Scholar

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Title of Scholarship Project:	Microglia Activation in Ageing and Neurodegenerative Diseases

Please describe the research you have conducted this period

My research project is titled 'Microglia Activation in Ageing and Neurodegenerative Diseases'. Microglia are found in the Central Nervous System (CNS) and their main function is to carry out an inflammatory response as a result of trauma or injury. Microglia are also known as 'scavengers' because of their nature to constantly search for debris or damages/dead paths and clear them away to avoid them becoming toxic to any other cells. Because of this, microglia are extremely dynamic. The microglia activate to maintain homeostasis after a traumatic brain injury (TBI). Here are known as M1 microglia. After inflammation and neurotoxicity, M2 microglia take over, which have the role of anti-inflammation. However, sometimes the microglia process gets stuck in the M1 phase, which causes constant activation and inflammation. This causes damage to the surrounding tissue which leads to cell death and eventually Neurodegeneration.

There are some drugs that are able to inhibit/ halt the activation of this microglia, but they exist with unwanted off-target effects. A class of drugs called HDAC inhibitors are also effective in inhibiting microglia activation but the mechanism by which they do so is unknown. Our aim was to test some of these HDAC inhibitors and find out which ones are the most effective in controlling microglia activity and explore the mechanisms by which they do so. The two drugs we used during our research period were Lipopolysaccharide (LPS) and Suberoylanilide Hydroxamic Acid (SAHA). It was also believed that when the cells are contained in a serum, a higher percentage of serum would result in an increase in cell growth, so we decided to check if reduced serum would lead to reduced activation.

The BV2 line of microglia were used rather than primary microglia cells because they can mimic the actions of microglia. They can trigger biological processes; the same way primary microglia can activate astrocytes. They are cheaper and easier to use and reduce the unnecessary overuse of animals.

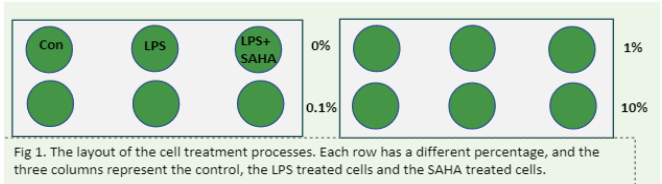


Fig 1. The layout of the cell treatment processes. Each row has a different percentage, and the three columns represent the control, the LPS treated cells and the SAHA treated cells.

The methods that were carried out involved counting the cells in a given volume of serum, splitting them into 6 well plates of different percentages of serum (Fig.1), treating the cells with LPS and SAHA and then carrying out cDNA synthesis and quantitative polymerase chain reactions (qPCR) (Fig.2) Some cells were treated and placed under an imaging microscope over time to obtain a time lapse of the cells after being treated and track how far each cell managed to move.

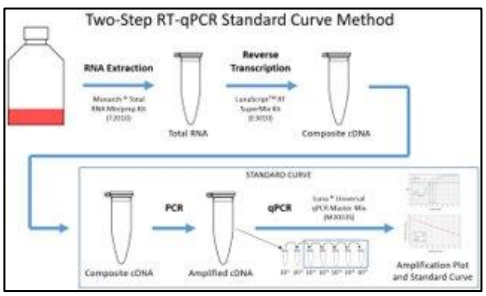


Fig.2 Process of two step RT- qPCR used to create a standard curve

After collecting the images from the microscope, I put the images together in sequence for each well to produce a time-lapse, I then put that into the Image J software and used the plugin trackmate to highlight and track the total distance moved by the cells. The results were then put into the coding system jupyter where graphs of my results were made.

	%CON 1	%CON2	%CON3	%CON4	MEAN	STANDARD DEV
CON0	8.9	15.5	26.5	15	16.4	7.32
LPS0	100	100	100	100	100	0
SAHA0	10.9	54.9	50.3	17.1	33.3	22.5
CON0.1	12.2	3.4	35.5	28.9	20	14.8
LPS0.1	100	100	100	100	100	0
SAHA0.1	25.2		48.2	27.5	33.6	12.7
CON1	16.2		23	35	24.7	9.5
LPS1	100		100	100	100	0
SAHA1	19.1		42.2	2.1	21.1	20.1
CON10	7.1	3.4	29.4	34	18.5	15.5
		12.3				
LPS10	100	100	100	100	100	0
SAHA10	82.9	2.5	21.3	7.8	28.6	37.0

Fig. 3 Table of qPCR results of treatment of cells with LPS and SAHA at different concentrations of serum

