Imaging Glomerular Signaling of Unrestrained Olfactory Search in mice

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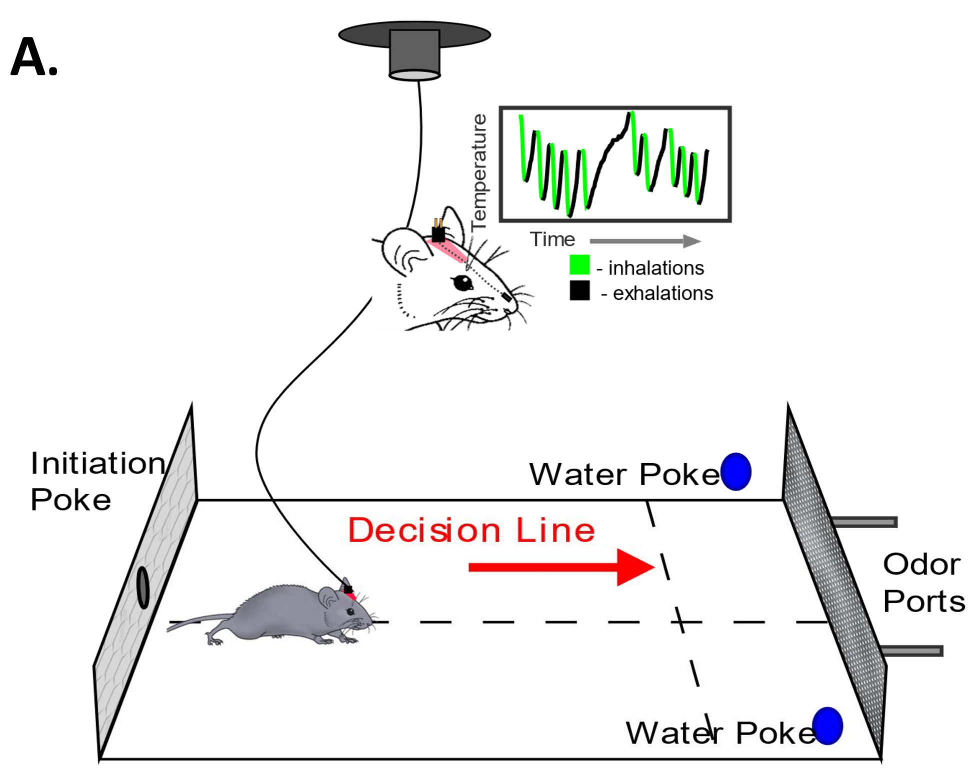
ABSTRACT

Olfaction is vital for many crucial animal behaviors such as social interaction, avoiding predators, and locating food. Our goal is to understand how an animal navigates toward the source of an odor. However, little is known about how odors are coded to inform olfactory searching behaviors. Air turbulence can cause odor distributions to be highly variable and unpredictable. Although we have previously characterized specific behavioral patterns in turbulent odor plumes, little is known about how odors are translated into movements.

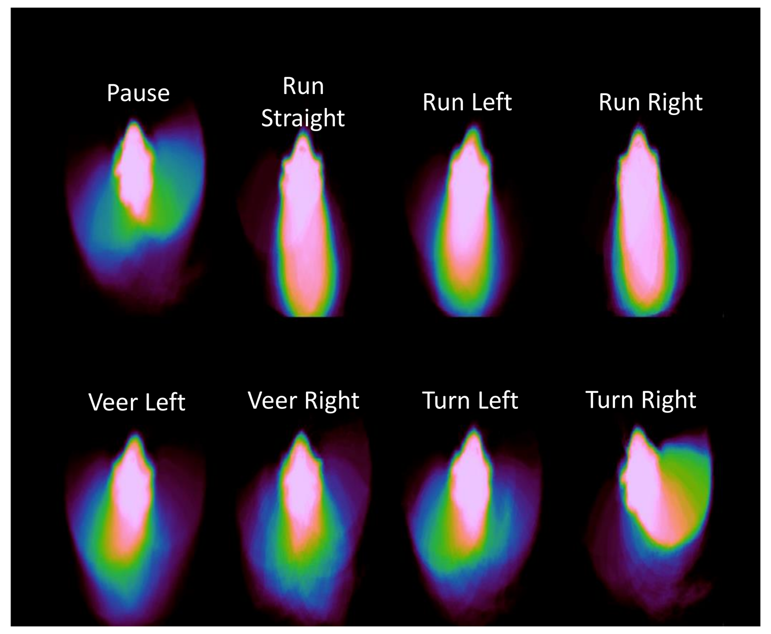
Our goal is to capture and understand the sensory input that informs these previously observed behaviors. We do this by injecting iGluSnFR, a fluorescent glutamate reporter, into the mitral cell layer of the olfactory bulb. This reporter tells us how glutamate released from olfactory sensory neuron terminals influences activity of mitral cells. iGluSnFR's fast kinetics allows us to observe and measure glutamate levels as the mouse performs olfactory navigation. By revealing activity in olfactory sensory neurons during olfactory navigation, this technique can tell us how odor informs the mouse's brain during active sampling. Following the development of this technique, we will image from iGluSnFR mice performing our olfactory search task to determine the neural computation that connects movement and sensation. Understanding how mice translate odor into behavior will inform our understanding of active sensory sampling behaviors in humans.

