**AUTHENTICATION OF KEY RESOURCES PLAN**

**Mice**

*ArcCreERT2 Mice*: The studies proposed in this grant application rely heavily on the use of the ArcCreERT2 x channelrhodopsin (ChR2)-enhanced yellow fluorescent protein (EYFP) transgenic mouse line. Dr. Denny previously developed the ArcCreERT2 line to allow for the permanent labeling of neurons activated during learning. We have since bred this line with the ChR2-EYFP mice and/or EYFP to study neuronal circuitry during disease progression. The ArcCreERT2 mice are now deposited at the Jackson Laboratory.

*Genotyping*: We confirm all genotypes using PCR. These mice have been characterized extensively and Cre expression in the hippocampus closely matches that of endogenous *Arc* protein (Denny et al., 2014, Neuron). The floxed strain (ChR2-EYFP) were obtained from the Jackson Laboratory. The genotypes were confirmed upon receipt using PCR genotyping protocols developed by Jackson Laboratory. The APP/PS1 mice were also obtained from the Jackson Laboratory and genotypes were also confirmed upon receipt using PCR genotyping protocols developed by the Jackson Laboratory. Genotypes of all mice bred in-house are identified using PCR on tail biopsies which are taken at approximately P7.

*cFos TetTag Mice*: All TetTag mice are obtained from Jackson Laboratory. These mice can be injected with various virus constructs under the control of the TRE promoter, thereby permitting doxycycline-regulated tagging of discrete sets of cells active in a defined period of time. We have utilized this intersectional transgenic/virus strategy to label neurons active during learning and retrieval of both positive and negative experiences, as well as to monitor the structural and functional integrity of these cells throughout a battery of behavioral paradigms.

*Genotyping*: We confirm all genotypes using PCR. These mice have been characterized extensively cFos-driven expression of a flurophore or rhodopsin closely matches that of endogenous *cFos* protein (Liu and Ramirez et al., 2012, Nature). Genotypes of all mice bred in-house are identified using PCR on tail biopsies which are taken at approximately P14.

*End Point*: Following the completion of behavioral experiments, mice are euthanized and brain tissue is examined to confirm expression of the expected construct(s).

**Viruses**

The viruses (e.g., ChrimsonR-tdT, mCherry, or GCaMP6) are obtained from an established core (UNC or U Penn Vector Core). Expression of the virus is confirmed in pilot animals before preparing larger cohorts of mice for surgery and experiments. Following experiments, brains from all mice undergoing viral injection are assessed for viral transduction in targeted brain areas and specified cell types.

Each viral construct will be confirmed for all subjects in all experiments with a combination of immunohistochemistry and in vitro physiology. In experiments utilizing custom viral vector constructs, such as the proposed c-Fos-tTA construct, the efficacy of transduction as well as functionality will be confirmed using standard immunohistochemical and in vitro physiological techniques. To ensure robustness and reproducibility, all viruses—custom and currently available—and their associated standardized protocols will be made available for use by external labs freely.

**Antibodies**

All antibodies for immunofluorescent staining are obtained from reliable sources (i.e., Synaptic Systems, Abcam, and Jackson Immunoresearch) and are identified in the Antibodies Online registry and the NIH’s Research Identification Portal. No-primary controls are used to verify specificity of secondary antibodies. The functionality of all acquired antibodies will be initially confirmed by staining against common proteins leveraged in this proposal, including ChR2-mCherry and c-Fos. The remaining biologicals and chemicals utilized throughout this proposal, including mouse strains, surgical resources, and tissue fixation chemicals, are standard laboratory reagents that are not expected to vary between labs or over time, and do not have qualities that could influence the research data.

**Behavior**

We make great efforts to ensure that our behavioral protocols can be reproduced by other researchers. Our publications include very detailed descriptions of our behavioral procedures and apparatus, and we regularly publish papers characterizing our behavioral procedures in detail (e.g., Ramirez et al. 2015, Perusini et al., 2017). All behavioral experiments use mice from the same background strain, and each group of mice is used in just one experiment to prevent hysteresis.