Name: N. Dalton Fitzgerald
PI Name: Jeremy Day

Email: ndf@uab.edu
PI Email: jjday@uab.edu

# Neuronal Activation by Cocaine Varies Across Molecularly-Defined Subpopulations of VTA Dopamine Neurons

N. Dalton Fitzgerald, Lydia Slocum, Jeremy J. Day

University of Alabama at Birmingham

Substance use disorder is a complex neurobiological disease characterized by a loss of control over drug-taking and drug-seeking behaviors. Drugs of abuse increase dopamine (DA) transmission from ventral tegmental area (VTA) neurons that densely innervate the nucleus accumbens. We previously profiled the VTA using single nucleus RNA sequencing and identified unique markers for two distinct subpopulations of DA neurons. Slc26a7, a gene encoding an anion transporter, serves as a selective marker for combinatorial neurons that express genes implicated in glutamate, GABA, and DA neurotransmission. Likewise, the GTP cyclohydrolase member Gch1 serves as a marker for DA-only neurons. Here, we used RNAscope to examine whether distinct DA neuron subpopulations respond differently to drugs of abuse. We identified unique induction of the neuronal activity marker Fos in Slc26a7+, but not Gch1+, cells in the VTA 1 hour following cocaine experience, but neither fentanyl nor fentanyl and cocaine co-administration in either subpopulation. Therefore, we designed and generated two novel adeno-associated viruses (AAVs) to express distinct fluorophores driven by cell-type specific promoters (Gch1-Cre-P2A-mCherry and Slc26a7-Cre-P2A-EGFP). Following bilateral VTA injection, 94.00% of mCherry+ cells were Gch1+ identified using RNAscope, and 90.24% of eGFP+ cells were Slc26a7+, suggesting high targeting efficiency. Using these AAVs, we performed ex-vivo whole-cell patch clamp slice electrophysiology to determine both passive and active properties of the two subtypes. These results suggest there are two distinct subpopulations of DA neurons within the VTA that respond in unique ways to cocaine and highlight novel tools to enable access to these populations.