UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2020/21

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

Mr Joshua Ludkin

Name of supervisor(s):

Associate Professor Mohammad Hajihosseini

Project Title: (no more than 220 characters)

Microtubule Dynamics in the Stem Cell Niche of Postnatal Hypothalamus

Project aims: (no more than 700 words)

The hypothalamus is a critical homeostatic regulator, connecting the endocrine and autonomic systems of the body. It is important in the control of several important processes, including body temperature, heart rate, circadian rhythms and appetite. Previous work on this region of the brain has identified the existence of a population of β-tanycytes at the base of the third ventricle, that act as the neural stem cells and that give rise to new appetite-regulating neurons (Goodman and Hajihosseini, 2015). This is achieved via a continual process of symmetrical and asymmetrical divisions, resulting in the production of intermediate progenitors, in the form of α-tanycytes, that differentiate into hypothalamic neurons and few glial cells (Goodman *et al.*, 2020).

These above processes of cell division, delamination and differentiation likely require extensive cytoskeletal rearrangements, involving numerous cellular components. Microtubules are important components of the eukaryotic cytoskeleton, involved in mitosis, intracellular transport, as well as cell shape and motility (Brouhard and Rice, 2018). Regulation of microtubule dynamics is performed by several proteins, including microtubule-associated proteins (MAPs) and plus-end-trafficking proteins (+TIPs). Of these groups, the end-binding (EB) family of +TIPs are particularly important in the polymerization and growth at the microtubule ends, whilst also preventing catastrophe (Komorova *et al.*, 2009). The three distinct members of this group, EB1, EB2/RP1 and EB3, are coded by the MAPRE1, MAPRE2 and MAPRE3 genes, respectively (Su and Qi, 2001). Recent Studies have shown the presence of EBs play in a number of neural cells, whilst EB2 has been shown to also play a particularly important role in microtubule reorganisation during early apical-basal epithelial differentiation in the intestinal crypts (Goldspink et al., 2013). However, the presence and role of EBs in hypothalamus has yet to be extensively investigated.

The aim of this study was to investigate the expression of EB proteins in the hypothalamic stem cell niche and compare this with other regions of the brain, as well as to consider their potential involvement in asymmetric cell division and cell remodelling. Particular attention was paid to the possible selective expression of EB2 in β-tanycytes at the base of the third ventricle and a hypothesised down-regulation in α-tanycytes.

References:

Brouhard, G. and Rice, L., 2018. Microtubule dynamics: an interplay of biochemistry and mechanics. *Nature Reviews Molecular Cell Biology*, 19 (7), pp. 451-463.

Goldspink, D., Gadsby, J., Bellett, G., Keynton, J., Tyrrell, B., Lund, E., Powell, P., Thomas, P. and Mogensen, M., 2013. The microtubule end-binding protein EB2 is a central regulator of microtubule reorganisation in apico-basal epithelial differentiation. *Journal of Cell Science*, pp. 4000-4014.

Goodman, T. and Hajihosseini, M., 2015. Hypothalamic tanycytes—masters and servants of metabolic, neuroendocrine, and neurogenic functions. *Frontiers in Neuroscience*.

Goodman, T., Nayar, S., Clare, S., Mikolajczak, M., Rice, R., Mansour, S., Bellusci, S. and Hajihosseini, M., 2020. Fibroblast growth factor 10 is a negative regulator of postnatal neurogenesis in the mouse hypothalamus. *Development*, 147 (13).

Komarova, Y., De Groot, C., Grigoriev, I., Gouveia, S., Munteanu, E., Schober, J., Honnappa, S., Buey, R., Hoogenraad, C., Dogterom, M., Borisy, G., Steinmetz, M. and Akhmanova, A., 2009. Mammalian end binding proteins control persistent microtubule growth. *Journal of Cell Biology*, 184 (5), pp. 691-706.

Su, L. and Qi, Y., 2001. Characterization of Human MAPRE Genes and Their Proteins. *Genomics*, 71 (2), pp. 142-149.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Project Outcomes

Reverse transcription polymerase chain reaction (RT-PCR) analysis using gene specific primers showed that EB1, EB2 and EB3 are expressed in the hypothalamus of post-natal mice (fig. 1). Control area used, the cerebral cortex, also showed a similar expression. Interestingly, two bands of approximately 500 bp and 450 bp were observed for EB2 in both regions, indicating possible expression for two differing EB2 isoforms, with possibly different functions. The bands for all EB proteins were more strongly observed in the cerebral cortex compared to hypothalamus indicating greater expression of these proteins in the former when compared to the latter.

