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Thesis abstract

The intersection of nitrosative stress, ferroptosis and aberrant calcium signalling in Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder characterised by the deposition of two protein aggregates, amyloid β and hyperphosphorylated tau, in conjunction with the breakdown of neuronal signalling pathways and cell death. It has been proposed that disruption to calcium signalling may be a central neurodegenerative mechanism driving AD, with several distinct pathogenic pathways contributing to the presentation of aberrant signalling phenotypes. Unfortunately, over 30 years of clinical trials targeting the removal of the protein aggregates have largely failed, however, short-term symptomatic relief has been observed when modulating signalling pathways. This highlights the need to further understand the pathophysiology of neuronal signalling during AD.

To address this knowledge gap, it was hypothesised that nitrosative stress and ferroptosis, two discrete pathogenic pathways implicated with AD, would alter calcium signalling dynamics. Nitrosative stress, the consequence of aberrant levels of the second messenger nitric oxide (NO), was examined due to the intricate relationship between NO production and glutamatergic calcium signalling. Ferroptosis, a cell death pathway associated with redox dyshomeostasis, was assessed due to its disruption of lipid membranes where calcium signalling receptors and channels are localised. To investigate this hypothesis, induced pluripotent stem cell (iPSC) derived neurons generated from AD donors were used as an *in vitro* model to understand if, and how, these alternative disease mechanisms may underlie the presentation of dysfunctional calcium signalling phenotypes.

Live cell calcium imaging demonstrated a complex dysfunctional calcium signalling phenotype in AD neurons, which consisted of fast spontaneous calcium transients in addition to reduced but prolonged glutamatergic calcium responses, a higher proportion of non-responsive neurons, and elevated intracellular calcium levels, when compared to the control neurons. This demonstrates the recapitulation of several distinct calcium phenotypes hypothesised to contribute to AD pathogenesis, representing the establishment of a humanised *in vitro* disease system to investigate mechanisms of AD.

Western blot quantification of the calcium activated enzyme responsible for producing NO, neuronal nitric oxide synthase (nNOS), demonstrated it was significantly increased in brain regions implicated with AD and iPSC-derived neurons from AD donors. Inhibition of nNOS activity, or scavenging of endogenous NO, decreased

the proportion of spontaneously signalling AD neurons, while significantly decreasing the glutamatergic calcium response in healthy neurons, in contrast to AD neurons, where this modulatory effect was lost. This suggests pathogenic modification of signalling receptors under conditions of elevated NO, implicating nitrosative stress in the presentation of aberrant calcium signalling phenotypes.

Evidence suggested that AD neurons had an increased susceptibility to nitrosative/oxidative stress damage, with an altered distribution of the lipid soluble antioxidant α-tocopherol (α-toc), and decreased levels of the antioxidant enzyme, glutathione peroxidase. This decreased resilience to redox dyshomeostasis likely increased neuronal vulnerability to the lipid peroxidation cell death pathway, ferroptosis. Induction of ferroptosis significantly reduced cell viability and the amount of phosphatidylethanolamine polyunsaturated fatty acid lipid species, in addition to abolishing glutamatergic calcium signalling. Supplementation with α-toc reduced the proportion of spontaneously signalling neurons and partially protected against the loss of select fatty acid species, implicating nitrosative/oxidative stress damage and alterations to the lipid membrane in the breakdown of neuronal calcium signalling.

Overall, the work presented in this thesis shows how the AD pathogenic alterations, nitrosative stress and ferroptosis, contribute to the presentation of calcium signalling phenotypes during AD. Identifying and understanding how different disease pathways modify neuronal calcium signalling is critical as effective treatments will likely need to target several mechanisms to ensure robust efficacy and minimise the contribution of alternative disease pathways that would continue to drive pathology.

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