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Presentation Abstract

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Presentation Title: [Autopatcher application to single cell RNA analysis and optogenetic cell type identification](#)

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Abstract: Much of the current work in neuroscience is aimed at revealing how different cell types of the brain work together in a circuit to implement brain computations as well as how different cell types go awry in brain disorders. Current techniques used to measure the activity of single cells in vivo are predominantly extracellular; and rely on spike timing and waveform characteristics, to determine the cell type of neurons being recorded. These are however, subjected to sampling biases and vary depending on brain state and region. Intracellular techniques such as whole cell patch clamping, on the other hand, enable the measurement of sub-threshold membrane potential deflections in individual cells. This property can thus be used along with optogenetics to identify cell types being recorded from by directly measuring induced photocurrents or lack thereof, due to light stimulation. Additionally, whole cell patch clamping allows access to the intracellular contents of the cell being recorded from, thus enabling the extraction of the cells cytoplasmic contents for single cell molecular and transcriptomic analyses via RT-PCR. We here present two extensions to the Autopatcher (Kodandaramaiah, S. B., et al, Nature Methods 2012). We have combined the Autopatcher with

optogenetic stimulation hardware to allow perturbation of specific cell types optically while recording distributed subthreshold membrane potential fluctuations. This technique can be used to reliably obtain whole cell recordings from Channelrhodopsin-2 (ChR2) and Archaeorhodopsin-3 (Arch) expressing neurons up to depths of 2 mm in the mouse brain in an automated fashion. Upon light stimulation, non-optogenetic molecule expressing neurons can be distinguished from optogenetic molecule expressing neurons by the lack of directly induced photocurrents upon light stimulation, making this a simple yet effective means of identifying the cell type of neuron being recorded.

Further, we are working on developing the automated patch clamping algorithms for programmed and optimized harvesting of cytoplasmic contents of the recorded cells in a manner which is compatible morphological analysis via biocytin staining. These developments will result in an easy-to-use tool that enables the integrative phenotyping of cells of the brain - namely, a robot that can acquire simultaneously the gene expression patterns, morphologies, and electrophysiological properties of single cells in brain tissue, in an automated fashion.

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PATCH CLAMP

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