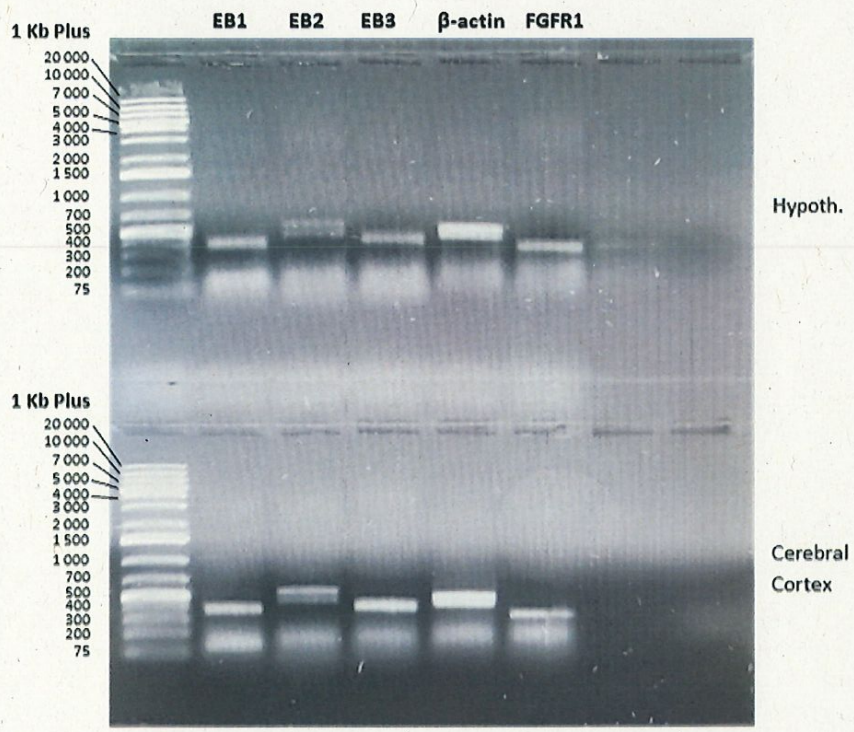

Figure 1. EB1, EB2 and EB3 expression in the cerebral cortex and hypothalamus of postnatal mice, using RT-PCR. Note the two bands of approximately 500 bp and 450 bp for EB2, indicating the possible presence of two isoforms of this protein.

To investigate the cellular compartments of EB expression, post-natal mice brains were fixed with either paraformaldehyde (PFA) or neutral buffer formalin (NBF) and sectioned using a vibratome or a cryostat at 60 and 20 µm, respectively. Sections were then stained with Rat-anti EB1-3 antibodies and detected with fluorescene-tagged secondary antibodies. Vibratome sections didn't show a clear signal, possibly due to poor penetration of antibodies. Subsequently, the protocols were refined and focusing on cryostat sections, immunostaining indicated EB expression in PFA and NBF-fixed thalamus sections treated with either methanol or citrate buffer (fig. 2). Of the treatments and fixative agents used, NBF sections treated with citrate buffer (G) provided the strongest indication of EB expression in the thalamus. EB expression was also detected in PFA-fixed hypothalamus sections treated with methanol. However, this pattern was not

consistent across all observed sections of this treatment and further testing is required to confirm the results. Little to no expression was observed in the base of the third ventricle, suggesting that EB2 and EB3 may not be present in the β-tanycytes of the hypothalamus, or that the antibodies used do not detect an isoform expressed in hypothalamus.

