Name: Rajtarun Madangopal Email: rajtarun.madangopal@nih.gov

PI Name: Bruce T. Hope PI email: bhope@intra.nida.nih.gov

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 IR;
[2]Janelia Research Campus, Howard Hughes Medical Institute, VA;
[3]Center for Translational and Computational Neuroimmunology, Department of Neurology, Columbia University, NY.

In abstinent drug users, cues previously associated with drug-taking can precipitate relapse long after the last instance of drug use. These maladaptive cue-drug associations are thought to be encoded in sparse patterns of strongly activated neurons (neuronal ensembles) typically identified using immediate early gene (IEG, e.g. Fos) expression. However, IEG-based approaches lack the temporal precision needed to label and characterize ensembles during short-lasting behavioral events (e.g, lever press / drug infusion). We employed a green-to-red photo-convertible calcium integrator, CaMPARI2, to permanently label cocaine-memory ensembles in infralimbic cortex (IL) of rats with sub-second temporal specificity during cocaine seeking.

We used male and female Sprague-Dawley rats, delivered AAVs for CaMPARI2 expression, implanted optical fibers for photoconversion, and inserted a jugular catheter for cocaine self-administration. We trained rats to self-administer cocaine (FR1, 0.75 mg/kg/infusion + light cue) during twice daily trial-based cocaine self-administration sessions (30 trials/ 3 h, 1 min lever-access/trial). Following training and 21 abstinence days, we used CaMPARI2-photoconversion to permanently label IL cocaine-memory ensembles during a 1 min cocaine-seeking test. We observed robust cue-induced cocaine-seeking and collected brains either immediately after test (0-min group) or waited 10 minutes for experience-induced gene expression (10-min group). We isolated individual red (active) and green (inactive) CaMPARI2 labeled neuronal nuclei and performed single-nucleus RNA sequencing to identify unique molecular alterations (differentially expressed genes, DEGs) within IL cocaine-memory ensemble cell-types following relapse.

Understanding the molecular and cell-type basis of cocaine-memory ensembles could help prevent relapse by selectively weakening persistent drug memories, without influencing other memories.