**BBSRC MIBTP Studentship Project**

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| **Project Title** | Dissecting the interplay between the kynurenine pathway and extracellular vesicle signalling in schizophrenia |
| **Project Summary**  |
| Increased levels of the kynurenine pathway (KP) metabolites L-kynurenine (KYN) and kynurenic acid (KYNA) have been found in the brains of patients with schizophrenia, and have been linked to cognitive impairment in these individuals. KYN and KYNA can bind and activate the transcription factor aryl hydrocarbon receptor (AHR), which has key roles in inflammation and cancer. Activation of the AHR by other ligands has been shown to induce extracellular vesicle (EV) release, in particular the release of small exosome-like vesicles from cells in vitro. However, the release of EVs from microglia and neurons – critical cell types in KP physiology - in response to AHR activation by KP metabolites has not been previously examined. We hypothesise that KP metabolites modulate the release of EVs from microglia and neurons, and that these EVs can then be transferred between cell types to regulate gene expression and modify cellular functions - ultimately impacting schizophrenia pathogenesis . For the initial part of this project we therefore propose to characterise the release of EVs from microglial cells and neurons in response to kynurenine and KYNA, and examine the involvement of AHR and downstream signalling pathways. We also will examine the miRNA and protein content of the released EVs, as activation of cells with different stimuli can result in the differential loading of cargo into EVs. This not only potentially alters the outcome of EV uptake on the function of the recipient cells, but also makes EVs useful biomarkers of disease as their miRNA or protein content reflects that of the cells they were released from. Therefore, this study will examine the cargo of the released EVs, the transfer of EVs between microglia and neurons and their effects on cellular functions such as cell survival/apoptosis and the regulation of gene expression. Cell-type specific responses such as the release of inflammatory cytokines from microglia, and dendrite formation and neurotransmitter receptor expression in primary neurons will also be assessed. Promising findings from this work validated in neuronally-enriched EVs derived from the plasma of individuals with schizophrenia and controls arising from ongoing work in the Giorgini group. This will permit us to refine/extend our current work and validate findings from the proposed project in a translationally relevant manner. Techniques that will be undertaken during the project:Studies will use a human microglial cell line, a human neuronal cell line and primary neurons isolated from rats. EVs released from these cells in response to KYN, KYNA and immunostimulation will be characterised by Nanoparticle Tracking Analysis (NTA), immunoblotting for EV markers, and electron microscopy. KYN levels will be assessed in cells using a FRET-based nanosensor developed by a PhD student currently co-supervised by Hudson and Giorgini. The involvement of AHR and downstream signalling mechanisms will be investigated using specific inhibitors and siRNA targeting AHR and key downstream signalling pathways. The miRNA and protein content of the EVs will be examined by QPCR based miRNA profiling and mass spectrometry respectively, and the transfer of EVs between cell types will be examined by confocal microscopy. The effect of EVs on cellular functions and gene expression will be assessed using cell proliferation/apoptosis assays and QPCR respectively, whereas cytokine release will be examined using Meso Scale multiplex cytokine assays. BBSRC Strategic Research Priority: Understanding the Rules of Life - Neuroscience and behaviour |
| **References** |
| Erhardt S, Pocivavsek A, Repici M, Liu XC, Imbeault S, Maddison DC, Thomas MAR, Smalley JL, Larsson MK, Muchowski PJ, Giorgini F, Schwarcz R. Adaptive and Behavioral Changes in Kynurenine 3-monooxygenase Knockout Mice: Relevance to Psychotic Disorders. Biol Psych, 2017; pii: S0006-3223(16)33112-2. Leung, G. C. H.; Fung, S. S. P.; Gallio, A. E.; Blore, R.; Alibhai, D.; Raven, E. L.; Hudson, A. J. Unravelling the mechanisms controlling heme supply and demand. P Natl Acad Sci USA 2021, 118, e2104008118; https://doi.org/10.1073/pnas.2104008118  |