FULLY-AUTOMATED IN VIVO SINGLE CELL ELECTROPHYSIOLOGY

Jamison Go[1], Aaron Fan[1], Coby Lu[1], Suhasa Kodandaramaiah[1,2], Gregory L. Holst[1], William Stoy[1], Ilya Kolb[1], Edward S. Boyden[2], and Craig R. Forest[1]
[1]Woodruff School of Mechanical Engineering
Georgia Institute of Technology
Atlanta, GA, USA
[2]Media Lab and McGovern Institute
Massachusetts Institute of Technology
Cambridge, MA, USA

Neurons communicate information through fluctuations in the electrical potentials across their cellular membranes. Although scientists have been recording the electrical activity of neurons for more than a century, it was the advent of intracellular recording technology, or whole-cell patch clamping, that allowed the measurement of neuronal membrane potential that enabled precise characterization of neuronal function, winning Hodgkin and Huxley the Nobel Prize in 1963 and Neher and Sakmann the Nobel Prize in 1991. However, whole-cell patch clamping is something of an art form, requiring great skill to perform and has thus been primarily limited to *in vitro* experiments, a select few *in vivo* experiments, and in very limited applications in the awake brain [1]. Our team has recently developed a robot that *automatically* performs patch clamping *in vivo,* by algorithmically detecting cells through analysis of a temporal sequence of electrode impedance changes. Using it, we have demonstrated good yield, throughput, and quality of electrophysiology recording in mouse cortex and hippocampus [2]. With this autopatching robot enabling routine access to electrical and molecular properties of neurons embedded in intact tissue, systematic and scalable *in vivo* patch clamping experiments have become possible.

This autopatching robot (See Figure 1) utilizes a glass pipette to establish electrical and molecular connections to the insides of single neurons embedded in intact tissue and exhibits excellent signal quality and temporal fidelity, useful for understanding not only how neurons compute during behavior, but how their physiology changes in disease states or in response to drug administration. While this automation is powerfully useful, it still requires manual pipette replacement between each 15 minute single cell electrophysiology experiment. This includes a 3-4 minute preparation process of back-filling the pipette with an ionic fluid, threading a conductive silver wire into the inner diameter of the pipette, and securing the pipette to the headstage. The frequency and duration of pipette swaps thus require constant human supervision of the autopatcher. However, if this automated patch clamp robot were to be integrated with pipette exchange hardware and storage magazine, one could perform *fully-automated* in vivo single cell electrophysiology without human intervention, enabling vastly increased througphput (e.g., hundreds of cells/day) by a single human operator.

In this work, we report progress in developing device allow fully autonomous sequential patch clamp experimentation. As shown in Fig. 2, the machine works by integrating a storage magazine of pre-filled pipettes that can be accessed, and swapped, by the headstage at the conclusion of each experiment. In operation, following each neuron measurement, the program enters “swap” state where a set of programmed actuator movements take place. First, the headstage translates towards the pipette storage assembly and deposits its used pipette. The storage assembly rotates to index a fresh pipette, its is grasped, and finally, the headstage returns to its previously designated home position in preparation of subsequent experiments.

The most novel aspect of this machine is the precision collet design, which in grasping the pipette, simultaneously engages electrical contact, mechanical alignment, and pneumatic seals against the pipette using a single linear actuator (See Fig. 3). Pipettes enter through the opening of the collet and are limited in travel by a hard stop. The clamp slider traverses down

the collet with the assistance of a linear actuator which compresses the collet flexure and deforms the rubber end, forming a seal. Simultaneously, two magnets constrained to the clamp slider control the position of a ferromagnetic bead levitating within the collet and thread the silver wire as the assembly descends.

Machine performance was predicted by error budgeting and measured within 197 µm radial repeatability, sufficient for patch clamping in a conventional craniotomy (a 1 mm diameter hole in the skull to access the brain). The total pipette exchange time was measured at 88 sec from end of experiment to home position. It has been observed to exchange a full magazine of 20 pipettes in sequence without failure or assistance. We will report progress in performing the first sequential patch clamping recordings without human intevention, which could usher in a new era of fully automated in vivo neuroscience.

REFERENCES

[1] Poulet JF, Fernandez LM, Crochet S, Petersen CC, Thalamic control of cortical states, *Nat Neurosci.* Vol 22;15(3):370-2.

[2] S. Kodandaramaiah, G. Franzesi, B. Chow, E. Boyden, C.R. Forest, Automated whole-cell patch clamp electrophysiology of neurons in vivo, *Nature Methods.* Vol 9, p. 585–587.

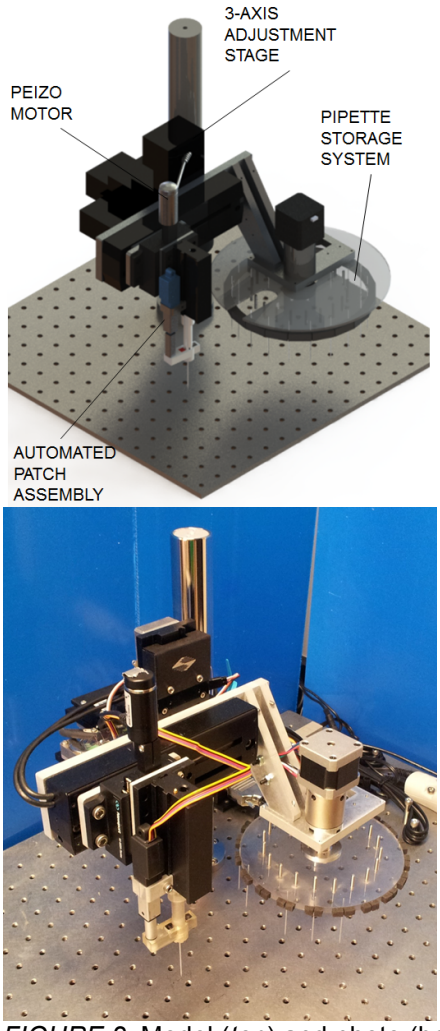

FIGURE 2. Model (*top*) and photo (bottom) of the machine for automated pipette swapping.

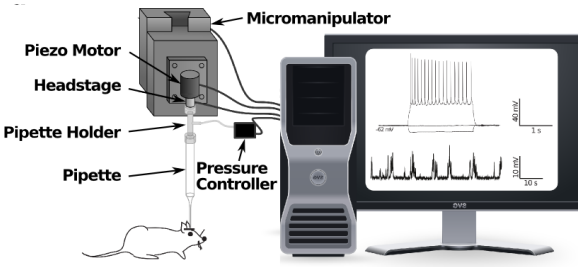

FIGURE 1. The autopatcher: a robot for *in vivo* patch clamping with representative current clamp traces during whole cell automated patch clamping of a cortical neuron.

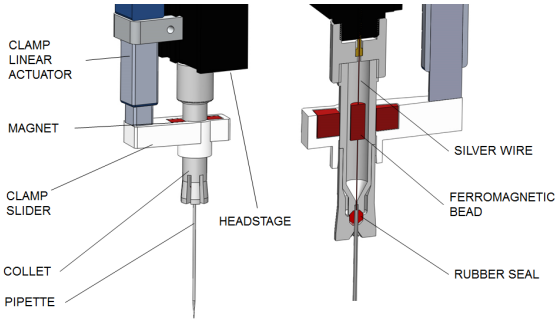

FIGURE 3. The pipette (See Fig. 1) is held by a novel collet design in the automated patch assembly (See Fig. 2) to simultaneously engage electrical contact, mechanical alignment, and pneumatic seals against the pipette using a single linear actuator.