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

Head of College Scholars List Scheme
Summer Studentship 2019
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: maureen.bain@glasgow.ac.uk **within four weeks of the end of the studentship**.

1. Student
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2. Supervisor: Professor Andrew Todd, Dr Maria Gutierrez-Mecinas

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3. Research Project Report

   1. Project Title (maximum 20 words):

Local and intersegmental axonal projections of GRPR cells in the superficial dorsal horn of the mouse spinal cord

   2. Project Lay Summary (copied from application):

The spinal cord receives information from nerve fibres that respond to various types of sensory stimulus, including those that are perceived as pain or itch. Processing of itch information is thought to involve a specific population of nerve cells that have a receptor known as GRPR (gastrin releasing peptide receptor). While investigating, a population of GRPR cells in laminae I-II of the spinal cord in the 3rd lumbar (L3) segment was found to innervate other GRPR cells in the same segment, but also to project axons into the L5 (but not L4) segment. The original project was thus changed to investigate these findings. The aims were to test whether these findings are replicable and to begin to investigate the features of this population as well as the secondary projection. Knowing more about the nerve cells involved and their synaptic connections can improve understanding of itch and pain pathways to help find therapeutics for chronic pain and itch.

3. Start Date:05/07/2019Finish Date: 14/08/2019

4. Original project aims and objectives (100 words max):

The aim of this project is to replicate the findings that GRPR cells in L3 have axons that innervate other GRPR cells in the same segment as well as projecting to the dorsal horn in L5.

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

Axons and dendrites belonging to GRPR cells were identified by injecting adenoassociated viruses (AAVs) with Cre-dependent constructs into the spinal cords of mice that expressed Cre recombinase in GRPR cells ($GRPR^{CreERT2}$). Two AAVs were used, one coding for tdTomato, and the other for both tdTomato and green fluorescent protein (GFP) fused to synaptophysin. The GFP-synaptophysin conjugate will be targeted to axonal boutons, whereas the tdTomato will be present throughout the cytoplasm of GRPR cells. The procedures were carried out under general anaesthesia, and mice received appropriate post-operative analgesia. After a survival time to allow virus expression and resultant labelling, mice were deeply anaesthetised and perfused with fixative. Appropriate spinal cord segments were removed and cut into sections for immunostaining. Antibodies against VGLUT2 and the postsynaptic density protein Homer were used to reveal excitatory boutons and synapses, respectively. Immunoreacted tissue was mounted and scanned with a confocal microscope with the use of ZEN visualising software. Excitatory synapses, neuronal boutons and axons were identified and marked on confocal image stacks of superficial dorsal horn, using Neurolucida software. We determined whether axons of GRPR cells in L3 consistently projected into L5 and whether the projections have the same pattern and characteristics of the cells they are assumed to project from by looking at the tdTom stained length of the spinal cord longitudinally under the confocal microscope.

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

In mice injected with the GFP-synaptophysin/tdTomato AAV, axonal boutons derived from the GRPR cells could be identified by the presence of GFP and VGLUT2. These formed a plexus throughout the upper part of the dorsal horn (laminae I-III). We found that these often contacted dendrites of other GRPR cells, which were identified by the presence of tdTomato. GFP-labelled boutons contacting GRPR dendrites accounted for 30% of all GFP boutons in lamina I, 47% of those in lamina II and 27% of those in lamina III. We looked for evidence that these contacts represented glutamatergic synapses by searching for the post-synaptic density protein Homer. Homer was identified at the majority (~85%) of the contacts between GRPR boutons and dendrites in lamina I, and at around half (53-59%) of those in laminae II and III.

In the mice that received injections of AAV coding only for tdTomato, the injection sites were targeted to the L3 and/or L4 segments. In both cases, we could identify axons extending both rostrally and caudally from the injection sites within the dorsolateral fasciculus (DLF) and these formed numerous boutons in the lateral spinal nucleus (LSN). However, we also noticed that there was a consistent projection from the DLF into the medial part of the dorsal horn of the L5 spinal segment. These axons showed a very similar laminar distribution (laminae I-III) as those seen in the L3 segment, and were present for ~500 μm length of the L5 segment.

Discussion (500 words max):

While some findings reflected known features, there are two important novel observations. Firstly, GRPR cells appear to innervate other GRPR cells. This could represent a feed forward excitation, involved in triggering pain and itch as the majority of these connections appear in lamina I and II. Recent unpublished data from the Spinal Cord Group has provided evidence for functional synapses between these cells, by showing that optogenetic activation of GRPR cells resulted in direct synaptic activation of other GRPR cells. Such a feed forward excitation pathway involving several of the GRPR interneuron cells in pain and itch circuits has not been documented, and suggests a reverberatory network that could be targeted in therapeutics for chronic pain and itch states. As the tdTomato in the GRPR cells appeared to fade in the deeper lamina, the real proportion of connections to other GRPR cells especially to lamina III and onward, could be higher than the presented estimate.

A second surprising finding was that while GRPR cells were found to project from L3 both rostrally and caudally in the DLF and LSN, there was a specific projection only in the caudal direction that targeted the L5 dorsal horn. This is apparently the first time that such a specific intersegmental projection has been identified for interneurons in the superficial dorsal horn. The most likely explanation for this connection is that even though the medial parts of the L3 and L5 segments are located a considerable distance apart, they both receive input from nearby skin regions, close to the axial line of the hindlimb. It is therefore likely that the GRPR cells in the medial part of L3 were projecting to a region of L5 that received cutaneous input from a very similar region. This would allow the network linking GRPR cells to be maintained for particular skin regions, even when these projected to separate regions of the dorsal horn. If this is correct, then it is likely that a reciprocal connection (from medial L5 to medial L3) would also be present, and this can be tested in future studies. It will also be important to confirm that the projection from L3 to L5 targets GRPR cells in the latter location. This can be demonstrated by making injections of AAVs coding for different fluorescent proteins into these two segments.

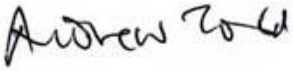
4. Reflection by the student on the experience and value of the studentship (300 words max):

This studentship has given me the opportunity to get a taste of my chosen career, with real responsibility and without having to compromise my quality of life as would be the case with unpaid laboratory assistances that would have been my alternative summer plans. I have been able to see scientists following their everyday life, with all the achievements and opportunities they are presented with, such as travelling the world to give talks or publishing exciting findings. I feel less intimidated by entering the world of research as all the staff have been incredibly welcoming, helpful and patient even when I asked questions or didn't understand the answer. I was taught how to prepare tissue, what steps to take to mark and preserve it, using a tissue slicing machine, immunolabelling techniques and others. I learnt the basics of using a confocal microscope and the software used with it to visualise different fluorescent stains. I also used marking software that could process the resulting image stacks. I was shown how to work with test animals, how they are cared for, how they are operated on to be injected with immunofluorescent markers, how the tissue is perfused and fixed in place ready to be viewed. I was encouraged to think about what I was doing and the results I was seeing. Working with a team of scientists with countless years of experience, published work and associated citations in many important studies within the field, I was also able to improve on my knowledge of nomenclature and on my report writing skills.

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

Further work will be done to try to pinpoint the origin of the projections found in L5 and the direction of their axons and dendrites by injecting tdTom and GFP into different lumbar segments and following the axons and dendrites of the neurons they label. Learning more about these neurons will help map the extent of GRPR neurons, their connections and the circuits they are involved in in superficial lamina, mainly in propagating excitation in pain and itch pathways.

6. Signatures:



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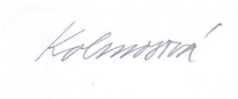

16/09/2019

Supervisor Date Student Date