0.49
Mean			0.4 (St Dev ± 0.2)