VERTEBRATE ANIMALS

Mice will be housed and used in the [redacted] Science Building at the [university]. All experiments will be conducted in compliance with the NIH guide (as appropriate) and following institutional approval. The Animal Welfare Assurance number for the [university] is xxxxxx.

We estimate the animal numbers based on the nature of experimental procedures and time required for individual projects.

Here are the estimated experimental numbers for each Aim:

Aim1-STORM: X animals per sex per group for 2 groups per project. We estimate for 10 projects per year. **Aim1**-Teaching: X mice per trainee and there will be 8 trainees per course for X courses per year.
Aim2-Scale S: X-XX animals for 1 project per year (~X weeks 2P time per yr).
Aim3-ex vivo experiments: X animals per sex per group for 2 groups and 1-2 projects per year (XX weeks 2P time per yr).
Aim3-in vivo experiments for pilot projects: X animals per sex per group for 2 groups and 1-2 project per year (~12weeks 2P time per yr).
Aim4-FLIM: X animals per sex per group for 2 groups and 1 project per year (6 weeks 2P time per yr).

1. Description of Procedures

Species: Mice
Strains: XXX, YYY, or XYY/XXYYY mixed
Age: early postnatal ages through adult (3-5 months) for testing or adult (3-10 months) for breeding
Sex: Male and Female
Number: XXXX mice (to provide ~XXXX mice for X years of proposed experiments)

A total of ~XXX mice will be used over X years for experimental and teaching procedures. These mice will be bred in [redacted]. The total number of mice required for all aspects of the study is estimated to be ~XXXX mice. This number includes breeders, isolated mice for line maintenance, littermates of the incorrect genotype for a specific study, sickness/death, experimental failure, sacrificed to maintain similar number of pups per dam, etc. ~50% mice will be transferred to [redacted] from C3A affiliates or approved PIs for MultiScale Imaging Core-approved projects a few weeks before experimental procedure. Thus, those mice won't require breeding. **Wildtype and transgenic strains for proposed example experiments:**

1. XY-1 wild-type mice
2. XXYY/Y
3. [Redacted] mice
4. Other transgenic mice selected for the approved projects. The service nature of the Multi-Scale Imaging Core makes it impossible to list out the transgenic mice that will be employed for selected projects over five years.

Mice will be treated as outlined in the experimental plan and then anesthetized prior to brain slice preparation or perfusion following approved protocols. Housing will be standard grouped housing.

This number of experimental mice (~XXX) reflects using an average of XX mice per month for 12 months per year for X years. Animals will be bred in [redacted] following IACUC-approved breeding protocols to generate mice for [redacted] treatment. CD1 mice will be purchased from Charles River and XXYY/Y mice from JAX to replenish our breeding stocks.

Below is a brief description of animal procedures. Please note that these methods are provided here in response to the PHS398 instructions which request "*Provide a concise description of the proposed procedures to be used that involve live vertebrate animals in the work outlined in the "Research Strategy" attachment. The description must include sufficient detail to allow evaluation of the procedures*." These procedures are also presented in the experimental plan and the references cited within and no new details are presented here.

Tissue harvesting. Brains will be harvested after utilizing an AVMA-approved euthanasia technique.

Administration of substances:
[Redacted] and other compounds will be given by subcutaneous (s.c.). or intraperitoneal (i.p.) injection in a volume of 5-10µl/g body weight as approved by our animal care and use committee.

Sterotaxic AAV injections:
P30-60 mice will be anesthetized and prepared for their aseptic surgical procedure. After stereotaxic determination of the coordinates of the medial dorsal thalamus, a single burr hole per injection site will be drilled in the skull while taking care not to damage the dura. Using an automated syringe pump and a 30 gauge needle mounted on the stereotaxic frame, the needle will be advanced to the appropriate coordinates and the desired amount of rAAV-containing solution (< 1 µl per injection) will be injected at a rate of 100 nL/min as described (1,2).

Newborn AAV injection procedure
For intraventricular (icv) injection, sterile saline containing recombinant AAVs will be injected freehand into both the left and right lateral ventricles of P0/P1 pups which have been anesthetized with hypothermia (5, 6). ~0.2-1 µL volume will be injected into each ventricle via a Hamilton syringe. After injections, pups will be placed on heating pad until they regain normal color and resume movement before being returned to their moms. For stereotaxic AAV injection, pups or young adult mice will be anesthetized and head-fixed on animal holder . A single burr hole will be drilled in the skull allowing the entrance of pulled-glass capillary or 30 gauge needle connecting to an automated syringe pump mounted on the stereotaxic frame to advance to S1 area according to the coordinates. ~0.2-1 µl rAAV will be injected at a rate of 100 nL/min as described.

Cranial window installation
This procedure is conducted as described (3,4). Briefly, an anesthetized mouse will be placed in an animal holder, secured with ear bars and kept warm by using a commercial mouse heating pad with temperature controller. After cutting open the scalp over the top of the skull, a ~4 mm in diameter hole will be drilled through the skull with a micro-drill. Next, a sterile glass cover slip will be placed on top of the dura mater. A drop of cyanocrylate-based glue will be applied to secure the cover slip. A customized head frame is placed on top of the cranial window and fixed in place with dental cement.

Two-photon imaging with an awake behaving mouse
To reduce stress during imaging, mice will be handled or trained multiple days to habituate to head-restraint and walking on an air-supported floating platform. Briefly, the mouse is taken from its home cage and gently restrained to secure the head bar on top of the mouse's head into the head holder on top of the stage connected to the Two-photon microscope while allowing it to move freely on the floating platform.

2. Justification for the Use of Animals
The use of animals (mice) is necessary for this project because the project seeks to elucidate the mechanisms underlying [redacted] and how it may be disrupted in the intact brain and the subsequent behavioral implications. Mice are chosen because of the similarities between mouse and human neurodevelopment (5,6) and the availability of numerous transgenic lines, which allows us to elucidate underlying molecular mechanisms. Mice are also the standard experimental animal for neural circuit mapping, brain slice electrophysiology, neuroanatomy, pharmacology, and immunohistochemistry. No existing computer simulations are sophisticated enough to address the questions to be studied in this proposal.

Breeding colonies for the mice that we will use for these studies have already been established in the [redacted] animal facility at [university]. The number of mice used will be the minimum necessary to validate the experimental observations and are guided by our extensive experience with these types of studies (Dr. [redacted] and Dr. [redacted] collectively have more than XX years of experience working with mice). As we are conducting these experiments, we will continuously monitor our results and adjust numbers of animals used as

appropriate. We will also perform interim power analyses to verify sample sizes once experiments are underway and as guided by initial effect sizes and observed variance.

3. Procedures to Minimize Pain and Distress
All procedures are consistent with the NIH Guide for the Care and Use of Laboratory Animals and follow the guidelines of the International Association for the Study of Pain. In all cases, mice will receive ad lib access to food and water in their home cages. Moreover, mice in our study will not experience undue pain, injury, or discomfort. For electrophysiology or anatomical studies, prior to decapitation, the animal will be deeply anesthetized with isoflurane administered via inhalation. The mouse will be visually monitored during this procedure and when unconscious, the depth of anesthesia will be assessed by the absence of a response to noxious stimulation (pinching of a paw).

If unexpected health findings are observed, we will consult with one of [the university's] veterinarians.

References:

1. References Redacted