ESTABLISHED OLFACTORY SEARCH TASK AND UNDERLYING BEHAVIORAL MOTIFS USED TO LOCATE ODOR

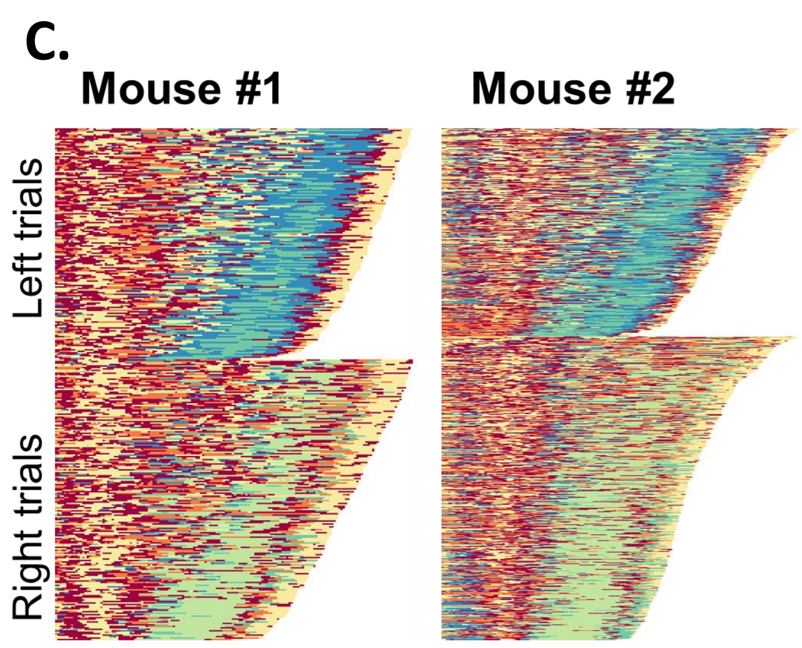


(Graphic by Teresa Findley)

A . Olfactory Search Task
Mice are presented with a two-choice olfactory task. Mice initiate trials by sticking their noise into initiation port and move toward side with higher odor concentration. Nose, head, and body movements are monitored by camera above in real-time. Sniffing behavior is recorded by an implanted thermistor.

