REPORT ON THE PEMRIG VISIT TO PROFESSOR JON LANE'S LABORATORY AT THE UNIVERSITY OF BRISTOL - JUNE 12th 2023

Background Information

Professor Jon Lane of Bristol University offered members of PEMRIG and other RIGs and anyone else interested the opportunity for a zoom visit to his labs to hear about his Parkinson's UK-funded research.

People with Parkinson's don't have enough of a chemical called dopamine because some of the brain cells that produce it have died. We still don't fully know what causes this, but one hypothesis is that a person's immune response may play a role. When we get an infection, our immune system responds by recruiting cells and producing signals which fight against it. This is known as inflammation. Sometimes inflammation can be wrongly activated and damage healthy cells, and this might be the case in Parkinson's. This research aims to understand more about the role of inflammation in brain cell death and may pave the way for new treatments to tackle some of the earliest changes in Parkinson's.

A Glossary is available at the end of this report with brief explanations of some scientific words.

Autophagy and neuroinflammation in Parkinson's.

Jon Lane welcomed PEMRIG to his lab and introduced members of his group. Dr Madhu Kollareddy briefly described his recent work on neuronal stress protection just published in the prestigious Journal of Cell Biology as is discussed later. Research students Shiza (funded by Parkinson's UK), Beth and David then introduced themselves.

Jon then took us on a virtual tour of his lab area which is housed in a 1970s building which was refurbished 4-5 years ago and is very well equipped. The lab's work on autophagy which is the regulated recycling of cytoplasmic material through delivery to a digestive organelle, the lysosome means that live-cell imaging, fixed cell microscopy and electron microscopy are essential techniques along with cell culture facilities essential for growing various human and mouse cell culture lines and induced pluripotent stem cells (iPSCs) derived from human skin cells. The iPSCs are differentiated into specific neural cell lineages (neurones, astrocytes and microglia) to understand how autophagy is regulated in the neurons which are damaged in Parkinson's. Jon explained that some of the capital equipment is bought on grants but problems arise in finding funds to repair or replace older equipment. Jon's research is funded by grants from The Wellcome Trust, the BBSRC and Parkinson's UK.

Jon then showed slides to explain the work in his lab. He reminded us that one of the main features of Parkinson's is the loss of dopaminergic neurones in the midbrain; these neurones normally provide essential dopamine for other neurones in the brain especially those controlling movement. The aim is to understand why these particular neurones are lost. These dopaminergic neurones have very long processes (axons) so need exceptional amounts of energy to function normally so any insult to these cells such as mitochondrial malfunction, oxidative stress, proteinopathies leading to misfolded proteins (e.g. alpha-synuclein in Lewey Bodies) will all contribute to neuronal cell death. All these insults are linked to produce a vicious cycle of failed homeostasis.

Next Jon explained that there is growing evidence that astrocytes and microglia, the glial cells of the brain, contribute to neuronal cell death in Parkinson's. Normally, astrocytes release 'good' cytokine and chemokine chemicals which support neuronal cell function but when microglia, the brain macrophages, become activated in the damaged brain, they release 'bad' cytokines and chemokines which can damage neurones and they also make astrocytes more aggressive. These aggressive astrocytes then release a new range of cytokines and chemokines which cause further neuronal cell

death. This is the basis of neuroinflammation. Jon explained that new evidence from studies on people with REM sleep disorder suggests that this inflammatory process involving activation of microglia may be taking place early on in Parkinson's well before any obvious symptoms are apparent.

At the heart of understanding neuroinflammation is the process of autophagy-the way in which cells dispose of damaged and misfolded proteins and other damaged cell chemicals – and mitophagy, in which damaged mitochondria are removed and recycled. Jon described the autophagy pathway in which damaged mitochondria and proteins to be removed are trapped within a membrane-bounded vesicle known as a phagosome. This phagosome matures into an autophagosome which fuses with a lysosome, an organelle full of digestive enzymes at low pH. These enzymes degrade the misfolded proteins and damaged mitochondria recycling their components for reuse. The lab is working to determine how autophagy influences neuroinflammation ín Parkinson's and if autophagy can be harnessed to improve the resistance of dopaminergic neurones to damage.

To do this the lab produces co-cultures of neurones and glial cells derived from human stem cells. Jon stressed the importance of working on human cells as opposed to animal models to get as close to the in vivo human situation as possible. He described how the lab produces IPSCs from human skin cells and how these IPSCs can be treated with a defined mixture of growth factors to produce ventral midbrain dopaminergic neurones-the neurones that are lost in Parkinson's. He showed some beautiful time lapse photos of the neurones with autophagosomes moving inside the cell bodies.

