Video Article

# A Craniotomy Surgery Procedure for Chronic Brain Imaging

Ricardo Mostany[1], Carlos Portera-Cailliau[1]

[1]Department of Neurology, University of California, Los Angeles

URL: https://www.jove.com/video/680
DOI: doi:10.3791/680

Keywords: Neuroscience, Issue 12, glass, cranial window, two-photon, 2-photon, cortex, dendrite, axon, green fluorescent protein, svoboda, cell

Date Published: 2/15/2008

Citation: Mostany, R., Portera-Cailliau, C. A Craniotomy Surgery Procedure for Chronic Brain Imaging. *J. Vis. Exp.* (12), e680, doi:10.3791/680 (2008).

## Abstract

Imaging techniques are becoming increasingly important in the study brain function. Among them, two-photon laser scanning microscopy has emerged as an extremely useful method, because it allows the study of the live intact brain. With appropriate preparations, this technique allows the observation of the same cortical area chronically, from minutes to months. In this video, we show a preparation for chronic in vivo imaging of the brain using two-photon microscopy. This technique was initially pioneered by Dr. Karel Svoboda, who is now a Howard Hughes Medical Institute Investigator at Janelia Farm. Preparations like the one shown here can be used for imaging of neocortical structure (e.g., dendritic and axonal dynamics), to record neuronal activity using calcium-sensitive dyes, to image cortical blood flow dynamics, or for intrinsic optical imaging studies. Deep imaging of the neocortex is possible with optimal cranial window surgeries. Operating under the most sterile conditions possible to avoid infections, together with using extreme care to do not damage the dura mater during the surgery, will result in successful and long-lasting glass-covered cranial windows.

## Video Link

The video component of this article can be found at https://www.jove.com/video/680/

## Protocol

1. Anesthetize mice with isoflurane (4% for induction, 1.5-2% for surgery) using IACUC approved procedures. It is important that tail and/or toe pinches are used in order to ensure the animal is fully sedated.
2. Using a rodent trimmer, shave the hair from the back of the neck up to the eyes.
3. Place the mouse in a stereotaxic frame, over a surgery water re-circulating blanket. Firmly secure the head with ear bars.
4. Apply eye ointment, in order to prevent the animal's eye from drying out.
5. Administer, subcutaneously, Dexamethasone (0.2 mg/Kg) and Carprofen (5 mg/Kg) to prevent swelling of the brain and/or an inflammatory response, respectively.
6. Before beginning the surgery, sterilize the operating area by wiping skin with three alternating swipes of 70% alcohol and betadine.
7. All surgical instruments have been pre-sterilized using a glass bead sterilizer. Using scissors that have been sterilized with ethanol, remove the skin over the top of the skull, starting with a horizontal cut all along the base of the head, followed by two cuts in the rostral direction, almost reaching the eyelids, then two oblique cuts that converge at the midline.
8. A drop of lidocaine + epinephrine solution is applied at this point onto the periosteum to avoid excessive bleeding or pain. With a scalpel, retract the periosteum to the edges of the skull. Also, lightly retract the musculature of the back of the neck.
9. Gently scrape the entire exposed area of the skull with the scalpel to create a dry surface. This is very important, as it will allow the glue to adhere better when applied later.
10. Once an imaging site has been chosen, one is ready to create the cranial window. First, gently "draw" a circle of about 4 mm in diameter with the pneumatic dental drill.
11. After a slight drilling, apply lidocaine + epinephrine solution again onto the skull surface. Stop the drilling when a very thin layer of bone is left. By pushing gently on the center of the craniotomy to feel how it gives way, one can usually know that this stage is reached.
12. Under a drop of saline and taking advantage of the bone trabeculae - the spongy structure of the bone - lift away the craniotomy from the skull with very thin tip forceps. The saline is important, as it will help lift up the skull and prevent bleeding of the dura.
13. Apply Gelfoam that has been previously soaked in saline to the dura mater to stop any small bleeding that occassionally occurs when the skull is removed.
14. After drying the dura mater surface and ensuring that there is no bleeding, gently lay a sterile 5 mm glass cover slip on top of the dura mater. (*Note*: other groups also place a drop of low melting point agarose (1.2%) over the dura and put the coverslip on top of the agar).
15. Apply a drop of cyanocrylate-based glue to the opposite hemisphere on the skull. With the help of a needle, gently apply the glue all around the window while being careful not to put it under the glass. Glue can now be applied in a thin layer over the entire surface of the skull.
16. Once the glue has dried, mix dental acrylic and apply it throughout the skull surface, covering also a small rim of the cover slip, to secure it.
17. After securing the cover slip, make a small well around the window with dental acrylic. Also, embed a titanium bar in the dental acrylic. This bar will later be used to attach the mouse securely on to the stage of the microscope for imaging. It is important to ensure that the bar is level, so that it is parallel with the cranial window. Placing a piece of paper under the bar can allow the bar to remain level while the acrylic hardens.
18. The dental acrylic is allowed to cure (harden) for 10 minutes, by which time the titanium bar is fixed in place. Place the animal in a warm cage until it recovers.

19. After recovery from anesthesia, the animal can be imaged on the same day.

## Discussion

As we have shown in the video and in the supplementary figures, the cranial window preparation, combined with the use of two-photon microscopy, is a very powerful tool to study in vivo the structure and function of the neocortex. The technique requires rigorous training to become familiar with the relevant anatomy and the fine surgical procedures and skills that this preparation requires. Only pristine surgeries can be used for chronic imaging. If the dura is manipulated excessively or punctured, the preparation should not be used for imaging.

## References

1. Svoboda K, Denk W, Kleinfeld D, Tank DW. In vivo dendritic calcium dynamics in neocortical pyramidal neurons. Nature. 85, 161-5 (1997)

2. Lendvai B, Stern EA, Chen B, Svoboda K. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature. 404, 876-81 (2000)

3. Trachtenberg JT, Chen B, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 420, 788-94 (2002)

4. Portera-Cailliau C, Weimer RM, DePaola V, Caroni P, Svoboda K. Diverse modes of axon elaboration in the developing neocortex. PLoS Biol. 3, e272 (2005)

5. Holtmaat A, L Wilbrecht, Knott GW, Welker E, Svoboda K. Experience-dependent and cell-type-specific spine growth in the neocortex.Nature.441,979-83 (2006)

 