Name: Katherine Savell
Email: katherine.savell@nih.gov
PI Name: Bruce Hope
PI email: bhope@intra.nida.nih.gov
Presentation preference: Oral

Molecular characterization of cocaine-seeking engram neurons in the infralimbic cortex using single nuclei RNA-sequencing in rats

Katherine E. Savell, Rajtarun Madangopal, Ryan G. Palaganas, Olivia R. Drake, Diana Pham, Megan B. Brenner, Toni Martin, Jae H. Choi, Madeline A. Merriman, Sophia J. Weber, Veronica A. Lennon, Lauren E. Komer, Elise Van Leer, Bruce T. Hope

Behavioral Neuroscience Branch, NIDA IRP, NIH, Baltimore, Maryland

The risk of relapse is a cardinal feature of addiction. Environmental stimuli previously paired with drug-taking can elicit drug seeking and precipitate relapse long after cessation of drug use. One strategy to combat relapse is to inactivate or weaken these persistent maladaptive drug-cue associative memories by targeting the neurons encoding this memory representation (engram). Engram neurons are commonly identified by their transient expression of immediate early genes (such as *Fos*) after the association experience, and Fos-expressing engram neurons play a causal role in drug-seeking behaviors. However, the molecular mechanisms and cell types that reinforce this drug-cue association are unknown. We hypothesized that the drug-cue engram in association cortices comprises multiple cell types with unique transcriptional signatures to support future cue-induced drug seeking.

Here we use single nuclei RNA sequencing and a Fos-based transgenic rat (Fos-mRFP) to characterize drug-cue engram neurons in the infralimbic cortex. We trained rats to self-administer cocaine during twice daily 3 h sessions. Following 21 days of abstinence, we tested rats for cocaine-seeking (30 min, extinction conditions) and collected brains 3 h after test (peak Fos-driven mRFP expression). We used fluorescence-activated nuclei sorting to isolate mRFP-positive (engram) and mRFP-negative (non-engram) nuclei as input for snRNA-seq. Using this unbiased approach, we characterize cell-type and engram-specific transcriptional signatures that contribute to drug-seeking behaviors. We will employ CRISPR-based transcriptional modulators in future experiments to assess causal roles for these cocaine memory-specific genes in relapse.