In vivo labeling and molecular characterization of cocaine memory-specific active neurons using the photo-convertible calcium integrator CaMPARI2

Rajtarun Madangopal[1], Katherine E. Savell[1], Olivia R. Drake[1], Megan B. Brenner[1], Veronica A. Lennon[1], Diana Q. Pham[1], Sophia J. Weber[1], Lauren E. Komer[1], Lihua Wang[2], Kathy Schaefer[2], F. Javier Rubio[1], Andrew Lemire[2], Eric R. Schreiter[2], Vilas Menon[3], Bruce T. Hope[1]

[1]Neuronal Ensembles in Addiction Section, Behavioral Neuroscience Branch, NIDA IRP.
[2]Janelia Research Campus, Howard Hughes Medical Institute, VA.
[3]Center for Translational and Computational Neuroimmunology, Department of Neurology, Columbia University, NY

Background: In abstinent drug users, cues previously associated with drug-taking can provoke drug craving and promote relapse long after the last instance of drug use. These maladaptive drug-cue associations are thought to be encoded by sparse patterns of neurons (neuronal ensembles) that are strongly activated during learning. However, previous ensemble labeling approaches used immediate early genes (IEGs; e.g. Fos, Arc) as activity markers and lacked the temporal resolution needed to selectively label active neurons during short-lasting behavioral events (e.g, lever press or drug infusion) or characterize them immediately after the event when important learning mechanisms are being activated. To address this gap, we developed procedures to label active neurons *in vivo* with sub-second temporal specificity using the photo-convertible calcium-based activity marker CaMPARI2. We delivered ultraviolet photoconversion (PC) light into the infralimbic cortex (IL) during cocaine seeking to rapidly convert CaMPARI2 protein in active IL neurons from green to red fluorescent state and thus permanently labeled these cocaine-memory specific neurons.

Methods: We used male and female Sprague-Dawley rats in all experiments. We delivered AAVs into IL for CaMPARI2 expression, implanted an optical fiber for PC light delivery and inserted a jugular catheter for cocaine self-administration. We trained rats to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/infusion cocaine paired with a 3.5 s light cue) during twice daily 3 h sessions before switching to trial-based cocaine self-administration (30 trials/ 3 h session, 1 min lever access/trial). Following training and 21 abstinence days, we tested rats for cocaine-seeking (1 min lever access, extinction conditions) and delivered PC light (1 min, 10 mW, 375 nm) to permanently label cocaine-memory specific active neurons in IL.

Results: We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking during the 1 min seeking test on abstinence day 21. We collected brains either immediately after the 1 min test (0-min group) or waited 10 minutes to allow for experience-induced gene expression (10-min group). We isolated red (active) and green (inactive) CaMPARI2 labeled neuronal nuclei using fluorescence activated nuclei sorting and performed single-nucleus RNA sequencing. Dimensionality reduction revealed distinct clusters corresponding to all known IL cell types, including glutamatergic and GABAergic neurons that subclustered into expected layer and subtypes. Further, IEGs were selectively induced in red ‘active’ neurons in the 10-min group in all IL cell types.

Discussion: CaMPARI2-snRNAseq allows unbiased transcriptional profiling of ensemble neurons following time-locked, permanent labeling during behavior. We will identify unique molecular alterations (differentially expressed genes, DEGs) induced specifically within cocaine memory ensemble neurons following relapse and investigate whether DEGs are restricted to specific cell type clusters within IL. Understanding the molecular and cell-type basis of how drug memories are maintained within ensembles could help prevent relapse by selectively weakening persistent drug memories, without influencing other memories.

This research was supported by funds from NIDA IRP (grant no. DA000467-17), NIH CCB Fellowship (RM), NIDA IRP SDFDR Fellowship (DP, OD), and the HHMI Janelia Visiting Scientist Program.