<Final Report>

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Title of Project: Do oligodendrocytes die by ferroptosis in ageing and disease?

Summary:

Oligodendrocytes are a specialised type of brain cell that is responsible for increasing the speed and reliability of information transfer in the central nervous system (CNS). They do this by wrapping an insulating substance, known as myelin, around axons and through this process they additionally provide metabolic support to sustain neuronal health. A number of CNS diseases involve the pathological loss of oligodendrocytes, including multiple sclerosis, schizophrenia, Huntington's disease and stroke. Following a pathological insult, such as infiltration of inflammatory immune cells or stroke, these information transfer areas (white matter tracts) can become damaged and dysfunctional contributing to the disability incurred by patients in these circumstances. It is the oligodendrocytes within these areas that die, but the way in which they die is unclear. At the beginning, **identifying how oligodendrocytes die in response to pathological stimuli and during ageing were the major aims of this project.** Since being awarded the Brain Foundation Research Gift and commencing this project, sophisticated research has been published that followed the fate of oligodendrocytes over time using two photon imaging techniques, providing compelling evidence that very few of these cells die during normal ageing of the healthy mouse brain (Hill et al., *Nat. Neurosci*., 2018; Hughes et al., *Nat. Neurosci.,* 2018). Accordingly, we have narrowed the scope of our project to focus on **identifying how oligodendrocytes die in response to pathological stimuli.**

Cells in the CNS are often considered to die by one of two distinct pathways - apoptosis or necrosis. Apoptosis is triggered by cellular events that result in a well characterised Caspase mediated cascade culminating in controlled cell death. Apoptotic cell death is a necessary process for normal development, as it removes unrequired cells from the body. However, increased apoptosis has been demonstrated in a number of conditions such as Parkinson's diease (Yalcinkaya et al., *Neurosci Letters*, 2016) and Huntington's disease (Petersen et al., *Exp. Neurol.* 1999). We (Pepper et al., unpublished) and others (Koenning et al., *J Neurochem,* 2013; Schneider et al., *Glia*, 2016) have examined the possibility that oligodendrocytes die by apoptosis, and find limited evidence to support this possibility. Oligodendrocytes expressing the apoptotic cell death marker cleaved Caspase-3 are extremely rare. The second major form of cell death is necrosis. While oligodendrocytes may die by necrosis in response to acute injury, for example

at the centre of an ischemic lesion where the blockage occurs and the deprivation of oxygen and glucose is most severe, it is extremely unlikely that oligodendrocytes die by necrosis in the ischemic penumbra (moderate to mild deprivation of oxygen and glucose) or during normal ageing. We therefore propose that oligodendrocytes are dying by an alternative mode of cell death.

This project aims to understand the mode of oligodendrocyte death induced by pathology, particularly by investigating a newly described mode of cell death triggered by inappropriate iron breakdown known as *ferroptosis* and determine the capacity for already developed therapeutics to rescue these cells. As oligodendrocytes have the highest intracellular stores of iron of any cell type in the CNS, **we hypothesise that oligodendrocytes die by ferroptosis**. By saving oligodendrocytes from death following a pathological event, we aim to reduce the lesion size, but also keep these critical cells in place to support nerve cell survival and function.

To determine whether oligodendrocytes or the immature cells that make them, known as oligodendrocyte progenitor cells (OPCs), were susceptible to death by ferroptosis following a pathological event we triggered inflammatory immune cell infiltration into the mouse CNS using a model of experimental autoimmune encephalomyelitis (EAE). We identified oligodendrocytes in this tissue by immuno-labelling for aspartoacylase (ASPA) and identified OPCs by labelling with platelet derived growth factor receptor alpha (PDGFRα). To identify cells that were potentially dying by ferroptosis we looked for expression of cyclo-oxygenase 2 (COX2), an enzyme that is commonly used as a biomarker for ferroptosis. We found that in response to EAE, oligodendrocytes had upregulated expression of COX2, while OPCs expressed relatively low levels of this enzyme (Figure 1). This suggests that **oligodendrocytes, but not the immature cells that form them, are susceptible to death by ferroptosis following a pathological insult**.

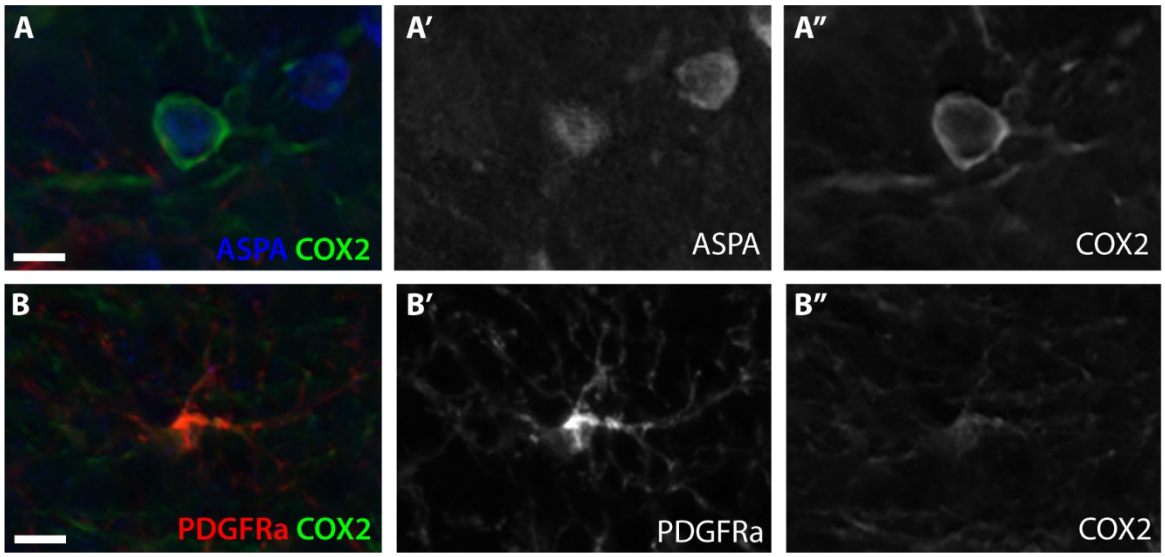

Figure 1. *COX2 expression by A) oligodendrocytes (ASPA) and B) Oligodendrocyte progenitor cells (PDGFRa) in the brain following EAE induction.*

To further validate and understand this finding, we are carrying out further experiments in culture. So far, we have treated cultured OPCs with Erastin - a known pharmacological inducer of ferroptosis and found that these cells do not upregulate COX2 expression or die in response to this treatment. Conversely, although we have not yet examined the response of myelinating oligodendrocytes in culture, immortal cell lines such as HEK and SHSY-5Y cells do die following similar treatment with Erastin. These initial observations suggest that OPCs are resilient to death by ferroptosis, yet questions still remain. For example, do oligodendrocytes die by ferroptosis in other pathological circumstances, such as following an ischemic stroke? Can oligodendrocyte death can be prevented using anti-ferroptotic drugs? Why are OPCs less susceptible to ferroptosis induced death following a pathological insult? Despite the remaining questions, these data provide vital proof-of-concept evidence for ferroptosis as a mechanism of oligodendrocyte death and open the door for ongoing research to explore potential therapeutic interventions, targeted at reducing death of oligodendrocytes following injury and thereby promoting the health and recovery of neurons supported by these cells.