Shiza then described how by using a different treatment iPSCs can be converted into midbrain astrocytes defined by specific markers. She uses these to analyse by mass spectrometry what proteins are secreted to the medium when the astrocytes become stressed. One protein of interest known as CCL2 can damage neurones. CCL2 is of interest because when autophagy in astrocytes is turned off, the levels of CCL2 released increase. Shiza is now working out how autophagy regulates CCL2 secretion from stressed astrocytes. Beth then described how she can convert iPSCs into mid brain microglia again as defined by specific markers. She showed fluorescence microscopy images showing that these microglia can digest fragments of dying neurones and that autophagy is involved in this process. Her work is to investigate whether taking up the nerve cell fragments leads to activation of the microglia with release of factors that can then stress astrocytes as described earlier.

Jon returned to emphasise that the process of autophagy keeps cells healthy but that the efficiency of autophagy declines with age. The aim is to understand how autophagy is controlled and why the efficiency of the process declines with age. He again stressed how important it is to work with midbrain neurones and astrocytes since experiments have shown that astrocytes from other brain regions release different types of cytokines and chemokines. It is apparently not yet clear whether microglia show regional variation in the brain.

Jon highlighted two recent papers from his group which explain some aspects of the work already described in more detail. One (1) covers the work described by Shiza that astrocytes from different brain regions vary in their secretory profile. The other paper (2) deals with how autophagy shapes the resilience of human midbrain dopaminergic neurones at the molecular level as mentioned earlier by Madhu. This work deals with how cells control which genes are switched on and off to specifically become ventral midbrain dopaminergic neurones. Jon reminded us of the DNA to RNA to proteins pathway and that it is the specific protein content of a cell that make it unique. Transcription factors are proteins which switch genes on or off and the paper is about the role of a transcription factor LMX1B which regulates the synthesis of proteins which control neuronal survival and resilience and in particular are involved in the autophagy pathway specific to ventral midbrain neurones. The group have shown that the activity of LMX1B bound to DNA is regulated by one of the autophagy proteins produced. This is LC3B, a protein involved in the formation of the autophagosome membrane. So the group have identified a feed-back mechanism whereby LMX1B is regulated by LC3B so promoting autophagosome formation so contributing to neuronal resilience. Mahdi has shown that blocking mitochondrial function in neurones lacking LMX1B leads to an increase in dying cells as autophagy

pathways cannot function. The work described in the paper shows that autophagy plays a key protective role in cells and notably that autophagy proteins are self-regulating to keep autophagy working efficiently. Jon then opened the meeting to questions which can be heard on the recording.

at: Recording of Jon Lane Virtual Lab Visit PEMRIG's Chair John Turner then proposed a vote of thanks for a most informative and stimulating lab visit.

1. Crompton L A et al Brain Communications (vol 5, issue 2, 2023)

2. Jimenez-Moreno N et al Journal of Cell Biology (2023) Vol222 (5) e201910133

GLOSSARY

Astrocytes: a type of cell in the Central Nervous System which supports nerve cell function and maintains the blood-brain barrier

Autophagy: a cellular mechanism for recycling damaged cell components like misfolded proteins for recycling

Axons: a long process from a nerve cell that sends signals to other nerve cells

BBSRC: Biotechnology and Biosciences Research Council-they give out grants for scientific research

Chemokines and cytokines: proteins released from glial cells which control the activity of nearby cells in a good or bad way

Dopaminergic neurones: nerve cells which use dopamine as a neurotransmitter

Glial cells: The astrocytes, microglia, oligodendrocytes, ependymal cells of the CNS which support nerve cell function in different ways

Homoeostasis: A state of balance among all the body systems needed for the body to survive and function

Lysosome: An organelle in many cells responsible for the breakdown and recycling of degraded cellular components. Lysosomes are full of digestive enzymes and their interior is at a low pH

Microglia: brain macrophages responsible for clearing up cellular debris formed during inflammation and disease

Neurone: the impulse transmitting cell in the central nervous system

Neuroinflammation: an activated state of nerve tissue initiated in response to infection, traumatic brain injury, toxic metabolites, or autoimmunity.

Oxidative stress: reflects an imbalance between the formation of damaging free radicals and other reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates and to repair the resulting damage to DNA and other cell components

Pluripotent stem cells: are derived from skin or blood cells that have been reprogrammed back into an embryonic-like pluripotent state that enables the development of an unlimited source of any type of human cell needed for therapeutic purposes. In this work iPSCs can be converted into dopaminergic neurones, astrocytes or microglia by providing the correct signals

Proteinopathies: an umbrella term for neurodegenerative disorders that are characterized by the accumulation of specific proteins within neurones or in the brain tissue, e.g. Alzheimer's and Parkinson's diseases.

Phagosome: a tiny membrane-coated vesicle in a cell in which damaged proteins and other cell components are trapped for transport firstly to an autophagosome which then fuses with a lysosome for degradation and recycling

Ventral midbrain dopaminergic neurones: Dopamine (DA) neurons of the ventral (lower part) midbrain (VM) which play vital roles in the regulation of voluntary movement, emotion and reward. These are the nerve cells which are lost in Parkinson's leading to the well-known motor and non-motor problems of the disease