Integration of automated patch clamp electrophysiology system with optogenetics for cell type identification *in vivo*

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Introduction: 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 there of, due to light stimulation. We here combine the Autopatcher [1]: an automated system for *in vivo* whole cell patch clamping with optogenetic stimulation hardware to allow perturbation of specific cell types optically while recording distributed subthreshold membrane potential fluctuations.

Materials and Methods: The optic fiber is aligned parallel to the glass electrode of an Autopatcher and fixed by wrapping heat shrink tubing around the fiber and electrode and heating it for 2-3 seconds (Fig. 1a). It allows the center of a 200 µm diameter optical fiber to be positioned ~800 µm from the tip of glass electrode that is used for patching and deliver light powers up to 20 mW/mm^2 at the recorded neuron for optogenetic stimulation. For patch clamping, the fiber coupled glass electrode is positioned 20-30 µm above the brain surface and automated whole cell patch clamping is carried out as described in [1].

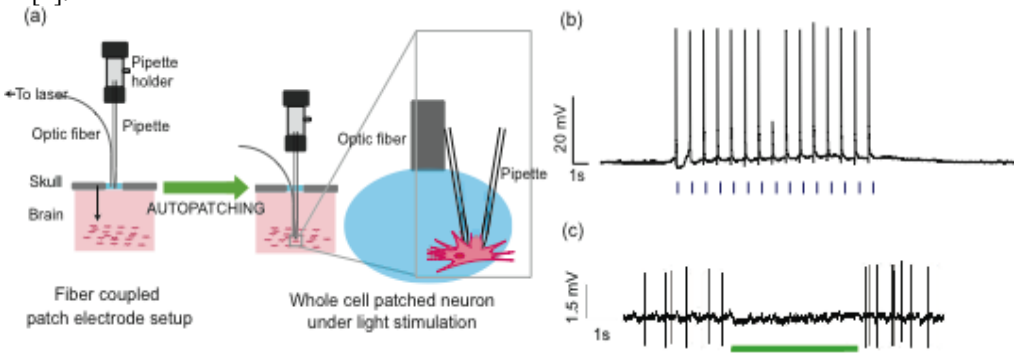

Figure 1: (a) Schematic of a fiber coupled patch electrode mounted on a robot for automated whole cell patch clamping [1]. (b) Current clamp recordings during 473nm blue light illumination for a ChR2 expressing cortical neuron which was whole cell patched using the Autopatcher in a Thy-ChR2 transgenic mouse. Blue bars indicate 10ms pulses of light stimulation. *In vivo* loose-cell attached mode of a SERT-Cre mouse, to 30 seconds of Arch-mediated silencing, green bar indicates time duration of light stimulation.

Results and Discussion: This technique can be used to reliably obtain whole cell recordings from Channelrhodopsin-2 (ChR2) and Archaeorhodopsin (Arch) expressing neurons up to depths of 2 mm in the mouse brain in an automated fashion. Shown in Figure 1b are current clamp recordings from a single layer-5 neuron in motor cortex Thy1-ChR2 transgenic mouse that expresses the blue light activated ChR2 molecule using the Thy1 promoter. We have also used this technique to get cell-attached recordings in the Dorsal Raphae Nucleus (depth of 3 mm) and demonstrated Arch mediated silencing of spontaneous firing activity of serotonergic neurons *in vivo* (Fig 1c). In both cases, non-optogenetic molecule expressing neurons can be distinguished from optogenetic molecule expressing neurons by the lack of directly induced photocurrents upon light stimulation.

Conclusions: The combination of automated patch clamping *in vivo* with optogenetic stimulation hardware acts a high-throughput tool for the characterization of optogenetic molecules *in vivo*. The ability to measure direct induced photocurrent due to light stimulation allows on-the-fly cell type identification of recorded neurons *in vivo*.

References: [1] Kodandaramaiah, S. B., et al, Nat Meth, 2012 (in press)