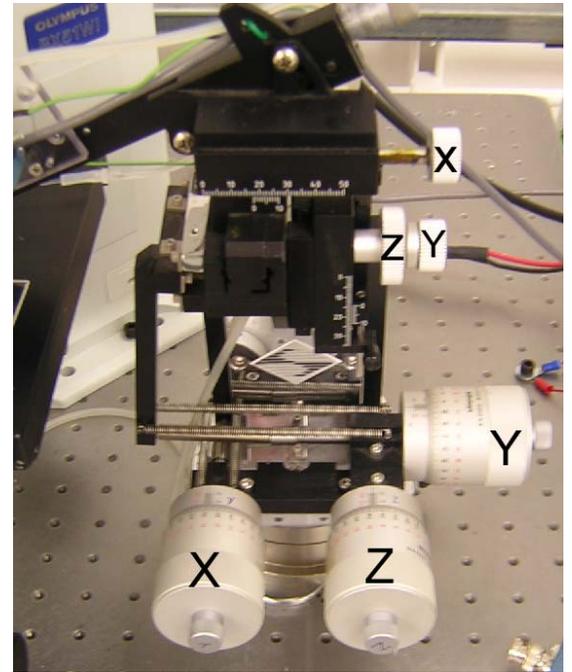
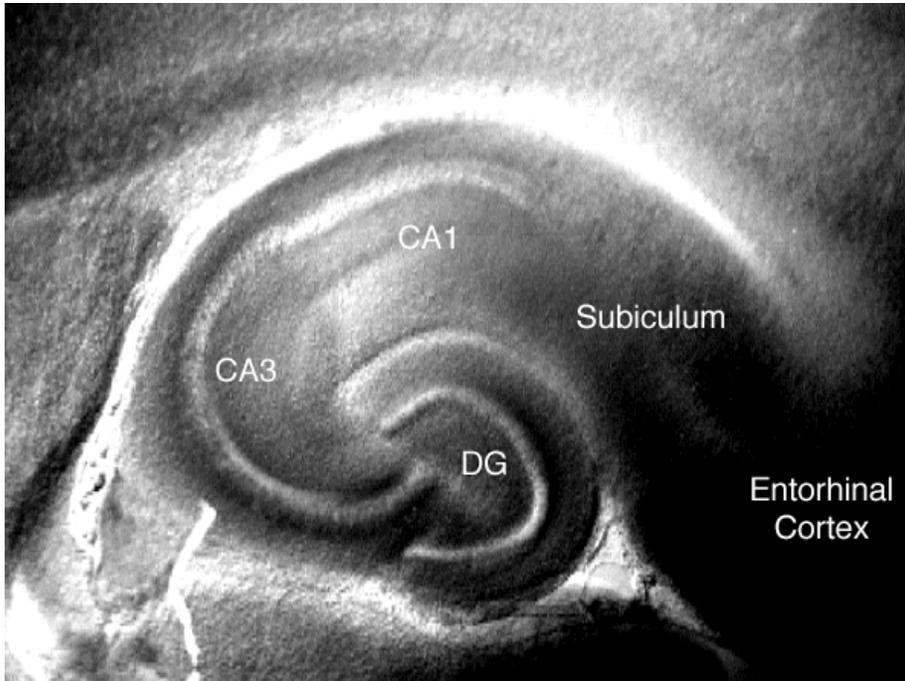


# Patch Clamp Guide



## Patch-clamp Guide: Step by step

1. Turn on the power supply to the light source, TV monitor, and amplifier
2. Insert the gas diffuser into your flask of ACSF. Begin bubbling the ACSF with oxygen.
3. Place your chamber onto the microscope stage, and tack the chamber down with tacky wax. Place the inflow and outflow in the chamber and start the flow of ACSF.
4. Allow ACSF to flow through the chamber for 5 minutes to equilibrate the perfusion pathway.
5. Stop the flow of ACSF, then remove the recording chamber and get a brain slice from your instructor or TA.
6. Return the chamber to the microscope stage, tack it down, and immediately resume the flow of ACSF.
7. Center the slice over the light source. Find the hippocampus under 10x magnification. (Refer to the labeled hippocampus figure). Center the pyramidal cell layer (it appears as translucent stripe) of the CA1 region in the field of view.
8. Move to 40x magnification. Find the cell bodies of neurons in or near the pyramidal cell layer (see hippocampal closeup figure). Select a healthy-looking cell to patch. Be careful not to move the microscope stage once you have located your target cell!
9. Fill a patch pipette, insert it into the holder, and put on positive pressure (about 0.6 psi).
10. Raise the 40x objective, and find the pipette tip at 40x magnification. You should see signs of fluid flowing out of the tip. If not, replace the pipette.

11. Amplifier initialization. Make sure the Capacitance Compensation, Bridge Balance, and Holding Voltage are set to 0 by rotating them fully counter-clockwise.

12. Amplifier check-out. Make sure the amplifier is in voltage-clamp mode

- a. Move the “mode” switch to “IClamp “
- b. Switch the “Source” switch next to the monitor to “Vm”
- c. Adjust the offset dial until the meter reads 0.00 mV.
- d. Move the “mode” switch to “Vclamp” make sure the meter still reads 0 mV.
- b. External Command Destination switch to “Vclamp”.

13. Make sure that the voltage clamp checkbox is checked in DataPro. Now start the test pulse in Data Pro. It should indicate a resistance of 5-7 MΩ

14. Move the pipette tip down toward the cell, first by using the coarse controls, then switch to the fine controls when you are near the slice surface.

15. Establish a giga-ohm seal. Form a “dimple” on the neuron, pushing the pipette down, and then to the left slightly, so that your pipette ends up on the center of the neuron. Release positive pressure quickly once you see the dimple, and monitor the seal test waveform on the computer screen.

16. Slowly increase suction to seal the membrane to the pipette (usually -0.3 psi is enough). Seal formation will be apparent as the current change from a 1 mV step pulse decreases to near 0 pA, leaving on the capacitive transients. Release suction.

17. Set the holding potential using the “Holding Voltage” to be about -65 mV. Flip the “Holding Voltage” switch to “NEG.” and turn the “Holding Voltage” dial until the meter reads -65 mV This improves seal quality, and anticipates the next step.

18. Increase the test pulse to 10 mV. Restart the test pulse

19. Establish a whole-cell intracellular recording (“break-in”). Begin applying suction to the pipette then slowly increase suction, watching the capacitive transients on the computer test pulse display. “Break-in” will be apparent as a sudden increase in the duration of the capacitive transients. Release suction immediately. Stop the test pulse.

20. Switch into current clamp mode.

- a. Move the “Mode” switch to “IClamp”.
- b. Change the “External Command Destination” switch to “IClamp”
- c. In DataPro, make sure the Current Clamp checkbox is checked.

Start your Experiment!