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Non-invasive primate head restraint using thermoplastic masks

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HIGHLIGHTS

- Individualized thermoplastic masks restrain awake monkeys' heads non-invasively.
- Masks suppress movement sufficiently for electrophysiology and eye-tracking.
- Compared to head-posts, masks cost less and better enable MRI and TMS.

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ABSTRACT

Background: The success of many neuroscientific studies depends upon adequate head fixation of awake, behaving animals. Typically, this is achieved by surgically affixing a head-restraint prosthesis to the skull.
New method: Here we report the use of thermoplastic masks to non-invasively restrain monkeys' heads. Mesh thermoplastic sheets become pliable when heated and can then be molded to an individual monkey's head. After cooling, the custom mask retains this shape indefinitely for day-to-day use.
Results: We successfully trained rhesus macaques (*Macaca mulatta*) to perform cognitive tasks while wearing thermoplastic masks. Using these masks, we achieved a level of head stability sufficient for high-resolution eye-tracking and intracranial electrophysiology.
Comparison with existing method: Compared with traditional head-posts, we find that thermoplastic masks perform at least as well during infrared eye-tracking and single-neuron recordings, allow for clearer magnetic resonance image acquisition, enable freer placement of a transcranial magnetic stimulation coil, and impose lower financial and time costs on the lab.
Conclusions: We conclude that thermoplastic masks are a viable non-invasive form of primate head restraint that enable a wide range of neuroscientific experiments.

1. Introduction

Neuroscientific studies in awake, behaving non-human primates make immense and unique contributions to our understanding of brain function. Intracranial electrophysiological recordings offer unparalleled spatial and temporal precision compared with techniques like functional magnetic resonance imaging (Wurtz and Sommer, 2006), and are of particular value in the monkey due both to structural and functional homologies with human brains and complex cognitive behavior (e.g. Adams et al., 2012; Chang et al., 2013; Goulas et al., 2014, 2014; Grefkes and Fink, 2005; Hutchison et al., 2012; Miranda-Dominguez et al., 2014; Tsao et al., 2008). Such recordings often require the animal's head to be immobilized to ensure stability of the recording electrode (but see (Roy and Wang, 2012; Schwarz et al., 2014) for examples of wireless recordings in freely-moving primates). Head immobilization further facilitates eye-tracking (Kimmel et al., 2012), a crucial component of many neuroscientific studies. Other techniques facilitated by head fixation include microstimulation (Tehovnik et al., 2006) and drug delivery via intracranial injection (Kurata and Hoffman, 1994; Roy et al., 2014; Sommer and Wurtz, 2006) or a nebulizer (Chang et al., 2012). Recent interest in the neural mechanisms underlying transcranial magnetic stimulation (TMS) has also focused attention on electrophysiological recordings in the monkey before and after TMS

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(Mueller et al., 2014), and TMS application requires the head to be stationary.

The wide array of neuroscientific methods that depend on primate head fixation has led to the development of several immobilization devices. The majority of these devices involve implantation of screws or bolts into the subject's skull, as well as the attachment of additional hardware – most commonly a head-post – that protrudes from the head and can be attached to the primate chair during experimental sessions (Adams et al., 2007; Betelak et al., 2001; Evarts, 1968; Foeller and Tychsen, 2002; Porter et al., 1971). An alternative technique is the halo device, in which an aluminum ring surrounds the skull and is attached by several skull pins which require a smaller amount of skin to be removed and shallower penetrations into the skull (Friendlich, 1973; Isoda et al., 2005; Pigarev et al., 1997, 2009).

While the stability and biocompatibility of such implants continues to improve, they still carry five main disadvantages. First, the animal typically needs to be placed under general anesthesia for the initial surgical attachment of the device to the skull. Although routine, anesthesia is a risky procedure, with a wide range of potential harmful side-effects like hypothermia, hemorrhage, aspiration, respiratory insufficiency, cardiovascular emergencies, and death (Thurmon et al., 1996). Second, post-operative recovery can be stressful for the animal since analgesic and antibiotic drugs need to be administered intramuscularly while the animal is awake, and socially-housed animals must be separated from one another. Third, implanted head-restraint devices carry the risk of failure and detachment from the skull, particularly when they are under pressure during repeated head fixation. Implant failure requires an emergency surgical procedure to close the wounds on the animal's head and/or re-attach the device. Fourth, the protrusion of these devices themselves, as well as the hardware on the primate chair to which they attach, impede the application of TMS. During TMS, the large stimulating coil must lie flush with the scalp, centered over the brain region of interest, and the thick wires attaching the coil to the stimulator must have space to emerge from the set-up. Finally, metal implants on an animal's head cast shadows during magnetic resonance imaging, thus limiting brain scan utility.

