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A non-invasive head-holding device for chronic neural recordings in awake behaving monkeys

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HIGHLIGHTS

- We developed a completely non-invasive head-holding device for behaving monkeys.
- The device is made from a head mold that is customized for each monkey's head.
- Accurate monitoring of ocular movements was possible during use of the device.
- Stable chronic recording of neuronal activity during behavior was also possible.

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ABSTRACT

Background: We have developed a novel head-holding device for behaving non-human primates that affords stability suitable for reliable chronic electrophysiological recording experiments. The device is completely non-invasive, and thus avoids the risk of infection and other complications that can occur with the use of conventional, surgically implanted head-fixation devices.
New method: The device consists of a novel non-invasive head mold and bar clamp holder, and is customized to the shape of each monkey's head. The head-holding device that we introduce, combined with our recording system and reflection-based eye-tracking system, allows for chronic behavioral experiments and single-electrode or multi-electrode recording, as well as manipulation of brain activity.
Results and comparison with existing methods: With electrodes implanted chronically in multiple brain regions, we could record neural activity from cortical and subcortical structures with stability equal to that recorded with conventional head-post fixation. Consistent with the non-invasive nature of the device, we could record neural signals for more than two years with a single implant. Importantly, the monkeys were able to hold stable eye fixation positions while held by this device, demonstrating the possibility of analyzing eye movement data with only the gentle restraint imposed by the non-invasive head-holding device.
Conclusions: We show that the head-holding device introduced here can be extended to the head holding of smaller animals, and note that it could readily be adapted for magnetic resonance brain imaging over extended periods of time.

1. Introduction

Single-electrode and multi-electrode recordings have been successfully applied in conscious monkeys to explore neural substrates of behavior, providing valuable information about the functions of the brain. Most of these experiments have been performed in monkeys that were head-fixed, as most recording systems depend critically on adequate fixation of the animal's head so that electrical activity from single and multiple units can be recorded without noise artifact and with a constant relationship of the subject's head position to experimental stimuli and monitoring equipment. For this purpose, series of methods for head holding have been developed (Adams et al., 2007; Davis et al., 2009; Evarts, 1968; Isoda et al., 2005; Pigarev et al., 2009; Srihasam et al., 2010), most of which, following the pioneering work of Evarts, require head-restraint bolts to be implanted into the skull (Adams et al., 2007; Davis et al., 2009;

Abbreviation: MRI, magnetic resonance imaging.

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Evarts, 1968). These methods all allowed excellent stabilization of the subject's head, but with prolonged times following implantation, head-bolt systems become increasingly at risk for intracranial infection or necrosis and softening of bone around the head post, where the principal torques are applied during experimental sessions. This situation can lead to instability and ultimately failure of the implanted devices. In an attempt to solve such problems, less invasive methods without bolts screwed on the skull have been developed in some studies (Isoda et al., 2005; Pigarev et al., 2009). They achieved rigid head fixation, but even with those methods, small incisions of the skin are still necessary at the time of installation.

In this study, we developed a completely non-invasive head-holding device to prevent head movement during experimental sessions. The device is constructed with a head mold, bar clamp and metal brackets for fixation. The head mold is made with a flexible plastic that readily conforms to the contours of each monkey's head, and, as a result, the device achieves a strong but gentle securing of the head. A bar clamp holds the head mold on both sides and can be attached to a primate chair by metal brackets. This head-holding device has allowed us to record from the neocortex and deeper subcortical structures in the forebrain and midbrain both with conventional single-electrode recording methods (Evarts, 1968) and with a multi-electrode recording method developed in-house (Feingold et al., 2012) as monkeys perform tasks that require eye movements and ocular fixation.

Here we present the procedure for making the head-holding device, demonstrate the precision of eye-tracking possible with this system, and illustrate the reliability of both acute and chronic recordings of neuronal activity from the neocortex and from subcortical structures performed with the head-holding device as the sole source of head restraint. The completely non-invasive character of this device should help researchers to maintain non-human primates in a robustly healthy condition even with multiple electrodes chronically implanted in multiple sites in the brain.