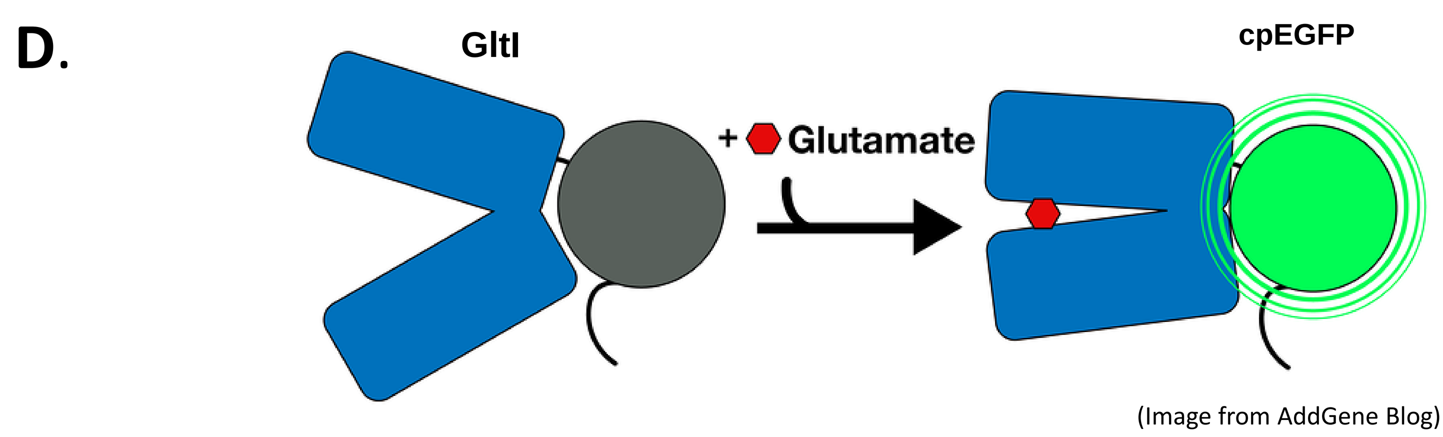


B. **Behavioral Motifs**
Heatmap of each behavioral motif that appears frequently in our olfactory search task averaged across multiple mice. Data extracted using Deeplab Cut

