SRF VACATION SCHOLARSHIP REPORT 2016

Student's Name:	James Healy	**Student's Institution/University:**	University College Cork
Degree Title and year of study:	BSc. Physiology Year 4 of 4		
Supervisor's Name:	Professor William H. Colledge	**Supervisor's Department and Institution:**	University of Cambridge
Project Title:	Validation of a Kiss1r antibody using Kiss1r knockout mice		

Briefly outline the background and aims of the project *(max 200 words)*

The background aims of this project were to validate commercially available antibodies to see if they were effective immunohistochemical agents in detecting GPR54. GPR54 or Kiss1r, a G-protein coupled receptor expressed by GnRH neurons is activated by the potent ligand Kisspeptin. Kisspeptin, the neuropeptide encoded by the *Kiss1* gene, is required for pubertal activation of the mammalian reproductive axis at puberty and maintenance of fertility in adults (d'Anglemont de Tassigny & Colledge, 2010). Kisspeptin stimulates GnRH release in many mammalian species and mice lacking kisspeptin or with mutations in GPR54/KISS1R fail to undergo puberty and are sterile (Seminara *et al.,* 2003, d'Anglemont de Tassigny *et al.,* 2007). Mutant mice have underdeveloped gonads (hypogonadism), low levels of gonadotropic hormone (hypogonadotropism) and low sex steroid levels. In the adult mouse, there are two regions in the hypothalamus where kisspeptin neurons are localized; the arcuate (ARC) and the anteroventral periventricular nucleus (AVPV). In females, the AVPV *Kiss1* neurons are required for the pre-ovulatory GnRH/LH surge and make connections with GnRH cell bodies. In contrast, the ARC *Kiss1* neurons rarely connect to GnRH cell bodies and probably mediate basal GnRH release by an action at the median eminence of the hypothalamus. Expression of GPR54 at GnRH nerve terminals in the median eminence has not yet been demonstrated because of the lack of a suitable antibody. There are several anti-GPR54 antibodies commercially available (Table 1) but these have not been properly validated.

Table 1: Test Antibodies.

Supplier	Cat. Number	Host Species	Antigenic Site
GeneTex	GTX54378	Rabbit	Not provided
MyBioSource	MBS242888	Rabbit	18 aa, 3rd Cytoplasmic Domain
Antibodies Online.com	ABIN741180	Rabbit	aa 139-157
AbCam	ab140839	Rabbit	17 aa, 2nd extracellular domain

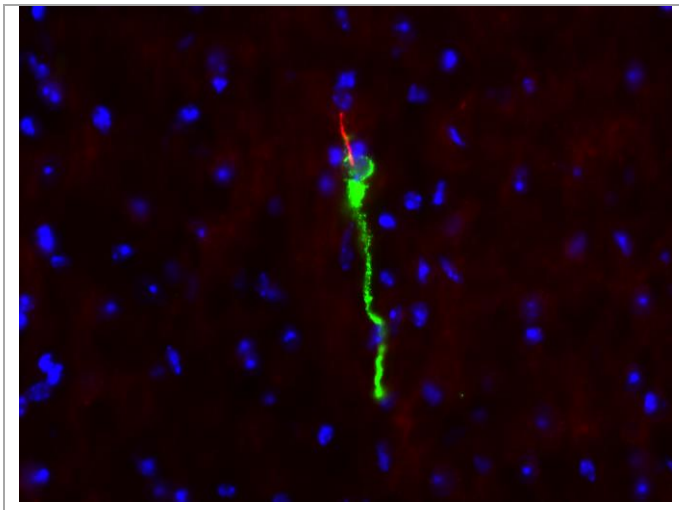
SantaCruz	sc-48218	Goat	C-terminal cytoplasmic domain
Strategic Diagnostics	Not provided	Rabbit	GPR54 -Amino Acid 348-396

Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

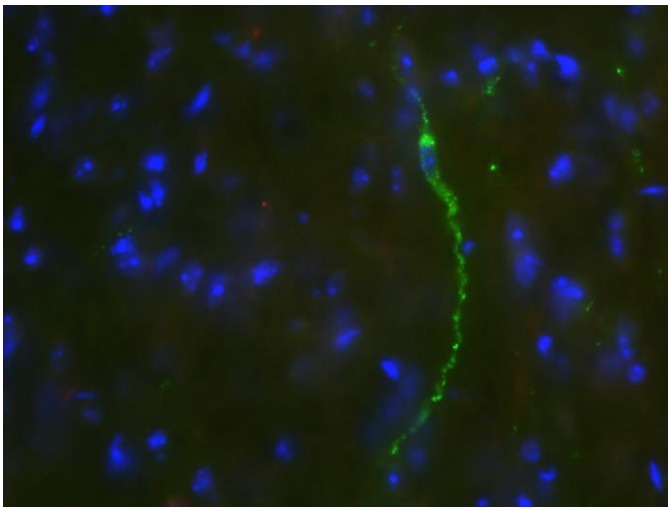
From analysis of the relevant literature, the antibody produced by Strategic Diagnostics proved to be the most successful in directly targeting GPR54. Our choice to solely test anti-GPR54 (Amino Acid 348-396) was reinforced by the data gathered from past research in the Colledge lab, which showed that the first five antibodies in table 1 showed no significant specificity to GPR54. The majority of the immunohistochemical work took place in the preoptic area of the brain, as the median eminence contained levels of background too high for accurate judgement of GPR54's presence in the nerve terminals. Time constraints of the project limited further confirmation of primary cilia attached to GnRH cell bodies in the preoptic area of the brain. Time was also a limiting factor for sufficient elimination of the background in the median eminence.

