Novel Methodology for Probing Microglial Metabolism *in situ*

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Background

- Microglia are brain resident macrophages that respond to their microenvironment by taking on adaptive or injurious phenotypes.
- Injurious phenotypes have been implicated in the pathogenesis and progression of NDs.
- Activation is coupled with a metabolic switch where activated microglia rely less on oxidative phosphorylation (OXPHOS) and more on glycolysis (Van den Bossche et al., 2018; Solito et al., 2012).
- An increased reliance on glycolysis rather than OXPHOS leads to an increased ratio of free:bound NADH
- Current methods for studying microglial activation and metabolic profile, like scRNAseq, are invasive and rely on *ex vivo* measurements. These methods could alter microglial phenotype.
- Understanding this metabolic switch could guide the development of novel metabolism-based therapeutics

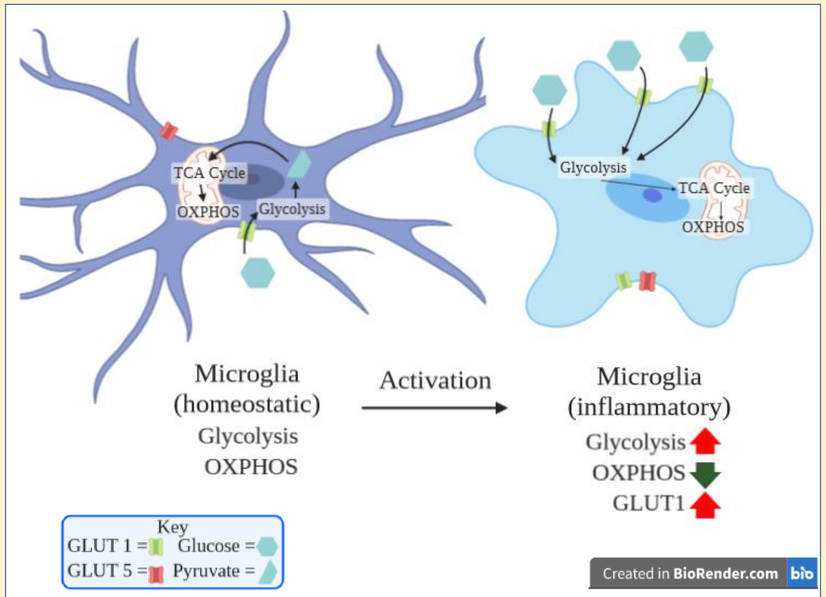


Figure 1 Upon activation, homeostatic microglia undergo a metabolic switch to a more glycolytic activation state. These activated microglia rely more on glycolysis and less on OXPHOS to meet metabolic demands. Activated microglia have also been found to upregulate GLUT1.

Hypothesis

- Fluorescence Lifetime Imaging Microscopy (FLIM) can be used to probe the microglial metabolic profile *in situ*, and ergo the activation state, without the need for labeling.
- Microglia from higher scoring EAE mice will exhibit shorter NADH fluorescence lifetimes indicative of more free NADH, suggesting more glycolysis and less OXPHOS.

Disclosures

None of the authors have any disclosures at this time.

Methods

FLIM:

- Fluorescence Lifetime Imaging Microscopy (FLIM) takes advantage of endogenous fluorophores to assess the metabolic profile and activation state of microglia.
- FLIM uses differences in fluorescence lifetime (FLT) to generate contrast in an image.

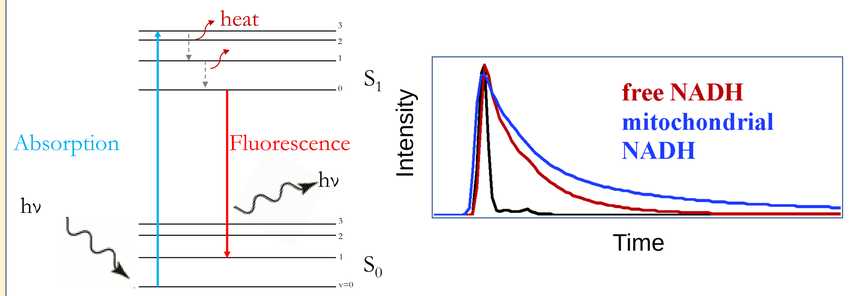
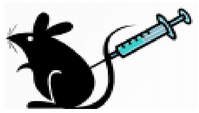
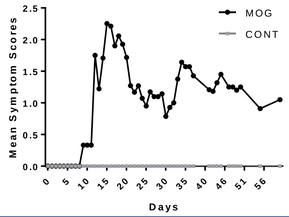


Figure 2 FLIM uses differences in FLT to generate contrast in an image. This also provides insight into the microenvironment of the fluorophore. In the case of NADH, the FLT can indicate if it is bound or free (Blinova et al., 2005).

