FLiACT PROTOCOL

ELECTROPHYSIOLOGY

Electrophysiology is the most direct method available to scientists for *listening to* the electrical activity of a single neuron or a group of neurons. It provides the ability to resolve sub- and supra- threshold activity neurons with theoretically infinite temporal resolution. In addition, recoding of local field potentials is convenient for the measurement of collective activity of a group of neruons. Thanks to genetic tractability of Drosophila electrophysiologists can target and record identified neurons. This combined with smart experimental design using systems neuroscience approaches has proven to be very effective in cracking the neural code in several Drosophila brain regions. In the following section, we provide blueprints for whole cell patch-clamp for neurons of adult central nervous system of *Drosophila*.

The location of neurons of interest would dictate the approach angle in the recordings. Accordingly the fly is pinned with a small syligard plate with 0.1mm minutiens insect pins to a chamber (Figure 1). The proboscis removed initially, and the brain is dissected in PBS as all remaining trachea is removed. The area of interest would be positioned facing-up. After the blood brain barrier of *Drosophila* is removed via de-sheathing. The prepared specimen is put under the microscope setup (Figure 2). The general structure is found with the 10X lens, while the 63X lens is used for locating individual cells (Figure 3).

Unobstructed, direct contact with the neuron to be recorded is essential and periphery of the contact should be cleaned. A cleaning pipette of 5-10 MΩ resistance, pulled beforehand, is filled with saline solution (K-aspartate 140mM, HEPES 10mM, 1mM KCL, MgATP 4mM, EGTA 1 mM, NaGTP 0.5mM). Subsequent to insertion of pipette in the head stage, the mouthpart is connected to a thin tygon tube to the headstage and blown smoothly on the area of ther sample that will be recorded. The solution coming out of the pipette will remove the tissue covering the cells to record (glia or connective tissue). Once the cells are clean move up the objective and remove the cleaning pipette. The clean cells will appear with smooth and round on the edges. Dirty cells will have small fragments on their surface. Sometimes a smooth suction to remove extracellular debris might be necessary.

Once the cleaning is performed, recording phase commences. The recording pipette of 8-12 MΩ resistance is pulled (If needed, the pipette is polished in a microforge). With help of a fine syringe, the pipette is filled with the saline solution. The pipette is placed and the neuron of interest is located in the fashion explained above. While the 63X lens is used to *park* the pipette next to the cell to record, the membrane clamping potential is measured. The membrane clamping potential at this point should be zero and seal test on the screen should show the deflection showing the resistance of the pipette. It is extremely important to apply constant and strong positive pressure to the recording electrode by blowing to keep the electrode clean.

Set your recording to the voltage clamp mode. Under strong positive pressure, the pipette is moved carefully closer to the cell of interest until it touches the border of the cell. As soon as the electrode touches the cell membrane relase the pressure by stop blowing. The pipette should adhere to the cell membrane instantly and form a giga ohm seal (infinite resistance). If the seal doesn't form instantly at this step, DO NOT apply any suction, blow the cell away stop the recording and re-start all from the beginning. If the giga ohm seal is formed instantly, wait a few seconds, back up your electrode gently to confirm that your electrode is sealed on the cell. If the seal is on your cell the electrode should gently pull the cell membrane One more time check the monitor, a flat line on the monitor shows that you have reached the Giga ohm seal (Figure 4). Change the camping potential to keep the membrane potential at -60mV and apply a gentle suction. The suction has to be very brief and not too strong. If the initial seal is good the electrode should break in the cell which can be visualized by increase in the capacitance transients and appearance of sub-threshold activity such as EPSCs and IPSCs. If you couldn't break in the cell in the first suction, gently increase the suction intensity until you break in the cell. If you cannot break in the cell by suction, this could have several reasons

1) Your initial seal was bad
2) Your seal was god but you sealed on a debris outside the cell
3) Your electrode is too small (too high resistance) to break in
4) Your prep was not clean enough
5) Your setup had a flaw
    a. Check the bath electrode
    b. Check the connectivity

If you cannot find a solution it is best always to clean the prep first and retry

Good luck

Acknowledgement:

