SPECIFIC AIMS: The accumulation of small hemorrhages in the brain, even microhemorrhages that can only be detected in post-mortem histology, has been linked to cognitive decline in aging patients[1-5]. However, the mechanisms by which these lesions cause injury to or dysfunction in brain cells remains unclear, and a lack of robust animal models of microhemorrhage has hampered progress. We recently used a novel approach that relies on femtosecond laser-induced rupture of targeted cortical vessels in mice[6] to explore the impact of microhemorrhages on brain cells. Surprisingly, we found that microhemorrhages do not lead to neural death or cause significant dendrite dystrophy[7] suggesting that they must affect brain function in a more subtle way. Our experiments did show a rapid response by inflammatory cells near the microhemorrhage that involved activation of brain-resident microglia and, likely, invasion of blood-derived macrophages[7]. Recent work has revealed that microglia play a critical role in cognitive processes through maturation and pruning of synapses during development[8-10], and in plasticity-associated synaptic changes later in life[11-13]. In response to brain injury, microglia undergo a drastic transformation in their phenotype and blood-derived macrophages may invade the brain[14-16]. This inflammatory response has been correlated to abnormally increased gain and loss of synaptic spines[17, 18]. Building on these ideas in preliminary experiments, we have found that the rate of spine turnover in the vicinity of a microhemorrhage was about double that of controls, but with no overall change in the density of dendritic spines. **In this proposal, we test the hypothesis that microhemorrhages lead to elevations in synaptic spine turnover that is driven by interactions with reactive inflammatory cells.** Such rewiring of neural circuits could underlie the cognitive impact of microhemorrhages. In addition, our work could establish a new mechanism of neural dysfunction that arises due to brain inflammation: abnormal synapse regulation by activated inflammatory cells. This process could play a role not only in the impact of microhemorrhages on brain cells, but also in a broad range of other neurodegenerative diseases.

Aim 1: Determine the relative role of microglia and macrophages in the inflammatory response to a microhemorrhage and test strategies to suppress the response of inflammatory cells to the lesion.

Hypothesis: The reactive inflammatory cells aggregating at a microhemorrhage consist of both brain-native microglia and macrophages recruited from vasculature. Because some data suggest that these cells may play a different role in brain diseases, such as amyloid-beta clearance in Alzheimer's disease[20], we will distinguish their behavior in our experiments. (a) The early inflammatory response will be dominated by activated microglia and will be attenuated by pharmacological inhibition of the P2Y purigenic receptors (see Fig. 5)[21]. (b) Blood-derived macrophages will play an increasingly important role over the following days (see Fig. 2) and their recruitment will be reduced by blocking leukocyte adhesion to the vascular wall[22].

Approach: Using bone marrow transplants between selected lines of fluorescent protein expressing mice and wild type animals, we will create animals where we can distinguish between brain-resident microglia and blood-derived macrophages. We will then use chronic *in vivo* two-photon excited fluorescence (2PEF) imaging to quantify the spatial scale and temporal dynamics of the response of these cells to a laser-induced microhemorrhage. We will repeat these experiments in animals where 1. P2Y inhibitors are infused to the cortex to block microglia activation, 2. integrin-blocking antibodies are intravenously administered to block macrophage recruitment, and 3. both microglia and macrophage responses are inhibited.

Aim 2: Quantify the elevation of synaptic turnover after a microhemorrhage and test the hypothesis that activated inflammatory cell interactions with synapses drive this accelerated spine turnover.

Hypothesis: Rates of spine appearance and disappearance will be increased near a microhemorrhage, where activated microglia and recruited macrophages reside. (a) Based on preliminary data (Figs. 3 and 4), we expect an approximately two-fold elevation in synaptic turnover rates, but no overall change in spine density, sustained for several weeks, within ~300 μm of a microhemorrhage. (b) We expect that blocking microglia activation or macrophage recruitment or both will eliminate or reduce the elevation in spine turnover. (c) Alterations in the dynamic interactions between microglia/macrophage processes and synaptic structures after a microhemorrhage, such as process morphology and contact time[17], will be associated with elevated synaptic turnover rates and thus will be modulated by inhibition of microglia activation and/or macrophage recruitment.

Approach: We will use two lines of transgenic mice that will allow us to visualize dendritic spines in excitatory and inhibitory cortical neurons, respectively. Using chronic 2PEF imaging to identify the same spines over time, we will quantify changes in spine density and the rate of spine turnover as a function of distance from a microhemorrhage, over time, and compared to controls. These experiments will then be repeated while blocking the activation of microglia, the recruitment of macrophages, and both (as above). Finally, using mice that express red fluorescent protein in excitatory neurons either crossed to or receiving bone marrow transplants from mice expressing green fluorescent protein in microglia and/or macrophages we will pilot experiments to image both synaptic spines and identified microglia or macrophages, and quantify changes in the dynamic interaction between inflammatory cell processes and synapses near a microhemorrhage.