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: Borland Forename: Shona

   E-mail address: 2024057B@student.gla.ac.uk

2. Supervisor:

   Surname: Cobb Forename: Stuart

   E-mail address: stuart.cobb@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Original title: Mapping the expression of MeCP2 in different peripheral cell types by fluorescence imaging.

   Final title: Measuring the transduction efficiency of SCAAV9MeCP2 at a high dose in the brain, liver and spinal cord using fluorescence imaging.

   3.2 Project Lay Summary (copied from application):

   Rett Syndrome (RTT) is a common genetic disorder affecting girls and results from mutations in the gene *MECP2*. Disruption of this gene in mice produces a constellation of 'symptoms' that mirror those seen in RTT quite closely. Significant efforts are being made to develop therapies for Rett syndrome based on targeting the nervous system with various

pharmacological and gene therapy-based interventions. As a foundation for such studies, it is important to identify the key neuronal and other cell types that contribute to different aspects of the disorder. We have made mice that express a fluorescent form of MeCP2 to map out the levels and distribution of MeCP2 in different cells. This information will help us discriminate functional deficits that are predominantly peripheral from those that are nervous system in origin.

3.3 Start Date: 4th August 2014 Finish Date: 12th September 2014

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

The original aim was to map the expression of MeCP2 in peripheral tissues. The aim, however, was changed and was to find the transduction efficiency of self-complementary adeno-associated virus 9 (SCAAV9) containing the MeCP2 gene at a high dose. Another aim, was to identify the proportion of transduced cells that were neurons.

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

Before my project started four mice (two mutant and two wild type) were intravenously injected with 10^{13} viral genomes per mouse of SCAAV9MeCP2. The mice were perfused about 10 days later. Tissue was dissected and put into 4% paraformaldehyde for three hours and then changed to a sucrose solution.

The brain, liver and spinal cord were sectioned using a vibratome to give 80µm sections. The sections were blocked using a 5% goat serum solution. The sections were washed and immunocytochemistry was carried out to label cells containing Myc (a label on the transgene) and to identify neurons (by labelling NeuN protein) in the brain and the spinal cord and Myc and MeCP2 protein in the liver to allow for confocal microscopy.

The tissues were incubated with DAPI to stain the nuclei of cells and then mounted on slides for confocal microscopy to be undertaken.

Image J software was used to analyse confocal stacks (taken on a confocal microscope) of CA1, motor cortex, brainstem, thalamus, liver and spinal cord for the four mice.

By counting the number of cells stained with DAPI and the cells containing the transgene (see appendix A and B), the percentage of cells which had been transfected in the liver, spinal cord and each part of the brain was calculated (the transduction efficiency). By counting the number of cells that were both positive for Myc and NeuN (see appendix B) in the spinal cord and brain sections the percentage of those cells that were transduced that were neurons was also calculated. Error was also calculated using standard deviation.

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

The transduction efficiencies for each mouse and each mouse part can be found in appendix C and D along with the average in appendix C. The average transduction efficient for the brainstem was 15.20±4.17%, the motor cortex was 12.85±2.43%, the liver was 38.22±23.00%, the spinal cord was 11.00±2.32%, the thalamus was 22.54±5.42% and the CA1 was 13.06±4.46%.

The percentage of cells containing the gene that were neurons for each mouse and for each part of the brain studied and the spinal cord are found in appendix E as well as averages. The average percentage of transduced cells that were neurons for each part was as follows: the brainstem 64.72±14.03%, the motor cortex 58.34±11.71%, the spinal cord 62.88±7.97%, the thalamus 71.32±7.07%, the CA1 71.40±4.46%. The proportion of cells that were NeuN positive and negative is also shown in appendix F.

3.7 Discussion (500 words max):

The results showed that over half of the cells that were transduced in the brain and spinal cord were neurons showing that the SCAAV9 mainly targeted neurons. In the brain, the thalamus had the highest transduction efficiency. There was a range of transduction efficiency of 11.00-22.54% in the brain and spinal cord. It also appeared that the thalamus and CA1 showed the highest proportion of neurons transduced. There was a range of 58.34-71.40% of transduced cells were neurons across the brain and the spinal cord.