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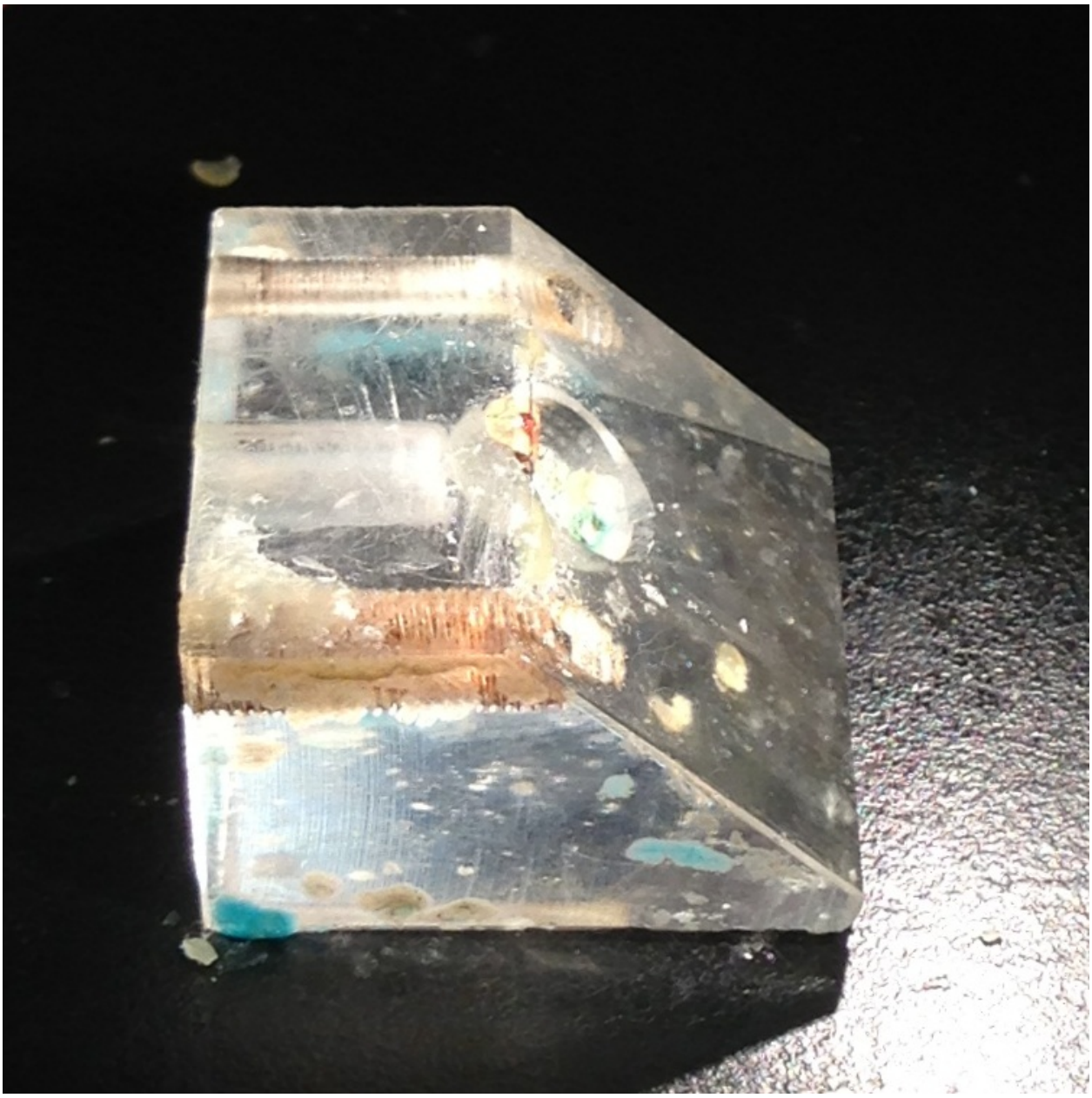

