**SPECIFIC AIMS**

Age-related cognitive decline affects nearly 50% of adults over the age of 85 in the United States (Bishop et al., 2010; Hebert et al., 2003). With ageing, decline in episodic memory functions are prevalent, though exact causes are unclear (Grady, 2012; Tulving, 1983). One working hypothesis from human work suggests that older adults use neural resources inefficiently, resulting in over-recruitment of cortical areas during cognitive tasks (Grady, 2012). A recent study found that hippocampal neural ensembles in aged mice exhibit less overlap between two related experiences compared to younger mice (Cai et al., 2016), suggesting that aged mice use more neural resources to represent similar memories which could explain memory impairments. Motivated by the lack of specific knowledge about the exquisite neural ensemble patterns associated with cognitive ageing, we propose to investigate how these ensembles in aged rodents store experiences and integrate novel information into existing networks compared to those in young adults. To do this, we will utilize state-of-the-art *in vivo* calcium imaging while simultaneously developing novel technologies for cell-specific stimulation protocols. The findings from these studies will substantially increase our understanding of how the ageing brain is impaired as well as bridge human and rodent work in cognitive ageing. Furthermore, these studies will also have the potential to inform the health community on how to deliver targeted treatments to the ageing population suffering from cognitive decline.

**Aim 1. Test the hypothesis that neural ensembles in aged mice display different integration strategies than those in younger mice.** Neural ensembles in aged mice integrate two experiences to a lesser extent than in young mice (Cai et al., 2016), though their fine-timescale activity patterns have yet to be explored. Here, we will study how ensembles in hippocampal CA1 incorporate new components of ongoing experience and compare them between aged and young mice. To this end, we will train mice using auditory trace fear conditioning with two conditioned stimuli (CS1 and CS2). We will measure CS-locked responses during learning using a newly-developed wireless *in vivo* calcium imaging microscope (Shuman et al., 2018). Previous reports have shown that CA1 ensembles can be divided into a stable “backbone” of cells as well as a plastic population (Grosmark and Buzsáki, 2016; Mau et al., 2018). We hypothesize that CA1 ensembles in young mice will exhibit properties characteristic of generalization (via an ensemble “backbone”) across CS1 and CS2. On the other hand, ensembles from aged mice will lack the generalizing backbone scaffold and will instead inefficiently over-recruit cells to represent each CS-shock association separately.

**Aim 2a. Test the hypothesis that aged mice behaviorally and neurally generalize knowledge differently than younger mice.** Previous reports have indicated that rodents can form generalized schematic representations of various behavioral tasks (McKenzie et al., 2014; Tse et al., 2007), though how this process is implemented in aged individuals is unknown. Here, we will study how previous training with auditory trace fear conditioning (CS-A in a familiar context) may be used to inform behavior during unfamiliar situations (CS-α in a novel context). We hypothesize that ageing impairs the ability to use previous knowledge for generalization, with aged mice demonstrating less freezing in the novel context and less neural similarity across the two conditions than younger mice.

**Aim 2b. Actuate generalization in aged mice via spatiotemporally-patterned optical stimulation.** Recent advances in optogenetics and genetic tagging have enabled optical induction of complex behaviors (Liu et al., 2012; Ramirez et al., 2013), however these techniques lack fine spatiotemporal resolution. Despite well-known sequential activity observed in the hippocampus (Buzsáki and Tingley, 2018; Cheng and Ji, 2013; Mau et al., 2018), the causal role of these temporal patterns remains unclear. Using *in vivo* calcium imaging, we will identify specific neurons that have sequential activity locked to CS-A and stimulate them using spatiotemporally-patterned light delivery via a modified miniature microscope with an incorporated spatial light modulator (Mini-SLM). We predict that patterned stimulation of CS-A-locked neurons in aged mice will elicit generalization to a novel condition, resulting in increased freezing and neural similarity. Though patterned single-cell optical stimulation via two-photon microscopy is possible, a huge limitation is that head fixation is required (Rickgauer et al., 2014). Thus, development of the Mini-SLM, which allows freely-moving behavior, will be a monumental contribution to behavioral neuroscience.