Video Article

In Vivo 2-Photon Calcium Imaging in Layer 2/3 of Mice

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Abstract

To understand network dynamics of microcircuits in the neocortex, it is essential to simultaneously record the activity of a large number of neurons. In-vivo two-photon calcium imaging is the only method that allows one to record the activity of a dense neuronal population with single-cell resolution. The method consists in implanting a cranial imaging window, injecting a fluorescent calcium indicator dye that can be taken up by large numbers of neurons and finally recording the activity of neurons with time lapse calcium imaging using an in-vivo two photon microscope. Co-injection of astrocyte-specific dyes allows one to differentiate neurons from astrocytes. The technique can be performed in mice expressing fluorescent molecules in specific subpopulations of neurons to better understand the network interactions of different groups of cells.

Protocol

1. Prepare the fluorescent calcium indicator solution. We use acetoxymethyl ester dyes, or AM dyes for short. These are dyes that can be taken up by cells when injected extracellularly. We typically use Oregon-Green BAPTA-1 AM or Fluo-4 AM at a concentration of 1 mM. AM dyes can be obtained from Invitrogen in 50 microgram vials. We first prepare a 20% solution of Pluronic F-127 in DMSO. We warm the solution prior to use every day until its clear. We dissolve the calcium indicator the 20% Pluronic F-127/DMSO for 15-20 minutes to a concentration of 10 mM. We then dilute the solution to 1mM in a solution containing, in mM: 150 NaCl, 2.5 KCl, 10 Hepes, pH 7.4. In addition, 100 μ M Sulforhodamine 101 is usually included to label astrocytes and visualize the pipette during the injection. We filter the solution prior to injection with a 0.49 micron centrifugal filter (Stosiek et al., 2003; Garaschuk et al., 2006).
2. Implant a cranial window over the area to be imaged. We have [described the general approach in JoVE](#), but the following modifications are important for calcium dye injection:
Make a smaller craniotomy of 2 mm in diameter over the cortical area of interest, taking extreme care not to damage the underlying dura. Secure a coverslip in place over the craniotomy, but only partially cover the craniotomy, leaving a small gap between the edge of the craniotomy and the glass coverslip to allow room for a pipette to enter the cortex obliquely. Prior to injection, make a shallow well with dental cement around the craniotomy. The well should extend as rostral in direction and be shallow enough to allow insertion of the micropipette for injection.
3. Transfer the mouse transferred to the stage of the microscope and immobilize the head, as shown in our [video on blood flow imaging](#).
4. Pull glass microelectrode pipettes to a tip diameter yielding 2-4 Mega Ohms in resistance, using a pipette puller. Load the calcium indicator dye mix onto the glass pipette using a microsyringe. Using a micromanipulator, gently lower the micropipette onto the surface of the brain using a 4X objective, which is then finely positioned under a 40X objective. Choose a suitable location for injection, such that there are few or no overlying blood vessels to obscure the imaging. Using two-photon microscopy, the tip of the pipette is visualized and advanced slowly into the cortex, to a depth of 200 micrometers below the dura. Then, pressure inject the dye mixture at 10 PSI for 1 minute using a Picospritzer. We usually deliver two to four injections of the AM calcium dye mix, separated in space by approximately 200-300 microns. This bulk loading approach of slightly overlapping injections ensures that an area as large as 600 x 600 microns is adequately stained for calcium imaging. Begin imaging 1 hour after the injection of the AM dye mixture, to allow for the dye to diffuse and be taken up by neurons.
5. Perform in-vivo two-photon imaging with a custom made two-photon microscope using a Chameleon Ti-Sapphire Laser (tuned to 800-880 nm) and Cambridge Technology galvanometer mirrors. We acquire images using ScanImage Software developed in Karel Svoboda's laboratory (Pologruto et al., 2003). Laser Power is typically well below 70 mW at the sample. Whole field images are acquired either using a 20X (0.95 NA) or 40X (0.8 NA) Olympus objectives, at 1.95-15.63 frames per second (512 x 256 pixels to 256 x 64 pixels). Line scans are acquired at 500 Hz. During imaging, animals are kept under light isoflurane anesthesia (0.6-0.9%), and are also kept at 37°C using a Harvard Apparatus Temperature Control Device. Care is taken to keep the respiratory rate of animals as close to 100 breaths/minute as possible. This level of anesthesia use is the lowest level that immobilizes the animal, but still permits spontaneous activity to be recorded.

Discussion

The advantage of this method over electrophysiological recordings is that it is less invasive and allows the recordings of activity in dense neuronal neuronal networks. When doing this procedure it's important to remember to take extreme care when performing the craniotomy not to damage the dura. Even a small amount of subdural bleeding with greatly obscure the imaging.

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References

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