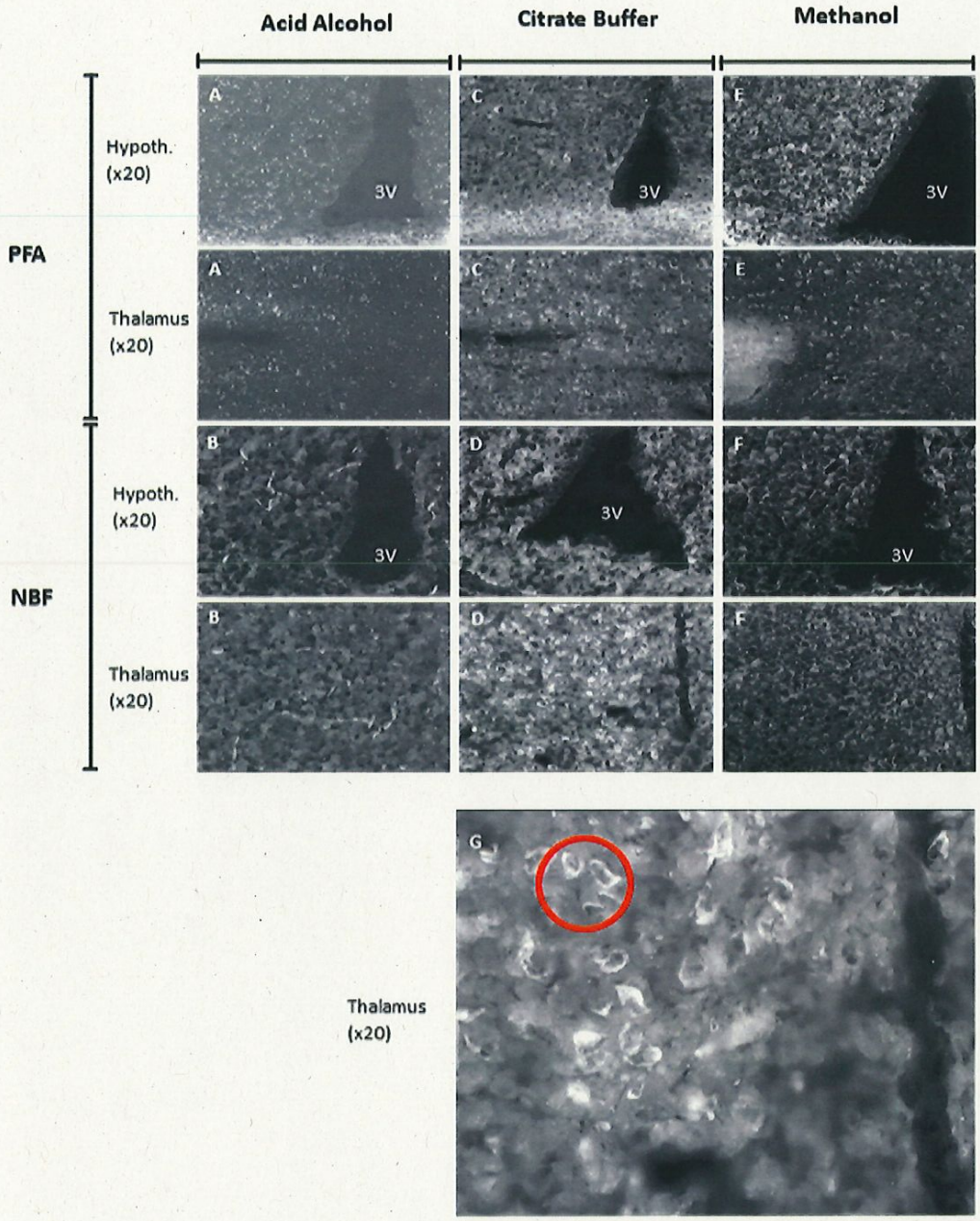

Figure 2. EB2/EB3 expression in PFA-perfused and NBF-fixed postnatal mice brains following treatment with acid alcohol (A-B), citrate buffer (C-D) and methanol (E-F). All tissue samples obtained using cryostat. (G) EB2/EB3 expression in NBF-fixed thalamus section treated with citrate buffer. Note perinuclear expression pattern consistent with EB expression.

