Rokos Award internship report

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This past summer, I organised a 6-week internship at the Blizard Institute which is part of the Barts and London School of Medicine and based on the Royal London Hospital campus. Dr Alex Clark was hosting me in the Neuroscience, Trauma and Surgery group: a collection of researchers whose focuses generally relate to neural health. My work, as an extension of research in my third year, was looking into the disease process of Hereditary Sensory Neuropathy type 1 (HSN1). I was investigating whether the key symptoms of HSN1, particularly a loss of sensation in the feet, are evident in 3-month-old mice.

HSN1 is an inherited condition that causes peripheral sensory fibres to die off. HSN1 most frequently presents in patients' 20s with decreased sensation at the feet. The negative symptoms are predominantly loss of pain and temperature sensation, and motor deficits can

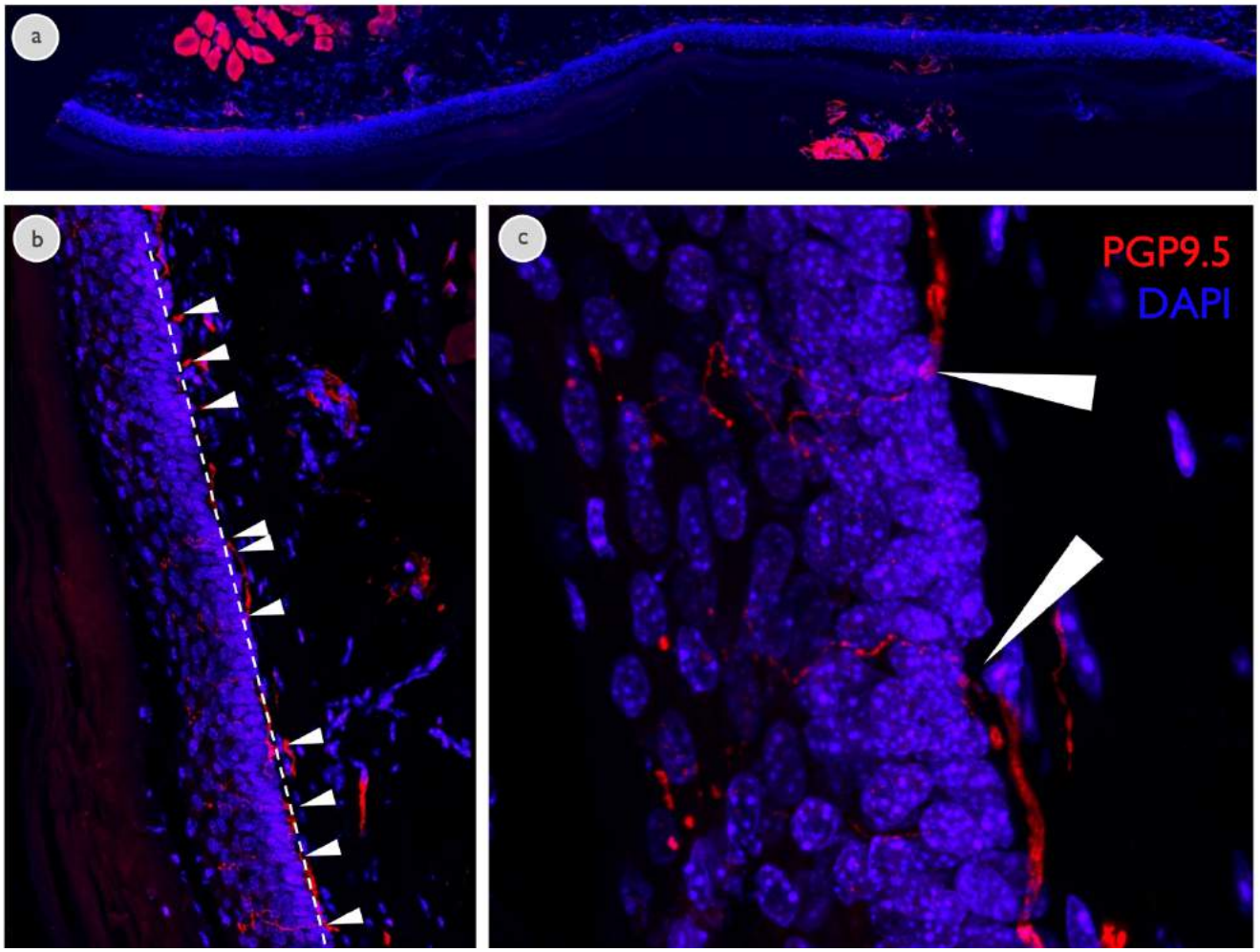

Figure 1a–c: *Confocal microscopy images of hind paw section from SPTLC1S331F mouse stained for PGP9.5 (non-specific neuronal marker, red) and DAPI (stains the dermal and epidermal cells, blue).* ***a*** *was taken at 10x magnification and shows the whole section* ***b*** *shows the section at 40x magnification, and the dashed line represents the dermal-epidermal barrier. Only fibres crossing this barrier were counted.* ***c*** *shows the section at 63x magnification showing two free nerve endings marked with arrowheads.*

arise in advanced disease states. Positive symptoms include lancinating pain and paraesthesia. HSN1 is clinically diagnosed by an assessment of the intraepidermal nerve fibre density (IENFD): a sample of skin is taken via punch biopsy from the feet of a patient with suspected HSN1. This method allows for all layers to be collected, including the outer epidermis, dermis, and some subdermal tissue. The density of fibres that enter the epidermis from the dermis [Fig. 1] is quantified and is expected to be reduced in a patient with HSN1.