What were the main results/findings of the project? *(max 300 words)*

The key finding of the project was the validation of a suitable antibody to identify GPR54. The primary antibody used was a Rabbit Polyclonal Anti Kiss1r/GPR54, specifically against the amino acid sequence 348-396. Successful location of GPR54 was found as part of a cellular appendage, a primary cilia like structure attached to GnRH cell bodies in the rostral preoptic area of the murine brain, specifically above the third ventricle. To validate the Kiss1r antibody, we performed dual immunofluorescent staining using coronal brain sections from adult mice. Specifically, four Kiss1r knockout mice, Kiss1r tm1Coll (Seminara et al., 2003) were compared to three wild types using equal volumes and concentrations of the rabbit polyclonal anti-Kiss1r primary antibody. The primary antibody was diluted in 1:5000, an Alexa Fluor 568-conjugated goat anti-rabbit secondary immunoglobulin was used for visualization of the Kiss1r immunoreactivity with red fluorescence. We also used a 1:2000 concentration of sheep polyclonal anti-GnRH antiserum followed by Alexa Fluor 488-conjugated donkey anti-sheep secondary immunoglobulin to label GnRH neurons with green fluorescence.



Wild type mouse, HG696, at a magnification of 40x. GnRH cell body in green, stained by Alexa 488, primary cilia like appendage containing GPR54 in red, stained by Alexa 568 and the nuclei, stained blue with DAPI.



Mutant knock out, HG762 -/-, at a magnification of 40x.

What do you conclude from your findings? *(max 150 words)*

That the Rabbit Polyclonal Anti Kiss1r/GPR54, specifically against the amino acid sequence 348-396, successfully located GPR54 in the rostral preoptic area of the mouse brain in primary cilia like cellular appendages. This matches the results of the novel study by (Koemeter-Cox et al. 2014), who first made this antibody.

How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? *(max 150 words)*

This studentship has been a lesson. From a scientific perspective I was hooked on the possibility of new discoveries and the ramifications of those novel findings. The idea that perseverance in the lab would equate to the elucidation of previously unknown knowledge or further the understand of a known process, was exciting. Though it was the frustrating days which confirmed that a career in scientific research was the correct decision for me. Those days where experimental error, followed backfired techniques and mechanical mishaps, tested my attributes of patience and optimism to great extents. What brought me back from these depths was not the goal of personal achievement or gaining recognition but it was the belief that this work was larger than myself. The belief that my perseverance in the lab could

eventually provide medical relief to an individual, allowed me to push past the hardships associated with research, and gave me the renewed optimism to troubleshoot and problem solve to achieve the appropriate solution. It was this feeling of selflessness and the motivation it gave me, which cemented research as my ideal career choice.

Please use the space below to add any other comments/thoughts about the SRF Vacation Scholarship *(max 100 words)*

Student: This studentship has allowed me to receive an invaluable experience, one which will stand with me for the rest of my life. For that reason alone I am very grateful for this opportunity and I hope the SRF stays facilitating undergraduates with these life changing experiences for years to come.

Supervisor: I think that James had a very productive time in the lab and mastered the techniques of cryosectioning and immunohistochemistry. He independently validated a GPR54 antibody and based on his data we will continue to use this antibody for future work. This data has been a great help to our research. James was very hard working and conscientious and I believe will make a great PhD student.

References:

Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK et al. 2007. "The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone." J Neurosci 27(44):12088–12095

Koemeter-Cox, Andrew I., Thomas W. Sherwood, Jill A. Green, Robert A. Steiner, Nicolas F. Berbari, Bradley K. Yoder, Alexander S. Kauffman, et al. 2014. "Primary Cilia Enhance Kisspeptin Receptor Signaling on Gonadotropin-Releasing Hormone Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 111 (28): 10335–40.

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK et al 2003. "The GPR54 gene as a regulator of puberty". N Engl J Med 349(17):1614–1627