**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). Despite rich literature from human functional imaging experiments, specific knowledge about the exquisite neural ensemble patterns associated with cognitive ageing is severely lacking. 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 are less efficient at linking similar memories which could explain memory impairments. Exploiting the well-known sequential activity patterns commonly observed in the hippocampal system (Buzsáki and Tingley, 2018; Cheng and Ji, 2013; Mau et al., 2018), we propose to investigate how neural sequences 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 sequences 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 sequences in hippocampal CA1 incorporate new components of a memory task and compare them between aged and young mice. To this end, we will train mice on a delayed cue-location association task with multiple different cues. We will also simultaneously measure behavioral-timescale neural sequences from CA1 during learning using a newly-developed wireless *in vivo* calcium imaging microscope (Shuman et al., 2018). Previous reports have shown that CA1 sequences 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 sequences in young mice will exhibit properties characteristic of generalization (via the sequence “backbone”) in addition to differentiation (via plastic cells) across multiple cues. On the other hand, sequences from aged mice will lack the generalizing backbone scaffold and will instead inefficiently over-recruit cells to represent each cue-location association.

**Aim 2. Develop and test novel technology for spatially-patterned optical stimulations to artificially generalize memories in aged mice.** Previous experiments have artificially linked distinct memories via temporally coarse manipulations (Cai et al., 2016; Rashid et al., 2016). However, these studies were agnostic to the functional signatures of the ensembles being manipulated. Here, we propose to manipulate functionally critical cells with patterned optical stimulation to affect learning in aged mice. In a trace fear conditioning task, we will train mice to avoid a spatially restricted zone in an environment by associating a cue with footshocks in that zone. Then we will reverse the safe and shock zones for a different cue, thus associating two different cues with two different shock zones. We predict that young mice will (1) learn the first association and then quickly learn the second association based on their prior training and (2) have significantly more ensemble overlap between the two experiences. By contrast, aged mice will (1) learn the second association at a slower rate and (2) demonstrate less ensemble overlap due to deficits in memory linking (Cai et al., 2016). Then using *in vivo* calcium imaging, we will identify specific neurons that have sequential activity locked to the cue and stimulate them using patterned light delivery via a modified miniature microscope within an incorporated spatial light modulator (Mini-SLM). We predict that specifically stimulating behaviorally-relevant neurons during learning in aged mice will accelerate and rescue age-related impairments in memory linking. 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.