PROJECT SUMMARY (See instructions):

A central goal in neuroscience is to identify cellular ensembles supporting mental and behavioral states. These ensembles cannot be defined *a priori*. The dentate gyrus (DG), for example, contains more than 1M granule cells, which are essentially indistinguishable from each other, but less than 5% of these seemingly identical neurons are active during any one behavioral event, suggesting that the associated mental states are mediated by a small subset of neurons. We propose to develop a novel method for identifying and gaining genetic access to such transient, behaviorally-relevant assemblies of neurons. The key unique features of our approach are (1) its temporal precision—it is the first neuronal tagging technique that matches the timescale of naturalistic behavior; and (2) its ability to label multiple cell populations in the same animal, enabling the comparison of state-specific cell ensembles.

Our molecular-genetic technique identifies activated neurons on the basis of elevated intracellular calcium, then tags them using light. Light application is especially attractive because it is temporally precise: just as other optogenetic methods have aided neuronal circuit analysis by approximating the timescale of cell activity, so too will a light-dependent labeling technique illuminate functional cell assemblies. The technique will be entirely virus-based, usable across species without relying on transgenic animals. Our near-term goal is to uncover hippocampal memory traces associated with contextual learning. Ultimately this approach will be used to elucidate the neuronal substrates of other mental states, such as fear, hunger, depression, anxiety, and addiction, advancing the exploration of critical brain networks.

RELEVANCE (See instructions):

To uncover the neural substrates of mental states, neuroscientists have traditionally relied on two strategies: cellular recordings correlated neural activity with behavior and the underlying mental states; lesions helped evaluate the necessity of particular brain regions or cell types for behavior. While these approaches have led to great advances, both carry severe limitations. Recordings can provide excellent temporal resolution but are purely correlational. Lesions can test necessity and sufficiency, but they offer poor temporal resolution and no means to specifically target the sparse subsets of cells associated with the behavior of interest. As a result, there is a critical gap in our knowledge: we know very little about the causal significance of activity patterns within and across sparse populations of neurons. This multidisciplinary project represents a groundbreaking way to distill neuronal activity *in vivo*, uncovering cellular assemblies operative during brief, experimenter-defined time windows. The functional role of each assembly can then be tested by direct molecular-genetic manipulations. Our method will enable active neurons to be tagged on a seconds-to-minutes time scale, followed by expression of fluorophores, heterologous actuators or virtually any other protein of interest in the tagged cells. In brief, the method enables the experimenter to identify the subset of neurons activated during a transient behavioral or mental event, and then to manipulate these cells in the living animal and/or to analyze these cells *ex vivo* using protein, gene or histological assays. The proposed method will provide a highly novel and powerful tool for functional brain mapping, congruent with the stated goals of the UT BRAIN Initiative to support the development of techniques for unbiased species-independent whole brain characterization.

The backbone of our proposal is a virus-based activity reporter system that has already been developed and validated in our labs. Under this proposal, we will produce a second-generation reporter system with greatly enhanced temporal precision and flexibility, and we will validate this system using *in vivo* behavioral experiments in mice. After achieving these milestones, we will be in a strong position to compete for NIH funding through the NIH BRAIN Initiative.

Three PIs from the College of Natural Sciences at UT Austin will lead this pilot project. Two are early career investigators at the forefront of systems neuroscience: Boris Zemelman is a co-inventor of optogenetics and an expert in the design and use of molecular-genetic reagents for *in vivo* neural circuit analysis; Michael Drew, is a behavioral neuroscientist focusing on memory mechanisms of the dentate gyrus. They will collaborate with Stephen Martin, a renowned synthetic chemist specializing in developing and optimizing biologically active compounds to target the brain.