Figure 1

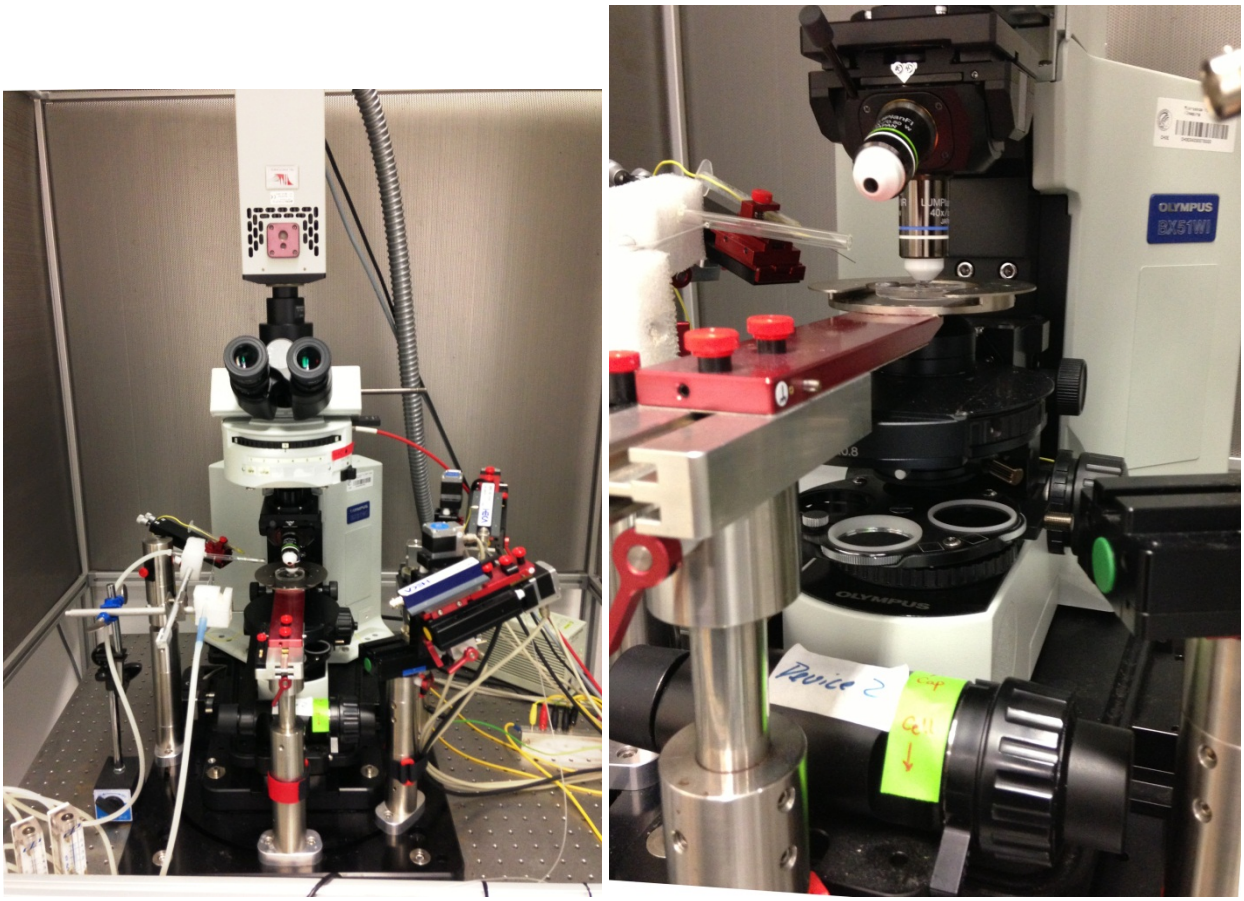

Figure 2

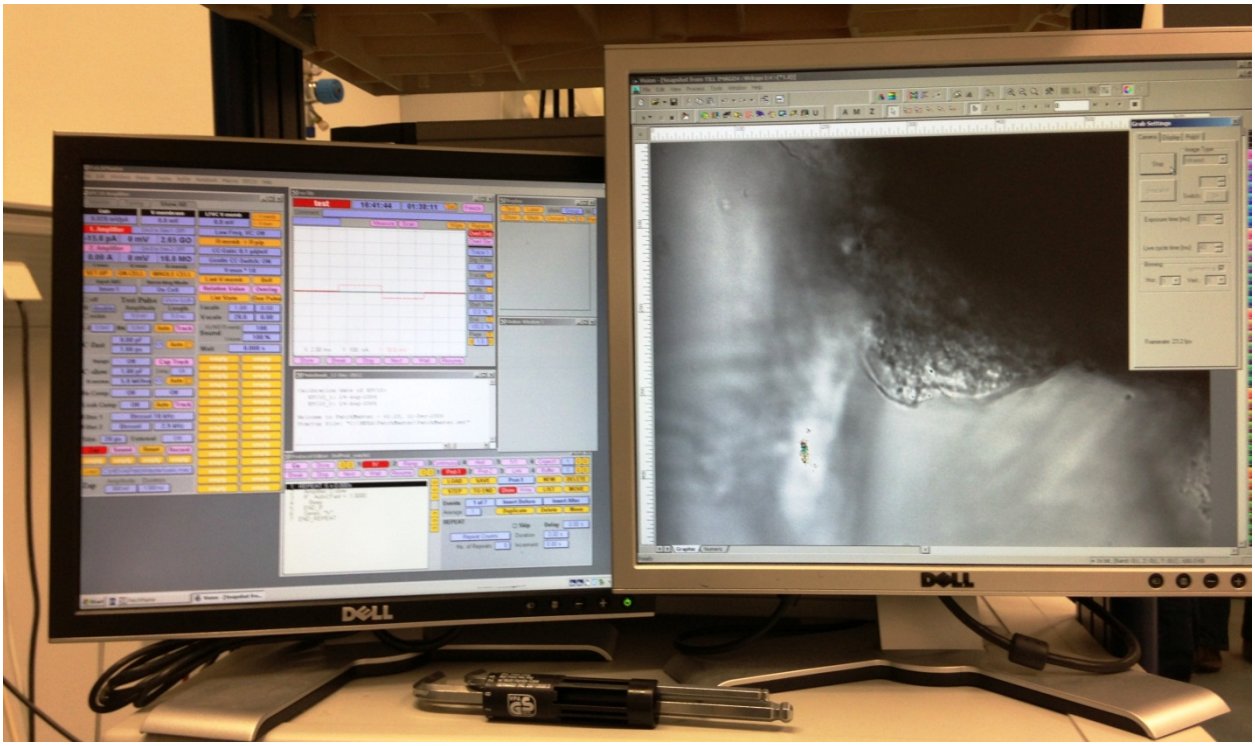

Figure 3