Experience Gained

During this project, I was able to gain an understanding of the skills required to plan and undertake a research project, and the need to successfully time manage. I also gained knowledge and experience in several laboratory techniques, including:

- Primer design and RT-PCR
- Vibratome and cryostat sectioning
- Immunolabelling, including the use of various fixative agents, treatments and antibodies.
- Light microscopy and image capture/ analysis

The requirements of these techniques also meant that I was able to regularly practice core skills, including the calculation of dilutions, pipetting and database usage.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer 2022

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

The hypothalamus is a critical homeostatic regulator, important in the control of several important processes, including body temperature, heart rate, circadian rhythms and appetite. Previous work on this region of the brain has identified the existence of a population of β-tanycytes that act as the neural stem cells and that give rise to new appetite-regulating neurons (Goodman and Hajihosseini, 2015). These cells undergo a process of delamination and migration away from the base of the third ventricle hypothalamic stem cell niche (Goodman et al., 2020), and likely require extensive cytoskeletal rearrangements. Microtubules are important components of the eukaryotic cytoskeleton, involved in mitosis, intracellular transport, as well as cell shape and motility (Brouhard and Rice, 2018). Microtubule dynamics are regulated by several important proteins, including the end-binding (EB) family of plus-end-trafficking proteins (+TIPs), that consist of EB1, EB2/RP1 and EB3 (Komorova et al., 2009). These proteins have been shown to be present in several neural cells. However, their presence and role in hypothalamus has yet to be extensively investigated.

In this study, EB expression in the post-natal hypothalamus of mice was compared to other regions of the brain, notably the cerebral cortex and thalamus. Reverse transcription PCR (RT-PCR) analysis indicated expression of the three EB families (EB1, EB2, EB3) in both hypothalamus and cerebral cortex, although expression was stronger in the latter. Interestingly, two bands were observed for EB2, indicating the possible presence of two spliced isoforms in both hypothalamus and cerebral cortex. Immunostaining indicated EB expression in the thalamus. However, little expression was observed in the hypothalamus, with no expression observed at the base of the third ventricle, suggesting that EBs may not be present in the β-tanycytes of the hypothalamus, or that the isoforms detected by antibodies used is not present.

References:

Brouhard, G. and Rice, L., 2018. Microtubule dynamics: an interplay of biochemistry and mechanics. *Nature Reviews Molecular Cell Biology*, 19 (7), pp. 451-463.

Goodman, T. and Hajihosseini, M., 2015. Hypothalamic tanycytes—masters and servants of metabolic, neuroendocrine, and neurogenic functions. *Frontiers in Neuroscience*.

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Brief Resume of your Project's outcomes: (no more than 200-250 words).
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Title: Microtubule Dynamics in the Stem Cell Niche of Postnatal Hypothalamus

The hypothalamus is a critical homeostatic regulator, important in the control of several important processes, including body temperature, heart rate, circadian rhythms and appetite. Hypothalamic neural stem cells, known as β-tanycytes, undergo a process of delamination and migration away from the base of the hypothalamus before forming new appetite-regulating neurons (Goodman et al., 2020). Cytoskeletal rearrangements and microtubule dynamics are likely to play an important part in these processes, requiring the involvement of the end-binding (EB) family of plus-end-trafficking proteins (+TIPs), consisting of EB1, EB2/RP1 and EB3 (Komorova et al., 2009). These proteins have been shown to be present in neural cells. However, their presence and role in hypothalamus has yet to be extensively investigated.

In this study, EB expression in the post-natal hypothalamus of mice was compared to other regions of the brain, notably the cerebral cortex and thalamus. Reverse-transcriptase PCR (RT-PCR) analysis indicated expression of all three EB families (EB1, EB2, EB3) in both hypothalamus and cerebral cortex, although expression was stronger in the latter. Interestingly, two bands were observed for EB2, indicating the possible presence of spliced isoforms in both hypothalamus and cerebral cortex. Immunostaining indicated low EB expression in the thalamus. However, little expression was observed in the hypothalamus, with no expression observed at the base of the third ventricle, suggesting that EB2 and EB3 may not be present in the β-tanycytes of the hypothalamus, or that the antibodies/protocols used are ineffective in detecting EB proteins.

References:

Goodman, T., Nayar, S., Clare, S., Mikolajczak, M., Rice, R., Mansour, S., Bellusci, S. and Hajihosseini, M., 2020. Fibroblast growth factor 10 is a negative regulator of postnatal neurogenesis in the mouse hypothalamus. *Development*, 147 (13).

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