Video Article

A Method for 2-Photon Imaging of Blood Flow in the Neocortex through a Cranial Window

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Abstract

The ability to image the cerebral vasculature (from large vessels to capillaries) and record blood flow dynamics in the intact brain of living rodents is a powerful technique. Using in vivo 2-photon microscopy through a cranial window it is possible to image fluorescent dyes injected intravenously. This permits one to image the cortical vasculature and also to obtain measurements of blood flow. This technique was originally developed by David Kleinfeld and Winfried Denk. The method can be used to study blood flow dynamics during or after cerebral ischemia, in neurodegenerative disorders, in brain tumors, or in normal brain physiology. For example, it has been used to study how stroke causes shifts in blood flow direction and changes in red blood cell velocity or flux in and around the infarct. Here we demonstrate how to use 2-photon microscopy to image blood flow dynamics in the neocortex of living mice using fluorescent dyes injected into the tail vein.

Protocol

Tail vein injections of fluorescent dextrans dyes:

1. In order to image cerebral vasculature, animals must be injected intravenously with a fluorescent dye. We use dextran-conjugated dyes because the dextran moiety prevents the dye from crossing the brain blood barrier and leaking out of the blood vessels.
2. Mice are anesthetized with isoflurane (4% for induction, 1.5-2% during the injection).
3. Tail is disinfected with 70% alcohol.
4. With a 26 gauge needle, 75-100 µl of a 5% v/v solution of rhodamine dextran dissolved in saline is injected through the tail vein, midway along the shaft of the tail.
5. Animals may be imaged immediately after tail vein injection until the dye is excreted in urine, which will turn pink if using a rhodamine dye in around 2 hours.

Imaging of blood flow using two-photon microscopy (total duration 30-60 min per session, depending on the number of vessels imaged):

1. Following the fluorescent dextran dye injection, the mouse is anesthetized with isoflurane (4% for induction, 1-1.5% for imaging).
2. Mouse is firmly attached using the titanium bar to the microscope stage, which contains a thermo-regulated heating pad to keep the animal warm. Some eye ointment is applied to keep the eyes moist.
3. The cover glass of the cranial window is cleaned with 70% alcohol.
4. The window is positioned parallel to the focal plane and centered in the field of view under the 4X objective.
5. It is best to take a photograph of the brain surface vessels with a digital camera. This picture will be used as the image of reference in following imaging sessions to find the imaged region repeatedly from day to day.
6. The 4X objective is replaced with the water immersion 40X objective without moving the stage. A digital picture of the field of view is taken again. The coordinates at the stage manipulator are set at zero.
7. We use ScanImage as the image acquisition software. This was written in MatLab by Tom Pologruto and Bernardo Sabatini in the laboratory of Karel Svoboda (Pologruto et al., 2003). We next turn on all the other equipment: the laser, the power meter, the photo multipliers tubes, the amplifiers, etc.
8. The area of the window suitable to be imaged is briefly scanned at low magnification to find the best regions. Once these are identified, a low magnification stack of the chosen region to image is taken, and its XY coordinates are annotated.
9. To record blood flow dynamics in small vessels or capillaries we use line scans along at least 40 µm of the vessel of interest. These single "sweeps" lasting one second are done using as little laser power as possible and the coordinates and angle of scanning are written down.
10. Once the imaging session is over, the animal is moved to a warm chamber where it can recover from the anesthesia.

Discussion

Cortical blood flow dynamics can be studied in vivo by imaging fluorescent dextran dyes injected into the tail vein of rodents with two-photon microscopy. This video showed a method for how to image blood flow dynamics in neocortex of mice through a glass-covered cranial window preparation.

References

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