Name: BaDoi Phan Email: badoi.phan@pitt.edu
PI Name: Andreas Pfenning PI Email: apfenning@cmu.edu

Cell Type Specific Enhancers for the Primate Dorsolateral Prefrontal Cortex

Jing He[1], BaDoi N. Phan[2,3,4], Willa G. Kerkhoff[1], Aydin Alikaya[1], Olivia R. Brull[1], J. Megan Fredericks[1], Tao Hong[1,5], Morgan Sedorovitz[6], Chaitanya Srinivasan[2,3], Michael J. Leone[2,3,4], Olivia M. Wirfel[1], Samuel Dauby[1], Rachel K. Tittle[1], Meng K. Lin[1], Andreea C. Bostan[1], Bryan M. Hooks[1], Omar A. Gharbawie[1], Leah C. Byrne[6], Andreas R. Pfenning[2,3], William R. Stauffer[1]

[1]Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15213;
[2]Computational Biology Department, Carnegie Mellon University, Pittsburgh, PA 15213;
[3]Neuroscience Institute, Carnegie Mellon University, Pittsburgh, PA 15213;
[4]University of Pittsburgh-Carnegie Mellon University Medical Scientist Training Program, Pittsburgh, PA 15213;
[5]Program in Neural Computation, Carnegie Mellon University, Pittsburgh, PA 15213;
[6]Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA 15213

Background: The primate dorsolateral prefrontal cortex (DLPFC) is essential for higher cognitive functions, but studying its complex circuits requires cell type specific molecular tools that are currently limited in nonhuman primates (NHPs).

Rationale: Enhancer sequences can drive precise cell type specific gene expression when delivered via viral vectors, potentially enabling targeted manipulation of DLPFC circuits. We hypothesized that combining single cell genomics with machine learning could identify enhancers for specific DLPFC neuron subtypes.

Methods: We performed single-nucleus RNA-Seq and ATAC-Seq on rhesus monkey DLPFC to define molecular phenotypes and identify open chromatin regions. Machine learning models were trained to recognize regulatory sequences that could drive cell type specific expression. Top enhancer candidates were screened using viral vectors in NHP brain tissue.

Results: We identified and validated enhancers using pooled and one-at-a-time injection experiments for two key neuron subtypes: layer 3 pyramidal neurons (L3PNs) and layer 5 extratelencephalic neurons (L5ETs). The performance of these enhancers was titer dependent. The top L3PN enhancer, RMacL3-01, successfully restricted expression to layers 2/3 with 77.2% specificity for target neurons. When used to drive channelrhodopsin expression, RMacL3-01 enabled reliable optical control of neural activity, demonstrating its utility for circuit manipulation.

Discussion: This work establishes effective enhancers for targeting specific DLPFC neuron subtypes in NHPs and provides a systematic pipeline for developing cell type specific tools. These resources will enable precise investigation of circuit-based mechanisms underlying primate cognition and potentially aid development of targeted gene therapies for neurological disorders.