I deployed the same diagnostic strategy to quantify the IENFD in 3-month-old mice who carry a mutation responsible for HSN1: a mutation in the SPTLC1 gene (specifically the S331F mutation, which relates to the position and code in the gene). The hind paw skin was dissected bilaterally from 6 mutated mice and 6 controls. After dissection, the hind paw skin fixed, washed, embedded and 14μm sections were made with a cryostat. The tissue was cultured overnight with an antibody staining for protein gene product 9.5 (PGP9.5), a non-specific neuronal marker. The tissue was washed three times and cultured for 2 hours with secondary antibodies. The intraepidermal nerve fibre count was measured under the eyepiece of a Leica confocal microscope using a tally counter, and the length of the sample was quantified using Leica Zen imaging software. The data was unblinded and the intraepidermal nerve fibre density (IENFD) was calculated as fibres mm^{-1}.

The IENFD assay demonstrates that peripheral processes are denervated [Fig. 2]. The measurements are made of free nerve fibre endings that penetrate the epidermis which are predominately unmyelinated pain fibres. Therefore, the reduced IENFD will likely present in hyposensitivity to pain and temperature — the key clinical feature of HSN1. My data is relevant to the overall phenotype of the mice as it is in concordance with (unpublished) behavioural data from my supervisor showing that mice exhibit less sensitivity to cool and warm temperature. The phenotype which I have shown at the tissue-level of reduced nerve fibre density is significant and is relevant to the behavioural phenotype of sensory impairment. Most importantly, given the association between IENFD and functional

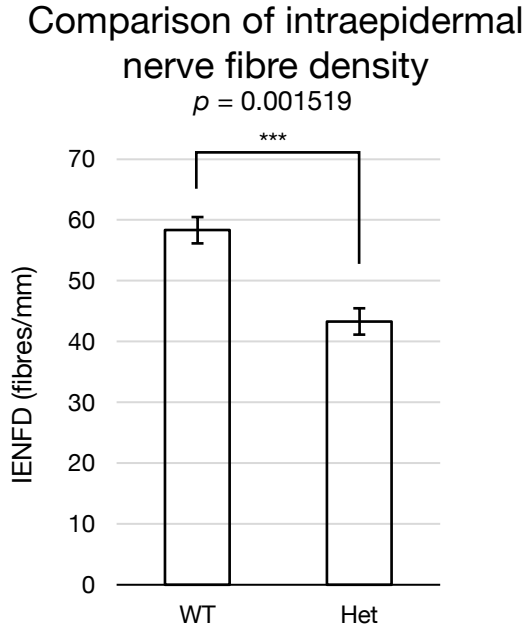

Figure 2: *IENFD data showing a significant reduction for mutated mice (Het) compared to controls (WT) at 3 months. Student's t-test performed, with n = 11. Error bars show SEM.*

outcome in humans, the mice exhibit the same marked neuropathy observed in HSN1 patients.

I have previously established that 12-month-old mice (relatively aged) show reduced IENFD. However, such a profound phenotype has never been demonstrated in 3-month-old mice that express the S331F mutation. Hence, my works is important as my results demonstrate that 3-month-old mice are likely valid as a model of HSN1 for future research. This will increase the rate at which research can be performed as researchers will not have to wait as long to age the mice; this is also ethically preferable as it means that the mice do not have experience this illness for the extra 9 months.

My work allowed me to hone my practical techniques in medical science as practiced at the bench. I also practiced specific research techniques including cryotomy, used for generating sections as thin as 4μm; immunohistochemistry, where antibodies are deployed as exquisitely specific markers; and confocal microscopy, whereby lasers at precise wavelengths scan across a sample to excite different fluorescent probes.

The most significant aspect of my research project would relate to the day-to-day experience of a research scientist. It involved a lot more — and I'm being frank here — drudgery than I expected. I spent lots of time waiting for my experiments to complete or waiting for supplies to arrive from across the globe. Much of this could perhaps have been mitigated by better planning of experiments before I arrived in the lab. However, I also had the insurmountable adversary and bureaucracy (by way of illustration, it took two months to get my access card approved). Unfortunately, academic research does not seem to mind about excessive red tape. The Institute also lacked a cohesive and collegiate feel which I have come to really treasure at Oxford.

Having spoken to many of the other researchers at the Blizard Institute, it also felt like much research is conducted for the sake of research; work rarely seems to have a translational focus that might impact the lives of patients. This research experience has pushed me towards thinking about a dual track academic-clinical training pathway, for example, though the Academic Specialised Foundation Programme. I have come to understand that I would need the added diversion and interest from clinical practice alongside any scientific research. I also would want my research work to have clear implications for clinical treatment and therefore improve the lives of my future patients.