Protocol

Preferential Loading of Bergmann Glia with Synthetic Acetoxyethyl Calcium Dyes
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The cerebellar cortex contains two astrocyte types: the Bergmann glia of the molecular layer and the velate protoplasmic astrocytes of the granule cell layer. In vivo, these cell types generate both subcellular calcium transients and trans-glial calcium waves. This protocol outlines a method for in vivo calcium imaging in cerebellar astrocytes of mice which have undergone a cerebellar craniotomy. Multicell bolus loading (MCBL) is performed using the synthetic calcium indicators Fluo-5F AM and Fluo-4 AM. In the cerebellum, a degree of cell-type specificity can be achieved by varying the depth of injection. This protocol describes a loading procedure following craniotomy which allows preferential labeling of Bergmann glia.

RELATED INFORMATION

Figure 1 illustrates glial cells of the cerebellar cortex: Bergmann glia (Fig. 1A–E) of the molecular layer and velate protoplasmic astrocytes (Fig. 1G) of the granule cell layer. Protocols are also available for Injection of Recombinant Adenovirus for Delivery of Genetically Encoded Calcium Indicators into Astrocytes of the Cerebellar Cortex (Kuhn et al. 2011a) and Cerebellar Craniotomy for In Vivo Calcium Imaging of Astrocytes (Kuhn et al. 2011b).

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution’s Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPE: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose, 1.5% in saline (Type III-A, Sigma-Aldrich)

Heat the agarose until it is completely dissolved, and keep in a 45°C water bath.

Calcium indicators: Fluo-5F AM (50 µg, Invitrogen) or Fluo-4 AM (50 µg, Invitrogen)

Dimethylsulfoxide (DMSO), desiccated by molecular sieves (3-Å pore size)

Mice or other experimental animal, prepared surgically as per Cerebellar Craniotomy for In Vivo Calcium Imaging of Astrocytes (Kuhn et al. 2011b)

All procedures must be approved by the local institutional animal care and use committee.


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Pluronic F-127 (Invitrogen or BASF)
Saline (0.9% NaCl in H2O) or HEPES-buffered artificial cerebrospinal fluid without CaCl2 <R>

Equipment

- Borosilicate glass tubing for pipettes
- Coverslips, glass #1 (~5 × 5-mm)
- Electrode beveler (Sutter Instruments or World Precision Instruments [WPI])
- Electrode puller suitable for preparing patch pipettes (Sutter Instruments or WPI)
- Microloader (Eppendorf)
- Micromanipulator with a position counter, an electrode holder with tubing, syringe, and pressure gauge on the two-photon microscope system
- Two-photon microscope
- Ultrafiltration device, 0.22-µm pore size (e.g., Ultrafree-MC, Millipore)
- Vortex mixer

METHOD

1. For the preparation of the dye stock solution, add 5 µL of 20% Pluronic F-127 freshly dissolved in H2O-free DMSO to 50 µg of Fluo-5F AM or Fluo-4 AM. Vortex, and then add 80 µL of saline. Vortex, and filter the dye stock solution through a low-volume 0.22-µm filter.

   We find that Fluo-5F AM as well as Fluo-4 AM generally allow for measurements with better signal to noise in astrocytes than does OGB-1 AM. Fluo-5F (KD ~ 1 µm) (Sarkisov and Wang 2008) is especially well suited for measuring calcium signals of the magnitude produced in transglial waves.
2. Pull a patch pipette, and backfill it with the dye solution using a microloader. Bevel the pipette at an angle of 20° to a resistance of 3–4 MΩ.

3. Mount the beveled pipette in the pipette holder on the micromanipulator, and apply 0.5 psi (0.035 bar) positive pressure to the pipette before entering the saline over the craniotomy.

4. Approach the dura under visual control by entering the saline over the craniotomy, and zero the manipulator when the brain surface is reached. Check under fluorescence viewing that the dye is coming out of the pipette.

5. Advance the pipette into the surface of the brain. After passing the dura, reduce the pressure to 0.3 psi (0.02 bar), and advance to a depth of \(\approx 50 \mu m\) below the dura. Verify that dye is ejected from the pipette by visualizing its fluorescence. To label Bergmann glia, eject the dye solution at \(\approx 2\) psi (0.14 bar). A similar injection into the granule cell layer (in mice >140 µm below pia mater) will result in relatively increased labeling of Purkinje cells. Apply pressure for 5 min.

6. Reduce the pressure to 0.1 psi (0.007 bar), and slowly retract the electrode.

7. Add 1.5% agarose at 45°C over the craniotomy, and seal it with a coverslip while the agarose is still liquid.

8. Wait for \(\approx 30\) min for dye uptake.


**DISCUSSION**

MCBL, which is reliable and quick, preferentially labels Bergmann glia if the dye is injected into the superficial molecular layer (Fig. 1E). When dye is injected into the granule cell layer, we have been able to simultaneously observe activity in Bergmann glia, interneurons, and Purkinje cells (Sullivan et al. 2005). The disadvantage of bolus loading is low contrast so that fine processes in the neuropil cannot be resolved; three-dimensional (3D) reconstructions of Bergmann glia are blurry, and signals from small structures cannot be assigned to particular cell types. To overcome the problem of nonspecific labeling, we use attenuated nonreplicating adenovirus to express the fluorescent calcium indicator protein (FCIP) G-CaMP2 in Bergmann glia and velate protoplasmic astrocytes, as described in Injection of Recombinant Adenovirus for Delivery of Genetically Encoded Calcium Indicators into Astrocytes of the Cerebellar Cortex (Kuhn et al. 2011a).

**RECIPE**

*Artificial cerebrospinal fluid, HEPES-buffered*

135 mM NaCl  
5.4 mM KCl  
5 mM Na-HEPES buffer  
1.8 mM CaCl₂  
1 mM MgCl₂  
Adjust to pH 7.3 with HCl.

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REFERENCES
