



ULTRATHIN CNS SLICES: ENHANCED VISUALIZATION OF NEURON STRUCTURE FOR ELECTROPHYSIOLOGY AND IMAGING. L. Song, M.

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Electrophysiological studies using patch clamp recordings in CNS slice preparations involve either 'blind' or visual targeting strategies. Recordings from visually-identified neurons generally require specialized upright microscopes equipped with Nomarski optics (DIC) (e.g. Konnerth et al Pflugers Arch. 414:600, 1989). Further image optimization to observe neuronal processes employ video-enhanced infrared wavelength illumination (e.g. Stuart et al Pflugers Arch. 423:511, 1993). Though powerful, these approaches require specialized, expensive equipment.

Since the ability to resolve detailed cellular features is generally limited to 40-50 μ m from the slice surface, we have developed an alternate strategy to visualize neurons using a semi-transparent 'ultrathin' slice preparation (20-50 μ m) visualized on an inverted microscope equipped with Hoffman modulation optics. Isolated cerebellum, hippocampus or spinal cord from neonatal rats (P1- P14) were embedded in AGAR (2.5% w/v) then sectioned with a Leica VT1000E vibrating blade microtome. After incubation at 32°C, slices were fixed to the bottom of the recording chamber and maintained at room temperature. The enhanced transparency due to reduced slice thickness permitted superior optical resolution of dendritic and axonal processes and cells were visible throughout the slice thickness. Live/dead cell staining revealed that many cells remained viable for imaging and electrophysiological experimentation. In all CNS regions examined, neurons were easily identified for successful patch recordings. While not yet tested, use of an inverted microscope should permit high numerical aperture oil-immersion objectives to be used for simultaneous imaging experiments and the reduced slice thickness would hasten drug equilibration and washout times. *Supported by the Canadian Neuroscience Network.*

Slice preparations are used to elucidate synaptic and cellular properties of CNS neurons in vitro.

In order to undertake studies which identify particular cell types or discern between distinct spatial regions within individual cells, procedures which enhance optical resolution are required.

Edwards et al (Pflügers Arch 1989) introduced technical procedures to allow for patch recordings from visually-identified neurons in 'thin' tissue sections (100-140 μ m). This approach generally requires specialized upright microscopes equipped with Nomarski optics (DIC) and long working-distance objectives (to permit pipette placement).

Demands for increased cellular resolution in living slices has lead to advances in optical imaging which includes video-enhanced infrared wavelength illumination (Dodt Adv Exp Med Biol 1993; Stuart et al Pflügers Arch 1993).

These techniques have been developed, in part, to circumvent limitations in slice thickness provided by existing methods for tissue sectioning.

We have chosen an alternate strategy for cell visualization in slice. Using the Leica VT1000S oscillating blade microtome, we have prepared 'ultrathin' sections of living CNS tissue (20 - 60 μ m) with superior cellular visualization and retained in situ organization. Procedures for slice placement in an experimental recording chamber are described. Photography and electrophysiology are undertaken using an inverted microscope equipped with Hoffman modulation optics.

The spinal cord, cerebellum or hippocampus of 1-21 day old rats were isolated in cold oxygenated (~40C) ACSF1.

Isolated tissues were embedded in agar (2.5% w/v), and sectioned into 20-60 (µm) slices using a Leica VT 1000E oscillating blade microtome.

In order to enhance viability during slicing, equiosmolar sucrose replaced NaCl and CaCl₂ was removed from ACSF solution² (Aghajanian & Rasmussen, Synapse 1989).

In order to affix slices to the coverslip bottom of the recording chamber, the sucrose-ACSF solution was drained from the dish until only a small drop remained covering the slice. Filter paper pieces cut into long wicks were used to remove additional solution so that only a meniscus was left covering the slice. A short period of time was allowed for the perimeter of the solution to evaporate creating a syrupy base for slice adherence. ACSF^{2,3} was then reintroduced into the chamber. This was achieved by gently applying drops of ACSF using a Pasteur pipette immediately over the tissue until the section was completely submerged in solution to prevent tissue lifting from hydrostatic forces.

Experimental solutions (in mM)

1. Standard ACSF

NaCl 125, KCl 2.5, MgCl₂ 1, NaH₂PO₄ 1.25, NaHCO₃ 26, D-glucose 25, CaCl₂ 2.

2. Modified ACSF

Sucrose 250, KCl 2.5, MgCl₂ 3, NaH₂PO₄ 1.25, NaHCO₃ 26, D-glucose 25.

3. HEPES-buffered extracellular solution

NaCl 150, KCl 5, MgCl₂ 1, CaCl₂ 2, HEPES 10, D-glucose 10

Histochemical procedures:

Slice Viability

1. Slices were incubated in 0.004-0.04% fluorescein diacetate (live cell assay) and 0.003% propidium iodide (dead cell assay) for 10 minutes in pre-oxygenated ACSF. (see Jones & Senft, J Histochemistry & Cytochemistry 33(1) 77, 1985)
2. Tissues were then washed 3 x 5 minutes in ACSF and photographed.

Immunohistochemistry for slice culture:

1. Cultured hippocampal slices were fixed for 2 hours in 4% paraformaldehyde with 0.1 M phosphate buffer, pH 7.4 at room temperature (RT).
2. All subsequent washes and incubations were performed in 0.1 M phosphate buffered saline containing 0.3% Triton X-100 (PBS-T).
3. Tissue was incubated in anti-panaxonal (Sternberger Monoclonals) 1:1000, anti-MAP 2 (Sternberger Monoclonals) 1:1000, and anti-GFAP (Chemicon) 1:1000 for 48 hours at 40C.
4. Tissue was washed 3 x 10 minutes at RT, then incubated for 1.5 hours at RT in Cy3 conjugated sheep anti-mouse (Sigma) and FITC conjugated horse anti-rabbit (Sigma), both diluted 1:100.

5. Tissue was washed for 20 minutes in PBS-T then 20 minutes in 50 mM Tris-HCl pH 7.4, dried and coverslipped using Aquamount (BDH).

Imaging - due to enhanced optical resolution

- * Tissue sectioned at 20 mm can provides a monolayer of living cells with in situ organization.
- Use of an inverted microscope permits imaging experiments to be performed with high numerical aperture immersion objectives without concern for working distance.

Electrophysiology

- The reduced section thickness permits visual patch clamp recordings using an inverted microscope and hence a greater ease and flexibility in the placement of experimental equipment (electrodes and local perfusion systems).

Organotypic Culture

- * Because sections are at near monolayer thickness, limited tissue topographical distortion occurs due to slice flattening.