EAE:

- Experimental autoimmune encephalomyelitis (EAE) mouse model used to model multiple sclerosis (MS).
- Mice are injected with myelin oligodendrocyte glycoprotein (MOG). This leads to the development of an immune-mediated demyelination of the CNS.
- Mice are scored based on the severity of their MS-like symptoms. Brains are isolated, frozen, and cut into 10 µm sections prior to FLIM analysis.





Clinical EAE Scores:
1. Limp tail
2. Hind leg weakness
3. Hind limb paralysis
4. Moving minimally
5. Death/Euthanasia

IHC:

- Since FLIM has yet to be used in the brain, these findings need to be validated with IHC.
- Activated microglia have been found to upregulate expression of GLUT1 to fuel a greater reliance on glycolysis.

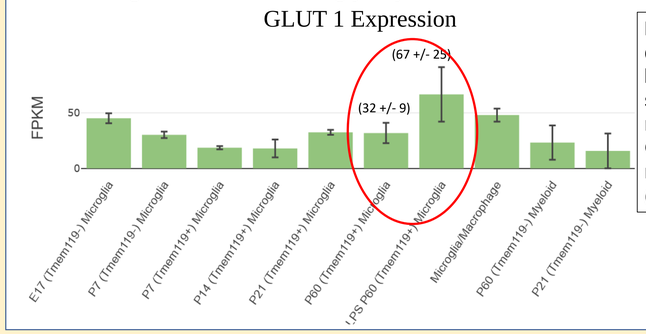


Figure 3 Expression data from Dr. Barres' lab found that upon stimulation with LPS, microglia upregulate GLUT1 expression by more than 2 fold (Bennett et al., 2016).

Results

- Preliminary data from the cortex of EAE mice suggest a higher EAE score is correlated with a shorter NADH FLT.

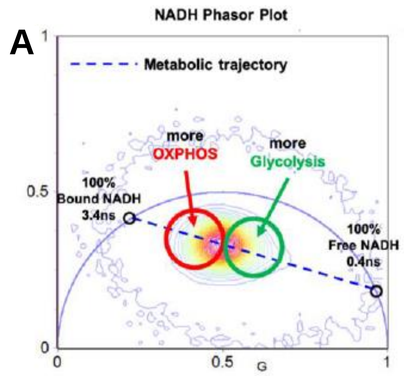
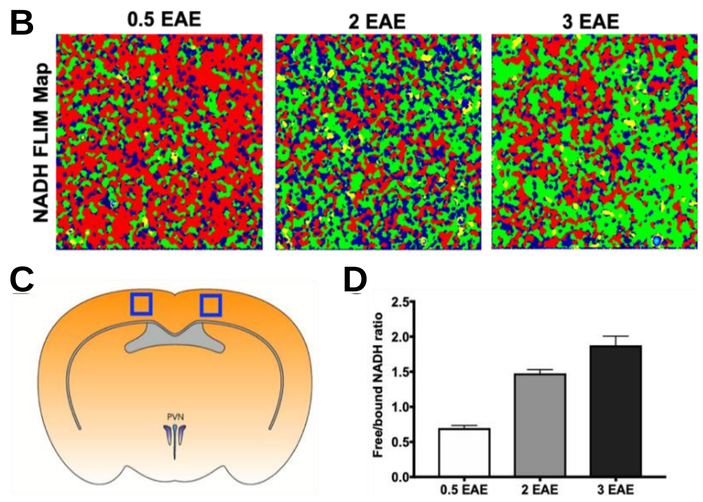


Figure 5 A. NADH phasor plot depicting FLTs corresponding to glycolysis (green) and OXPHOS (red) (Bruce et al., 2020). **B.** NADH FLIM analyses in microglia of male mice with low to moderate EAE scores. **C.** M2 region of the cerebral cortex where measurements were taken. **D.** There is more free NADH in mice with higher EAE scores, suggesting more glycolysis.



Summary and Future Directions

- Preliminary data suggests a higher EAE score is correlated with a shorter NADH FLT and therefore more free NADH and more glycolysis.
- These findings still need to be validated with IHC looking at GLUT1 expression with a microglial specific marker (Iba1).
- Look at higher and lower scoring EAE mice as well as female mice to see if sex plays a role in activation state.

References

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