

Video Article

A Craniotomy Surgery Procedure for Chronic Brain Imaging

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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.

Protocol

1. Mice are anesthetized 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, the hair from the back of the neck up to the eyes is shaved.
3. Following shaving, the mouse is then placed in a stereotaxic frame, over a surgery water re-circulating blanket, and the head is firmly secured with ear bars.
4. Eye ointment is then applied, in order to prevent the animal's eye from drying out. Next, Dexamethasone (0.2 mg/Kg) and Carprofen (5 mg/Kg) are administered subcutaneously, in order to prevent swelling of the brain and/or an inflammatory response, respectively.
5. Before beginning the surgery, sterilize the operating area by wiping skin with three alternating swipes of 70% alcohol and betadine.
6. All surgical instruments have been pre-sterilized using a glass bead sterilizer. Using scissors that has been sterilized with ethanol, the skin over the top of the skull is removed starting with a horizontal cut all along the base of the head followed by two cuts in the rostral direction, almost reaching the eyelids, and then two oblique cuts that converge at the midline.
7. At this point, a drop of lidocaine + epinephrine solution is applied onto the periosteum to avoid excessive bleeding or pain. With a scalpel, the periosteum is retracted to the edges of the skull. The musculature of the back of the neck is also lightly retracted.
8. The entire exposed area of the skull is gently scraped with the scalpel to create a dry surface. This is very important because it will allow the glue to adhere better when it is later applied.
9. Once an imaging site has been chosen, one is ready to create the cranial window. First, a circle of about 4 mm in diameter is "drawn" gently with the pneumatic dental drill.
10. After a slight drilling, lidocaine + epinephrine solution is applied again onto the skull surface. The drilling is stopped when a very thin layer of bone is left. Usually, one knows this stage is reached by pushing gently on the center of the craniotomy to feel how it gives way.
11. Under a drop of saline and taking advantage of the bone trabeculae - the spongy structure of the bone - the craniotomy is lifted away from the skull with very thin tip forceps. The saline is important because it will help lift up the skull and prevent bleeding of the dura.
12. Gelfoam that has been previously soaked in saline is applied to the dura mater, in order to stop any small bleeding that sometimes occurs when the skull is removed.
13. After drying the dura mater surface and ensuring that there is no bleeding, a sterile 5 mm glass cover slip is gently laid 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).
14. A drop of cyanoacrylate-based glue is applied to the opposite hemisphere on the skull. With the help of a needle the glue is applied gently all around the window with care not to put it under the glass. Glue can now be applied in a thin layer over the entire surface of the skull.
15. Once the glue has dried, dental acrylic is mixed and applied throughout the skull surface, covering also a small rim of the cover slip, to secure it.
16. After securing the cover slip, a small well is made around the window with dental acrylic. A titanium bar is also embedded 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.
17. The dental acrylic is allowed to cure for harden for 10 minutes at which time the titanium bar has been fixed in place. The animal is then placed in a warm cage until it recovers.
18. 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.

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