

Video Article

Methods for Patch Clamp Capacitance Recordings from the Calyx

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Abstract

We demonstrate the basic techniques for presynaptic patch clamp recording at the calyx of Held, a mammalian central nervous system nerve terminal. Electrical recordings from the presynaptic terminal allow the measurement of action potentials, calcium channel currents, vesicle fusion (exocytosis) and subsequent membrane uptake (endocytosis). The fusion of vesicles containing neurotransmitter causes the vesicle membrane to be added to the cell membrane of the calyx. This increase in the amount of cell membrane is measured as an increase in capacitance. The subsequent reduction in capacitance indicates endocytosis, the process of membrane uptake or removal from the calyx membrane. Endocytosis, is necessary to maintain the structure of the calyx and it is also necessary to form vesicles that will be filled with neurotransmitter for future exocytosis events. Capacitance recordings at the calyx of Held have made it possible to directly and rapidly measure vesicular release and subsequent endocytosis in a mammalian CNS nerve terminal. In addition, the corresponding postsynaptic activity can be simultaneously measured by using paired recordings. Thus a complete picture of the presynaptic and postsynaptic electrical activity at a central nervous system synapse is achievable using this preparation. Here, the methods for slice preparation, morphological features for identification of calyces of Held, basic patch clamping techniques, and examples of capacitance recordings to measure exocytosis and endocytosis are presented.

Video Link

The video component of this article can be found at <https://www.jove.com/video/244/>

Protocol

Patch Clamp Setup:

Optics: The optics are very important to find a good cell, and to establish the position of it.

Try to get a clear image of the cells: sharp edges, soft-looking cell.

Scan the whole field to look for good MNTB principal cells with attached calyx. Rotate dish to look for calyces from different angles. At first, it is OK to practice on balloon-shaped terminal.

Pick cell at surface of slice, or in the 2nd cell layer. Target the first 1/3rd of cell with pipette

Identifying Calyces: Look for a double membrane (see Figure 1), and rotate the slice dish to see it from different angles to help confirm that it is a calyx.

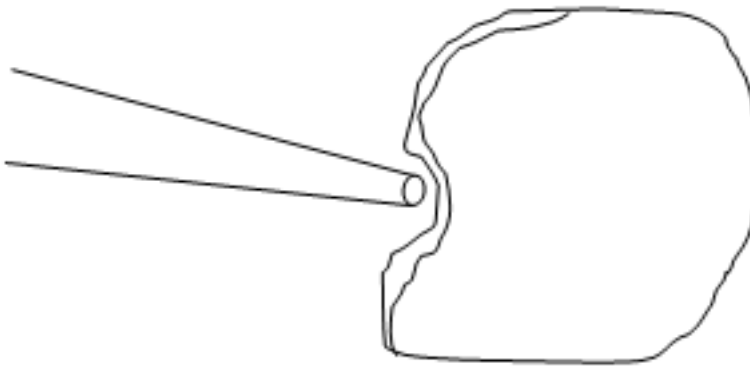


Figure 1

Depth of Calyx: Calyx should be on the surface or as close to the surface as possible.

Surface available for patching: Use fine-focus knob on microscope to determine how "wide/deep" the calyx is. For example, ~10% of a full turn on the fine focus will give you more than enough surface area to patch onto.

Sealing onto a presynaptic cell: To get to **cell attached**, look for a "small" deformation on the cell surface due to pos pressure (~0.15psi) in the pipette, use manipulator to run pipette up, down and across, and even into the surface of the presyn terminal to get a good deformation, then release pressure and apply suction, if and as necessary.

PULSE, data acquisition program:

Need to increase buffer size before first use, and whenever required by sampling capacity.

Need to load modified macros each time.

Liquid Junction Potential: Presyn Recording Solution is -11mV (negative 11mV).

Presyn: Cslow~15 pF

Rs comp for presyn: 10 μ sec, 65 % (more compensation may possible w/out oscillations)

Before recording capacitance: Test current relaxation (10 mV, 10 msec jump) to see whether relaxation is monoexponential, i.e., whether presyn term can be modelled by a single compartment.

Filters: Set at 30 kHz and 10 kHz (Bessel filter 1 and 2 respectively) during relaxation measurement, to make sure a fast component isn't missed out on.

During mini recordings, Xinsheng used 10 kHz and 5kHz. LGW asked me to use only filter 1 set at 30 kHz to make sure we can measure rise-time of mini.

Files naming: 7 digits 000mmxx, where mm is month (or month and year) and xx is cell number.

Cleaning Solution Lines: Lines cleaned at end of experiment with 20 ml of 0.1 M HCl followed by 200 ml ddH₂O.

External Solutions are recycled, pump speed is 20-30 rpm.

(solution returning from bath chamber flows back into the graduated cylinder).

Set up 3 outgoing lines to ensure sufficient suction.

Pipette Presynaptic Recording Solution: ~310 milli Osmolar

Presyn Solution needs to be ~310 mOsm to prevent R-access increase during experiment.

Presyn pipette solution is different from postsyn pipette solution (Presyn has ATP & GTP for ex.).

TTX-TEA Solution (to isolate Ca currents):

- Try to apply after sealing, or even patching, onto the cell, as TEA in solution blocks K-channels and depolarizes the cell. This is not good for the cell. For the same reason, it might not be possible to reuse the slice after TTX is applied.
- Compare the first and last response of the cell, or slice to check for any effect of prolonged depolarization.

- TTX is used at 1 μ M instead of 0.5 μ M so the effect is faster. Need to wait less time after changing solutions.

Cutting Slices

Vibratome settings:

- 70Hz lateral vibrations
- Amplitude: 1.0 mm
- Forward speed 0.01-0.02 mm/sec while cutting slices with calyx, 0.1 otherwise thickness: 180 μ m (~150 μ m in older animals)

Slices incubate at 37°C for 1 hour (includes cutting time), i.e., leave in bath 15-20 minutes after cutting.

Pipettes:

Presyn: 3.5-4.5 M Ω at first, 3.0-3.5 M Ω when feel more confident

Use puller manual to set settings. Use delay feature because glass is thick (delays of 200 or 1 work)

Glass type: Borosilicate, standard wall, no filament

Outer Diameter: 2.0 mm

Inner Diameter 1.16 mm

Length: 7.5 cm