

# In vivo dynamics of retinal microglial activation during neurodegeneration: confocal ophthalmoscopic imaging and cell morphometry in mouse glaucoma

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Microglia, which are CNS-resident neuroimmune cells, transform their morphology and size in response to CNS damage, switching to an activated state with distinct functions and gene expression profiles. The roles of microglial activation in health, injury and disease remain incompletely understood due to their dynamic and complex regulation in response to changes in their microenvironment. Thus, it is critical to non-invasively monitor and analyze changes in microglial activation over time in the intact organism. In vivo studies of microglial activation have been delayed by technical limitations to tracking microglial behavior without altering the CNS environment.

This has been particularly challenging during chronic neurodegeneration, where long-term changes must be tracked. The retina, a CNS organ amenable to non-invasive live imaging, offers a powerful system to visualize and characterize the dynamics of microglia activation during chronic disorders. This protocol outlines methods for long-term, in vivo imaging of retinal microglia, using confocal ophthalmoscopy (cSLO) and CX3CR1(GFP/+) reporter mice, to visualize microglia with cellular resolution.

Also, we describe methods to quantify monthly changes in cell activation and density in large cell subsets (200-300 cells per retina) . We confirm the use of somal area as a useful metric for live tracking of microglial activation in the retina by applying automated threshold-based morphometric analysis of in vivo images. We use these live image acquisition and analyses strategies to monitor the dynamic changes in microglial activation and microgliosis during early stages of retinal neurodegeneration in a mouse model of chronic glaucoma.

This approach should be useful to investigate the contributions of microglia to neuronal and axonal decline in chronic CNS disorders that affect the retina and optic nerve.

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