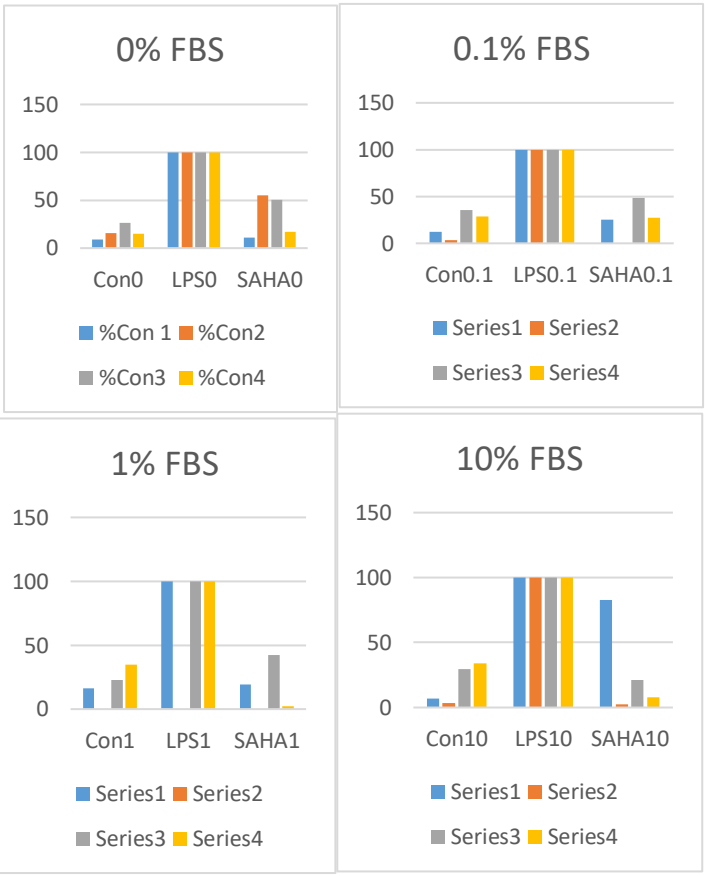


Fig. 4 graphs of qPCR results of treatment of cells with LPS and SAHA at different concentrations of serum

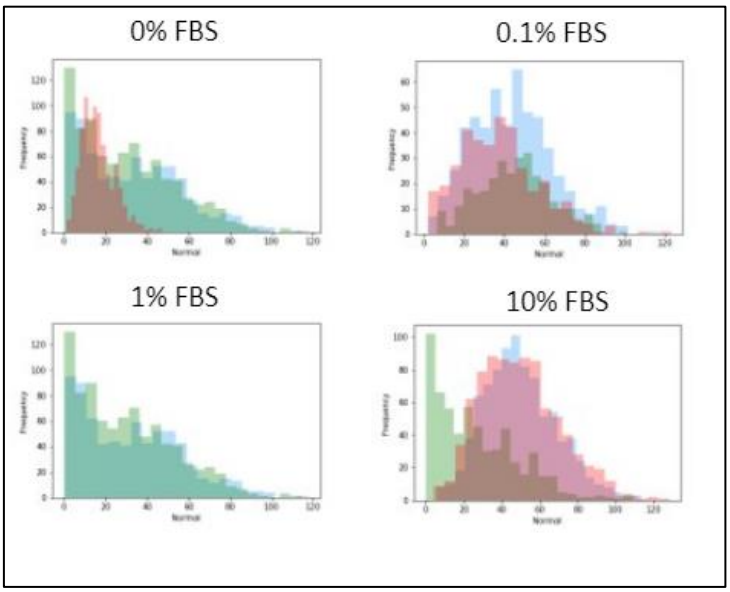


Fig. 5 Histograms of the total distance moved by BV2 cells under the microscope at different percentages of media. Cell images taken on

The results showed that SAHA was successful in inhibiting the activation of microglia. However, the concentration of the media used did not seem to make any difference on the effect of the drug. The different concentrations of serum did manage to prove that the movement of cells increases when a higher concentration of serum was used, which was expected.

How is the research work you have been undertaking impactful or important?

I think that the research I have undertaken is important because no matter how small I was able to contribute something to the research of science. When it comes to scientific research, it takes a while to get adequate results, so although I would have loved to do more, and expand my research, I am very happy that I was able to produce results in only 6- weeks. The research I have done will hopefully be of aid to anyone who wants to investigate microglia and neurodegenerative diseases, and I feel as though I have been able to help us move a step forward in research. Over 1 million people within the UK alone suffer with neurodegenerative diseases. One in 6 of the world's population suffer from neurological disorders. In 2016 they were the leading cause of Disability-Adjusted life years (DALYs). It is a huge issue globally and it affects so many people's day to day lives apart from those suffering from the diseases, their loves ones around them. Hopefully one day the research done will be able to find the main cause for these diseases and put us on the right path towards stopping them.

What impact has conducting research had on your degree course and university experience?

Doing this research projects over the summer was an amazing experience for me because it was neuroscience based so I was able to gain understanding of my course to a new level. Spending 6 weeks in the lab allowed me to learn so many things that might not be available to most people at my level of study. It also prepared me for things that I may be facing in my second year. I was able to learn processes such as counting cells, cell passaging, RNA extraction, coding, as well as venturing through the complex system that is Image J. I was extremely thankful that I was given the opportunity to be independent with most aspects in my research because it is an extremely rewarding experience once you reach your goa. Processes such as qPCR and cDNA synthesis, I had learnt about in lectures, however, being able to actually carry out the processes in a lab and multiple times at that was challenging at first but very educating. I was able to understand what it takes to carry out scientific research and putting my work together, making diagrams and my poster has helped me process all the information I got from my 6- weeks. I am eternally grateful to my supervisor for all his efforts and for being patient with me but also challenging me and helping me do the best that I can in the few weeks I was working with him.