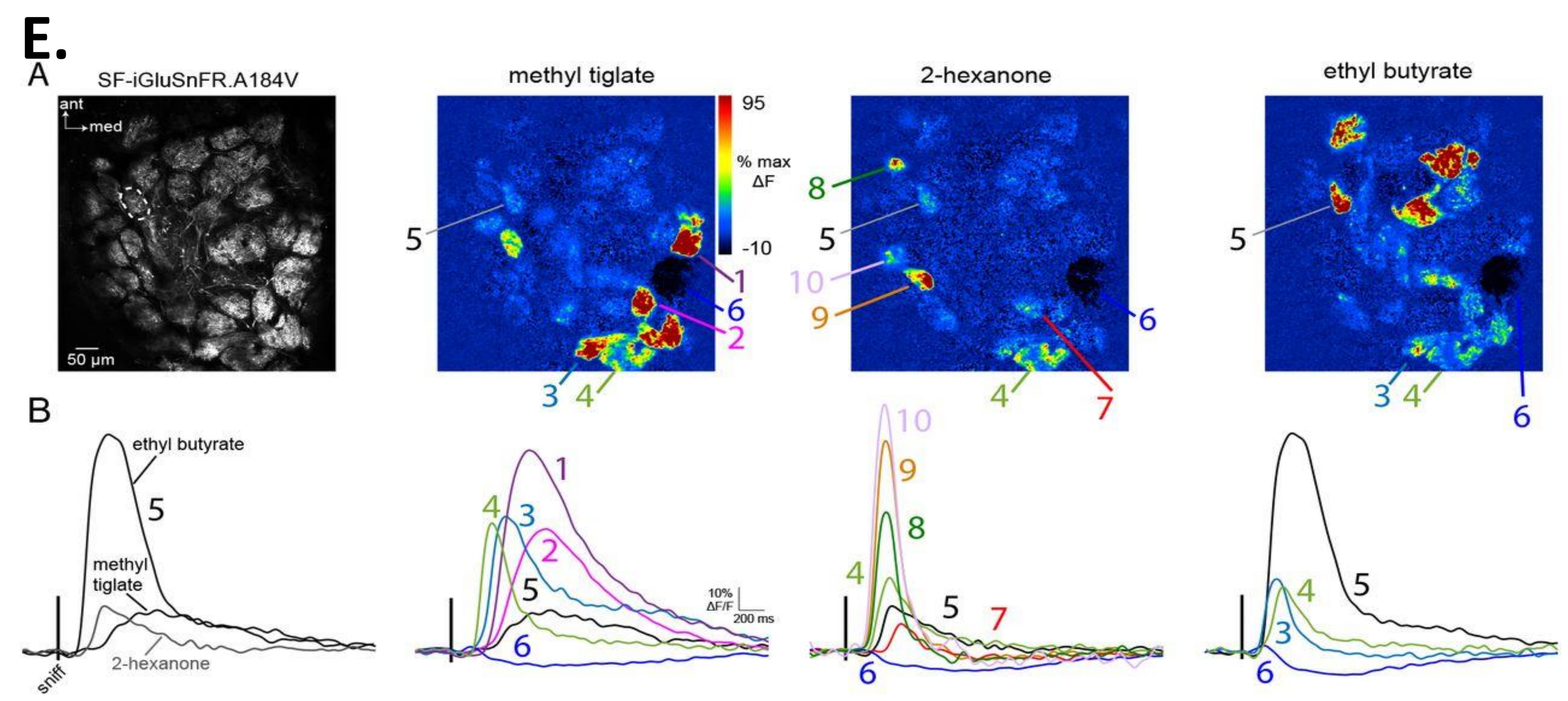


C. Behavioral Motifs across Trials
All trials for two example mice separated by correct side and sorted by trial length. Each color represents a discrete behavioral motif. Behavioral structure is similar across different mice.

NEXT STEPS:: iGluSnFR GENE CONSTRUCT

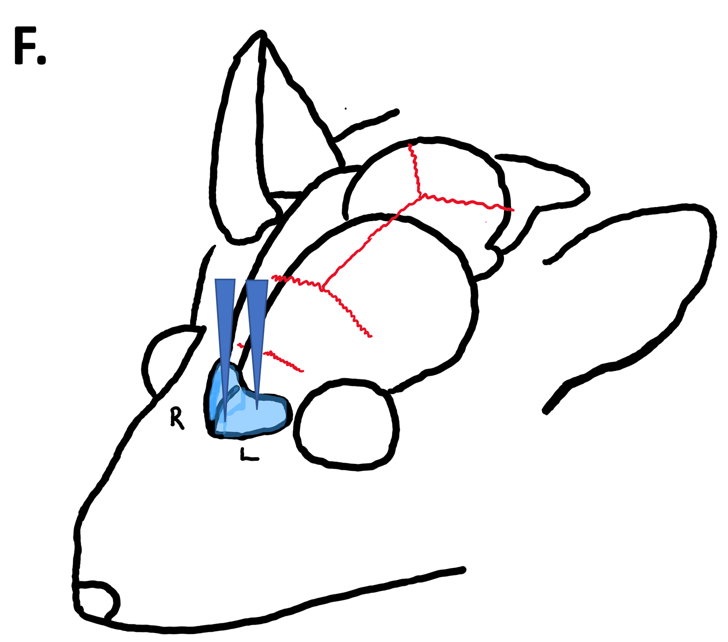


D. Green Fluorescent Protein Construct attached to Glutamate Binding Protein
Presence of glutamate induces conformational changes in the glutamate binding protein (Gltl), which enhances the fluorescent of the permutated green fluorescent protein (cpEGFP)

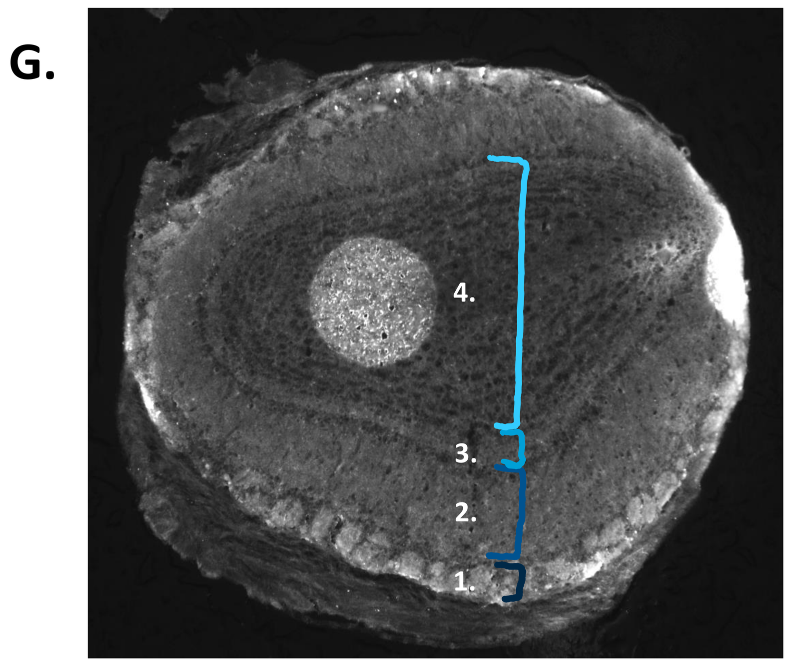


iGluSnFR Expression in Mitral Cells of the Olfactory Bulb (OB)
Image taken from Wachowaik Lab (University of Utah) In vivo expression of iGluSnFR in OB. Change in fluorescent (ΔF) across presentation of three odors: methyl tiglate, 2-hexanone, and ethyl butyrate (Moran, Eiting, Wachowiak 2019)