The liver showed higher transduction efficiency than the brain and spinal cord in two of the mice. This increase was consistent with previous studies. In a previous study biomarkers in the liver with high SCAAV9 were studied and it was shown that marker were present for acute liver damage, however further studies looking at this will be carried out (Gadalla et al, 2013). It appeared that in the wild type mice there was not an increase and in the mutant mice there did appear to be, however due to the small sample size no conclusion could be made from this.

It also appeared that the Myc in neurons appeared brighter than in other cells. This could have been due to multiple viral genomes entering the cell and could show a further affinity to neurons over other cells, however further studies would have to be undertaken to accurately quantify and show this.

The mice became ill or lost 20% of their body weight and therefore had to be perfused earlier than planned at around 10 days after injection. This suggests that 1×10^{13} viral genomes per mouse is a lethal dose. This could be due to sudden expression of MeCP2 as this was seen when the gene was silenced and reactivated suddenly and was not seen at all when the process was done gradually (Guy et al, 2007). In the future the laboratory group will carry out studies at lower doses.

Gadalla, K, Bailey M, Spike R, Ross P, Woodward K, Kalburgi S, Bachaboina L, Deng J, West A, Samulski R, Gray S, Cobb S. 2013. Improved Survival and Reduced Phenotypic Severity Following AAV9/*MECP2* Gene Transfer to Neonatal and Juvenile Male *Mecp2* Knockout Mice. *The American Society of Gene and Cell Therapy* **21**:18-30

Guy J, Gan J, Selfridge J, Cobb S, Bird A. 2007. Reversal of Neurological Defects in a Mouse Model of Rett Syndrome. *Science* **315**: 1143-47

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

   I feel that the studentship has been a valuable experience. From the studentship, I have learnt a variety of new skills in particular in data and image analysis as well as immunocytochemistry and tissue sectioning- all of which I hope to be able to use again in the future. I got the opportunity to present my findings at the weekly lab meeting. I have experienced the environment of a working research laboratory (which you do not get from simply attending university classes) and learnt a lot about the research they are doing, have previously done and plan to do in Dr Cobb's lab. I saw how much work goes into working in a lab. My experience in the laboratory has helped me think more about the future and the possibility of doing a PhD after I have graduated and having a career in research.

   I saw the importance of research to people's lives when I attended a day trip with the research group to the Rett Syndrome Association Scotland weekend away in Badaguish and met families with their daughters who had Rett Syndrome. From talking to the families, I realised how important the research in Dr Cobb's laboratory is to the families as in the future the findings could change and improve their lives and this made me think more about the great importance of research overall.

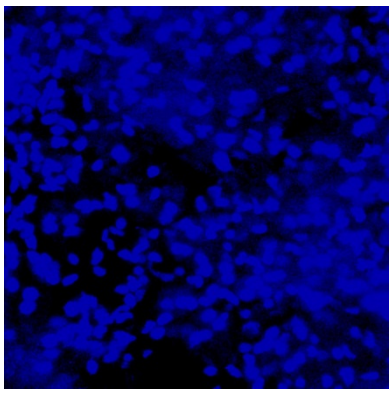
5. Dissemination: (note any presentations/publications submitted/planned from the work):

The work contained in this report will be pooled with a larger body of work in relation to our gene therapy studies. As such, the work will ultimately be disseminated through published papers and meeting presentations.