What leadership skills do you believe you have gained from the research period? (Please refer to the leadership attributes below)

I feel as though I have gained a wide variety of skills over this research period. Firstly, I think I was able to lead without supervision at times. As stated earlier, the research we did was mostly independent. Of course, there would be times where I would come to a bump in the road and would be stuck at what I should do. In situations like this I would try my best to overcome the issues independently and most times I would be able to. I believe I was able to listen with understanding. Some of the processes that we had to carry out in the lab were rather complicated at first glance, but once I had them explained to me by either my supervisor or another student working in the lab, things would start to click into place. I think one thing I did very well was turning ideas into action. After I had received the time lapse of my cells, I needed to figure out a way to track them using Image J. I had only used image J once before, and even at that we had an entire protocol to follow, so essentially, I was going into this task blind. It was an extremely challenging task because of the number of cells in each image, I knew I couldn't track them individually, so I had to find a way to track them all at once. This task really helped me hone my independent research skills. I brainstormed with a lot of different ideas and there was a lot of trial and error, but I was eventually able to track them which was so rewarding. This situation could also fall under the

ability to navigate new and foreign environments. Apart from ImageJ, I had to use a coding software called jupyter which I had never done before, and I have never been very strong in IT related subjects, so it was quite a challenge for me, but I was able to slowly gain an understanding as I went along. I had a lot of data, since we took repeat experiments, and we were dealing with a lot of cells, so I had to be able to analyse all of that data and make something of it. That is one skill I am very grateful to have gained because I know in my next academic year, we will be dealing with more data sets in which we will have to code and use statistics etc. So having that background understanding will be helpful.

Please talk about activities you've been involved in to disseminate your research, including but not limited to attending conferences, producing research posters, and promotion of the scholarship

I have created a poster to post onto the Laidlaw Network and give other scholars an insight of the work I have done over those 6-weeks. I have tried my best to make it as detailed as possible but also make it easy to understand for people who don't do biomedical sciences so may find the information somewhat complicated. I am very happy to be able to share my work with others and to see the work that they have done. Apart from this, I also made a guide to using Image J to track cells. The method I used was a mix of multiple methods that I had found online put together. It was a very complicated process trying to find a way to track all my cells at once as they moved so I felt as though making a small guide would be a great opportunity for others if they ever decided to try something similar to what I have done. I also have multiple video clips of my time lapses with my tracking tool on top, so that it is clear to see where each cell is moving and what path it takes to get there. The University of Leeds has provided us as scholars with a lot of opportunities to meet up and attend talks where we learn about each other and ourselves, and we are able to gain and sharpen those skills that we need to be successful leaders.

What are your future career or educational plans?

As of right now, I have two different paths that I am contemplating taking after I complete my degree in Neuroscience. The first path would be after completing my undergraduate studies, I would go on to study post- graduate medicine, as I have always had an interest in medicine, especially the brain, thus my course. I would love to eventually become a Neurosurgeon and be able to study the brain further whilst hopefully saving people's lives. My second option would be to study a master's degree, use my knowledge to become a Clinical Scientist and continue to research new techniques that can help prevent and treat Neurological diseases. I would also love to do clinical work in 3rd world/developing countries that may not have the same resources that are available in first world countries and make treatments more accessible. I would love to start with my home country, Nigeria because they still lack many modern medical facilities that are required to be effective institutions.

References

Donat, CK, Scott, G, Gentleman, SM, Sastre, M. 2017. Microglial activation in Traumatic Brain Injury. *Front Aging Neurosci*. Pp.9:208.

Henn, A et al.2009. The Suitability of BV2 Cells as Alternative Model System for Primary Microglia Cultures or for Animal Experiments Examining Brain Inflammation. *ALTEX.* Pp83-94

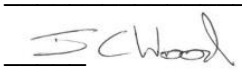
Wake, H, Moorhouse, AJ, Nabekura, J. 2012. Functions of Microglia in the central nervous system—beyond the immune response. *Neuron Glia Biol*. **7**(1), pp.47-53.

Supervisor

Please comment on your scholar's research period, what you consider to be your scholars' strengths and which leadership attributes (please refer to the leadership attributes below) you feel your scholar has demonstrated and is particularly skilled in. You could also identify areas which your scholar can develop further.

Yasmin is an excellent scholar. She showed a really strong ability to be able to work independently and learn some complex programming ideas that are not like anything she has done before. Her drive to achieve results from our experiments and find a way to analyse the data we collected was exemplary. Her work ethic to get this done and her ability to keep pushing to find a solution and not give up after the first method she attempted did not work was excellent. Yasmin also has very good people skills, is a very personable individual and is able to interact well with fellow students and staff. Yasmin has very good initiative but maybe at times this is held in check through insufficient self-belief which would be the area that I think she could develop further.

Signature of Scholar  Date: 19/9/2022

Signature of Project Leader  Date: 20_/_9_/ 2022