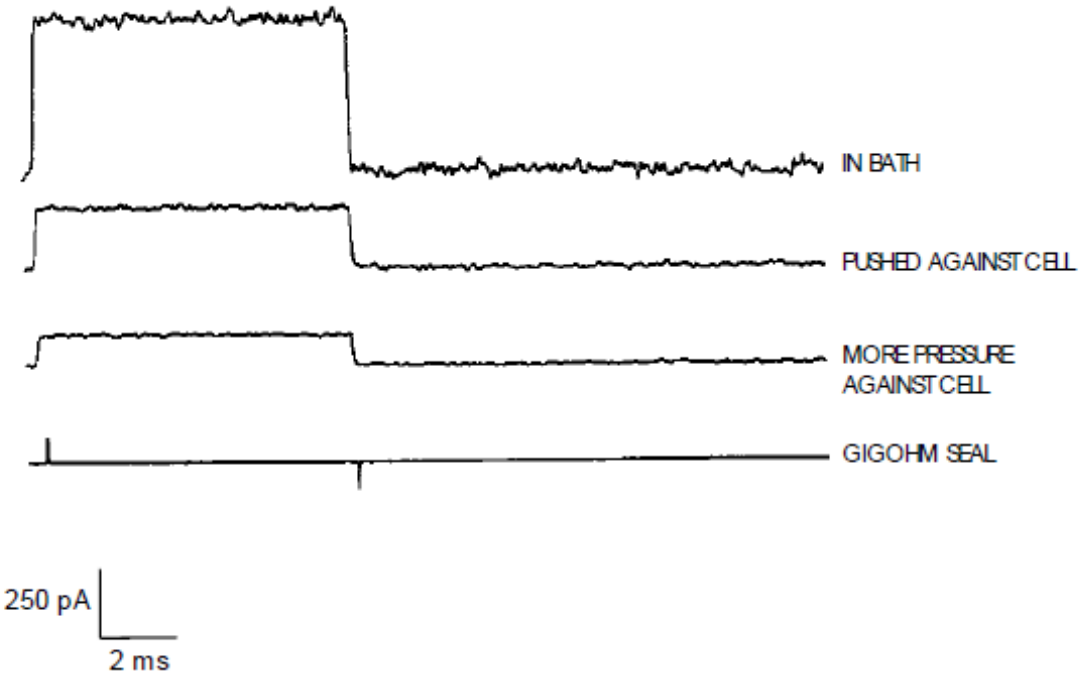
IN BATH
PUSHED AGAINST CELL
MORE PRESSURE
AGAINST CELL
GIGOHM SEAL
250 pA
2 ms

Figure 4