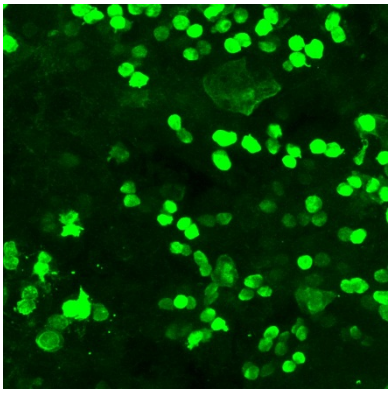
6. Signatures:

Supervisor	Date
	29/9/2014
Student	Date
	16/9/14

Appendix A: Example images of liver cells (mouse 83) used for counting

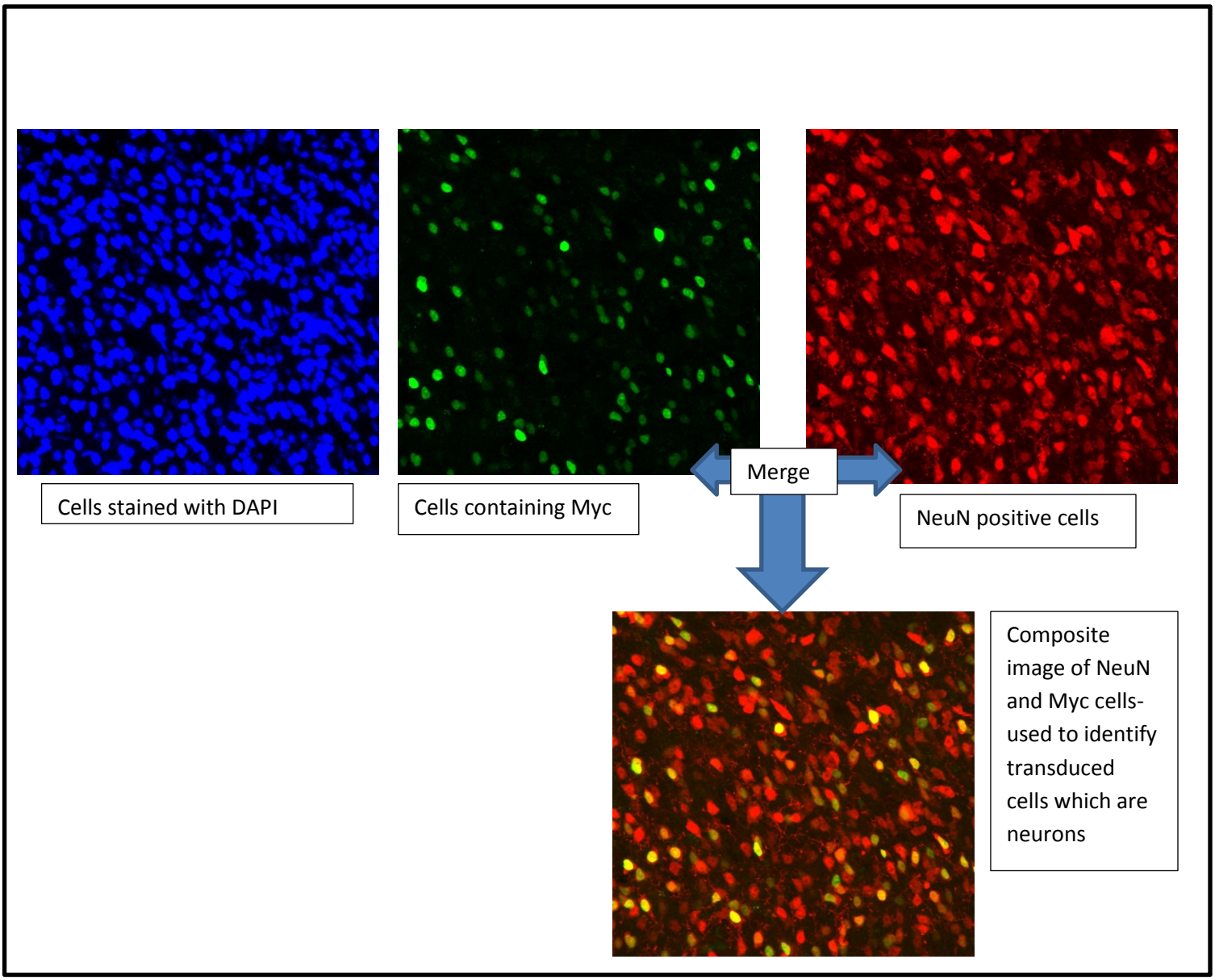


Cells stained with DAPI



Cells containing Myc

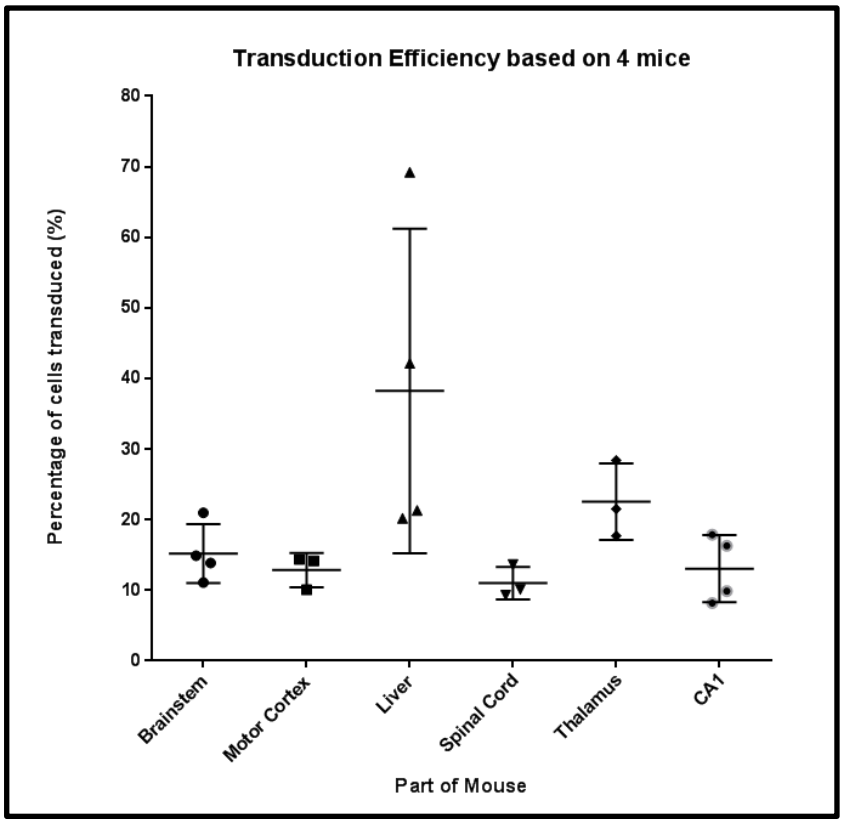
Appendix B: Example images from the thalamus (mouse 95) used for counting



Appendix C: Table showing the percentage of cells that were transduced for each part of the brain for each mouse.

	Percentage of cells transduced (%)					
	Mouse 82 Mutant	**Mouse 83 Mutant**	**Mouse 94 Wild type**	**Mouse 95 Wild type**	**Average**	**Standard deviation (%)**
Brainstem	14.91	11.07	20.97	13.86	15.20	±4.17
Motor Cortex	10.04	14.14	14.37	-	12.85	±2.43
Liver	42.16	69.22	21.31	20.21	38.22	±23.00
Spinal Cord	9.27	13.63	10.10	-	11.00	±2.32
Thalamus	21.53	17.70	-	28.40	22.54	±5.42
CA1	9.88	8.18	17.88	16.31	13.06	±4.46

Appendix D: Scatter plot showing the range of transduction efficiency including error bars



Appendix E: Table showing the percentage of transduced cells that were NeuN positive

	Percentage of transduced cells that were NeuN positive (%)					
	Mouse 82 Mutant	Mouse 83 Mutant	Mouse 94 Wild type	Mouse 95 Wild type	Average	Standard deviation (%)
Brainstem	44.8	77.56	66.95	69.57	64.72	±14.03
Motor cortex	48.27	71.20	55.55	-	58.34	±11.71
Spinal cord	60.12	71.87	56.66	-	62.88	±7.97
Thalamus	64.52	78.63	-	70.81	71.32	±7.07
CA1	65.03	72.22	75.43	72.90	71.40	±4.46

Appendix F: Graph showing the average transduction efficiency and the proportion of cells that were NeuN positive and negative