**PROJECT SUMMARY / ABSTRACT**

A tremendous amount of research has provided us with an understanding of how neurons work in concert during the formation and retrieval of individual memories. While we understand how memories are stored in a limited number of brain regions, we do not yet understand how multiple memory traces are stored across whole-brain neural networks, as well as their real-time physiological dynamics, genetic landscape, and preferential wiring. What is needed now is technology to bridge the gap in our understanding between microscopic interactions at the neuronal level and macroscopic structures that perform computations across networks involved in learning and memory. Using a combination of two activity-dependent tagging systems that utilize the immediate early genes (IEG) *Arc* and *c-fos*, the aim of this proposal is to address the critical need for obtaining a map of multiple memories and provide the dynamic states of the brain in the context of behavioral performance and memory expression. We will first utilize behavioral assays and whole-brain imaging to provide unprecedented insight on how multiple memories (e.g., positive and negative memories) are stored with single-cell resolution in a brain-wide manner. Identification of similarities and differences between populations and projections of positive and negative memory ensembles will be quantified and correlated with behavioral performance by using neuronal modeling developed in the Denny laboratory. Tagged cells will also be pulled down and sequenced to delineate the genetic landscape differentiating positive and negative memories. We will then use *in vivo* Ca2+ imaging to resolve the real-time dynamics (e.g., Ca2+ activity) of neural ensembles as they participate in positive and negative memory encoding and retrieval. Moreover, we will use optogenetic modulation to manipulate the positive or negative ensembles in a within-subject manner during behavioral performance to identify key nodes involved in memory expression. Finally, we will use viral tracing strategies to determine how these ensembles are structurally wired across brain, thereby providing a wiring diagram for multiple experiences in the brain. In summary, comprehensive molecular biology, immunohistochemistry, network modeling, Ca2+ imaging, and optogenetic techniques will be utilized. As most studies have narrowed their analyses to a single brain structure, these studies will expand this scope exponentially by analyzing whole-brain memory traces mediating multiple memories. This combinatory system will result in a whole-brain atlas for individual memories, including positive and negative memories, with single-cell resolution.