A recent innovation uses a two-piece plastic head mold and a bar clamp holder to restrain monkeys' heads during experiments, and does not require surgery or metal implants (Amemori et al., 2015). However, the thick plastic mold and large bar clamp create potential barriers to TMS delivery. The design may also limit intracranial recordings or injections in far posterior and temporal regions. Furthermore, the plastic mold covers the animal's ears, potentially muffling sound and interfering with auditory testing. Finally, while the authors report that they were able to record neuronal activity and gaze position stably using this head-fixation technique, they note that animals do still move their heads during reward delivery, a potential confound for studies of reward-related processing.

Here we describe a non-invasive means of primate head restraint that mitigates the problems described above. Thermoplastic masks are plastic mesh sheets that become soft upon heating and can be molded to any shape, which they retain when cooled. Machado and Nelson (2011) adapted thermoplastic masks, which are used in human radiotherapy to stabilize the head, for eye-tracking in macaques. We expand upon their innovation by using these masks for electrophysiological recordings and TMS in macaques. We present a comprehensive comparison of the performance of thermoplastic masks and traditional head-posts on multiple measures relevant to neurobiology, including single-unit recording stability, eye-tracking accuracy, TMS coil placement, MRI clarity, and financial cost to the lab. We find that thermoplastic masks compare favorably with implanted head-posts on each of these metrics.

2. Materials and methods

All procedures were approved by Duke University Medical Center's Institutional Animal Care and Use Committee.

2.1. Subjects

Subjects were 24 adult rhesus macaque monkeys (*Macaca mulatta*), 10 female and 14 male, housed individually or in pairs at the Duke University Medical Center. 18 monkeys had head-posts, 5 monkeys had thermoplastic masks, and 1 monkey had a head-post that failed and was subsequently given a thermoplastic mask. Information from all monkeys was used in calculating cost to the lab. Eye-tracking data were collected from one mask monkey (Monkey Fe: female, 19 years old, 7.6 kg) and one post monkey (Monkey Br: male, 17 years old, 13.4 kg). Neuronal recording data were collected from one mask monkey (Monkey Sc: female, 17 years old, 6.8 kg) and one post monkey (Monkey Da: male, 17 years old, 10 kg). Implant size data were collected from two mask monkeys (Monkey Fr: female, 7 years old, 5.2 kg; Monkey Sc: female, 17 years old, 6.8 kg) and two post monkeys (Monkey Go: female, 7 years old, 5.4 kg; Monkey Br: male, 17 years old, 13.4 kg). Magnetic resonance images were collected from one mask monkey (Monkey Sc: female, 17 years old, 6.8 kg) and one post monkey (Monkey Br: male, 17 years old, 13.4 kg).

2.2. Thermoplastic masks

2.2.1. Fitting

The process of fitting a thermoplastic mask and applying it to an awake monkey is shown in Fig. 1. We used a commercially-available reinforced thermoplastic mask (Type-S IMRT Reinforced Style 22 Mask, product number MTAPUID2232, from CIVCO Medical Solutions, Coralville, Iowa, USA). Since the mask would be used while the monkey was seated in a primate chair, it needed to be molded while the monkey was in a chair, since the angle of the monkey's head relative to the chair neckplate needed to be replicated. For mask fitting, the animal was sedated with ketamine (3 mg/kg, intramuscular (IM)) and dexdomitor (0.075–0.15 mg/kg, IM), as well as ondansetron (0.15 mg/kg, IM) in cases where animals were known to become nauseated due to sedation. The monkey's head and face were shaved to achieve a closer fit, and petroleum jelly or vitamin E oil was applied to the animal's head and any existing implant (i.e. a recording chamber), as well as the top of the primate chair, to make the mask easier to remove. Ophthalmic lubricant was applied to the animal's eyes. The thermoplastic mask was submerged in hot water (at least 165 °F) until soft. It was then removed from the water and allowed to cool until it was comfortable to touch with a bare hand. A hole was cut near the center of the mask where the animal's nose would be to enable breathing. The monkey was then held in place in a primate chair by an experimenter who ensured that the animal's head and neck stayed in a proper position for normal breathing. Another experimenter placed the thermoplastic mask on the monkey's head and molded it to fit the contours of the face and head, with an emphasis on the bridge of the nose and the brow ridge. Adequate space was left at the back of the head and sides of the muzzle and neck such that the mask could be removed once it hardened. Locations for eye and mouth holes were marked on the mask. Once the mask had hardened, it was removed and the animal was returned to its cage and sedation was reversed with antisedan at the same dose volume as dexdomitor. Holes were cut in the eye and mouth regions of the mask such that the animal would be able to see and drink while wearing it. This entire procedure typically lasted 20–30 min. If any further modifications to the mask were needed, such as widening areas that seem too tight on the animal, a heat gun or boiling water was used to soften and reshape parts of the