PROJECT SUMMARY: Brain microhemorrhages have been linked to cognitive decline and increased dementia risk, but the mechanisms by which small bleeds affect the function of neural cells remains unclear, in part due to a lack of good animal models. In recent work, we used a novel laser-based approach to rupture targeted arterioles in the brain of mice and imaged the impact of these lesions using two-photon microscopy. Surprisingly, we found no evidence of neural cell death or pathological changes in dendrites, both of which are observed after occlusion of small arterioles. Instead, we observed a rapidly-initiated and locally-sustained activation of inflammatory cells. These data suggest that microhemorrhages do not lead to cognitive dysfunction by killing brain cells or driving neurite dystrophy, but rather impact neural function in more subtle ways. Recent work has revealed that microglia play an active role in surveillance and pruning of synapses in development and adulthood. In addition, activation of these cells has been associated with elevated rates of synaptic spine turnover, but without a change in overall spine density. Here, we propose to test the hypothesis that microhemorrhages cause aberrant increases in the rate of spine turnover and that this increase is caused by the interaction of activated inflammatory cells with synapses. Elevations in synaptic turnover have been associated with plasticity that facilitates functional recovery after brain injury, such as stroke. For the microhemorrhages studied here, however, there is no indication of significant neural injury, suggesting that any rewiring of neural circuits is more likely to cause dysfunction. In Aim 1, we use chronic two-photon imaging after laser-induced microhemorrhage to distinguish the role of brain-resident microglia from blood-derived macrophages in the inflammatory response. These experiments use chimeric animals created through bone marrow transplants to achieve specific labeling of either microglia or macrophages. We then test approaches to block microglia activation (inhibition of P2Y receptors) and macrophage recruitment (blocking of leukocyte adhesion). This work will quantify the spatial scale, temporal dynamics, and cellular players in brain inflammation after microhemorrhage as well as establish tools to modulate this inflammatory response. In Aim 2, we quantify changes in the rates of spine turnover and density in excitatory and inhibitory neurons near a microhemorrhage as compared to controls. Our preliminary data suggests a two-fold elevation in turnover rates that is sustained beyond two weeks. We then determine if inhibition of microglial activation and/or macrophage recruitment shifts the spine turnover rate back toward baseline levels. If true, this would support our hypothesis that the action of inflammatory cells after a microhemorrhage elevates spine turnover. Finally, we pilot experiments to directly image alterations in the interaction of inflammatory cell processes with synaptic spines after a microhemorrhage. In addition to microhemorrhage, brain inflammation occurs in neurodegenerative diseases, such as Alzheimer's disease, and inflammatory cell-mediated increase in synapse turnover may also contribute to the cognitive impact of these conditions.