iGluSnFR INJECTION PROCEDURE

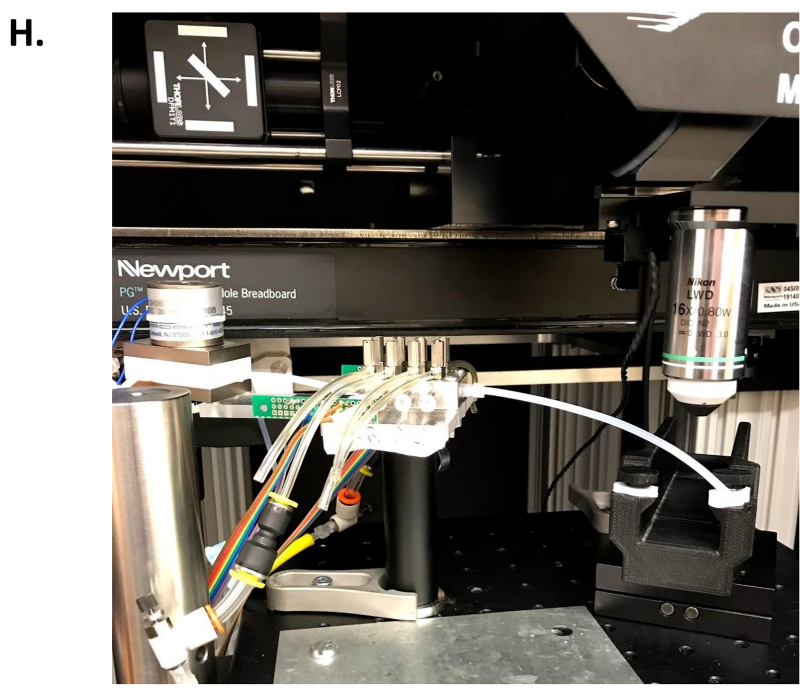


F. **Surgery Image of Viral Injection**
Image adapted (White et al. 2016). Injection of GCamp7f into Left Olfactory Bulb. Image altered to show suture lines and injection areas.

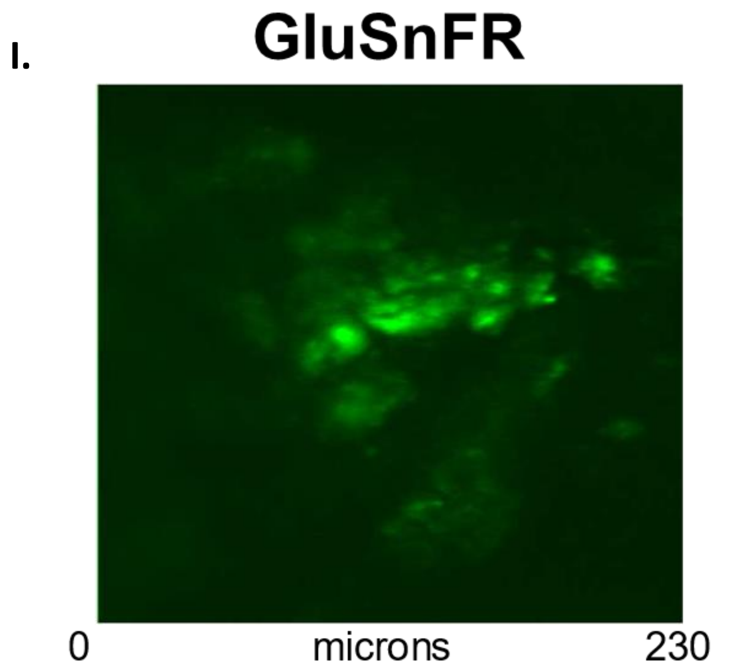


G. Figure of Olfactory Bulb cell layers
1) Glomeruli
2) Periglomerular/Tufted Cell Layer
3) Mitral Cell Layer
4) Interneurons

