Novel Methodology For Probing Microglial Metabolism In Situ

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Background: Microglia play a role in the pathogenesis of multiple sclerosis (MS) and other neurodegenerative diseases (ND) by taking on adaptive and injurious phenotypes in response to their environment. Understanding these phenotypes may guide the development of novel therapeutics. Recent single-cell RNA sequencing (scRNAseq) analyses of microglia *ex vivo* found that genes associated with lipid and lipoprotein metabolism are tightly regulated in ND. We hypothesize that microglial activation is paired with a metabolic switch key in the development of ND. However, our current understanding of microglial metabolism draws on what is known about peripheral macrophages and invasive techniques like scRNAseq that could alter microglial phenotype and metabolism. Therefore, there is a need for methodologies that allow probing of microglial metabolism *in situ*, as a first step to develop metabolism-focused interventions.

Methods: We use the endogenous fluorophore NADH to probe the metabolic profile of microglia *in situ* via fluorescence lifetime imaging microscopy (FLIM). By exciting NAD in the sample and tracking its fluorescence lifetime (FLT), we can determine whether it is free or enzyme-bound. More free NADH suggests more glycolytic flux and presents as a longer NAD FLT, whereas bound NAD indicates more oxidative phosphorylation. We use an experimental autoimmune encephalitis (EAE) mouse as a model of MS. Mice were scored based on their MS-like symptoms. Brains were frozen and sectioned before analysis.

Results: Preliminary data suggest that a higher score is associated with a shorter NAD FLT, suggesting a higher reliance on glycolysis in an EAE mouse. This also suggests that microglia have adopted an injurious phenotype.

Conclusions: The data support our hypothesis that microglial activation in the setting of MS is paired with a metabolic switch towards more glycolysis. This study also supports FLIM being used to probe microglial metabolism *in situ* to better understand other NDs.