Investigating the neurotoxicity of polystyrene nanoplastics on mitochondrial function in the brain.

Introduction and background

Micro and nanoplastics (MNPs) are becoming increasingly present in our lives and our bodies, with 360 million tonnes of plastic being produced annually[1]. MNPs can enter the brain by crossing the blood brain barrier in fish, rodents and shockingly humans[2]. Mitochondria are highly important components of all human and animal cells, that produce energy from food, mainly on the form of ATP. Mitochondrial dysfunction causes severe disease including neurodegenerative diseases such as Alzheimer's and Parkinson's disease[3].

Reactive Oxygen Species (ROS) are a dangerous form of oxygen also known as free radicals that can damage our cells. Our body can deal with a normal amount of ROS, however, if production increases above normal then our cells can become damaged; this can ultimately lead to cell death[3]**. I am hypothesising that excess reactive oxygen species will be produced in the presence of polystyrene nanoplastics.** I will be investigating the mechanisms of the neurotoxic effects in the mitochondria by examining the electron transport chain (ETC) as shown in Figure 1.

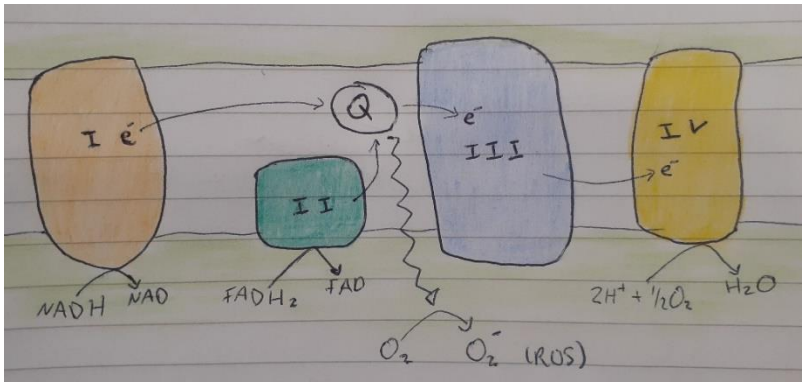

Figure 1: The four complexes of the ETC and their donors.

MNPs have been shown to cause harm in many organisms especially in the mitochondria however, the neurotoxic effects of nano plastics still remain largely a mystery especially in mammals[2]. I have searched the literature for experiments that involve the neurotoxicity of MNPs[1,2] and found that most experiments looked at neuroblastoma cells or developing cells. These experiments showed that the MNPs caused damage to the cells. To the best of my knowledge nobody has investigated the neurotoxic mechanisms in mitochondria extracted directly from mammal brains. This research is particularly important because every new generation is coming in contact with higher levels of plastic pollution, and we know very little about its effect on our brain.

References:

1 Lee, Yi, Moon, Yoon, Park. "Impact of Micro- and Nanoplastics on Mitochondria". *Metabolites*. (2022). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9612075/

2 Prüst, Meijer and Westerink. "The plastic brain: neurotoxicity of micro- and nanoplastics". *Part Fibre Toxicol*. (2020). Available at: https://doi.org/10.1186/s12989-020-00358-y

3 Wang, XU, Musich and Lin. "Mitochondrial dysfunction in neurodegenerative diseases and the potential countermeasure". *Wiley Online Library*. (2019). Available at: https://onlinelibrary.wiley.com/doi/10.1111/cns.13116

Methods

The purpose of this research is to investigate the mechanisms of neurotoxic effects of polystyrene nanoplastics on mitochondria from rat brains.

I will complete my experiments in Professor Gavin Davey's laboratory, his work focuses on mitochondria in the brain and will therefore be suitable for my research. The lab is located in TBSI, which is hub of interdisciplinary research including Biochemistry and Neuroscience. This offers a great opportunity to meet researchers from many different backgrounds and learn from them.

Firstly, the rat brains will be processed via centrifugation techniques to isolate the mitochondria. They will be separated into; non-synaptic mitochondria that come from many different cells in the brain such as astrocytes and oligodendrocytes, and synaptic mitochondria which come from the synaptic terminals of neurons. A sub-objective is that synaptic mitochondria may be preferentially susceptible to damage by nanoplastics, which may underlie a loss in synaptic function and neurodegeneration.

To prepare the cells I will treat them with a range of concentrations of the polystyrene nanoplastics and freeze them. I will have one batch that has no plastics which will be my control. The other batches will have 1, 10, 50 and 100 mg/L of nano plastics added. I will remove the nano plastics from half of the cells and leave the others in to investigate lasting effects. Freezing the mitochondria breaks them open and allows me to assay the different complexes individually.

<u>The neurotoxic mechanism through the ETC</u>

I will investigate which of the ETC complexes (I to IV) are affected by the nanoplastics. I will use the following inhibitors in these experiments as control toxins to demonstrate inhibition of the ETC activities; Rotenone, Antimycin A and KCN. I will examine the complex function by measuring the absorbance of a dye at 340nm in the presence of; NADH, $FADH_2$, Ubiquinone and O_2 respectively, in a spectrophotometer.

<u>Measuring the production of ROS</u>

I will quantify the production of dangerous free radicals by measuring how much hydrogen peroxide is produced using Amplex Red. Hydrogen peroxide is produced when ROS are made, it can then be converted to water and Resorufin when coupled with Amplex Red as seen in Figure 2. Resorufin can be quantified as it fluoresces.

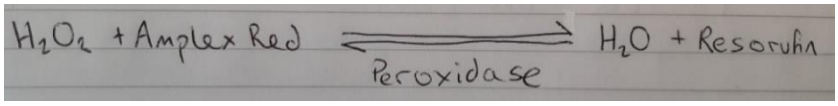

Figure 2: reaction involving Amplex Red

Intended outcomes

My hypothesis predicts that the mitochondria will produce more dangerous ROS when they have been treated with polystyrene nanoplastics. If these nanoplastics cause mitochondrial dysfunction or the production of ROS, they will most likely be a contributing factor to neurodegenerative diseases such as Alzheimer's or Parkinson's disease. Understanding the mechanism of mitochondrial damage

by these nanoplastics is imperative if we are going to be able to find a treatment for long term exposure to plastic particles.

Following this research, it would be important to look at the neurotoxic effects of different types of micro and nano plastics on the human brain. Researching the prevention of MNPs consumption would also be valuable. Scientific links between long-term exposure to MNPs and neurodegenerative diseases would need to be solidified.