FUTURE DIRECTIONS:: TWO-PHOTON IMAGING AND FREELY MOVING IMAGING



H. Imaging iGluSnFR
Mice are head-fixed and placed under the laser. They are presented odors passively and changes in fluorescence can be tracked via Mathlab software



I. iGluSnFR in Vivo with Odor Presentation
Fluorescent signals from head-fixed B6 mouse injected with iGluSnFR under two-photon microscopy

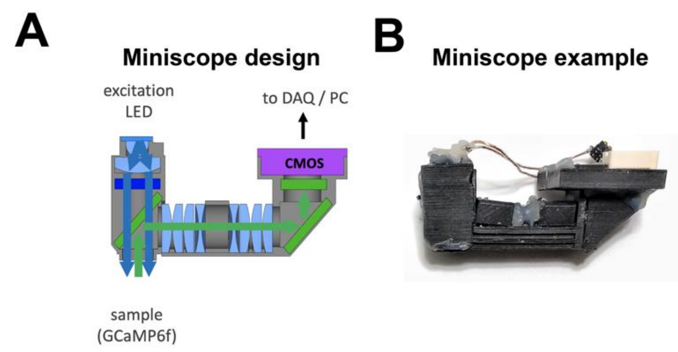
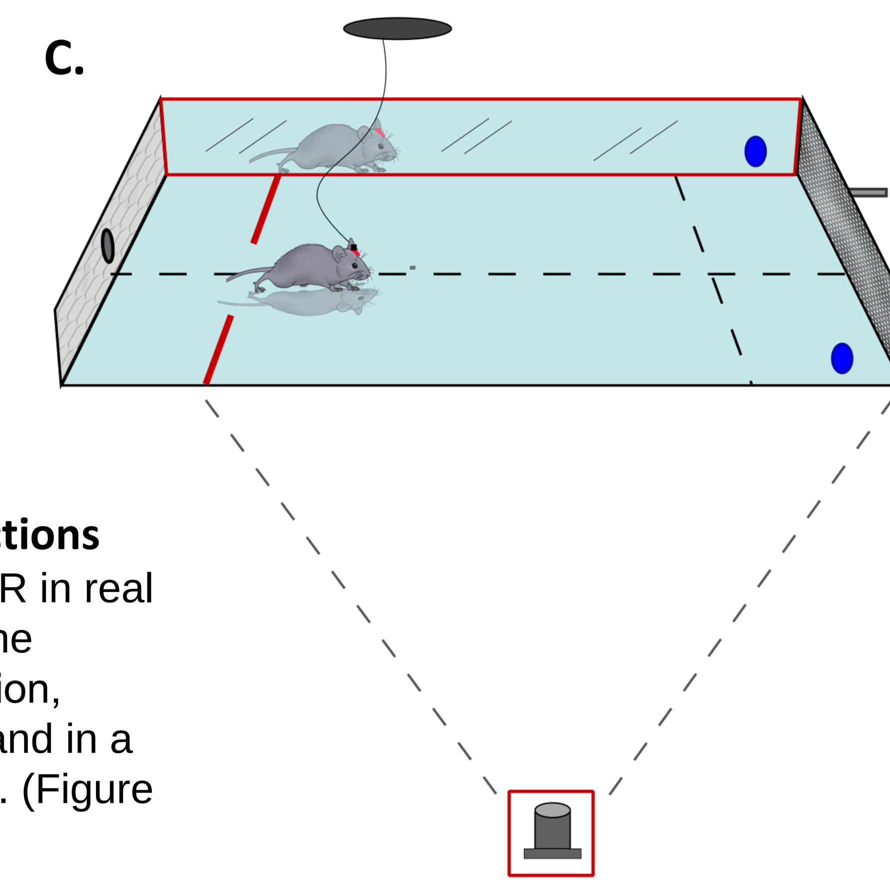


Figure 3. Reading and writing glomerular input patterns. A. Schematic of the miniscope. **B.** An example miniscope (provided by Davison lab)



J. Miniscope Design and Future Directions
Future experiments will record iGluSnFR in real time through a miniscope mounted to the mouse's head. (Figures JA,JB) In addition, tracking will be done from underneath and in a larger arena for more accurate tracking. (Figure JC)

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