2. Materials and methods

2.1. Design of the head-holding device

2.1.1. Description of the head-holding device

Each part of this head-holding device and its overall configuration are shown in Fig. 1. The device is made of five parts: (1) a bar clamp (21 cm long) with a left arm and an adjustable right arm with a large screw (Jorgensen #3908, Chicago, IL, labeled 1 in Fig. 1A), (2) a custom-made extender (2 in Fig. 1A), (3) a custom-made clamp attachment with screws (3 in Fig. 1A), (4) a holder for the extender (4 in Fig. 1A), and (5) three sheets of "Aquaplast" moldable plastic (Patterson Medical, Bolingbrook, IL, not shown). One sheet is about 23 cm × 36 cm, and the other two are about 15 cm × 20 cm.

Fig. 1B–F illustrates the assembled device. The tips of the left arm of the bar clamp and the large screw on the right arm of the bar clamp (1 in Fig. 1A) are covered by the smaller Aquaplast sheets (Fig. 1B–F). The covered left arm and right screw are connected to the left and right sides of the head mold, which was formed with the larger sheet of Aquaplast and touches the monkey's head (Fig. 1B–F). The head molds are customized to each monkey's head (Fig. 1B). The bar clamp is attached to the clamp attachment (3 in Fig. 1A), which is inserted into the extender (2 in Fig. 1A). This extender is held on the primate chair by means of the holder (4 in Fig. 1A). All of the attachments and the extender are secured by tightening screws.

2.1.2. Manufacture of the head-holding device

The entire procedure for making the head-holding device takes only a few hours. During the procedure, the monkey sits in a primate chair under mild sedation with ketaset (5 mg/kg i.m.). Important points are (1) preventing the hot Aquaplast from touching directly to the monkey's head; (2) molding the device to fit the monkey's neck as well as the monkey's occipital, parietal, temporal, zygomatic and mandible bones for secure holding of the head; (3) shaping the mold quickly before the Aquaplast hardens; and (4) preventing the mold from covering the face, including the regions around the mouth, nose, eyes and forehead, to allow comfortable task performance.

During the procedure, we cover the monkey's face, neck, chin, and ears with a thin and soft underpad (<1 mm thickness, McKesson #477563, Waltham, MA) that is waterproofed outside and has fluffy filler inside to prevent heat from the Aquaplast from reaching the monkey's skin. We attach the bar clamp to the primate chair for preparation. We boil water in a flat pan and put a 23 cm × 36 cm sheet of Aquaplast into the hot water. At >70 °C, the Aquaplast becomes soft and transparent. We then wrap the softened Aquaplast sheet around the monkey's head as shown in Fig. 1B. We mold and cover the monkey's neck as well as the monkey's occipital, parietal, temporal, zygomatic and mandible bones precisely with gloved hands. This step is essential for secure fixation of monkey's head. The Aquaplast hardens in about 15 min. Just before this time, the head mold is separated into left and right halves by carefully cutting the center of the mold with heated or non-heated scissors. After the head mold becomes hard, we then make a joint with the bar clamp. We cover and wrap the tip of the left arm and the tip of the large screw on the right arm of the bar clamp with the smaller sheets of the Aqaplast, which have been heated in the hot water bath. While these smaller Aquaplast sheets are still transparent and flexible, we slide the right arm of the adjustable bar clamp and tighten the large screw into the head mold. We push the head mold tightly from both sides and make sure that it attaches to the bar clamp, and we then allow the Aquaplast to harden. Finally, we wrap the underpad to the head mold with tape to make a cushion between the monkey's head and the device.

2.1.3. Attachment procedures

We first place an attachment bracket (3 in Fig. 1A) on a horizontal metal bar of the bar clamp (1 in Fig. 1A). We then insert the projecting part of the bracket (3 in Fig. 1A) into the holding pocket of the extender (2 in Fig. 1A). The extender is held by a holder (4 in Fig. 1A) which is attached to the primate chair. We tighten the screws to make all parts of the head-holding device stable. In each session, with the monkey in the primate chair, the head-holding device is carefully attached to the chair from the back. After the head mold is put on the sides of the monkey's face, we first slide the right arm of the clamp (1 in Fig. 1A) to adjust roughly the distance, and we then tighten the large screw. When we remove the device, we loosen the large screw, and then slide the right arm to release holding.

2.1.4. Adaptation of the head-holding device for squirrel monkeys

We also made a smaller version of the macaque head-holding device to fit to squirrel monkeys (see Fig. 1G). We used a 10-cm-long bar clamp from the same company (Jorgensen 3904-LD, Chicago, IL). This head-holding device has the same design as the device for macaques and achieved successful stabilization of the squirrel monkey's head.

2.2. Experimental application of the head-holding device

We collected behavioral and neuronal data while two macaque monkeys, fitted with the head-holding device, performed